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Review

Tongue carcinoma in young adults: a review of the literature

Il carcinoma della lingua nei giovani: revisione della letteratura

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SUMMARY

A recent reduction in the number of smoke-related tumours has been observed thanks to the diffusion of anti-tobacco campaigns carried out in the majority of developed countries. Nevertheless, as demonstrated by recent global epidemiologic studies, squamous cell carcinoma of the mobile tongue appears to be progressively increasing in incidence, particularly among young adults and especially in females. The driving mechanism responsible for such changes is still to be precisely defined. Several genetic studies have compared the mutational pattern of tongue squamous cell carcinoma in young adults to that of more elderly patients, without identifying significant differences that may help in better characterising this subgroup of subjects. Tongue squamous cell carcinomas in young adults have been historically considered as particularly aggressive clinical entities, with a high risk of loco-regional relapse, survival rates inferior to those of the general head and neck cancer group and need for a more aggressive therapy. However, considering the most recent studies, prognostic results in this patient group are heterogeneous and it is not possible to confirm this tendency. Thus, it is not justified to embrace different therapeutic approaches according to patient age. Eventually, an additional element to consider when examining young subjects affected by tongue cancer is the possibility of genetic predisposition. Alterations affecting pathways involved in DNA repair, surveillance of genetic stability or regulation of cellular growth may determine an increased likelihood of developing head and neck cancers.

KEY WORDS: Tongue carcinoma • Young • Epidemiology • Prognosis • Therapeutic approach

RIASSUNTO

Grazie alla diffusione delle campagne anti-fumo che sono state portate avanti dalla maggior parte delle nazioni sviluppate, negli ultimi anni è stata osservata una riduzione del numero dei tumori ad esso correlati. Ciononostante, come dimostrato da recenti studi epidemiologici mondiali, l'incidenza dei carcinomi squamosi della porzione mobile della lingua è progressivamente aumentata, in particolare nei giovani e specialmente nelle donne. Il meccanismo responsabile di questi cambiamenti epidemiologici non è ancora stato precisamente definito. Diversi studi genetici hanno confrontato il pattern mutazionale dei carcinomi squamocellulari dei giovani rispetto a quelli dei soggetti più anziani, senza identificare differenze significative che permettano una migliore caratterizzazione di questo sottogruppo di pazienti. Storicamente, il carcinoma squamocellulare nei giovani è stato considerato come un'entità clinica particolarmente aggressiva, con un rischio maggiore di recidiva loco-regionale ed un tasso di sopravvivenza inferiore rispetto alla popolazione generale dei pazienti con carcinomi testa e collo, che necessiterebbe di trattamenti più aggressivi. Considerando i più recenti studi, i risultati prognostici di questo gruppo di pazienti sono eterogenei e non è possibile confermare tale tendenza. Non sembra quindi al momento giustificato un approccio terapeutico differenziato in base all'età del paziente. Un altro elemento da prendere infine in considerazione quando si valutano pazienti affetti da carcinoma della lingua in giovane età, è la possibile presenza di una predisposizione genetica. Mutazioni che interessano i sistemi di riparazione del DNA, la sorveglianza della stabilità genetica o la regolazione della crescita cellulare possono determinare un aumento della probabilità di sviluppare un carcinoma del distretto testa-collo in giovane età.

PAROLE CHIAVE: *Carcinoma della lingua • Giovani • Epidemiologia • Prognosi • Approccio terapeutico*

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Introduction

Head and neck tumours are typically characterised by a peak of incidence in the elderly and a strong correlation with chronic exposure to risk factors such as smoking and alcohol abuse [1]. This is a consequence of the typical pattern of initiation and neoplastic progression which is encountered in these anatomical sites, characterised by an incremental accumulation of mutations leading, in

the long-run, to frank neoplastic transformation [2]. In this sense, the adult-elderly age has a central role in allowing the accumulation of a sufficient rate of genetic alterations to promote carcinogenesis [3 4].

Nevertheless, around 5% of patients suffering from head and neck cancer are diagnosed before they reach the age of 45 years. The incidence in this subgroup appears to be consistently growing, a trend that is in contrast to what observed in the general population. This phenomenon seems to be mainly linked to an increase of oropharyngeal carcinomas (mainly human papilloma virus [HPV]-related) and of the oral cavity (apparently not related to any known virus), paralleled by a reduction of laryngeal and hypopharyngeal tumours [5].

The aim of this review is to collect evidence published in the recent literature and pertinent to such an epidemiological trend, with special emphasis on oral tongue squamous cell carcinoma (SCC) in the young population. Due to the remarkable heterogeneity of studies assessing the molecular, epidemiological and clinical characteristics of tongue SCC, a non-structured review on all evidence available to date will be presented for each of these aspects.

Epidemiology

In oral cavity SCCs, gender distribution is different according to the age of onset taken into consideration: while in the elderly, males represent over 70% of cases, this percentage falls to 50-65% under 45 years of age. This difference has increased evidence when considering that the majority of non-smokers and non-drinkers young patients are females [6 7]. These epidemiological features may thus represent the expression of aetiopathogenic differences distinguishing the young subgroup of patients from the elderly.

Thanks to the diffusion of anti-tobacco campaigns carried out in the majority of developed countries, it has been possible to observe a recent reduction in the number of smoke-related tumours. Nevertheless, oral cavity SCC, in particular of the mobile tongue, as well as those of the oropharynx, appear to have a progressively increasing incidence [8]. While in oropharyngeal carcinomas this may be explained by an increasing exposure to the HPV, in the oral cavity, a specific pathogenic role for viral infections has yet to be demonstrated [9-11].

Tongue SCCs demonstrate an increasing incidence in the young population, as previously reported in the 1990s by a group of American authors [12-14]. These data were further confirmed by a global study specifically investigating tongue carcinomas and including a total of 22 tumour registries. This study highlighted a yearly increase ranging from 0.4% to 3.3% that was significantly higher in young patients (< 45 years) in 14 out of 22 registries [8]. In this comprehensive analysis, the greatest increase observed in females, particular in the younger cohort, was found only in some registries, while in others a homogenous distribution or even a male preponderance was shown [2 6 9 10 14 15]. In this context, it is however important to point out the difficulties in sorting out precise data for particular subgroups with homogenous clinical characteristics. Considering these recent epidemiological changes, some authors have hypothesised the involvement of a viral agent in the carcinogenetic process which may be known or yet to be defined, even though no specific viral genetic material has been consistently isolated in tongue SCCs of young patients [16 17].

Peculiarities of tongue tumours in young adults

From a clinical standpoint, tongue SCCs in the under-45 year-old group, particularly if non-smokers and non-drinkers, are found to prevalently originate from the tongue edge [16]. The concept of "field cancerisation", typically seen in the general head and neck cancer group, in these patients appears less evident, with a pattern of relapse/second tumour generally characterised by the involvement of the residual tongue in continuity with the primary neoplastic lesion [16 18 19]. However, no evidence of differences in histopathological features have been reported [20]. Some authors have also described the relapse tendency in the first two years of follow-up, with a significant decrease in risk following this lapse of time [21].

Considering the varied epidemiological characteristics, some genetic studies have compared the mutational pattern of tongue SCCs in young adults to that of more elderly patients. In the vast majority of publications, the two types of tumours have been shown to be similar and generally comparable to head and neck carcinomas [22]. The most frequently mutated genes have been reported to be TP53, CDKN2A, NOTCH1, CASP8, FAT1, PIK3CA and MLL2 in both groups of patients [23]. In the literature, however, heterogeneous results have been reported in relation to the prevalence of p53 mutations in young patients, with a tendency to a significant increase in some studies [23] and a decrease in others, in particular for non-smokers [24 25]. A recent analysis performed by Knopf and colleagues [26] has shed further light on the molecular mechanisms linked to the starting of tongue SCCs in patients below 45 years of age, highlighting a predominant alteration of the WNT-CTNNB1-STK11 and CDKN2A-HGF-MET pathways, as well as increased expression of ATM, BRCA1, E2F1,

and FHIT. Such a biological molecular landscape may be associated with greater radio-sensitivity in this patient population, which has significant consequences from the therapeutic and prognostic points of view [27-30].
However, these differences in genes mutation/expression may be mainly related to heterogeneous study populations: when analysing the extensive "The Cancer Genome Atlas" (TCGA) database, it is not possible to confirm any significant difference in relation to age, even considering the most recent genomic data [31 32]. Thus, tongue SCC in young patients appears to be comparable to those in elderly patients even when taking into account their genetic alterations.

Prognosis

Historically, tongue SCCs in young adults have been considered by many authors as particularly aggressive clinical entities, with a high risk of loco-regional relapse, and survival rates inferior to that of the general population and resulting need for a more aggressive course of therapy [12 33-35]. However, considering the most recent studies, the prognostic results in this patient group are heterogeneous and it is not possible to confirm this tendency [12 13 19 24 33 35-41]. This heterogeneity of results may also depend on the variable definition of "young age", with a wide range of cut-offs going from 30 to 45 years of age. In the future, to make the data more homogeneous and comparable, it has been suggested that it might be useful to consider as young patients only those below the age of 30 [42].
In the specific field of tongue SCCs, the analysis of a wide case series of 276 patients (66 below and 210 over the age of 45) performed by Knopf and colleagues [26] has revealed a significant prognostic advantage in younger patients. Regardless of this consideration, it is crucial to interpret this result as likely influenced by tumour extension, and thus T category, which is significantly lower in patients below 45 years of age (without differences in N and M categories). This selection bias is partly mitigated by case-matched studies characterised by a homogenous patient distribution in relation to tumour characteristics and comorbidities. Considering these types of studies, a broad cohort of 185 patients below the age of 45 affected by head and neck SCC, 80 of which of the oral cavity, compared with an analogous group of elderly subjects, demonstrated better results in terms of overall survival and second tumour occurrence in young patients [13]. Focusing the analysis on tongue carcinomas, 20 cases under-45 years compared with the same number of older subjects, matched by stage and gender, highlighted better overall and disease-related survivals in the younger subgroup [43].
On the other hand, some studies have demonstrated the absence of prognostically significant differences, taking into account 87 patients ≤ 45 years of age in a study by Lassig and colleagues [44] and 31 patients ≤ 40 years analysed by Pytynia and coworkers [45], both case-matched and including head and neck tumours in general. Finally, Blanchard and co-authors [46] have recently confirmed the absence of significant differences in 50 patients ≤ 40 years affected by tongue carcinomas versus older subjects matched by stage at presentation. It is nevertheless important to point out the outlier results on tongue SCC reported by at least three different studies [47-49], which showed a significant increase of relapses in young adults and a related negative impact on overall survival within this cohort. A non-significant trend towards poorer prognosis has also been reported by the group led by Kourelis [50] which considered only patients with early stage tongue SCCs. In particular, in young women, there has also been evidence of a significant increase in the risk of relapse compared to matched cases [50].

Genetically-determined conditions of increased susceptibility to tongue cancer

An additional element to take into consideration when examining patients affected by tongue SCC in the young age is the possibility of some sort of genetic predisposition [2]. Alterations affecting pathways involved in DNA repair, surveillance of genetic stability or regulation of cellular growth may determine an increased likelihood of developing head and neck cancers. This is clearly exemplified in rare hereditable syndromes linked to specific genetic mutations leading to a dramatically increased incidence of head and neck cancers and tumours in general. These are represented, in particular, by Fanconi anaemia, xeroderma pigmentosum, Bloom, Li-Fraumeni, familial atypical multiple mole melanoma, and ataxia telangiectasia syndromes. In all these diseases, the increased risk in developing tongue SCC (that may reach an incidence up to 700-1000 greater than that for the general population) can be associated with that reported for other neoplasms and syndromic presentations. The suspicion of a genetically-determined alteration should arise from a detailed family history of the proband positive for: 1) first-degree relative with the same kind of tumour and same clinical presentation; 2) two or more first-degree relatives with a tumour in the same location; 3) two or more first-degree relatives affected by rare tumours.
It is, however, important to note the existence of increased susceptibility to head and neck cancers even in the absence of syndromic presentations. Retrospective studies

have, in fact, demonstrated a relative risk that is increased 3.6-fold in subjects with first-degree relatives affected by other head and neck cancers [51-53]. In addition, young adults affected by tumours in these locations have been shown to be increasingly susceptible to genotoxic stress as shown by the bleomycin test, strongly suggesting the development of an increased number of chromosomal alterations in respect to older subjects [53]. In a similar manner, young adults affected by such neoplasms have shown an increased vulnerability to the negative effects of smoking (compared to healthy volunteers), a feature likely linked to an increased prevalence in this population of single nucleotide polymorphisms in genes involved in the repair of alcohol- and smoke-related mutations and in regulation of the cell cycle [54-56].

Conclusions

Tongue SCC in young adults represents a rare disease, characterised by an incidence that has been recently reported as increasing, particularly in females. However, no specific aetiopathogenic agent has been identified to date that may provide a univocal explanation for such a trend. As of today, it is not possible to demonstrate significant differences with respect to the general head and neck cancer patients group in terms of prognosis and, thus, a comprehensive therapeutic approach according to current guidelines should be recommended. In this view, thanks to the overall fitness of young patients, the "optimal treatment" according to tumour stage and characteristics may be frequently obtained, while it is essential to avoid unjustified overtreatments on the basis of patient's age alone.

In consideration of the patients' life expectancy, functional impairment and risk of recurrence, a long-lasting follow-up should be considered, with the aim of allowing an early diagnosis of persistent/recurrent disease, monitoring and optimising the patient's quality of life, and promoting awareness of adjunctive risk factors such as tobacco or alcohol consumption.

Patients presenting with associated syndromic characteristics, a suggestive family history and age of onset below 20-30 years might also require detailed genetic counselling to better assess the general disease framework.

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HEAD AND NECK

Electrochemotherapy: a well-accepted palliative treatment by patients with head and neck tumours

Elettrochemioterapia: trattamento palliativo ben accettato da pazienti con tumori della regione testa e collo

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SUMMARY

Electrochemotherapy (ECT) is a well established treatment strategy for skin tumuors. The aim of this study was to evaluate the feasibility and efficacy of electrochemotherapy in the palliative setting in patients with head and neck malignancies, in terms of improvement of quality of life and in control of pain and bleeding. Twenty-four patients with a loco-regional M0/M1 relapse not suitable for cure with radical intent by surgery or radiotherapy (RT) and not suitable for systemic therapy and/or already treated with it, were admitted to ECT protocol treatment. Clinical features, treatment response, and adverse effects were evaluated. An overall response of 100% was observed. Overall survival probability at 24 months was 46.5% (median OS: 9 months). The multiple application of ECT was associated with improved survival ($p = 0.02$). Pain, need for medical assistance or dressing and bleeding events was significantly reduced at 1 month after ECT ($p < 0.001$). ECT is effective as palliative treatment of non-resectable head and neck malignancies. Its main advantages are improved quality of life, local tumour control and limited side effects.

KEY WORDS: Electrochemotherapy • Electroporation • Head and neck cancer • Palliation • Supportive care

RIASSUNTO

L'elettrochemioterapia (ECT) è un trattamento palliativo ben noto dei tumori della cute. Lo scopo di questo studio è quello di valutare la fattibilità e l'efficacia dell'ECT in termini di miglioramento della qualità di vita intesa come riduzione del dolore e del sanguinamento. Ventiquattro pazienti con recidiva loco regionale e con +/– metastasi a distanza non suscettibili di trattamenti sistemici con intenti curativi né chirurgici, né radioterapici né chemioterapici sono stati sottoposti ad elettrochemioterapia in accordo alle line guida ESOPE. I pazienti sono stati seguiti per le caratteristiche cliniche, la valutazione della risposta al trattamento, e la comparsa di eventi avversi. È stata osservata una risposta globale del 100%. La sopravvivenza globale a 24 mesi è stata del 46,5% (mediana 9 mesi) Un incremento della sopravvivenza è stato associato a trattamenti multipli di ECT ($p = 0,02$). Si è osservato inoltre ad 1 mese dal trattamento con ECT una riduzione del dolore, della necessità di assistenza medica e di medicazione e del sanguinamento locale statisticamente significativo ($p < 0,001$). L'ECT si è rivelata un trattamento efficace nell'ambito della palliazione dei tumori ricorrenti/metastatici della testa-collo. I maggiori vantaggi sono il miglioramento della qualità di vita, il controllo loco-regionale con limitata tossicità ed effetti collaterali.

PAROLE CHIAVE: *Elettrochemioterapia • Elettroporazione • Tumori testa-collo • Palliazione • Terapia di supporto*

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Introduction

Worldwide, carcinomas of the head and neck (HNC) account for more than 5% of all malignancies, which are squamous cell carcinomas in 90% of cases [1]. Despite multimodal treatment, 50-60% of patients with stage III or IV disease relapse locoregionally. Of these, most are not suitable for salvage treatment and are eventually candidates for palliation [2].

In case of unresectable recurrent or persistent disease, Head and Neck 2015 NCCN guidelines recommend re-irradiation +/– systemic therapy, systemic therapy, clinical trial, or best supportive care [3]. These treatment options should be evaluated considering the patient's performance status (PS) and life expectancy.

Electrochemotherapy (ECT) is a well established treatment for cutaneous tumours consisting in the combination of elec-

troporation and chemotherapy [6]. Electroporation has been studied for approximately 20 years as a means of facilitating the transport of normally non-permeant molecules into cells. By applying an electric field to the cells, the membrane become permeable, allowing chemotherapeutic agents such as bleomycin to enter the cell[7], increasing its toxicity [8 9].

There are four different possible clinical applications of ECT: palliative treatment in case of advanced stage of disease; neoadjuvant role in the form of cytoreductive therapy; organ and function sparing treatment in patients in which conventional therapies cannot be performed; treatment of highly vascularised nodules [10]. The interest for ECT in treatment of the tumours in the H&N area has increased because specific clinical problems may arise due to failure or expected disfigurement of standard treatments. Many clinical reports described results of electrochemotherapy in treatment of H&N tumours [11-14].

The aim of this prospective study was to evaluate the feasibility and efficacy of ECT in the palliative setting in patients with recurrent inoperable head and neck malignancies not suitable for standard palliation by systemic chemotherapy.

Materials and methods

From April 2012 to April 2015 a total of 24 patients, observed at the Department of Otolaryngology Head and Neck Surgery of the National Cancer Institute Regina Elena, Rome, Italy (21 males, 3 females, age 37-88 years; mean: 69.9 years; median 70 years), with recurrent inoperable head and neck malignancies were submitted to ECT treatment. The clinical trial was approved by the institutional ethics committee and ECT indication was agreed by a multi- disciplinary tumour board for each patient (registration code RS 362/13). Each patient was asked to give written informed consent to participate to the study.

All patients had already been treated by multimodal therapy and, at the time of inclusion, presented with a locoregional M0 or M1 relapse not suitable for a cure with radical intent by surgery or RT, and not suitable for systemic therapy and/or already treated with it. Patient demographics and staging characteristics are shown in Table I. The histological characteristics of tumour were assessed.

Twenty patients (84%) were affected by squamous cell carcinoma (SCC), 1 skin melanoma (4%), 1 synovial sarcoma (4%), 1 adenocarcinoma (4%) and 1 mucoepidermoid carcinoma (4%). In 14 cases (58%), ECT was delivered at the primary tumour site, in 7 cases (29%) at laterocervical lymph nodes and in 3 cases (13%) at the primary site and laterocervical lymph nodes (Table I). Only palpable lymph nodes were treated, as revealed by staging. Cervical nodes metastases were all voluminous and palpable. All patients affected by recurrent, metastatic, or primary HNC not suitable for surgery or chemo/radiotherapy on the basis of poor general condition, age, cardiac deficit not related to electrical malfunction, reduced lung performance, comorbidities, high risk of major intra-postoperative complications, risk of anaesthesia, previous treatments, and when the surgery would be too aggressive to be curative, were eligible.

The technical procedure and patient selection were based on the ESOPE guidelines [15 16]. Inclusion criteria were: life expectancy longer than 6 months; measurable cutaneous or mucosal tumour lesions. Exclusion criteria included: clinically manifested arrhythmia, interstitial lung fibrosis, epilepsy, active infection, known allergy to bleomycin, kidney failure, previous treatment with bleomycin at the maximum cumulative dosage and different anticancer therapies administered within 2 weeks of the ECT [16 17]. Before treatment all patients underwent radiologic evaluation with CT and/or MRI to define the widest diameter of the lesion. RECIST criteria (Response Evaluation Criteria in Solid Tumors, version 1.1) were applied for evaluation of the results after ECT: *complete response* (disappearance of all target lesions; any pathological lymph nodes must have reduction in short axis to < 10 mm); *partial response* (at least a 30% decrease in the sum of diameters of target lesions); *progressive disease* (at least a 20% increase in the sum of diameters of target lesions; *stable disease* (neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease) [18].

All lesions were documented by photographs to evaluate aesthetic and functional results after treatment.

ECT protocol

The procedure was done under mild sedation. When feasible, local anaesthesia consisted of tissue infiltration with 2% lidocaine.

All patients received an intravenous bolus injection of 15,000 IU/m^2 of bleomycin. Eight minutes after the infusion electric pulses were delivered by different types of needle electrodes (hexagonal, or finger) chosen according to the site, volume and shape of the lesions to be treated, and generated by a Cliniporator™ (IGEA srl, Carpi, Italy). We used hexagonal electrodes in 20 patients (83%) and finger electrode in 4 cases (17%). Needles were inserted into and around the tumour lesions including 1 cm of safe margin. ECTs were completed within 25 minutes after intravenous administration of bleomycin.

Post-operative evaluation

Patients were evaluated one month after the treatment and every 3 months until 24 months. Tumour response was

evaluated according to RECIST criteria [13] (Table I). Pain was evaluated using a visual analogical scale (VAS) [19] varying from 0 (no pain) to 10 (extreme pain) before ECT and 1 months after ECT. At each visit, patients were submitted to the same examinations used during pre-operative evaluation (clinical and radiological, photographic). Furthermore, we evaluated hospitalisation time, number of dressings performed by medical staff and bleeding events 1 month before and after ECT. We delivered a single ECT treatment in 14 patients, 2 in 6 patients, 3 in 3 patients and 4 in 1 patient (Table I). In the poration column of Table I, the number of electrodes insertion for each treatment is also indicated. At each follow up visit, in case of evident and symptomatic persistence in terms of pain and/or bleeding, a new treatment with ECT was planned.

Statistical considerations

Endpoints included overall survival (OS), disease specific survival (DSS), safety, tolerability, pain control and post-operative care that impact on quality of life. DSS and OS were measured from the time of treatment until death and analysed using the Kaplan-Meier method. A Wilcoxon test was used where appropriate for continuous variables. A multivariate analysis was performed using the Cox proportional hazards model. Statistical significance was considered when $p < 0.05$. The SAS software was used for the statistical analyses (SAS for Windows, version 9.3, SAS Institute Inc., Cary, NC).

Results

ECT was completed successfully in all patients. All patients were discharged within 24 hours from admission. Post-operative bleeding events never occurred. No major complications were encountered. Post-operative fever was observed in 1 patient (patient 6) and was successfully managed using paracetamol. Post-operative pain was successfully managed at home using oral paracetamol in 23 patients (1 gm every 8 hours) for 5 days. Only in 1 patient were opioid medications required for pain control.

The mean follow-up time was 7.6 months (range 2-18 months). Main oncological results are summarised in Table I. OS probability at 12 months was 46.5%, while median OS was 9 months. DSS probability at 12 months was 63%. In 2 of 24 patients a complete response (CR) was observed, while a partial response (PR) was obtained in 22 patients. Twenty of 24 patients were histologically classified as SCC. Repeated treatment was planned at each follow-up visit in case of evident and symptomatic persistence and was necessary in 4 patients as pain and bleeding was still present.

Univariate and multivariate analysis were performed considering as prognostic factors age, gender, ECOG performance status, histology, TNM, application site, number of treatments received per month and use of adjunctive treatment. The only factor significant at univariate and multivariate analysis was the total number of treatments received (HR:0.20, CI:0.05-0.83, $p = 0.02$; HR:0.05, CI:0.00-0.71, $p = 0.02$, respectively; Table II).

Pain evaluation using the VAS scale showed significant pain reduction after ECT. Mean VAS score before treatment was 6.65 vs 2.77 at 1 month after ECT ($p < 0.001$, Table III).

Before treatment, patients referred to our centre a mean of 6.8 times in the last month for the local management (dressings). After treatment, patients referred to our center for a mean of 1.29 times per month. The difference was statistically significant ($p < 0.001$, Table III). Before ECT, 11 patients (46%) experienced local bleeding at least once per week. One month after treatment, only 2 patients (2%) complained of occasional bleeding ($p < 0.001$).

Discussion

Even if the majority of patients presenting with an early HNC will remain disease-free after single modality treatment, many patients presenting with an advanced HNC, relapse either locoregionally only, at distant sites only or both. A few patients with a locoregional recurrence can be salvaged by surgery or reirradiation, while most patients with recurrent or metastatic disease only qualify for palliative treatment [20].

Goals of treatment in these circumstances are mainly symptom control, prevention of new cancer related symptoms, improvement in quality of life, disease stabilisation and possibly prolongation of OS. Often it is necessary to combine local and systemic treatments to achieve the objective of yielding higher cure rates and lower toxicities in head and neck cancers [21].

In this setting, systemic chemotherapy remains a palliative alternative to best supportive care. Only the cisplatin/5-fluorouracil regimen (PF) and more recently the EXTREME trial (cetuximab + PF regimen, then followed by cetuximab as maintenance therapy) have been demonstrated to improve the OS rate [5 22].

According to NCCN guidelines, systemic therapy can be used in association only in case of good PS (0-1) (e.g. EXTREME [5]). The limitations of systemic therapy are in its toxicity.

The head and neck is a particularly complex anatomical region due to the presence of critical structures, such as carotid and cranial nerves, compacted in a small space. Thus,

Table I. Demographics and staging.

Patient	Gender	Age	ECOG PS	Histology and T site	TNM (ryc)	Application site	Response	Porations	Electrode	Treatment No.	Adjunctive treatment
1	M	52	4	Larynx SCC	T4aN2cM0	Tongue + LN	PR	20	Hexagonal	1	None
2	M	79	3	Ear melanoma	T4bN0M1	Retroauricular skin	PR	40	Hexagonal	1	None
3	M	59	2	Oral cavity SCC	T0N2cM0	LN	PR	31 - 50 - 57 - 90	Hexagonal	4	None
4	M	83	3	Oral cavity SCC	T0N2bM0	LN	PR	14	Hexagonal	1	None
5	M	71	2	Oral cavity SCC	T0N2bM0	LN	PR	20 - 20	Hexagonal	2	Cetuximab
6	M	75	2	Preauricular skin SCC	T1N2M1	LN + preauricular area	PR	48 - 59 - 50	Hexagonal	3	None
7	F	83	3	Oral cavity SCC	T4aN0M0	Buccal mucosa	PR	35	Hexagonal	1	None
8	M	69	2	Oral cavity SCC	T0N2cM0	LN	PR	36	Hexagonal	1	None
9	F	62	3	Oral cavity SCC	T4aN0M0	1/3 posterior tongue	PR	13	Finger	1	None
10	M	64	2	Parotid adenocarcinoma	T4aN0M1	Preauricular area	PR	13	Hexagonal	1	None
11	M	74	3	Oral cavity SCC	T4aN0M0	Chin skin/ oral cavity	PR	22	Hexagonal	1	None
12	M	88	4	External ear SCC	T0aN3M0	External ear	PR	52	Hexagonal	1	None
13	M	67	2	Larynx SCC	T4aN0M0	Peristomal recurrence	PR	67 - 50	Hexagonal	2	None
14	M	69	2	Oral cavity SCC	T4aN2bM0	Oral cavity + LN	CR	45	Finger	3	Methotrexate
15	F	37	1	Maxillary sinovial sarcoma	T4aN0M1	Maxillary area	PR	74 - 55 - 10	Hexagonal	3	None
16	M	79	2	Parotid mucoepidermoid carcinoma	T4aN0M0	Preauricular area	PR	65 - 82	Hexagonal	2	None
17	M	62	1	Frontal area skin SCC	T0N3M0	LN	PR	53	Hexagonal	1	Extreme
18	M	75	3	Oropharynx SCC	T0N2bM0	LN	PR	30 - 32	Hexagonal	2	None
19	M	87	3	Oral cavity SCC	T0N3M0	LN	PR	21	Hexagonal	1	None
20	M	85	2	Frontal area skin SCC	T0N2M0	Preauricular area	PR	25	Hexagonal	2	None
21	M	62	4	Chin skin SCC	T4aN0M0	Chin skin	PR	96	Hexagonal	1	None
22	M	68	3	Oral cavity SCC	T4aN0M0	Floor of the mouth + lips	PR	52	Finger	2	PDT
23	M	75	3	Oral cavity SCC	T4aN0M0	Oral cavity mucosa	PR	35	Finger	1	None
24	M	53	2	Oral cavity SCC	T4aN0M0	Buccal mucosa	CR	16	Hexagonal	1	None

SCC: squamous cell carcinoma; LN: laterocervical lymph nodes; PR: partial response; CR: complete response; PDT photodynamic therapy; ECOG PS: Eastern Cooperative Oncology Group Performance Status; TNM ryc= Tumour Nodes Metastases recurrent, persistent, clinical

Table II. Univariate and multivariate analysis of prognostic covariates.

Covariate	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Age	0.87	0.23-3.28	0.84	1.13	0.12-10.43	0.90
Gender	2.16	0.42-11.07	0.35	0.66	0.05-7.95	0.74
ECOG PS	1.97	0.68-5.69	0.20	0.24	0.02-2.88	0.26
Histology	0.50	0.06-3.99	0.51	0.11	0,00-7148.48	0.69
T	1.02	0.73-1.44	0.87	1.24	0.26-5.89	0.78
N	0.85	0.50-1.45	0.56	1,07	0.195.94	0.93
M	0.36	0.04-2.92	0.34	0.63	0.00-31421.06	0.93
Application site	0.55	0.18-1.63	0.28	0.91	0.01-42.14	0.96
Treatment No.	0.20	0.05-0.83	0.02	0.05	0.00-0.71	0.02
Adjunctive treatment	0.45	0.05-3.64	0.46	0.95	0.07-12.54	0.97

HR: hazard ratio; CI: confidence interval; PS: performance status; ECOG PS: Eastern Cooperative Oncology Group Performance Status

Table III. Pain and post-treatment management.

Pain (VAS)	Mean	Mean difference	SD	SD difference	p
Time 0	6.65		1.16		
PostT 1 month	2.77	3.87	1.01	1.21	< 0.001
Dressings					
PreT/month	6.83		1.16		
PostT/month	1.29	5.54	0.55	1.17	< 0.001

VAS: visual-analogic scale; PreT: pre-treatment; PostT: post treatment; SD: standard deviation.

tumours in head and neck cancer patients are hard to manage [13 23-29]. ECT results in a minimal or no functional impact and leads to healing of treated tumour lesions without damage to healthy tissues. For these reasons, ECT is described as an alternative to palliative chemo- or radiotherapy and partial and complete remission rates have been reported in various clinical trials with a low frequency of side effects [11 13 30-32].

Landstrom et al. carried out a phase II trial enrolling patients with early stage (T1-2) oral and base of tongue carcinomas treated with upfront ECT, followed by adjuvant radiation therapy. They observed no recurrence during the entire follow-up period and all patients were alive at 5 years and reported only mild local toxicity [33].

Bertino et al. in 2016 (actually the largest clinical trial focused on melanoma and non-melanoma skin cancers of the HN area treated with bleomycin electrochemotherapy) demonstrated that better responses are obtained with small lesions (≤ 3 cm), that primary tumours responded better than secondary (recurrent or metastatic) tumours and that treatment of naïve lesions responded better than pre-treated lesions. Interestingly, for recurrent tumour nodules, previous surgery least affected the outcome compared to (chemo) radiotherapy or multiple treatments [34].

Di Monta et al., in their retrospective, single-centre study, obtained a overall response after ECT treatment of stage III cSCC of 81% and CR of 22.7%. ECT is confirmed to display more effectiveness of other therapeutic options in locally advanced cSCC treatment [35].

In our study, a PR of 83.3% was observed, while only 2 patients (1.2%) presented a CR. Twenty patients (84%) were affected by SCC and in this group of patients we observed 10% CR and 100% PR. The low CR observed in comparison of literature data might be due to heterogeneity of our cohort including cutaneous or mucosal tumour localisation and for this reason not perfectly comparable to other studies. In our study, we performed univariate/multivariate analysis (Table II) considering as prognostic factors age, gender, ECOG performance status, histology, TNM, application site, number of treatments received per month and the use of adjunctive treatment. The only factor that resulted significant was the total number of treatments received. This result could be justified either as an ECT objective survival benefit or as a normal consequence derived from the prolonged survival by some patients. Hopefully, to better investigate the impact of the considered prognostic covariates, a more numerous sample is probably needed.

There are many potential benefits with the use of ECT, the most important of which is its reliability and versatility. The treatment can be performed in virtually every patient independently of the performance status. In fact, it does not require general anaesthesia: we performed all procedures in mild sedation, in an office-based setting, without performing a tracheostomy, even in patients with PS 4. The avoidance of intubation allowed us to better manage patients suffering from microstomia, oral cavity oedema, pain and trismus.

ECT is very simple to be performed, does not require a long learning curve and is repeatable every 30 days [13]. Probably the best indicator that electrochemotherapy is not too demanding or painful procedure is that among the interviewed patients, 20 of 24 (83.3%) would be willing to accept the treatment again if it indicated. From the first case, we were able to efficaciously complete every treatment without delays or complications. While systemic chemotherapy requires multiple administrations, each ECT treatment is completed in less than 25 minutes because after a single dose of intravenous bleomycin administration, the maximal plasmatic concentration is reached in 8 minutes, is maintained for about 20 minutes and then is gradually reduced. Additionally, any systemic chemotherapy using single or multiple agents imply a certain degree of toxicity that can result in a general impairment (e.g.: anaemia, leukopenia). In our study, we did not experience any major complications or prolonged hospitalisations. This means that ECT can be performed safely even in PS 3-4 without deterioration of quality of life. The main ECT contraindication is represented by lung fibrosis which could be exacerbated by bleomycin. In order to prevent this possibility, all patients performed arterial blood gas analysis the day before the application.

Even in case of partial response, ECT resulted in improvement, especially in terms of pain and bleeding reduction, and need for medical/paramedical care.

ECT by itself is responsible for a certain degree of post-operative pain which was successfully managed using oral paracetamol at home. Only in 1 patient were opioid medications required for pain control. Pain evaluation through the VAS scale resulted in a significant pain reduction after ECT ($p < 0.001$, Table III). This resulted in reduction in the administration of pain medications, which are considerably used and often abused by advanced stage HNC patients. A recent study showed that pre-operative pain, previous irradiation, large tumour size and high current values are predictors of post-operative pain. Knowing the risk factors, pain treatment can be better planned in advance [36].

The utility of ECT was also evident from the significant reduction of medical assistance (dressings, Table III). Patients were able to take care by themselves of the treated area by simple disinfection and dressings. They requested medical assistance only 1.29 times per month. These results depend partially on the significant reduction of bleeding events after ECT. In fact, bleeding was one the main reasons for medical assistance. One month after treatment, only 2 patients (8%) complained of occasional bleeding.

Reducing the need for the medical support results undoubtedly in an improvement in quality of life both from a psychological point of view (able to self-care) and from a practical point of view (fewer trips to the hospital, costs reduction).

ECT cicatrisation and pain/bleeding reduction is probably due to the 'vascular lock phenomenon'. In fact, blood flow changes occur after the delivery of electric pulses in vivo [37]. In the case of normal tissues, these effects appear as a transient hypoperfusion. In the case of tumour tissues, the vascular lock is much longer than in normal tissues and restoration of the initial blood flow levels may take hours. The mid-term and long-term antivascular effects of ECT could thus result from the killing of tumour endothelial cells, which could prevent the rapid reorganisation of tumour vasculature. Consequently, an almost permanent, extremely hypoxic situation is created after ECT [38].

A limitation of our study is the relatively small sample size and patient heterogeneity in terms of clinical features and treatment. Our study includes both primary and lymph node recurrences and patients who received concomitant palliative systemic chemotherapy. Our investigation on ECT is still ongoing and we aim to publish more data in the future with more representative samples, stratified by histology, recurrence site and concomitant treatments.

Conclusions

ECT was shown to be effective in the palliative treatment of non-resectable HNC. The advantages of ECT include improved quality of life and local tumour control, no damage to healthy tissue and limited side effects. We advocate its use in the palliative treatment of HNC, especially in patients with unfavourable PS.

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Head and neck

Clinical utility of an ultrasensitive thyroglobulin assay in the follow-up of patients with differentiated thyroid cancer: can the stimulation test be avoided in patients with an intermediate recurrence risk?

Utilità clinica del dosaggio ultrasensibile della tireoglobulina nel follow-up dei pazienti con carcinoma differenziato della tiroide: il test di stimolazione potrebbe essere evitato nei pazienti con rischio di recidiva intermedio?

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SUMMARY

Serum thyroglobulin (Tg) measurement during suppression with levothyroxine (LT4) using an ultrasensitive assay (OnT4-Tg) has been proposed as a replacement of TSH-stimulated Tg measurement (OffT4-Tg) in management of patients with differentiated thyroid cancer (DTC). The aim of this study is to evaluate the capacity of an ultrasensitive Tg assay in predicting an OffT4-Tg > 2.0 ng/mL based on the OnT4-Tg in patients with DTC and an intermediate recurrence risk. We analysed 101 patients with DTC and an intermediate (n = 92) or high risk of recurrence (n = 9) who were treated with total thyroidectomy and ablation with ^{131}I, and followed for an average of 6 years. OnT4-Tg was undetectable in 64 of 101 patients; OffT4-Tg was < 2.0 ng/mL in 61 of these 64 patients, all with negative imaging results. Furthermore, 37 of 101 patients had detectable OnT4-Tg; 32 of these 37 patients also presented OffT4-Tg > 2.0 ng/mL, and only 3 of these 32 patients had metastases detected by neck ultrasound. Considering a cutoff point of 0.1 ng/mL for OnT4-Tg, the assay had a sensitivity of 91%, specificity of 92%, positive predictive value (PPV) of 86% and the negative predictive value (NPV) of 95% when predicting an OffT4-Tg > 2.0 ng/mL (biochemical disease). The use of an ultrasensitive Tg assay allows prediction of which patients will remain disease-free even if they are at an intermediate risk of recurrence, and to decrease the need for stimulated Tg assays in two-thirds of these patients.

KEY WORDS: Thyroid cancer • Thyroglobulin • Highly sensitive thyroglobulin assay • TSH-stimulated thyroglobulin test • Neck ultrasound

RIASSUNTO

Il dosaggio della tireoglobulina (Tg) durante soppressione con levotiroxina (LT4) utilizzando la metodica ultrasensibile (OnT4-Tg) è stato proposto per sostituire il dosaggio della Tg dopo stimolazione con TSH (OffT4-Tg) nella gestione dei pazienti con carcinoma differenziato della tiroide (DTC). L'obiettivo dello studio è stato valutare la capacità del dosaggio ultrasensibile della TG di predire un valore di OffT4-Tg > 2,0 ng/mL, sulla base del valore di OnT4-Tg, nei pazienti con DTC e rischio intermedio di recidiva. Sono stati analizzati 101 pazienti con DTC, di cui 92 a rischio intermedio di recidiva e 9 ad alto rischio, tutti trattati con tiroidectomia totale e ablazione con ^{131}I, e seguiti mediamente per 6 anni. La OnT4-Tg è risultata indosabile in 64 pazienti su 101, mentre la OffT4-Tg è risultata inferiore a 2,0 ng/mL in 61 di questi 64 pazienti, tutti con imaging negativo. La OnT4-Tg è stata rilevabile in 37 pazienti su 101; 32 pazienti di questi 37 presentavano un valore di OffT4-Tg superiore a 2,0 ng/mL, e solo 3 di questi 32 avevano metastasi linfonodali apprezzabili all'ecografia del collo. Prendendo come valore cut-off 0,1 ng/mL per la misurazione della OnT4-Tg, tale dosaggio ha mostrato una sensibilità del 91%, una specificità del 92%, un valore predittivo positivo dell'86%, un valore predittivo negativo del 95%, nel predire un valore di OffT4-Tg > 2,0 ng/mL. L'utilizzo della metodica ultrasensibile del dosaggio della Tg ci permette di predire quali pazienti rimarranno liberi da malattia, anche se hanno un rischio intermedio di recidiva, e di evitare il dosaggio della Tg dopo stimolazione con TSH in due terzi di questi pazienti.

PAROLE CHIAVE: *Cancro della tiroide • Tireoglobulina • Dosaggio ultrasensibile della tireoglobulina • Test della tireoglobulina dopo stimolazione con TSH • Ecografia del collo*

Introduction

In recent years, the incidence of differentiated thyroid cancer (DTC) has notably increased worldwide [1]. It is the most frequent endocrine neoplasia and accounts for 1-2% of all cancers overall; it is more common in females than in males (4:1) [2]. After total thyroidectomy (TT) and ^{131}I therapy, most patients achieve disease-free status, but at follow-up approximately 15-20% have evidence of persistence and/or recurrence of DTC [3].

Thyroglobulin is a glycoprotein produced only by normal or well-differentiated malignant thyrocytes, and after TT and ^{131}I therapy, Tg levels should be undetectable; otherwise, detectable Tg levels would suggest disease recurrence or persistence [4]. The diagnostic precision of Tg as a tumour marker is high when the patient is treated with levothyroxine (OnT4-Tg), and it increases after stimulation with TSH either as a result of LT4 withdrawal or the administration of human recombinant TSH (rhTSH) [5], the availability of which is limited in Mexico. Usually, Tg is considered endogenously stimulated after LT4 withdrawal (OffT4-Tg), or exogenously stimulated (rhTSH administration), if levels are above 2.0 ng/mL or 1.0 ng/mL, respectively, and these values suggest that the patient is at risk of disease recurrence or persistence [6 7].

The measurement sensitivity of Tg kits has increased in the past few years whereby the functional sensitivity (FS) of immunometric assays improved from ~1.0 ng/mL to a FS < 0.1 ng/mL; this has opened the possibility of measuring OnT4-Tg and detecting persistent or recurrent disease without the need for stimulation with TSH.

Although some studies have reviewed the functionality of these new ultrasensitive assays by measuring Tg during patient follow-up and in the prediction of TSH-stimulated Tg, most have been conducted in cases of low-risk DTC; in general, the results suggest that TSH stimulation may be unnecessary when basal Tg is undetectable, thus causing fewer unwanted effects in patients and lowering overall costs [3 8-11]. Serum Tg measurement and neck ultrasound (US) are standard procedures in follow-up of patients with DTC [3].

The aim of this study is to evaluate the capacity of a serum Tg ultrasensitive assay in predicting an OffT4-Tg > 2.0 ng/mL based on OnT4-Tg in patients with DTC and an intermediate recurrence risk (ATA), using an OnT4-Tg cutoff of 0.1 ng/mL.

Materials and methods

Patient selection

One hundred and one patients with DTC were retrospectively evaluated; the group included 88 females and 13 males, with an average age at diagnosis of 39.9 years (range 10-75). They were diagnosed and treated between 1985 and 2010 at the Thyroid Clinic of the Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán"; the analysed serum Tg values were obtained between January 2010 and May 2015. The study was conducted in a tertiary care hospital and approved by the Ethics Committee.

All patients underwent TT and dissection of the central compartment (except 4 cases); 99 papillary and 2 follicular (2.0%) carcinomas were detected and all were also treated with ^{131}I for thyroid remnant ablation at doses between 100 and 150 mCi (3.7 to 5.5 GBq); in case of local recurrence or lymph node metastases, 200 to 250 mCi (7.4 a 9.2 GBq) were administered. The median cumulative ^{131}I dose was 150 mCi (IQR 150 to 300 mCi). Only 29 patients (28.7%) received a second dose or more of ^{131}I therapy, with a cumulative dosage of 250 to 750 mCi (9.2 to 27.7 GBq) due to local recurrence in cervical chain lymph nodes or distant metastases. Thirty of the 101 patients underwent cervical lymphadenectomy due to tumour recurrence. Most patients, 60 (59.4%) in clinical stage I, 7 (6.9%) were classified as stage II, 21 (20.8%) as stage III and 13 (12.9%) were stage IV (AJCC) [12]. After TT, whole body scan-post-ablation/treatment (WBS-PT) and initial neck US, patients were classified according to the modified 2009 risk stratification system of the American Thyroid Association (ATA) [3]. According to the ATA, 91.1% of participants had an intermediate risk and only nine cases (8.9%) were at high risk of recurring. The mean length of follow-up before the Tg stimulation test was 6.0 ± 4.5 years (range 3-30 years).

None of the 101 patients had structural evidence (neck US, WBS, neck and chest CT) of disease recurrence when Tg values were determined (OnT4-Tg/OffT4-Tg), and their OnT4-Tg levels were below 1.0 ng/mL and anti-Tg antibodies (TgAb) were negative. All patients underwent Tg stimulation (OffT4-Tg) by LT4 withdrawal for at least 4 weeks; before withdrawal, a basal Tg sample was obtained (OnT4-Tg) and both determinations also included TSH and TgAb values. The results of OffT4-Tg were considered accurate when combined with TSH value above 30 mIU/L.

All patients with a negative stimulated Tg, an OffT4-Tg < 2.0 ng/mL and a negative neck ultrasound, were considered disease-free and followed for 6 months with annual OnT4-Tg determinations and an annual neck US. Patients with a positive stimulated Tg, an OffT4-Tg > 2.0 ng/mL and suspicious neck images on US, underwent fine needle biopsy (FNAB) and Tg was measured in the needle washout (FNAB-Tg). In case the presence of

metastases or local lymphatic recurrence was documented, lymphadenectomy was performed and if necessary a therapeutic ^{131}I dose was administered. If suspicious images were absent in the neck US and the OffT4-Tg value was > 2.0 ng/mL, additional imaging studies such as WBS or neck and chest CT were requested. Positron emission tomography with fludeoxyglucose (18F) (18FDG-PET/CT) was only obtained in patients in whom ^{131}I refractivity was suspected [13].

Tg, TgAb and TSH determination assays

Since 2005, our institution has used a 2nd generation immunoassay to determine chemiluminescent Tg (Access Thyroglobulin Assay; Beckman Coulter, Fullerton, CA) with a FS < 0.1 ng/mL; the intra-assay coefficient variation (CV) was < 2.2% and the inter-assay CV was < 4.0% with a cutoff limit of 0.1 ng/mL. TSH was measured with a 3rd generation chemiluminescent immunoassay (Access HYPERsensitive hTSH assay; Beckman Coulter, Fullerton, CA) and TgAb values were determined by competitive radioimmunoassay with an analytic sensitivity of 2.0 IU/mL; values below 30 IU/mL were considered negative (TGAB ONE STEP; Cisbio Bioassays, France).

Imaging

Cervical ultrasound (US) was performed with a multi frequency (7.5-10 MHz) lineal transducer, integrated to colour Doppler. USs were obtained within 1-3 months before or after the OffT4-Tg; they were repeated every 6 months throughout routine follow-up. All suspicious findings [14] were biopsied with a fine needle (FNAB; gauge 23 to 25); tissue was aspirated with a 10 ml syringe and Tg was measured in the needle's washout (Tg-FNAB). WBS was performed in a SPECT-CT (Symbia T2; Siemens) with a high energy collimator and a scanning speed of 10 cm/min. Diagnostic WBS were obtained 3 to 5 days after the oral administration of 3-5 mCi ^{131}I (111 to 185 MBq) and the WBS-PT were performed on day 5 to 7 after radioiodine administration; the SPECT-CT was utilised since 2011. Chest and mediastinal computed tomography was performed in 5- to 10-mm thick sequential sections with a non-iodine contrast agent.

Statistical analysis

All results are expressed as median, interquartile range (IQR), mean ± standard deviation or percentages. The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were determined, as well as the accuracy of the OnT4-Tg at a cutoff point of 0.1 ng/ml. Data analysis was performed with IBM SPSS, version 21 (Armonk, NY, USA) statistical package.

Results

One hundred and one OffT4-Tg determinations were analysed, and the TSH values obtained during stimulation were > 30.0 mIU/L in all cases. The general characteristics of the study population are shown in Table I. The average TgAb concentration was 5.75 ± 3.6 IU/mL, the measured TSH level during LT4 withdrawal and Tg stimulation averaged 106.3 ± 72.6 mIU/L, with a median of 87.0 mIU/L (IQR 58.2 to 129.7). Median OnT4-Tg and OffT4-Tg were 0.05 ng/mL (IQR 0.02 to 0.21) and 0.61 ng/mL (IQR 0.10 to 4.3), respectively.

OnT4-Tg was < 0.1 ng/mL in 64 of the 101 patients, and in 61 of these 64 patients, OffT4-Tg was < 2.0 ng/mL; in all cases, the neck US was negative for nodal metastases.

Table I. General characteristics of the 101 patients.

	N	Percentages
Age at diagnosis (yr)	39.9 ± 14.9*	10-75**
Females	88	87.1
Males	13	12.9
Histological variety		
Follicular carcinoma	2	2.0
Papillary carcinoma	99	
Classic variant	55	54.4
Follicular variant	31	30.6
Tall cell variant	3	3.0
Diffuse sclerosing variant	6	6.0
Oncocytic variant	1	1.0
Solid/trabecular/insular growth pattern	3	3.0
AJCC stage		
T Stage		
T1	30	29.7
T2	17	16.8
T3	53	52.5
T4a	1	1.0
N Stage		
N0	17	16.8
N1a	32	31.7
N1b	48	47.5
Nx	4	4.0
M Stage		
M0	93	92.0
M1	8	8.0
ATA risk [3]		
Intermediate	92	91.1
High	9	8.9

**: average and SD; **: range; AJCC: American Joint Committee on Cancer, 7 ed.; ATA: American Thyroid Association.*

Thirty-seven patients had an OnT4-Tg > 0.1 ng/mL, and in 32 of these cases the OffT4-Tg was > 2.0 ng/mL. Among the 35 patients with evidence of biochemical disease (OffT4-Tg > 2.0 ng/mL), 13 had a Tg value > 10.0ng/mL during stimulation and only 3 had suspicious lymph nodes on US, subsequently confirmed by FNAB and Tg-FNAB; 2 patients underwent therapeutic lymphadenectomy and radioactive iodine was administered as single therapy in only one case. In the remaining patients in this group, all imaging studies (neck US, neck and chest CT, WBS or 18FDG-PET/CT) were negative; among the remaining 22 patients with a positive but < 10.0 ng/mL OffT4-Tg, further imaging studies were obtained aside from the neck US, but no structural or functional disease could be identified. No therapeutic procedures were performed and patients are being followed clinically with a "wait and see" attitude.

Using these cutoff limits, the test's sensitivity was 91%, the positive predictive value (PPV) was 86%, the specificity was 92%, the negative predictive value (NPV) was 95% and diagnostic accuracy was 92% (see Table II).

Discussion

In the last few decades, follow-up strategies of patients with DTC have changed. They are currently based on measurement of serum Tg and a neck US that together, yielded a NPV of 99 to 100% [11 15]. The high sensitivity of the second generation assays (functional sensitivity < 0.1 ng/mL) used to measure Tg appears to confidently predict increases in basal Tg and results of the TSH stimulated Tg either with rhTSH or LT4 withdrawal (OffT4-Tg), according to the two most often used cutoff limits for stimulation, 1.0 and 2.0 ng/mL [5].

Unlike previous studies, ours only included patients at intermediate and high risk of recurrence. We corroborated the fact that in this patient group, a negative stimulation test (OffT4-Tg) can be predicted if their basal Tg (OnT4-Tg) is < 0.1 ng/mL, if based on endogenous TSH stimulation and an OffT4-Tg cutoff limit of 2.0 ng/mL [3]. We found 64 patients with an OnT4-Tg < 0.1 ng/mL, and 61 had an OffT4-Tg < 2.0 ng/mL; the remaining 3 cases had an OffT4-Tg > 2.0 ng/mL, slightly above the cutoff limit but all below 3.1 ng/mL and with no evidence of structural disease. The NPV was very high (95%), similar to that previously reported [8-11 16 17].

Biochemical disease was detected in 32 of 37 patients with an OnT4-Tg > 0.1 ng/mL, but structural disease requiring treatment was identified in only 3 cases. The remaining patients in this group were reclassified as an incomplete biochemical response. The PPV was higher (86%) than that reported by other groups [9 15 17 18].

Controversies have arisen in terms of the use of Tg ultrasensitive assays: one in particular is the cutoff limit of unstimulated or basal Tg and although no consensus has been reached, most authors use a value of ≤ 0.1 ng/mL, as in our study. Most publications agree that ultrasensitive Tg is highly sensitive (88 to 97%) and has a NPV of 97 to 99%, suggesting that undetectable Tg in the absence of TgAb would preclude the need for a stimulated Tg test [5]. However, a recurring problem with which all publications coincide is its low specificity and PPV, with a resulting high percentage of false positive results, ranging between 15 and 20%, depending on the study [5 19]. Thus, if a patient has a Tg value ≥ 0.1 ng/mL, the possibility of a concomitant positive stimulated Tg test is, on average, slightly below 50% (PPV between 32 and 72%) [5]; this could expose a great percentage of patients to unnecessary testing and treatments [17]. However, perhaps the number of false positive results reported in other studies is directly related to the origin of the TSH used for Tg stimulation. Most studies have used rhTSH which neither reaches the levels nor the stimulus intensity provided by endogenous TSH. Spencer et al. measured Tg after stimulation with rhTSH in 1,029 samples after 72 hours. The median TSH was no greater than 23 mIU/L TSH [9]; although there are several studies in which rhTSH was used and the serum TSH peaks were above 30 mIU/L, it appears that the rhTSH-mediated peak is significantly affected by the patient's age, sex, height, weight, body surface area, body mass index, blood urea nitrogen, creatinine and glomerular filtration rate [20 21]. The fact that stimulation with rhTSH is inferior to that with endogenous TSH is a well-known fact and the Tg values obtained with rhTSH are also lower [22-24]; this has prompted some authors to reconsider the traditional cutoff limits originally used in OffT4-Tg and that were directly transferred to results using rhTSH. Recently, Kowalska et al. conducted a study in 63 DTC patients, comparing in the same subjects Tg values obtained after rhTSH stimulation

Table II. Sensitivity, specificity and predictive values of the OnT4-Tg with a cutoff point of 0.1 ng/mL.

Tg measurements (101 patients)	OffT4-Tg > 2 ng/ml N patients	OffT4-Tg < 2 ng/ml N patients	
Basal Tg > 0.1 ng/mL	32 TP	5 FP	PPV 86 % (71-95)
Basal Tg < 0.1 ng/mL	3 FN	61 TN	NPV 95% (87-99)
	Sensitivity 91% (77-98)	Specificity 92% (83-97)	

TP: true positive; FP: false positive; FN: false negative; TN: true negative; PPV: positive predictive value; NPV: negative predictive value. In parentheses, 95% CI.

and after OffT4-Tg; they also analysed the cutoff values for rhTSH-stimulated Tg (rhTSH/Tg). They determined that the rhTSH/Tg value with the greatest sensitivity and specificity equivalent to 2 ng/mL in the OffT4-Tg was 0.6 ng/mL, while a value of 10.0 ng/mL was 2.3 ng/mL [23]. One of the characteristics of our study is the predominance of individuals with an intermediate recurrence risk (91.1%), an unusual variable in this type of study since most include patients with a lower risk of recurrence. As in previous studies that included patients with a high risk of recurrence, we have confirmed that in most cases it is possible to predict the OffT4-Tg response based on the OnT4-Tg using an ultrasensitive immunoassay to determine Tg, in spite of an undetectable serum Tg < 0.1 ng/mL [15 17 18 25]. For this reason, several authors recommend replacing the stimulated Tg test by ultrasensitive basal Tg measurement, but only in patients with DTC and a low recurrence risk, since they mainly recruited DTC patients with a low risk [5 19].

One of our study's limitations is the relatively low number of patients, but this was the result of the exclusion of low risk patients and the participation of only one medical centre; however, it is compensated by the inclusion of patients with an intermediate risk and the use of endogenous TSH to stimulate Tg, a clearly unusual condition in published studies [25]. The obtained stimulation was significant, with a median TSH of 87.0 mIU/L (IQR 58.0-130.0), and reaching the recently recommended cutoff limit > 80 mIU/L or > 100 of TSH mIU/L, which leads to intense Tg stimulation in the OffT4-Tg test > 2.0 ng/mL [26]. Although low-risk patients were excluded, we must emphasise that 63% of patients had an OnT4-Tg < 0.1 ng/mL, with no evidence of structural involvement at the time of the study; therefore, in the reclassification system dependent on response to therapy ("dynamic risk"), they would be classified as excellent responders with a predicted risk of persistent/recurrent disease that is decreased in comparison with their initial estimated risk [27 28]. Although this argument may affect one of this study's strengths, we believe that this will become a daily clinical scenario when following patients with DCT and an initial high-risk for recurrence. This situation can be expected whenever only the initial or static risk is determined without considering the dynamic or delayed risk stratification [29]; few authors have made this point clear in recently published studies on the subject [17 25]. Nevertheless, 35% of our patients had biochemical evidence of disease, which is greater than the 22% reported in the intermediate risk group (this high number could be explained by the exclusion of patients with structural disease), and much greater than the 11% recorded in the low-risk group, evaluated 24 months after initiating treatment and with a dynamic risk stratification [27]. This should be analysed and taken into account when analysing our data.

Conclusions

The use of ultrasensitive Tg immunoassays allows prediction of which patients will be disease-free (NPV 95% and PPV 86%), even if they have an intermediate recurrence risk. Therefore, if individuals are TgAb (-), clinical follow-up may be simplified by measuring Tg during suppressive treatment with LT4 and a cervical US; these measures would optimise the endogenous TSH-stimulated Tg test, a costly test that is also uncomfortable for the patient; its use could be decreased in two-thirds of patients.

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Laryngology

Efficacy of microsurgery in Reinke's oedema evaluated by traditional voice assessment integrated with the Vocal Extent Measure (VEM)

Efficacia della microchirurgia nell'edema di Reinke analizzata con la valutazione vocale tradizionale integrata con la Vocal Extent Measure (VEM)

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SUMMARY

There are few data analysing to what specific extent phonomicrosurgery improves vocal function in patients suffering from Reinke's oedema (RE). The recently introduced parameter vocal extent measure (VEM) seems to be suitable to objectively quantify vocal performance. The purpose of this clinical prospective study was to investigate the outcomes of phonomicrosurgery in 60 RE patients (6 male, 54 female; 56 ± 8 years ([mean ± SD]) by analysing its effect on subjective and objective vocal parameters with particular regard to VEM. Treatment efficacy was evaluated at three months after surgery by comparing pre- and postoperative videolaryngostroboscopy (VLS), auditory-perceptual assessment (RBH-status), voice range profile (VRP), acoustic-aerodynamic analysis and patient's self-assessment using the voice handicap index (VHI-9i). Phonomicrosurgically, all RE were carefully ablated. VLS revealed removal or substantial reduction of oedema with restored periodic vocal fold vibration. All subjective and most objective acoustic and aerodynamic parameters significantly improved. The VEM increased on average from 64 ± 37 to 88 ± 25 ($p < 0.001$) and the dysphonia severity index (DSI) from 0.5 ± 3.4 to 2.9 ± 1.9. Both parameters correlated significantly with each other ($rs = 0.70$). RBH-status revealed less roughness, breathiness and overall grade of hoarseness (2.0 ± 0.7 vs 1.3 ± 0.7). The VHI-9i-score decreased from 18 ± 8 to 12 ± 9 points. The average total vocal range enlarged by 4 ± 7 semitones, and the mean speaking pitch rose by 2 ± 4 semitones. These results confirm that: (1) the use of VEM in RE patients objectifies and quantifies their vocal capacity as documented in the VRP, and (2) phonomicrosurgery is an effective, objectively and subjectively satisfactory therapy to improve voice in RE patients.

KEY WORDS: Vocal Extent Measure (VEM) • Phonomicrosurgery • Reinke's oedema • Voice function diagnostics • Voice range profile quantification

RIASSUNTO

Esistono pochi dati che mostrino di quanto specificatamente la microfonochirurgia migliori la funzione vocale in pazienti affetti da edema di Reinke (RE). Il parametro Misura di Estensione Vocale (VEM) recentemente introdotto sembra in grado di quantificare oggettivamente la performance vocale. Lo scopo di questo studio clinico prospettico era di investigare l'outcome della microfonochirurgia in 60 pazienti con RE (6 maschi, 54 femmine; 56 ± 8 anni [media ± SD]) analizzando il suo effetto su parametri vocali soggettivi e oggettivi con particolare riferimento al VEM. L'efficacia di trattamento è stata valutata tre mesi dopo la chirurgia confrontando la videolaringostroscopia (VLS), la valutazione percettiva-uditiva (stato RBH), il voice range profile (VRP), l'analisi acustica-aerodinamica e l'autovalutazione del paziente usando il voice handicap index (VHI-9i). Tutti gli RE sono stati attentamente rimossi con microfonochirurgia. La VLS ha mostrato la rimozione o la sostanziale riduzione dell'edema con ripristino della periodica vibrazione cordale. Tutti i parametri soggettivi e la maggior parte dei parametri oggettivi sono migliorati in maniera significativa. La VEM è aumentata in media da 64 ± 37 a 88 ± 25 ($p < 0,001$), il dysphonia severity index (DSI) da 0,5 ± 3,4 a 2,9 ± 1,9. Entrambe i parametri correlavano significativamente fra di loro ($rs = 0,70$). Lo stato RBH ha mostrato minore raucedine, voce soffiata e grado generale di disfonia (2,0 ± 0,7 vs 1,3 ± 0,7). Il punteggio del VHI-9i è diminuito da 18 ± 8 a 12 ± 9 punti. Il range vocale totale medio si è allargato di 4 ± 7 semitoni, la frequenza media del parlato è aumentata di 2 ± 4 semitoni. Questi risultati confermano che (1) l'utilizzo della VEM nei pazienti con RE rende oggettiva e quantifica la loro capacità vocale, come documentata dal VRP, e che (2) la microfonochirurgia è una terapia efficace e soddisfacente dal punto di vista soggettivo e oggettivo per migliorare la voce nei pazienti con RE.

PAROLE CHIAVE: *Misura di Estensione Vocale (VEM) • Microfonochirurgia • Edema di Reinke • Diagnosi della funzione vocale • Quantificazione del voice range profile*

Introduction

Reinke's oedema (RE) is a disease of the superficial lamina propria (SLP), also known as Reinke's space [1 2] and describes a pronounced chronic swelling of the SLP [3 4]. Reinke's space is very important for voice production and quality as it is responsible for vocal fold (VF) vibration. Impaired structural integrity reduces VF function and results in symptoms such as dysphonia [1]. The disease can be uni- or bi-lateral [5-8], and is one of the most common benign lesions of the larynx, showing very low tendency to malignancy [1 5 6 8-10]. Histopathologically, RE reveals epithelial basement membrane thickening in combination with oedematous lakes and increased submucosal vessel wall thickness, while the epithelium itself is smooth [5 11]. Fluid accumulates under the epithelium, an oedematous transudate which turns gelatinous later [8 12]. The bleach-white swelling is sessile and floating during phonation [5 6]. The initiating trauma remains unknown [3 5 8], although there is a strong association with smoking [1 3 7-10 13-15]. Further potential factors are vocal overuse and reflux [3 7 8 10 15]. Mostly there are several risk factors existent at the same time, which have a close interrelation [16].

RE is especially common among women > 40 years of age [4 10 17 18]. The voice sounds deep and hoarse [4 8 10 17 19]. Since there exists no efficient drug therapy, conventional microlaryngoscopic surgery with subsequent vocal rehabilitation is considered the best RE treatment [8]. Cessation of smoking alone is insufficient [20], and for long-term therapy success it is important to have voice therapy and abstinence from smoking post-operatively [3 8 17 19 20]. Most patients with RE consult a physician at an advanced stage, so that the illness usually lasts months or years [5]. Surgery is indicated in case of symptomatic dysphonia, airway obstruction and non-response to anti-reflux management, voice therapy and smoking cessation. Continued smoking is a relative contraindication for surgical intervention, as it induces RE recurrence [12].

There are scarse data investigating to what extent phonomicrosurgery in RE affects vocal function, and hence the specific benefits that patients can expect from the operation. Thus, this study examined phonomicrosurgical outcomes in RE by evaluating its impact on subjective and objective voice parameters. Particular attention was given to the vocal extent measure (VEM), a recently introduced and easy-to-use parameter, which seems to be suitable to objectively quantify vocal performance [21]. The intention was to investigate VEM changes with phonomicrosurgical treatment in RE patients and to compare its performance to that of established vocal parameters including the dysphonia severity index (DSI).

Materials and methods

Study design and participants

In a clinical prospective study, RE patients were treated phonomicrosurgically via direct microlaryngoscopy in intubation narcosis. Clinical examination and data collection was done at the initial pretherapeutic visit, during the operation and at regular follow-ups at 2 weeks and 3 months after surgery. Assessment of operation outcomes took place at the 3-month interval by comparing the pre- and post-operative data. In patients who missed this appointment, follow-up examination was set at a later date.

A total of 60 patients consecutively presenting at the Department of Audiology and Phoniatrics of Charité, University Medicine Berlin, Germany were included in the study. Selection criteria comprised: clear VLS-based finding, suffering from dysphonia or dyspnoea, absence of spontaneous RE resolution, lack of improvement under conservative therapy, complete treatment documentation and informed consent. The trial was conducted in accordance with the Declaration of Helsinki and approved by the local ethics review board.

Surgical procedure and post-operative care

RE were ablated phonomicrosurgically using the microflap technique. Surgery was performed bilaterally during the same session, preserving the anterior commissure to avoid web formation [8 12]. After inspection and palpation under the operation microscope, the superior/lateral VF aspect was incised with a sickle knife. The microflap between the epithelium and the polypoid material was raised using a 30° flap elevator. The exposed oedematous and gelatinous material was removed, mainly by suction. We left some material in the SLP behind to regenerate Reinke's space and preserve vibratory characteristics [3 12]. Finally, redundant epithelium was trimmed so that the edges of the flap kept closely with minimal mucosal dehiscence [12]. Post-operatively, patients were ordered voice rest for three days, followed by logopaedic vocal reestablishment. In addition, all patients received vocal hygiene counselling and were instructed to reappear in case of recurring voice impairment.

Examination instruments and criteria

A high-resolution rigid videolaryngoscope (10 mm; 70°) with integrated microphone was used to perform digital VLS (XION medical, Berlin, Germany) [22]. Laryngoscopy allowed RE classification according to Yonekawa [23]. RE type I is described as an oedematous swelling of the upper VF surface when the glottis is widely open. In RE type II,

the oedema extends from the upper to the lower surface and the VF touch only in the front section. RE type III is so extensive that there is only a small remaining space in the posterior part of the glottis. Patients with more localised findings too small to fit to type I were classified as marginal oedema. Furthermore, it was recorded whether the swelling was uni- or bi-lateral.

Standardised voice range profile (VRP) registrations and acoustic-aerodynamic analyses were performed with the DiVAS software (XION medical, Berlin, Germany) to obtain objective quantitative measurements of speaking and singing voice [21 24]. The following frequency, intensity and derived parameters were measured: lowest vocalisation (I_min), highest tone (F0_max), lowest tone (F0_min), mean speaking pitch (MSP_dB(A), MSP_Hz), maximum phonation time (MPT), jitter and DSI [25]. For better comparison of (logarithmical) frequency data in both genders (F0_max, F0_min, MSP_Hz), the corresponding amount of semitones related to the human hearing threshold of 16 Hz was used for further evaluation (ST_max, ST_min, ST_MSP). Derived from that, the total vocal range (VR) was assessed ([ST_max] – [ST_min]). Additionally, the VEM, a recently introduced measure for quantification of the patient's dynamic performance and frequency range, was calculated as a relation of area and perimeter of the VRP. The underlying idea is that VRP shape should not show abrupt differences in the dynamic range of notes along the frequency range. Well balanced dynamic extent approximates the VRP shape to a circle where the area is biggest for a given perimeter compared to other geometric figures. Each deviance from the idealised circular shape, where the dynamic range is evenly distributed over the tonal extent, indicates a decrease in vocal performance. Thus, the VEM multiplies the VRP area by the quotient of the VRP perimeter and the theoretical perimeter of a circle with the same area as the profile itself. The detailed mathematical derivation is explained elsewhere [21]. As a result, the VEM is scaled one-dimensionally to a range of 0 to 150, whereas these limits may be exceeded on both sides. A small vocal capacity is characterised by a small VEM; conversely, a large VRP results in a high VEM.

The RBH-system was used for auditory-perceptual voice evaluation when patients were reading the standardised text "The north wind and the sun" [26]. The perceived roughness (R), breathiness (B) and overall hoarseness (H) of the patient's voice was scored on a scale from 0 to 3 (0: normal, 1: mild, 2: moderate, 3: severe). To achieve objectivity, all 120 audio recordings were shuffled and blinded for patient allocation and pre-/post-operative status. Six raters (3 phoniatric physicians, 2 medical students, 1 medical technical assistant) independently rated all audio files. Further analysis was done using the mean group rating of each audio recording.

The Voice Handicap Index VHI-9i was applied for patient's self-assessment of voice [27]. They rated nine questions on a scale from 0 to 4 (0: never, 1: almost never, 2: sometimes, 3: almost always, 4: always), and one question regarding the self-perceived voice impairment at the present time (VHIs) on a scale from 0 to 3 (0: normal, 1: mild, 2: moderate, 3: severe). The questionnaires were filled out pre- and post-therapeutically to record subjective perception of vocal changes and to quantify the impact of the voice disorder on the patient's quality of life.

Outcome measures

The primary outcome measure was change of VEM at three months post-operatively. Secondary outcomes included surgery-induced differences of VLS as well as established objective and subjective vocal parameters assessing the scale of benefits which can be expected from the operation.

Statistical analysis

Descriptive statistics were calculated for all vocal function parameters before and after surgery, as well as their changes. Spearman's rank-order correlation (rs) was applied to examine the strength and direction of association between the pre- and post-operatively measured characteristics and their differences. The reliability of agreement among all six raters when assigning categorical RBH ratings to the blinded patient's voices was checked using Fleiss'kappa. The Mann-Whitney-Wilcoxon test served to analyse whether vocal function parameters significantly improved due to the treatment. Mean values and 95% confidence intervals for these changes were calculated. Significance was set at $p < 0.05$. All statistical tests and graphics were done with R version 3.2.2 (GNU project, Free Software Foundation, Boston, MA).

Results

Sample description and pre-operative assessment

The study cohort (n = 60) included 54 females and 6 males with an age ranged between 30 and 74 years (56 ± 8 years [mean ± SD]). Regarding medical history, 53 patients gave information about smoking, with only one RE patient being non-smoker. While 57% of participants (34/60) had no reflux, 8 patients indicated frequent symptoms and 18 patients suffered from reflux disease. Thirty-three individuals used their voice in a non-professional manner (e.g., business (wo)men, clerks, labourers), whereas 20 patients had

a high vocal strain in their profession (e.g., teachers, lecturers), 4 of them as elite vocal performers (singers). Subjects of both sexes were comparable in terms of age and sociodemographic characteristics (see Table I for details).

Laryngoscopy showed that 40 participants had bilateral and 20 patients unilateral RE (n = 10 at each side). Nineteen RE were classified as Yonekawa type I (32%), 29 as type II (48%) and 8 as type III (13%). Notably, none of the professional voice users had a RE type III. Four patients were grouped as marginal oedema (7%). Pre-operative analysis revealed significant differences between subgroups ($p < 0.05$), showing increasingly poor voice parameters with higher grades of oedema. Exemplarily, Figure 1 (left side) presents the pre-therapeutic findings for VEM, DSI and MSP_Hz in all subgroups.

Subjective auditory perception of voices in the total RE group was assessed with a mean of R2 B1 H2 (range 0-3). The VHI-9i had an average score of 18 ± 8, corresponding to moderate self-assessed complaints. Moderate impairment was further affirmed by the obtained acoustic-aerodynamic parameters (e.g., VEM 64 ± 37; DSI 0.5 ± 3.4; MPT 9 ± 5 sec.). The correlation analysis revealed that both DSI and VEM correlated with age (rs = – 0.39 and rs = – 0.36), R (rs = – 0.50 and rs = – 0.35), H (rs = – 0.49 and rs = – 0.40), VR (rs = 0.44 and rs = 0.74) and with each other (rs = 0.47). R and H correlated nearly perfectly (rs = 0.97), both criteria further correlated with B (rs = 0.36 and rs = 0.40), VHI (rs = 0.29 and rs = 0.28), and MSL_Hz (rs = – 0.42 and rs = – 0.38).

Post-operative assessment

All RE were completely ablated (39/60 patients) or significantly reduced (21/60). VLS showed no VF scarring. Within the mean post-operative observation period of 231 ± 287 days, no side effects or recurrences became apparent. Regarding functional aspects, patency of the glottis was regained and mucosal wave propagation was restored. The VF edge was fully straight in 39 cases with complete ablation (65%). All 21 cases with residual oedema (35%) showed substantial improvement with Yonekawa type I in 18 patients (30%) and type II in three patients (5%). Figure 2 gives a typical impression of pre- and post-operative objective findings in a RE patient initially classified as Yonekawa type III.

Regarding vocal function, all acoustic and aerodynamic parameters apart from MSP_dB(A) ($p = 0.29$) signifi-

Table I. Patient characteristics. Unless otherwise specified, data are expressed as number of patients and percentage of group.

Characteristics	No. of all patients	% of total group (n = 60)	No. of male patients	% of male group (n = 6)	No. of female patients	% of female group (n = 54)
Gender						
Male	6	10%	-	-	-	-
Female	54	90%	-	-	-	-
Age						
Years (mean ± SD)	56 ± 8	-	57 ± 11	-	56 ± 8	-
Main voice use						
Non-professional	33	55%	4	67%	29	54%
Professional	20	33%	2	33%	18	33%
Not stated	7	12%	NA	NA	7	13%
Reflux symptoms						
Reflux	26	43%	3	50%	23	43%
No reflux	34	57%	3	50%	31	57%
Edema classification						
Marginal oedema	4	7%	NA	NA	4	7%
Yonekawa type I	19	32%	3	50%	16	30%
Yonekawa type II	29	48%	2	33%	27	50%
Yonekawa type III	8	13%	1	17%	7	13%
Oedema occurrence						
Left vocal fold	10	17%	NA	NA	10	18%
Right vocal fold	10	17%	2	33%	8	15%
Both vocal folds	40	66%	4	67%	36	67%
Tobacco smoking						
Smoking	52	86%	6	100%	46	85%
Non-smoking	1	2%	NA	NA	1	2%
Not stated	7	12%	NA	NA	7	13%

NA: not applicable; No: number.

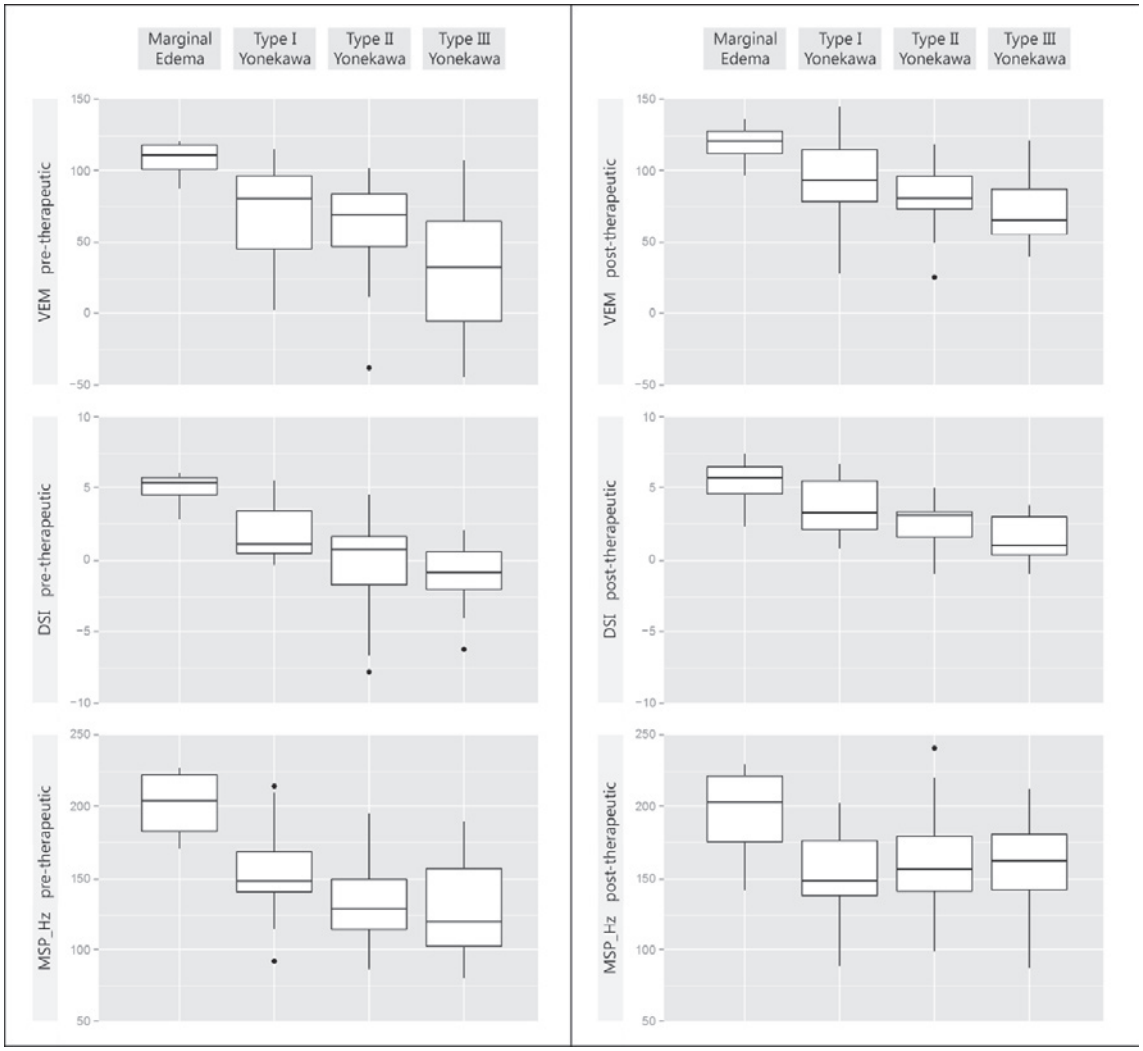

Fig. 1. Pre-therapeutic initial data (left) and post-therapeutic results (right) in all patient subgroups for selected vocal parameters (VEM, DSI, and MSP_Hz). The boxplots display the median, quartiles, range of values covered by the data and any outliers (single spots).

cantly improved at the 0.1% level (i.e. $p < 0.001$; pre vs post). The mean VEM increased by 25 ± 30 (64 ± 37 vs 88 ± 25), the DSI by 2.4 ± 3.1 (0.5 ± 3.4 to 2.9 ± 1.9). Both parameters correlated significantly with each other also post-operatively (rs = 0.70). Mean MPT increased by 2 ± 5 seconds (9 ± 5 vs 11 ± 4), MSP_Hz rose by 2 ± 4 semitones (142.6 ± 34.6 Hz vs 159.5 ± 33.0 Hz), and VR enlarged by 4 ± 7 semitones (17 ± 7 vs 21 ± 6). Selected objective parameters before and after RE removal are graphically displayed in Figure 3. Post-therapeutic findings for VEM, DSI and MSP_Hz were also itemised according to oedema subtypes (Fig. 1, right side). Inspection indicates convergence of therapeutic results, whereas pre-operative status affects the post-operative outcome. Analysis revealed significant differences between groups ($p < 0.05$), with greatest mean improvement in Yonekawa type III patients. To evaluate the magnitude of improvement and thus the extent of the operation-related benefit, Table II presents the mean differences between pre- and post-therapeutic values of both objective acoustic/aerodynamic parameters and subjective VHI-9i/VHIs vocal parameters and the 95% confidence intervals. These

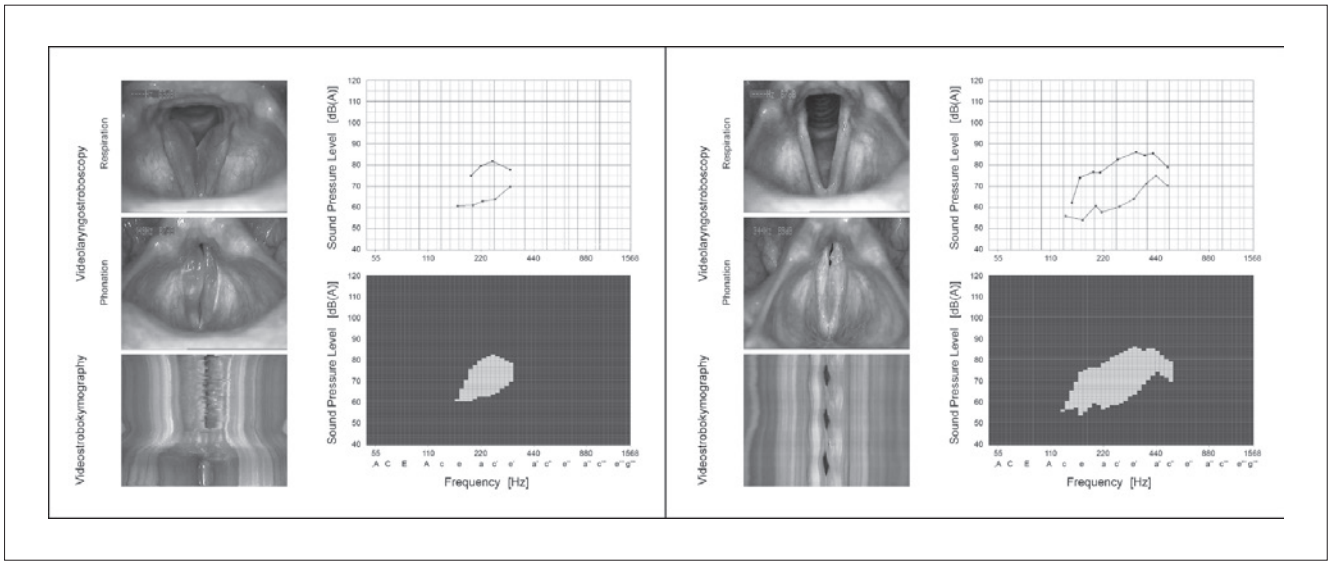

Fig. 2. Phonomicrosurgery-induced changes of objective findings in videolaryngostroboscopy [VLS], videostrobokymography, and VRP measurements in a 52-year-old female office worker with chronic manifestation of Reinke's edema (RE). Left: the preoperative status reveals bilateral RE (Yonekawa type III) with irregular vocal fold oscillations and absent mucosal wave propagation. The VRP envelope curves of loudest (black line) and softest singing (blue line) show small dynamic and frequency range. Polygon visualisation (green squares) depicts small VRP area, and VEM calculation results in a low value (VEM = 60). Right: three months postoperatively, the vocal folds present slim and irritation-free with a straight margin. The RE on both sides are completely removed, the healing process is finished (scar-free). The glottal closure is complete, vocal fold oscillations have normalised (mucosal wave propagation regular and symmetric). The VRP shows improved dynamic and frequency range with larger VRP area, hence VEM calculation results in a higher value (VEM = 100).

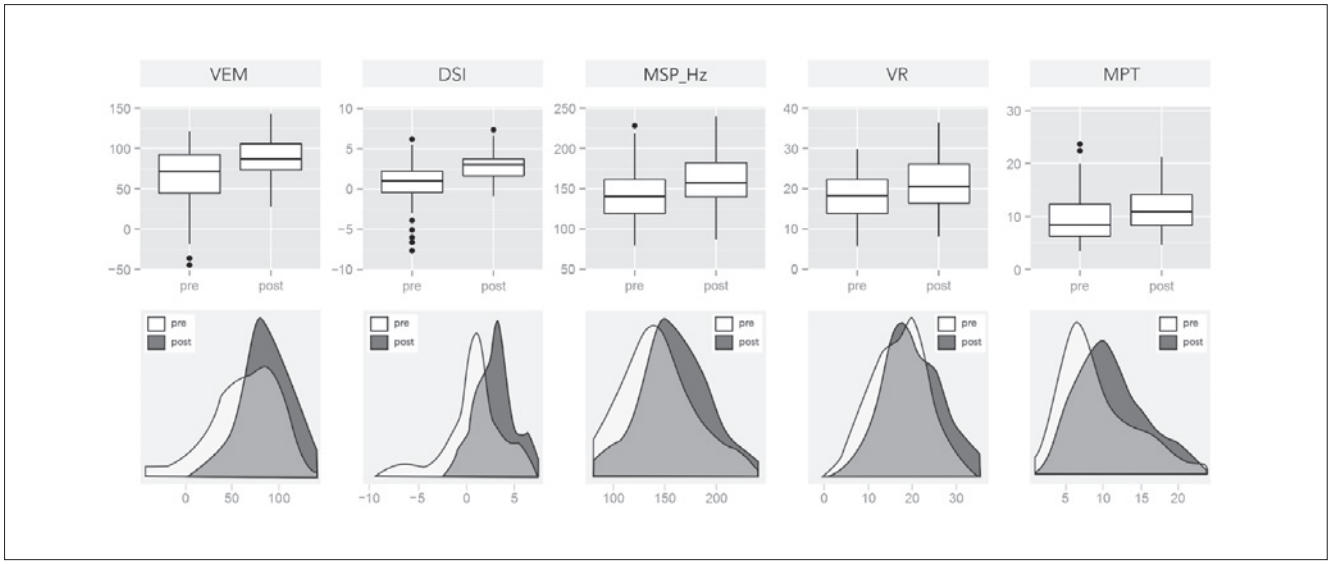

Fig. 3. Selected objective acoustic and aerodynamic parameters before and after RE removal (VEM, DSI, MSP_Hz, VR [semitones], and MPT [sec]) for all RE patients. Upper row: pre-/post-operative comparison via boxplots, which display the median, quartiles, range of values covered by the data, and any outliers (single spots). Lower row: pre-/post-operative comparison via kernel density curves (Gaussian smoothing), in which histograms were shifted and overlaid with smooth density estimates to illustrate the different distributions.

measures were calculated for the total RE group as well as separately for all oedema subtypes.

Concerning the auditory-perceptual evaluation using the RBH-system, blinded pre- vs post-operative comparison showed that voices were less rough (2.0 ± 0.8 vs 1.3 ± 0.7), breathy (1.2 ± 0.6 vs 0.7 ± 0.6) and hoarse (2.0 ± 0.7 vs 1.3 ± 0.7). The interrater reliability exposed fair agreement between the six raters ($\kappa = 0.35$). The VHI-

Table II. Changes in vocal measures after RE removal for all patients and oedema subgroups. Data expressed as mean differences of pre- and postoperative values (upper line), with 95% confidence intervals (lower line, in parentheses).

Vocal measure	RE total group (n = 60)	Marginal oedema group (n = 4)	Type I Yonekawa group (n = 19)	Type II Yonekawa group (n = 29)	Type III Yonekawa group (n = 8)
VEM	24.57 (16.89; 32.24)	9.41 (– 0.57; 19.38)	26.24 (12.59; 39.90)	20.77 (9.43; 32.10)	41.93 (12.23; 71.63)
DSI	2.41 (1.62; 3.20)	0.32 (– 1.06; 1.69)	1.87 (1.05; 2.69)	3.00 (1.53; 4.46)	2.61 (0.37; 4.84)
MPT	1.68 (0.48; 2.87)	2.10 (– 1.48; 5.68)	1.67 (0.51; 2.84)	2.20 (0.27; 4.13)	– 0.45 (– 6.58; 5.68)
VR	3.55 (1.79; 5.31)	1.23 (– 5.22; 7.67)	6.54 (1.75; 11.33)	1.86 (0.32; 3.41)	3.76 (– 0.42; 7.94)
I_min	– 2.50 (– 4.08; – 0.92)	– 0.25 (– 2.64; 2.14)	– 3.74 (– 6.83; – 0.64)	– 1.76 (– 4.15; 0.63)	– 3.38 (– 8.74; 1.99)
F0_max	112.55 (84.15; 140.95)	31.75 (– 116.55; 180.05)	118.26 (56.89; 179.64)	110.17 (72.59; 147.76)	148.00 (55.99; 240.01)
F0_min	16.72 (8.99; 24.44)	– 5.75 (– 27.85; 16.35)	– 0.74 (– 12.42; 10.94)	24.83 (16.29; 33.36)	40.00 (3.27; 76.73)
ST_max	6.28 (4.37; 8.19)	0.50 (– 4.78; 5.78)	6.26 (1.64; 10.89)	6.03 (3.95; 8.12)	10.12 (3.51; 16.74)
ST_min	2.70 (1.53; 3.87)	– 1.00 (– 3.25; 1.25)	– 0.21 (– 1.88; 1.46)	4.14 (2.71; 5.56)	6.25 (1.48; 11.02)
ST_MSP	1.92 (0.95; 2.88)	– 0.75 (– 3.14; 1.64)	– 0.47 (– 1.48; 0.53)	3.24 (2.03; 4.45)	4.12 (– 0.59; 8.84)
MSP_Hz	16.82 (9.26; 24.37)	– 7.00 (– 30.57; 16.57)	– 1.89 (– 11.36; 7.57)	28.14 (18.69; 37.59)	32.12 (– 1.05; 65.30)
MSP_dB(A)	0.55 (– 0.65; 1.75)	– 0.75 (– 6.32; 4.82)	– 0.05 (– 2.41; 2.31)	0.62 (– 1.23; 2.47)	2.38 (– 0.94; 5.69)
Jitter	– 0.83 (– 1.37; – 0.29)	0.00 (– 0.02; 0.03)	– 0.09 (– 0.18; 0.00)	– 1.42 (– 2.45; – 0.38)	– 0.86 (– 2.44; 0.73)
VHI	– 6.43 (– 8.57; – 4.30)	– 10.75 (– 19.79; – 1.71)	– 4.79 (– 9.04; – 0.54)	– 7.14 (– 10.46; – 3.81)	– 5.62 (– 10.56; – 0.69)
VHIs	– 0.72 (– 0.98; – 0.45)	– 0.75 (– 1.55; 0.05)	– 0.68 (– 1.19; – 0.17)	– 0.69 (– 1.10; – 0.28)	– 0.88 (– 1.82; 0.07)

VEM: vocal extent measure; DSI: dysphonia severity index; MPT: maximum phonation time; VR: vocal range; I_min: lowest vocalization; F0_max: highest tone; F0_min: lowest tone; ST: semitones related to the hearing threshold of 16 Hz (ST_max, ST_min, ST_MSP); MSP: mean speaking pitch [MSP_dB(A), MSP_Hz); VHI: voice handicap index; VHIs: self-perceived impairment of voice at the present time.

9i questionnaire showed a decrease from an average of 18 ± 8 to 12 ± 9 points. The VHIs demonstrated an improvement of the self-perceived impairment of the voice from moderately (2 ± 1) to mildly disturbed (1 ± 1). All these improvements were found significant at the 0.1% level. The pre- and post-therapeutic subjective vocal parameters are graphically displayed in Figure 4.

Discussion

Our pre-operative data confirms that RE most often develops in smoking and middle-aged women (> 40 years), with association to the risk factors vocal overuse and laryngopharyngeal reflux [1 3 7-10 13-15]. The initial distribution of oedema subtypes according to severity (Yonekawa classification) was conspicuous: type III occurred exclusively and type II more often in non-professional voice users, whereas in professionals (singers and speakers) a mild form of marginal oedema was seen more frequently. It seems that people who are reliant on their voice in their job are more sensitive to vocal changes, feel affected faster and therefore go earlier to see a voice doctor.

This study aimed at examining the outcome of phonomicrosurgery in RE by evaluating the changes in subjective and objective vocal parameters. Our results revealed a considerable improvement of vocal function. This corresponds to other studies concluding that microlaryngoscopic RE ablation has a beneficial effect on perceptual and acoustic voice parameters. However, most RE studies

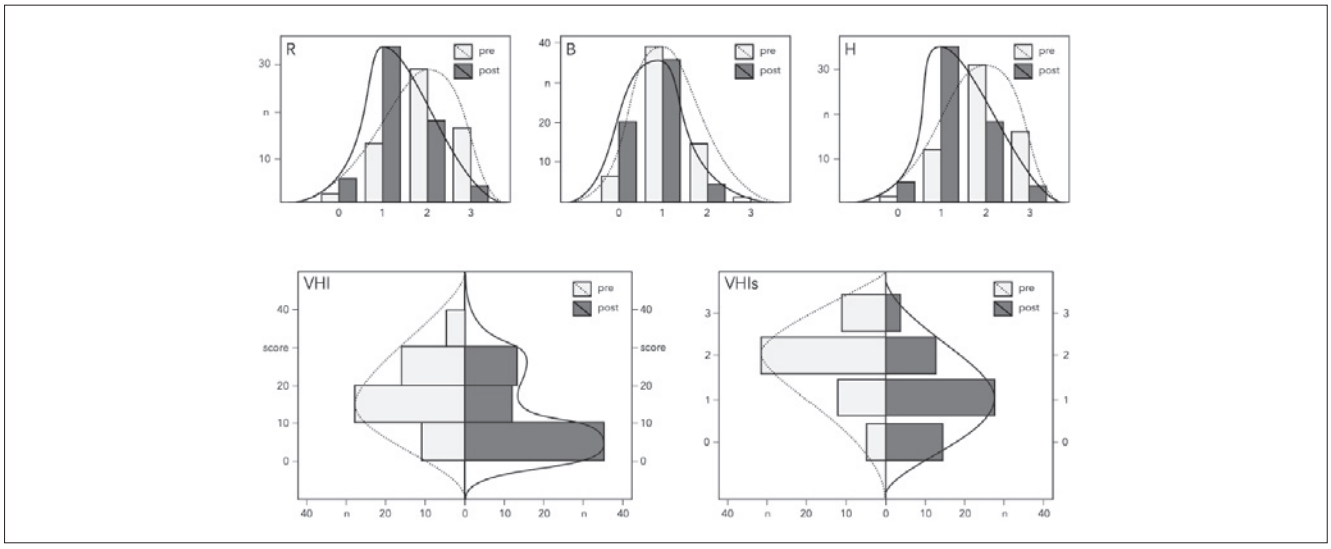

Fig. 4. Subjective vocal parameters before and after RE removal. Upper row: comparison of pre- and post-operative voice parameters according to the RBH-system. Lower row: comparison of pre- and post-operative VHI-9i and VHIs scores.

analysed the change in speaking fundamental frequency and subjective vocal parameters [28-30 32].

Lim et al. (2006) [29] studied 61 RE patients and 30 controls. They detected that the mean fundamental frequency was lowered: the more severe the oedema, the greater the difference with the control group. Re-evaluation of 23 patients two months after surgery showed, that the mean fundamental frequency was increased to almost normal values and vocal efficiency had improved. Šiupšinskienė and Skumanienė (2002) [30] compared different surgical techniques in the treatment of 62 RE patients. They showed that surgical preservation of VF medial edges mucosa yields best benefit for voice quality. As in our subjective results, reductions in grade of H and R were found and the voices were judged as improved in most cases. Still, the mean values of many voice parameters in early postsurgical period significantly differed from normal, but the evaluation took place on average after 24 days, which is quite short according to our experience. Tan et al. (2010) [31] presented an "M" shaped microflap for the treatment of bi-lobular and also centrally located RE using CO_2 laser and/or cold micro-instruments, allowing maintenance of an appropriate amount of SLP and easy coverage of the potentially exposed part of the ligament. This procedure was carried out in 11 patients with very good results in terms of fast recovery and improvement of voice quality; however, specific vocal parameters were not described in detail. Sant'Anna and Mauri (2000) [32] showed in a small population of 5 patients that resection of RE using the microdebrider also led to a satisfying vocal outcome. There was recovery in loudness and pitch, and voices changed from rough and breathy to normal or near-normal. This results from taking great care with the mucosa, SLP and vocal ligament to avoid iatrogenic scarring.

In our study, all subjective and objective parameters except for MSP_dB(A) improved significantly. MSP_dB(A) slightly increased post-operatively, suggesting that patients were able to speak louder. As many factors influence loudness of mean speaking voice, this parameter does not reliably reflect vocal capability. Most patients felt their voice to be more clear and stable, which was visually confirmed by VLS which showed regular and symmetric VF oscillations. Furthermore, MSP_Hz increased, and R and H decreased, so that the main characteristics of RE, a deep and hoarse voice, changed to the direction of a normal voice. The limited reliability of agreement between all six raters when assigning categorical RBH ratings to the blinded patient's voices seems to result from the reduced experience and training level in three of the participants (2 medical students, 1 medical technical assistant). According to the self-perception recorded with the VHI-9i-questionnaire, patients reported their voice to be significantly better after surgery. However, our study is lacking a control group to compare if patients in fact reached normal levels of MSP_Hz or if they remained below.

As a novelty, we collected a number of vocal parameters which can give further information about the magnitude of vocal improvement induced by phonomicrosurgery. Among the acoustic measurements, we especially inves-

tigated the VEM, which is not yet commonly established in phoniatric diagnostics, but has been demonstrated to be a suitable parameter for objective quantification of vocal performance in patients with VF polyps [21]. Even in RE patients, VEM and DSI correlated positively with each other and negatively with age as well as R and H. There was no correlation with B. This is understandable as R and H strongly correlated with each other indicating that roughness determines the impression of overall hoarseness in RE. The measurements VEM and DSI can be seen as comparable parameters that are related to each other. Dysphonia reduces the vocal capacity that can be measured by both parameters. However, whereas the DSI reflects the severity of dysphonia as a negative criterion, the newly developed VEM describes the vocal abilities and enables a classification of voice performance as a positive criterion. However, our investigations also confirmed differences between both parameters. Some patients had comparable DSI values, but revealed different VRPs with different values for the VEM. This example shows the significant influence of the recorded acoustic and aerodynamic parameters in the multidimensional DSI calculation. Previous studies also indicated that the DSI is influenced by differences of measurements in the registration programs as well as by age or gender [33 34]. Therefore, we saw the need to develop and investigate the VEM as an objective parameter unimpaired by these interacting factors. The VEM calculation avoids the inclusion of elements that are highly susceptible to interference (e.g. jitter). The VEM is also independent of pitch (i.e. women's and men's voices are evaluated in the same way) and vocal intensity (i.e. microphone distance has no influence on the assessment of the VRP). It represents a comprehensible and user-friendly objective measure of voice function and quantifies the VRP one-dimensionally by a single concrete value, instead of estimating it by visual perception and a few exposed values. The VEM is calculated automatically from the VRP and may be easily implemented into existing clinical protocols and evaluated. The resulting new parameter of vocal capacity provides additional information about voice function. Therefore, the introduction of the VEM in practical objective voice diagnostics is appropriate and desirable, complementing the established DSI.

Moreover, we examined the quantitative changes in vocal parameters caused by microlaryngoscopic excision of RE. The degree of improvement and the concrete extent of benefits are revealed by the mean differences between pre- and post-therapeutic voice parameters and 95% confidence intervals. The range of improvement of VEM (17 to 32), DSI (1.6 to 3.2), MPT (0.5 to 2.9 seconds), VR (2 to 5 semitones), MSP (1.0 to 2.9 semitones) and VHI (– 9 to – 4) could serve for quality control after phonomicrosurgical removal of RE as well as reference range for subjective and objective expectation values.

Conclusions

The use of VEM in RE patients objectifies and quantifies vocal capacity as documented in the VRP. Phonomicrosurgery is an effective and safe, subjectively and objectively satisfactory therapy to improve vocal function in patients suffering from RE. Essential requirements comprise a competent and precise microlaryngoscopic excision leaving some gelatinous material in the SLP, a normal post-operative course with regular wound healing, vocal rehabilitation and avoiding smoking to reduce the risk of RE recurrence.

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Laryngology

Efficacy of trans-nasal fiberendoscopic injection laryngoplasty with centrifuged autologous fat in the treatment of glottic insufficiency due to unilateral vocal fold paralysis

Efficacia della laringoplastica iniettiva fibroendoscopica trans-nasale con grasso autologo centrifugato nel trattamento dell'insufficienza glottica da paralisi cordale monolaterale

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SUMMARY

The objective of this work is to evaluate the safety, feasibility and efficacy of trans-nasal fiberendoscopic injection laryngoplasty (IL) with centrifuged autologous fat, performed under local anaesthesia, in the treatment of glottic insufficiency due to unilateral vocal fold paralysis (UVFP). It is a within-subject study with follow-up 1 week after phonosurgery and after 6 months. A total of 22 patients with chronic dysphonia caused by glottic insufficiency due to UVFP were enrolled. Each patient underwent trans-nasal IL with centrifuged autologous fat through flexible operative endoscope under local anaesthesia and was evaluated before and twice (1 week and 6 months) after phonosurgery, using a multidimensional set of investigations. The assessment protocol included videolaryngostroboscopy, perceptual evaluation of dysphonia, maximum phonation time and patient's self-assessment on voice-related quality of life (QOL) with the Voice Handicap Index-10 and the comparative self-assessment on vocal fatigue and voice quality pre-post treatment. Trans-nasal IL with centrifuged autologous fat was performed in all 22 patients and there were no complications in any case. Significant improvements in videolaryngostroboscopic findings, perceptual evaluation of dysphonia, maximum phonation time and QoL self-assessment were reported after 1 week and were maintained at 6 months. In one patient, the result after 6 months was not satisfactory and this patient then underwent a medialization laryngoplasty (thyroplasty type I) with satisfactory long-term results. In conclusion, trans-nasal fiberendoscopic IL with centrifuged autologous fat seems to be a safe, feasible and efficacious phonosurgical procedure for treatment of glottic insufficiency due to unilateral vocal fold paralysis.

KEY WORDS: Injection laryngoplasty • Autologous fat injection • Phonosurgery under local anaesthesia • Trans-nasal fiberendoscopic phonosurgery • Vocal fold paralysis

RIASSUNTO

L'obiettivo di questo lavoro è quello di valutare la sicurezza, la fattibilità e l'efficacia della laringoplastica iniettiva (LI) fibroendoscopica trans-nasale con grasso autologo centrifugato, effettuata in anestesia locale, nel trattamento della insufficienza glottica da paralisi cordale monolaterale (PCML). È uno studio intra-individuale con controlli a una settimana e 6 mesi dopo la fonochirurgia. Riguardo ai metodi, sono stati arruolati nello studio 22 pazienti con disfonia cronica causata da insufficienza glottica da PCML. Ogni paziente è stato sottoposto a LI trans-nasale con grasso autologo centrifugato mediante endoscopio flessibile operativo in anestesia locale ed è stato valutato prima e a distanza di una settimana e 6 mesi dall'intervento di fonochirurgia, utilizzando una serie di indagini multidimensionale. Il protocollo valutativo comprendeva la videolaringostroboscopia, la valutazione percettiva della disfonia, il tempo massimo fonatorio e l'autovalutazione da parte del paziente sulla qualità della vita in relazione al problema vocale mediante il Voice Handicap Index-10 e l'Autovalutazione Comparativa pre-post trattamento riguardo alla fatica fonatoria e alla qualità della voce. I risultati hanno mostrato che la LI trans-nasale con grasso autologo centrifugato effettuata nei 22 pazienti non ha avuto complicanze. Sono stati riportati miglioramenti significativi dei rilievi laringostroboscopici, della valutazione percettiva della disfonia, del tempo massimo fonatorio e dell'autovalutazione sulla qualità della vita ad una settimana dall'intervento di fonochirurgia, che si sono mantenuti anche a distanza di 6 mesi. In un paziente il risultato dopo 6 mesi non è stato soddisfacente ed è stato operato di laringoplastica di medializzazione cordale (tiroplastica di tipo I) con soddisfacenti risultati a lungo termine. In conclusione, la LI fibroendoscopica trans-nasale con grasso autologo centrifugato sembra essere una procedura fonochirurgica sicura, fattibile ed efficace per il trattamento dell'insufficienza glottica da paralisi cordale monolaterale.

PAROLE CHIAVE: *Laringoplastica iniettiva • Iniezione di grasso autologo • Fonochirurgia in anestesia locale • Fonochirurgia fibroendoscopica trans-nasale • Paralisi della corda vocale*

Introduction

Unilateral vocal fold paralysis (UVFP) is a frequent complication of surgical manipulation of the thyroid gland and mediastinal structures, which are located along the pathway of the recurrent laryngeal nerve [1-3]. Less than 50% of cases of vocal fold immobility caused by injury during surgery recover spontaneously within one year, while the remaining patients show persistent UVFP [1]. A growing number of idiopathic UVFP is documented [2-4]. In case of UVFP glottic insufficiency (GI) often occurs, which causes dysphonia and dysphagia and reduces patient's quality of life [5]. In addition, GI due to UVFP may significantly compromise postoperative recovery in patients with decreased pulmonary reserve or inability to protect their airways [6].

Voice therapy is usually the first choice in the treatment of GI due to UVFP. Its aim is to improve glottic compensation by the contralateral mobile vocal fold [7].

When voice therapy does not provide a positive effect, in particular when the glottic gap is wide because the immobile vocal fold (VF) lies in abducted (lateral) or intermediate position, phonosurgery may be indicated [8]. Several phonosurgical techniques for the treatment of GI due to UVFP have been proposed, which include medialisation laryngoplasty (thyroplasty type I) and/or arythenoid adduction [9] and neurorrhaphy [10]. Injection laryngoplasty (IL) is another widely applied phonosurgical technique [8]. IL is a safe and well-established procedure, frequently performed in the early treatment of GI [8 10]. Previous studies have shown that the improvement of glottic valvular function obtained with IL could improve symptoms of dysphonia and dysphagia [6 11-14]. In addition, early medialisation IL within 6 months of onset of UVFP may reduce the need of permanent external phonosurgery [6 14].

IL can be performed either under local or general anaesthesia using various alloplastic materials [10 14] or autologous fat [8]. The advantages of the local anaesthesia (LA) are noteworthy. First of all, evaluating and treating patients when they are awake and able to phonate allows a "functional" visualisation of the operative field, allows the phonosurgeon to monitor the effects of IL during and after the intervention. Moreover, IL under LA reduces costs of the procedure compared to a direct microlaryngoscopy procedure [15-17]. Finally, IL under LA may also be performed in patients with difficult laryngeal exposure and in patients with reduced neck extension or abnormal neck anatomy [8 11]. Several routes to reach the vocal fold (VF) during IL have been described: through the cricothyroid membrane or through the thyrohyoid membranes, or through a trans-oral approach [10]. These procedures may be difficult to perform, require great patient tolerance and may be impossible to perform when anatomic or physiologic barriers exist [15]. In order to overcome these limitations, a phonosurgical approach using a trans-nasal flexible fiberendoscope has been proposed by the CELF group (Cirugia EndoLaringea Fibroscopica) of Santander (Spain) [18] and further developed in Italy [8 19 20]. This phonosurgical technique is also used for the removal of laryngeal polyps, granulomas and papillomas with flexible cold instruments or with fibre lasers [20-22] as well as to perform IL [8]; it was named "fiberendoscopic phonosurgery" (FEPS) [8 19 20]. In particular, regarding IL under FEPS, Ricci-Maccarini et al. [8 19] developed a completely trans-nasal fiberendoscopic IL procedure, using a high-pressure injection pistol connected to a flexible injection needle, injecting centrifuged autologous fat (with modified Coleman technique) [5 8 19 23 24]. This procedure can also be employed for the IL with other materials and in particular hyaluronic acid [8]. The use of autologous fat reduces the risk of allergic reactions and local granulomatosis that could be caused by synthetic materials [8 11]. The centrifuged autologous fat as an IL material gives the advantage of less reabsorption compared to other injectable materials like hyaluronic acid, because the stem cells contained in the adipose stroma regenerate new lipocytes which replace the lipocytes deteriorated by the injection procedure [23-25].

Preliminary results on the efficacy of trans-nasal IL in the treatment of glottic insufficiency due to UVFP [11 15] seem promising, although only scarce information regarding its safety, feasibility and efficacy in the treatment of secondary dysphonia caused by UVFP both in the short- and in the long-term period are available.

The aim of this study is to evaluate the safety, feasibility and efficacy of trans-nasal IL with centrifuged autologous fat under fiberendoscopy in the treatment of glottic insufficiency due to UVFP. The underling hypothesis is that this phonosurgical technique is simple, safe, reproducible and could provide satisfactory results, in both the short- and in the long-term periods. The importance of this study is related to the need for a simple and effective procedure to treat GI due to UVFP which does not recover with voice therapy.

Materials and methods

Participants

A total of 22 patients, 11 females and 11 males, with at least 6 months history of dysphonia caused by GI due to UVFP were enrolled. The mean age was 60 ± 13 years

(range 32-79). The aetiology of UVFP is reported in Table I. Each patient underwent voice therapy for at least 2 months after the onset of dysphonia caused by GI due to UVFP, with the aim of improving glottic compensation by the contralateral mobile vocal fold (VF). When voice therapy was unable to obtain a satisfactory result, the patient underwent trans-nasal fiberendoscopic IL with centrifuged autologous fat (CAF). Each procedure was performed in the operating room (OR) after formal written consent was obtained. In our institution, IL under fiberendoscopy is usually performed in an office-based setting using hyaluronic acid, but when autologous fat is injected, an OR setting is preferred as centrifuged autologous fat needs to be manipulated in a sterile environment; an anaesthesiologist is not required, and the patient is monitored to control for eventual vagal nerve reflex problems.

Surgical procedure

IL was performed under LA on a day hospital basis. All procedures were performed by two surgeons: one manoeuvered the fiberscope and the other one manoeuvered the flexible injection needle and high-pressure pistol.

Table I. Demographic characteristics of the patients, aetiology of unilateral vocal fold paralysis.

Patient N.	Sex	Age (years)	Aetiology of UVFP	Side of injection
1	F	65	T	Left
2	F	77	I	Right
3	M	65	P	Left
4	F	38	I	Right
5	F	73	T	Right
6	F	32	T	Right
7	M	57	P	Left
8	M	53	P	Left
9	F	54	T	Left
10	F	47	T	Left
11	M	59	P	Left
12	F	38	I	Left
13	M	78	T	Left
14	M	69	T	Right
15	M	64	T	Right
16	M	64	T	Right
17	M	70	P	Left
18	M	51	T	Left
19	M	58	T	Right
20	F	71	T	Right
21	F	79	I	Left
22	F	49	T	Left

T: post-thyroidectomy; P: post-pneumonectomy; I: idiopathic) and side of injection.

Thirty minutes before the surgical procedure each patient received intramuscular atropine (0.5 mg) to reduce salivary secretions on the vocal folds and vagal nerve reflexes (bradycardia, lipothymia). The patient was initially placed in a supine position. An infiltration of local anaesthetic and vasoconstrictor solution was administered into the low peri-umbilical region, using two 20 cc syringes connected to a 22 gauge needle, 9 cm long. After 20 minutes, intravenous injection of 1 mg of midazolam was administered and liposuction was performed in the sub-cutis of the low peri-umbilical region, using a 10 cc disposable autostatic syringe, connected via luer-lock to a 14 gauge Chiba needle, 9 cm long (instead of the Coleman cannula). Lipoaspirate concentration was achieved by centrifugation for 3 minutes at 3000 rpm as described by Coleman [23]. This technique also concentrates the stromal stem cells contained in the adipose tissue, which may favour the regeneration of the infiltrated fat tissue, maintaining its volume [24]. The serum was eliminated from the centrifuged material and the concentrated fat (without mixing it with other substances) was placed into a disposable 3 cc luer-lock polycarbonate syringe, which was then inserted into a high-pressure injection pistol (modified Uroplasty pistol) [8]. Once this task was completed, the patient was placed in a semi-seated position, with his/her head tilted slightly backwards, to facilitate the sight of the glottic plane. Local anaesthesia was administered with vaporisation of 10% lidocaine into the nose and pharynx. The operative flexible endoscope was inserted trans-nasally; lidocaine 2% and then 10% was instilled into the larynx through the working channel of the fiberscope, using a plastic catheter (BTC Medical Europe). In difficult patients also a trans-cutaneous infiltration of lidocaine into the site of the superior laryngeal nerve, as in a thyroplasty procedure [9] can be helpful.

A flexible endoscope with a 2 mm working channel, an outer diameter of 5 mm and working length of 23 cm (Storz 11001UD1, Tuttlingen, Germany) was used in all surgical procedures. For fat injection, a 19 gauge endoscopic flexible needle, 80 cm in length (BTC Medical Europe, Valeggio sul Mincio, Verona, Italy) was used. The endoscopic needle was linked to the high-pressure injection pistol manoeuvered by the second operator. A single injection was performed laterally to the vocal process of the immobile vocal fold to obtain a vocal fold medialisation and augmentation (Figs. 1, 2). Because up to 50% of the injected fat might be reabsorbed within some months, double amount of fat than the amount needed to correct glottic insufficiency was injected [8 19]. In none of the cases was a bilateral VF injection performed.

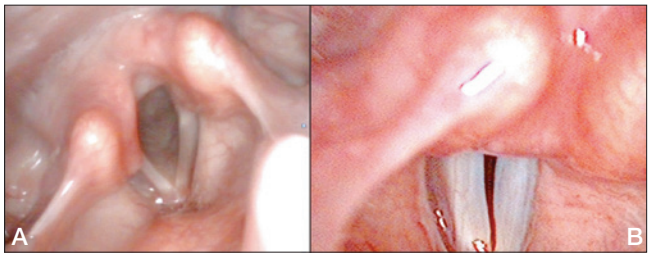

Fig. 1. Clinical case, Female, 32 years old. pre-op. GRBS scale: G2, R1, B2, A2, S0. Laryngostroboscopy: a) VF during breathing, the right VF is atrophic and immobile in abducted position; b) VF during phonation, glottic closure is incomplete with large gap, periodicity of glottic vibration is irregular; MPT: 6 sec.; VHI-10: 21.

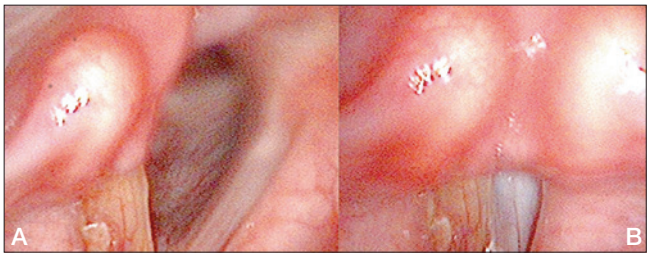

Fig. 3. Same clinical case of Figs. 1 and 2; one week post-op. GRBS scale: G0. Laryngostroboscopy: a) VF during breathing, the right VF is hypertrophic and immobile in a paramedian position; b) VF during phonation, glottic closure is complete, periodicity of glottic vibration is regular; MPT: 14 sec.; VHI-10: 8; comparative self-assessment: great improvement both for voice quality and vocal fatigue.

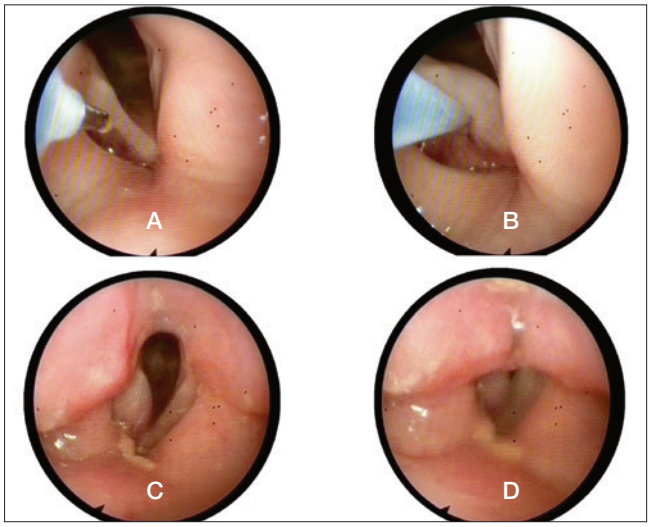

Fig. 2. Same clinical case of Fig. 1, Phonosurgery: injection laryngoplasty with centrifuged autologous fat; a) approximation of the fiberscope to the immobile right VF with the 19 Gauge flexible needle outside the working channel; b) injection of centrifuged autologous fat into the immobile right VF; c) VF during breathing at the end of intervention; d) VF during phonation at the end of intervention, glottic closure is almost complete with a small posterior gap due to the convex profile of the injected VF.

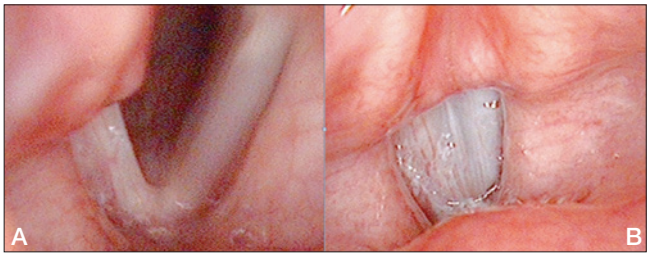

Fig. 4. Same clinical case of Figs. 1, 2 and 3; six months post-op. GRBAS scale: G0. Laryngostroboscopy: a) VF during breathing, the right VF has a normal morphology and it is immobile in an intermediate position; b) VF during phonation, glottic closure is complete although the immobile right VF is less medialised than in the first post-op. control, periodicity of glottic vibration is regular; MPT: 12 sec.; VHI-10: 5 ; comparative self-assessment: great improvement both for voice quality and vocal fatigue.

Patient evaluation

The number and type of complications during and after phonosurgery were analysed. Each patient was evaluated before, after 1 week (first post-operative-period) (Fig. 3) and after 6 months (second post-operative-period) (Fig. 4) from phonosurgery using a multidimensional set of measurements according to the guidelines of the European Laryngological Society [26]. In particular, each patient underwent videolaryngostroboscopy with either a rigid or flexible endoscope. The exam was recorded on video, stored in the host computer and then scored jointly by two phoniatricians blind to timing of the surgical procedure, who finally discussed the results of the examination in order to assign an univocal score. During videolaryngostroboscopic examination, glottic closure (which was scored as: complete, incomplete with small gap, incomplete with large gap), the position of the immobile vocal fold (which was scored as: immobile in median, paramedian, intermediate, abducted position or it could be normally mobile), the morphology of the vocal folds (which was scored as: normal, atrophic, hypertrophic) and the periodicity of glottic vibration (which was scored as: regular, irregular, inconsistent) were assessed, according to Hirano's nomenclature [27] and its modified updated version [28]. For the perceptual evaluation of dysphonia the GRBAS scale was used [27-29]. The conversational speech on standard sentences of each patient were recorded on the host computer. The recordings were rated jointly by an experienced phoniatrician and an experienced speech pathologist, both blind to the timing of the phonosurgical procedure and assessment, who discussed the results of the analysis in order to assign a univocal score to each of

the items included in the GRBAS scale. For the recording of voice signal, a microphone approximately 15 cm from the voice source was used to avoid an airflow effect. For aerodynamic evaluation, the maximum phonation time (MPT) was measured: each patient was asked to utter an/a/ in modal register, as long as he/she could. MPT was determined by measuring the sustained/a/ in 3 productions on the basis of the oscillogram signal (abnormal values < 10 seconds [27]). To obtain self-assessment data on perceived QOL, each patient completed the Italian version of the VHI-10 [30] (abnormal values > 11 [31]). Moreover, to obtain information from the patient regarding his/her perceived modification of voice after phonosurgery, each patient was asked to rate the "comparative self-assessment pre-post-treatment" regarding the changes of voice quality and vocal fatigue by means of a 5 points scale (– 2 = great worsening; – 1 = slight worsening; 0 = no difference; + 1 = slight improvement; + 2 = great improvement) [28].

Statistical analysis

The results are presented as arithmetic mean ± standard deviation. The Kruskal-Wallis test with Mann-Whitney post hoc for continuous variables and Fisher test for categorical variables were used to test the mean difference among the pre- and the post-operative periods. A significance level of 0.05 for all testing was used. Statistical analyses were performed using the SPSS 21.0 package (SPSS Science, Chicago, IL, USA).

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

Trans-nasal fiberendoscopic IL with CAF was performed in all 22 patients. The liposuction and the phonosurgical procedure were well tolerated by all patients. In none of the cases was any complication during the procedure or difficulties in the trans-nasal passage of the fiberscope reported. In particular, no haemorrhage in the nose or at the injection site was noted intra- or post-operatively; no granuloma was visible at the injection site after 1 week and 6 months. The amount of the injected fat was ranged between 2 cc to 3 cc for each injected VF; IL was always unilateral in all the treated patients. As far as categorical variables are concerned, Fisher's test demonstrated a significant difference in the videolaryngostroboscopic findings in the pre- and the post-operative periods for all variables (Table II). In particular, in the pre-operative period the morphology of the immobile vocal fold was considered normal in 1 of the 9 immobile right VF and in none of the 13 immobile left VF, while VF atrophy was found in the remaining 21 immobile VF of the 22 patients. In the first post-operative period 7 of the 9 immobile right VF and 9 of the 13 immobile left VF had a normal morphology; VF hypertrophy was found in 2 of the 9 immobile right VF and in 4 of the 13 immobile left VF, while in none of the cases vocal fold atrophy was detected. This difference was significant by Fisher's test ($p = 0.001$ and $p = 0.000$ for the right and left morphology, respectively). In the second post-operative period, 8 of the 9 immobile right VF and 12 of the 13 immobile left VF were considered normal, while VF atrophy was detected in only 1 right and 1 left immobile VF. No significant difference of vocal fold morphology between the first and the second post-operative periods was demonstrated on Fisher test. The position of the immobile VF and the periodicity of glottic vibration significantly improved after phonosurgery and no significant differences between the first and the second post-operative periods were found on Fisher's analysis.

Glottic closure also significantly improved after phonosurgery. In the first post-operative period complete glottic closure was achieved in 20 patients while an incomplete glottic closure with small gap was found in 2 patients, where a large gap was reported in the pre-operative period. In the second post-operative period, complete glottic closure was achieved in 15 patients, while in the remaining 7 patients incomplete glottic closure with a small gap was found. These differences were significant by Fisher's test ($p = 0.047$).

As far as continuous variables are concerned, the Kruskal-Wallis test demonstrated a significant difference in the scores obtained among the pre- and the post-operative periods for all variables. In particular, the perceptual evaluation of dysphonia demonstrated significant improvement in both the first and in the second post-operative periods for all the GRBA parameters (Table III). The S parameter was never altered even in the pre-operative period ($p = 0.317$) because all patients had an asthenic voice before phonosurgery and never a strained voice. No differences in the GRBAS scale were seen between the first and the second post-operative periods, with the exception of the R parameter, which was worst at one week after phonosurgery and was further improved in the second post-operative period (Table III).

The MPT score was significantly increased both in the

Table II. Videolaryngostroboscopic examination of patients: VF morphology, position of the immobile VF, periodicity of glottic vibration and glottic closure are reported. The results of Fisher's test as well as those of the post-hoc analysis are reported.

		Pre	1st post	2nd post	Fisher test		
					Pre *vs* 1st post	Pre *vs* 2nd post	1st *vs* 2nd post
Morphology Right VF	Normal	14 (1)	20 (7)	21 (8)	0.001	0.002	0.230
	Atrophic	8 (8)	0	1 (1)			
	Hypertrophic	0	2 (2)	0			
Morphology Left VF	Normal	9 (0)	18 (9)	21 (12)	0.001	0.001	0.084
	Atrophic	13 (13)	0	1 (1)			
	Hypertrophic	0	4 (4)	0			
Position Right VF	Mobile	13	13	13	0.006	0.010	0.399
	Immobile Median	0	2	0			
	Immobile Paramedian	1	7	8			
	Immobile Intermediate	4	0	1			
	Immobile Abducted	4	0	0			
Position Left VF	Mobile	9	9	9	0.001	0.003	0.690
	Immobile Median	0	5	3			
	Immobile Paramedian	2	8	9			
	Immobile Intermediate	6	0	1			
	Immobile Abducted	5	0	0			
Periodicity of glottic vibration	Normal	3	11	16	0.001	0.001	0.158
	Irregular	14	11	6			
	Inconsistent	5	0	0			
Glottic closure	Complete	0	20	15	0.001	0.001	0.047
	Incomplete small gap	6	2	7			
	Incomplete large gap	16	0	0			

Pre: pre-operative period; 1st post: post-operative period 1 week; 2nd post: post-operative period 6 months (bracketing the number of the immobile VF).

first and in the second post-operative periods (p = 0.001 at Kruskal-Wallis test). In particular, the MPT was scored 5.63 ± 4.44 in the pre-operative period, while it was 8.82 ± 3.25 and 10.55 ± 4.97 in the first and in the second post-operative periods, respectively. Post-hoc analysis demonstrated significant differences between the pre-operative and the first post-operative periods (p = 0.001) and between the pre-operative and the second post-operative periods (p = 0.001). No differences between the MPT results obtained in the first and second post-operative peri-

Table III. Results of perceptual evaluation of dysphonia in the pre- and the 2 post-treatment periods. The results of the Kruskal-Wallis test as well as those of the post-hoc analysis (performed using Mann-Whitney test) are also reported.

	Pre	1st post	2nd post	Kruskal-Wallis	Mann-Whitney		
					Pre *vs* 1st post	Pre *vs* 2nd post	1st *vs* 2nd post
G	2.64 ± 0.49 (2-3)	0.91 ± 0.53 (0-2)	0.82 ± 0.96 (0-3)	0.001	0.001	0.001	0.504
R	1.50 ± 0.74 (0-3)	0.77 ± 0.53 (0-2)	0.18 ± 0.39 (0-1)	0.001	0.001	0.001	0.001
B	2.59 ± 0.50 (2-3)	0.41 ± 0.50 (0-1)	0.68 ± 0.57 (0-2)	0.001	0.001	0.001	0.098
A	2.64 ± 0.49 (2-3)	0.46 ± 0.59 (0-2)	0.68 ± 0.57 (0-2)	0.001	0.001	0.001	0.249
S	0 ± 0 (0-0)	0.45 ± 0.21 (0-1)	0.45 ± 0.21 (0-1)	0.602	0.317	0.317	0.946

Pre: pre-operative period; 1st post: post-operative period 1 week; 2nd post: post-operative period 6 months.

ods were found (p = 0.224). The VHI-10 score significantly improved in both the short- and long-term periods (p = 0.001 at Kruskal-Wallis test). In the pre-operative period, the VHI-10 score was 17.77 ± 3.76; in the first post-operative period it was 7.09 ± 5.27, while in the second post-operative period it was 4.05 ± 4.46. Post-hoc analysis demonstrated significant differences between the pre- and the first post-operative periods (p = 0.001), between the pre- and the second post-operative periods (p = 0.001) and between the first and second post-operative periods (p = 0.020). Only in one patient the VHI-10, which improved one week after phonosurgery passing from the pre-operative score of 15 to 9, it return to 15 after 6 months.

All patients reported a slight to large improvement of voice quality and vocal fatigue one week after phonosurgery, while at 6 months after phonosurgery one patient reported no differences in vocal fatigue and voice quality compared to the pre-operative situation (as for the VHI-10). In particular, in the first post-operative period, a slight improvement in voice quality and vocal fatigue was reported in 7 of 22 patients, while substantial improvement was reported in the remaining 15 patients. These results were maintained after 6 months since in the second post-operative period a slight improvement in voice quality and vocal fatigue was reported in 6 of 22 patients, and large improvement was reported in 15 patients, while one patient reported no differences in vocal fatigue or voice quality compared to the pre-operative situation. However, no significant differences in perceived modification of voice between the first and second post-operative periods was found by Fisher's test (p = 0.919 and p = 0.891 for voice quality and vocal fatigue, respectively). In the patient who reported unchanged self-assessment of dysphonia 6 months following IL, we then performed a medialisation laryngoplasty (thyroplasty type 1) [9] which resolved the problem of glottis insufficiency in the long-term.

Discussion

In the present study, the safety, feasibility and efficacy of trans-nasal fiberendoscopic IL with CAF in the treatment of GI due to UVFP was evaluated in a group of 22 patients. This is the first report on voice modification in a relatively large group of patients with UVFP treated with this phonosurgical technique, and assessed with a multidimensional set of measurements at 1 week and at 6 months after phonosurgery. The results appear promising and further support the applicability of this phonosurgical technique in the treatment of UVFP.

Specific findings are noteworthy. In particular, no complication during the surgical procedure or difficulties in the trans-nasal passage of the fiberscope were reported in any case. This could be related to several factors. First of all, autologous fat causes less inflammation in the injection site because it is less likely to cause an allergic reaction or local granuloma than synthetic materials [8 11 32]. In addition, since the FEPS technique provides direct access to the surgical site and does not require external injection, oedematous complications are infrequent. As for the feasibility of IL under FEPS is concerned, the surgical procedure was performed in all patients, and each patient tolerated well both the liposuction and the phonosurgery. The intravenous injection of 1-2 mg midazolam decreases the anxiety of the patient, improving his/her collaboration, as in oesophageal-gastric and colon endoscopic procedures. The use of an 80 cm long flexible needle is possible because a 14 gauge Chiba needle is used for liposuction, instead of the Coleman cannula which draws very large blocks of fat tissue; moreover, the high-pressure pistol and 19 gauge flexible needle allow easy and safe passage of adipocytes.

As far as the efficacy of this phonosurgical technique in the treatment of GI due to UVFP is concerned, our results suggest that this IL provides good results not only in the short-term period, but also 6 months afterward. Previous

studies using magnetic resonance [33] showed that the injected fat that remains after 6 months may be considered permanent; therefore, the results at 6 months after IL may suggest that a positive voice outcome is maintained in the long-term. This could be explained by the fact that the stem cells contained in the autologous fat, in particular when it is centrifuged [23-25], may develop into new adipocytes which replace the adipocytes that are deteriorated by the injection procedure. In this way, after the volume decrease due to partial reabsorption of the injected fat, the remaining fat is alive at the site of injection. In order to compensate for the partial reabsorption of the injected fat, twice the amount of material sufficient for achieving complete glottic closure is injected.

Regarding laryngostroboscopic findings, the results demonstrated significant improvement of all parameters (morphology of the vocal fold, position of the immobile vocal fold, periodicity of glottic vibration, glottic closure) both one week and 6 months after phonosurgery, with no significant changes between the first and the second post-operative periods. Also, for perceptual evaluation of dysphonia and in particular for the G, R, B, A parameters of the GRBAS scale (the S parameter was obviously normal also before surgery) and for the maximum phonation time (MPT), the results showed significant improvement at both one week and 6 months after phonosurgery, with no significant changes between the first and the second post-operative periods.

As far as self-assessment of voice related QoL with the VHI-10 and of the perceived modification of voice after phonosurgery with the comparative self-assessment pre-post treatment, our results demonstrated a positive evolution of dysphonia after phonosurgery. Moreover, we found a significant difference between the VHI-10 obtained in the first and second post-operative periods; this seems to suggest progressive improvement of voice-related QoL in the long-term and might be related to the regenerative potential of the stem cells included in the injected centrifuged autologous fat [8 11 24 25]. In the second period, 6 months after phonosurgery, one patient reported an unchanged situation of voice quality and voice fatigue compared to the pre-operative situation; the score of his VHI-10, which improved one week after phonosurgery, at 6 months returned to the same pre-operative score. In this patient, we then performed a medialisation laryngoplasty (thyroplasty type 1) [9], which resolved the problem of GI in the long-term; this is a more invasive phonosurgical procedure, but has to be considered as a permanent technique.

The results reported herein appear similar to those of Saibene et al. [11] who reported complete glottic closure in 2 of 3 patients with UVFP after trans-nasal IL using micro-fractured (non centrifuged) autologous fat; regarding perceptual evaluation of dysphonia with the GRBS scale, MPT and VHI-10, he reported an improvement in all 3 patients in his preliminary study. Trask et al. [15] reported a reduction in medialisation of 20% to 40% as the injected aqueous fluid of the Cymetra was absorbed, in a group of 20 patients with UVFP treated with trans-nasal IL using Cymetra. Recently, Benninger et al al. [34] reported good long-term results for VHI and MPT in 50% of 25 patients with UVFP treated with trans-oral IL with non-centrifuged autologous fat under general anaesthesia.

Pagano et al. [35] treated 18 patients with UVPF with trans-oral IL with non-centrifuged autologous fat under general anaesthesia, reporting significant improvement of glottic closure, MPT, VHI and GRBAS scores both in the short- and in the long-term after phonomicrosurgery. Umeno et al. [36] treated 41 patients with UVFP with the same phonosurgical technique reporting significant improvement of MPT. Havas and Priestley [37] followed 45 cases treated for UVFP with the same phonosurgical technique during a mean follow-up of 33 months, reporting a near normal voice in 87% of the patients. Fang et al. [38] treated 33 patients with UVFP with the same phonosurgical technique, reporting an improvement of both voice and quality of life in 89% of patients after 12 months of follow-up. Cantarella et al. [25] noted a significant improvement in glottic closure, MPT, VHI and GRBAS scores over a follow-up of 10.6 months in a group of 14 patients with GI due to UVFP or VF atrophy and treated with trans-oral IL under general anaesthesia with centrifuged autologous fat.

Our results appear similar to the results obtained by other authors using a trans-oral approach under general anaesthesia for IL with autologous fat in the treatment of UVFP. Unlike prior phonosurgical techniques for IL with autologous fat, this approach is carried out in the awake patient under LA, safely and with good efficacy, without the added risks of general anaesthesia or direct microlaryngoscopy. The use of autologous fat suggests performing IL in the OR in day-hospital (while IL with hyaluronic acid is office-based) because sterile manipulation of fat is required, although an anaesthesiologist is not required. By the fact that the patient is in the OR, the monitoring is recommended to control eventual vagal nerve reflexes during the procedure. This phonosurgical technique requires an additional surgeon (or a trained nurse), equipment and training in injection techniques. It has benefits especially in patients with anaesthesia risks or with anatomical problems of the neck and/or teeth.

There are several limitations to this study. First, the number of patients is relatively small. Second, the pre-therapy

post-therapy study design does not allow direct comparison of IL under FEPS with others using different surgical techniques to improve voice in patients with GI due to UVFP. Third, follow-up was relatively short; thus, the long-term results of this phonosurgical technique should be investigated in a future study. Finally, this is a single institution study; thus, feasibility, safety and efficacy should be analysed in other voice centres as well.

Conclusions

In conclusion, trans-nasal fiberendoscopic IL with centrifuged autologous fat appears to be a safe, feasible and efficacious phonosurgical technique for the treatment of GI due to UVFP and seems to provide stable outcomes over time. The procedure is minimally invasive, without significant morbidity or complications, and is relatively easy to perform. However, the patient's collaboration is required, because the larynx is not immobile and control of swallowing is essential. This procedure does not require general anaesthesia and can also be performed in patients with abnormal neck anatomy or dental problems. The use of a flexible operative endoscope provides a direct approach to the glottic plane, allowing the surgeon continuous evaluation of glottic patency and voice quality modifications during the phonosurgical procedure. Therefore, trans-nasal fiberendoscopic injection laryngoplasty with centrifuged autologous fat can be considered as a possible option for the treatment of glottic insufficiency due to unilateral vocal fold paralysis.

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OSAHS

Moderate OSAS and turbinate decongestion: surgical efficacy in improving the quality of life and compliance of CPAP using Epworth score and SNOT-20 score

OSAS di grado moderato e decongestione dei turbinati: efficacia chirurgica nel migliorare la qualità di vita e la compliance della CPAP utilizzando l'Epworth score e lo SNOT-20 score

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SUMMARY

Drug-induced sleep endoscopy (DISE) is an important procedure in diagnostic pathway of patients affected by moderate OSAS. However, the Italian National Health System does not provide any compatible Diagnosis-related-group (DRG) code codification for DISE, which makes it impossible to obtain regional reimbursement. In order to overcome this problem, DISE is usually associated with other codified surgical procedures. The aim of our study is to assess the association of turbinate decongestion (TD) and DISE in order to combine in a single operating session diagnostic and therapeutic procedures. The objective of our work is to assess the role of nasal surgery on symptoms of moderate OSA. Recent studies have confirmed that isolated nasal surgery improves quality of life (QOL), but not the apnoea hypopnoea index (AHI) during polygraph registration. We enrolled 30 patients, aged between 29 and 64 years (mean 50.53 ± 9.20), 26 males and 4 females, with a mean BMI of 26.07 ± 2.81 kg/m^2, who were affected by moderate OSAS. All patients underwent otolaryngologycal pre-operative evaluation, home respiratory polygraph and subjective evaluation through Sino-Nasal-Outcome Test (SNOT-20) and Epworth Sleepiness Scale (ESS). During the same surgery session, they underwent DISE and TD. Patients were re-evaluated six months later using the same questionnaires. We observed a significant improvement ($p < 0.05$) in both the mean ESS index (6.03 ± 2.75 vs 4.16 ± 4.63) and total SNOT score (22.53 ± 12.16 vs 13.23 ± 10.82). Significant differences ($p < 0.05$) were also identified for partial SNOT questions 1-11 (9.1 ± 5.11 vs 6.13 ± 4.12) and 11-20 (13.36 ± 10.20 vs 7.13 ± 9.644). The results of the present study confirm that TD alone can improve sleepiness, QOL and nasal symptoms. Thus, in absence of a National Health System recognition for DISE, the association of this procedure with TD can be useful for diagnostic and therapeutic management of OSAS, improving CPAP compliance and adherence, reducing sleepiness, ameliorating nasal symptoms and therefore QOL.

KEY WORDS: DISE (Drug Induced Sleep Endoscopy) • OSAS (Obstructive Sleep Apnoea Syndrome) • Epworth score• SNOT-20 score • QOL (Quality of Life)

RIASSUNTO

La DISE (Drug Induced Sleep Endoscopy) è una procedura importante nella gestione diagnostica dei pazienti affetti da OSAS moderata. Il Sistema Sanitario Nazionale, tuttavia, non riconosce alcun DRG per tale metodica e ciò non consente di ottenere il rimborso regionale. Per superare questo problema di gestione di spesa sanitaria, di solito si associa la DISE a un'altra procedura chirurgica codificata. L'obiettivo del nostro studio è definire se l'associazione della decongestione dei turbinati alla DISE possa garantire un risultato terapeutico in aggiunta alla procedura diagnostica e migliorare la sintomatologia dei pazienti affetti da OSAS moderata. Recenti studi confermano che la sola chirurgia nasale migliora la qualità di vita ma non l'AHI (Indice Apnea-Ipopnea) durante la polisonnografia. Abbiamo arruolato 30 pazienti di età compresa tra 29 e 64 anni (media 50,53 ± 9,20), 26 maschi e 4 femmine con un BMI medio di 26,07 ± 2,81 kg/m^2. Tutti i pazienti sono stati sottoposti a valutazione obiettiva ORL pre-operatoria, registrazione polisonnografica domiciliare e ai questionari SNOT-20 e scala della sonnolenza di Epworth. Durante la stessa sessione chirurgica i pazienti sono stati sottoposti a DISE e in seguito a decongestione dei turbinati inferiori. I pazienti sono stati rivalutati a sei mesi tramite i medesimi questionari. Abbiamo osservato un miglioramento significativo ($p < 0,05$) sia nella media dei punteggi del questionario di Epworth (6,03 ± 2,75 vs 4,16 ± 4,63) sia nel punteggio totale dello SNOT-20 (22,53 ± 12,16 vs 13,23 ± 10,82). Sono state riscontrate differenze significative ($p < 0,05$) anche nei punteggi parziali delle domande 1-11 dello SNOT-20 (9,1 ± 5,11 vs 6,13 ± 4,12) e 11-20 (13,36 ± 10,20 vs 7,13 ± 9,644). I risultati del nostro studio confermano che la decongestione dei turbinati può migliorare sia la sonnolenza che la qualità di vita ed i sintomi ostruttivi nasali. Ciononostante, nell'assenza di un riconoscimento da parte del Sistema Sanitario Nazionale per la procedura DISE, l'associazione tra questa indagine e la decongestione dei turbinati può essere utile per l'iter diagnostico-terapeutico del paziente OSAS, migliorando la compliance verso la CPAP e la qualità di vita, nel contempo riducendo la sonnolenza e i sintomi ostruttivi respiratori nasali.

PAROLE CHIAVE: *DISE (Drug Induced Sleep Endoscopy) • OSAS (Obstructive Sleep Apnoea Syndrome) • Epworth score • SNOT-20 score • QOL (Qualità di Vita)*

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Introduction

Sleep-disordered breathing and obstructive sleep apnoea syndrome (OSAS) are common and increasingly important conditions in modern society. OSAS can influence general health status, occupational and family management. It can affect both cardiovascular and neurological systems. OSAS arises from obstruction of the upper airway during sleep at multiple levels, such as nasal cavity, pharyngeal cavity, and retroglottic and glottis regions. Among these, the nasal cavity accounts for 1/2 to 2/3 of general airway resistance. Nasal obstruction can result from multiple causes, including mucosal inflammation and structural abnormalities, such as septal deviation, nasal valve compromise and turbinate hypertrophy. The prevalence of OSAS is estimated to range from 3.1% to 7.5% in the adult male population. Diagnosis of OSA usually requires overnight polysomnography (PSG) to detect the frequency of apneic and hypopnoeic events. Continuous positive airway pressure (CPAP) is currently considered the gold standard for treatment of OSA.

Drug-induced sleep/sedation endoscopy (DISE), first described by Croft and Pringle in 1991 [1], enables exploration of upper airways during induced sleep. It is relatively quick and simple, targeting possible obstruction sites. A recent review by Blumen et al. [2 3] discusses the role of DISE as gold standard in diagnosis of OSAS, but this role is not yet well codified.

However, although DISE certainly has an important role in the diagnostic pathway of patients affected by moderate OSAS [4], at present the Italian National Health System does not provide any compatible Diagnosis-Related-Group (DRG) code codification. In order to overcome this economic and organisational problem, DISE can be associated can be associated with well codified surgical nasal procedures and refunded by the Regions.

Regarding the role of nasal surgery in OSAS, a recent meta-analysis by Wu et al. [5] pointed out that surgery on the nose can significantly improve the quality of life in patients affected by OSAS, especially those with nasal obstruction. The authors concluded that both apnea-hypopnea index (AHI) and Epworth Sleepiness Scale (ESS) (Fig. 1) significantly improve after isolated nasal surgery, but the improvement of AHI is slightly significant.

Moreover, other studies have evaluated the effect of nasal surgery on CPAP. Camacho et al. [6] conducted a systematic review and meta-analysis evaluating the effects of isolated nasal surgery on therapeutic CPAP device pressures and use in adults with OSA. The authors demonstrated that isolated nasal surgery in patients with OSAS with nasal obstruction reduces therapeutic CPAP device pressures and also increases use of.CPAP in selected patients. Ishii et al also investigated if nasal surgery can improve OSAS in patients with nasal obstruction and OSAS. They showed that isolated nasal surgery in these patients led to significant improvements in ESS and Respiratory Disturbance Index (RDI), but no significant improvements in AHI. One bias of these reviews and related papers is related to the absence of a specific analysis of the different nasal surgical approaches, and especially for turbinate decongestion.

It has been demonstrated by Powell et al. [8] that nasal surgery synergises the effects of CPAP treatment, but the majority of studies that involve the nasal district focus on nose surgery, combining various approaches without separately analysing the impact of septal surgery and turbinate decongestion [9].

Finally, regarding costs, Kempfle et al. [10] evaluated the cost-effectiveness of two types of nasal surgery versus no surgery in patients with OSA and nasal obstruction undergoing CPAP. The authors followed the contributor citizen viewpoint, comparing the cost of untreated OSAS patients, cost and complications of surgery and annual cost of CPAP. They concluded that the cost-effectiveness of nasal surgery is in improving CPAP compliance in the OSAS population with nasal obstruction, indicating the value of surgical intervention for non-adherent CPAP users or partially adherent users, as part of a multifaceted approach to improving overall compliance.

We currently associate turbinate decongestion with DISE, considering that the nasal cavities can affect the pathophysiology of OSAS in multiple ways [11] providing a stable airway while oral cavity and larynx collapse [12].

On the basis of these scientific studies of the literature and Italian and non-Italian economic considerations, the aim of this study is to assess the benefits of associating TD and DISE in ameliorating the diagnostic and therapeutic tools of OSAS, and in improving quality of life (QOL), sleepiness and nasal symptoms.

Materials and methods

We enrolled 30 patients, aged between 29 and 64 years (mean 50.53 ± 9.20), 26 males and 4 females, with a mean BMI of 26.07 ± 2.81 kg/m^2, admitted to the Department of Head and Neck Surgery "A. Gemelli Hospital" Catholic University School of Medicine and Surgery in Rome. Patients were affected by moderate OSAS 15 < AHI < 30 events/h (mean AHI 21.04 events/h DS: ± 4.79 considering a 3% dip of saturation) diagnosed with polysomnography. They underwent DISE and turbinate decongestion during the same session. The study was approved by the Ethics Com-

Epworth Sleepiness Scale

Use this scale to determine your patient's level of sleepiness.

Choose the most appropriate number for each situation:

0 = no chance of dozing
1 = slight chance of dozing or sleeping
2 = moderate chance of dozing or sleeping
3 = high chance of dozing or sleeping

Situation	Chance of dozing or sleeping
Sitting and reading	_____
Watching TV	_____
Sitting inactive in a public place	_____
As a passenger in a motor vehicle for an hour or more	_____
Lying down to rest in the afternoon when circumstances permits	_____
Sitting and talking to someone	_____
Sitting quickly after lunch without alcohol	_____
In a car, while stopped for a few minutes in traffic	_____
Total score (add the scores up) (This is your Epworth score)	_____

If your patient scores 10 or more, we recommend your patient consult one of our physicians to treat a sleep disorder, address an underlying condition affecting sleep and develop proper sleep hygiene.

Fig. 1. Epworth Sleepiness Scale (Vignatelli L, et al., 2003 [7]).

mittee of the Catholic University School of Medicine and Surgery in Rome. All patients were willing to participate and consented to their inclusion with formal written consent when recruited.

We performed a longitudinal prospective evaluation of our patients.

Inclusion criteria were: age > 18 y, moderate OSAS (15 < AHI < 30) and nasal obstructive symptoms that compromised QOL. Exclusion criteria were: previous upper airways surgery and weight loss > 10% body weight between surgery and follow-up, since it has been demonstrated that weight loss inferior to 10% of total body weight has no polysomnographic effects on moderate OSAS patients [13].

All patients underwent pre-operative otolaryngological evaluation including fibre optic nasopharyngoscopy with Muller's maneuver, home respiratory polygraph (Somntè Recording Unit©, Somntè Compumedics, Australia). Subjective evaluation was obtained using the Italian translation of strongly validated questionnaires,, namely the Sino-Nasal Outcome Test (SNOT-20) [14 15] (Fig. 2) and Epworth Sleepiness Scale (ESS) [7] (Fig. 1).

During the same surgical session, all patients underwent DISE with the presence of an anaesthesiologist, neuroelectrophysiology technician and ENT specialist. Patients, already prepared for polygraphic intraoperative recording, received drug sedation. For drug sedation, we used an increasing dose of propofol (3 mg/kg/h) until continuous BiSpectral Index (BIS) monitoring by Aspect A-2000 BIS monitor© (Apect Medical Systems, Natick, MA), was between 45-65. During snoring, we introduced flexible nasopharyngoscope trough the nasal cavity evaluating the pattern and degree of obstruction (nasopharynx, oropharynx, hypopharynx and larynx) and continuously monitored sleep with a polygraph (Somntè Compumedics System©, Somntè Compumedics, Australia). We also performed a bimanual pull-up mandibular advancement manoeuvre, advancing from 4 to 5 mm [16 17], evaluating masseter muscle activation [18] and visualising the effect on airway obstruction.

After these procedures, starting ventilation using a cuffed laryngeal mask, we performed TD with a quantic molecular resonance scalpel (Teleamedical ENT Quantum Bipolar Scalpel).

Patients were re-evaluated at six months after surgical

I.D.:_____ **SINO-NASAL OUTCOME TEST (SNOT-20)** DATE:_____

Below you will find a list of symptoms and social/emotional consequences of your rhinosinusitis. We would like to know more about these problems and would appreciate your answering the following questions to the best of your ability. There are no right or wrong answers, and only you can provide us with this information. Please rate your problems as they have been over the past two weeks. Thank you for your participation. Do not hesitate to ask for assistance if necessary.

1. Considering how severe the problem is when you experience it and how frequently it happens, please rate each item below on how "bad" it is by circling the number that corresponds with how you feel using this scale: →	No problem	Very mild problem	Mild or slight problem	Moderate Problem	Severe Problem	Problem as bad as it can be	5 Most Important Items
1. Need to blow nose	0	1	2	3	4	5	○
2. Sneezing	0	1	2	3	4	5	○
3. Runny nose	0	1	2	3	4	5	○
4. Cough	0	1	2	3	4	5	○
5. Post-nasal discharge	0	1	2	3	4	5	○
6. Thick nasal discharge	0	1	2	3	4	5	○
7. Ear fullness	0	1	2	3	4	5	○
8. Dizziness	0	1	2	3	4	5	○
9. Ear pain	0	1	2	3	4	5	○
10. Facial pain/pressure	0	1	2	3	4	5	○
11. Difficulty falling asleep	0	1	2	3	4	5	○
12. Wake up at night	0	1	2	3	4	5	○
13. Lack of a good night's sleep	0	1	2	3	4	5	○
14. Wake up tired	0	1	2	3	4	5	○
15. Fatigue	0	1	2	3	4	5	○
16. Reduced productivity	0	1	2	3	4	5	○
17. Reduced concentration	0	1	2	3	4	5	○
18. Frustrated/restless/irritable	0	1	2	3	4	5	○
19. Sad	0	1	2	3	4	5	○
20. Embarrassed	0	1	2	3	4	5	○

2. Please mark the most important items affecting your health (maximum of 5 items)_____ ↑

Fig. 2. SNOT-20 questionnaire (Mozzanica F, et al., 2017 [15]).

procedures and the SNOT-20 and ESS questionnaires were administered.

Data obtained by SNOT and ESS are expressed as mean ± standard deviation (SD). We compared the means of ESS results, total SNOT score and sums of questions 1-10 and 11-20 as representative of nasal symptoms and QOL, respectively.

Statistical analysis of data was performed using Microsoft EXCEL and IBM SPSS 24 software packages, comparing the means with T-test for paired samples. Data were considered positive if there was a decrease in SNOT and ESS scores after treatment. Differences were considered statistically significant if the p value was less than 0.05.

Results

Table I shows the results of ESS, total SNOT scores and partial SNOT scores for each patient.

We observed a significant improvement ($p < 0.05$) in both the ESS medium index (6.03 ± 2.75 vs 4.16 ± 4.63; Fig. 3) and in the total SNOT score (22.53 ± 12.16 vs 13.23 ± 10.82; Fig. 4). A significant difference ($p < 0.05$)

Table I. Per patient values, pre- and post-operative for ESS, the total Snot score and the partial scores for question 1-10 and 11-20. At bottom provided means and standard deviations.

ID	EpworthPre	EpworthPost	TotalSnotPre	TotalSnotPost	1-10 SnotPre	1-10 SnotPost	11-20 SnotPre	11-20 SnotPost
1	8.00	4.00	54.00	11.00	16.00	7.00	38.00	4.00
2	4.00	0.00	32.00	0.00	18.00	0.00	14.00	0.00
3	4.00	4.00	17.00	15.00	11.00	14.00	6.00	1.00
4	6.00	4.00	20.00	18.00	15.00	7.00	5.00	11.00
5	5.00	3.00	20.00	11.00	12.00	9.00	8.00	2.00
6	6.00	19.00	19.00	37.00	7.00	9.00	12.00	28.00
7	3.00	0.00	21.00	10.00	10.00	10.00	11.00	0.00
8	7.00	3.00	17.00	9.00	9.00	9.00	8.00	0.00
9	3.00	0.00	4.00	0.00	2.00	0.00	2.00	0.00
10	6.00	6.00	46.00	29.00	8.00	5.00	38.00	24.00
11	6.00	8.00	17.00	19.00	7.00	9.00	10.00	10.00
12	9.00	8.00	18.00	23.00	1.00	2.00	17.00	21.00
13	13.00	3.00	26.00	2.00	5.00	2.00	21.00	0.00
14	10.00	4.00	23.00	25.00	3.00	4.00	20.00	21.00
15	12.00	2.00	44.00	1.00	20.00	1.00	24.00	0.00
16	3.00	3.00	5.00	9.00	2.00	7.00	3.00	2.00
17	8.00	1.00	15.00	5.00	11.00	5.00	4.00	0.00
18	3.00	2.00	15.00	2.00	4.00	2.00	11.00	0,00
19	4.00	4.00	16.00	2.00	4.00	2.00	12.00	0,00
20	8.00	8.00	35.00	19.00	11.00	8.00	24.00	11,00
21	5.00	0.00	31.00	0.00	17.00	0.00	13.00	0,00
22	3.00	3.00	18.00	16.00	11.00	15.00	7.00	2,00
23	5.00	3.00	19.00	17.00	16.00	8.00	6.00	12,00
24	5.00	3.00	20.00	11.00	10.00	7.00	6.00	1,00
25	6.00	4.00	19.00	37.00	7.00	9.00	12.00	28,00
26	3.00	0.00	21.00	10.00	10.00	10.00	11.00	0,00
27	7.00	3.00	17.00	9.00	9.00	9.00	8.00	0,00
28	3.00	0.00	4.00	0.00	2.00	0.00	2.00	0,00
29	10.00	4.00	46.00	29.00	8.00	5.00	38.00	24,00
30	6.00	4.00	17.00	19.00	7.00	9.00	10.00	10,00
	Means and standard deviation							
	6.03 ± 2.75	4.17 ± 4.63	22.53 ± 12.16	13.16 ± 10.81	9.10 ± 5.11	6.13 ± 4.11	13.37 ± 10.20	7,07 ± 9,68

was also identified for partial SNOT questions 1-11 (9.1 ± 5.11 vs 6.13 ± 4.12) and 11-20 (13.36 ± 10.20 vs 7.13 ± 9.644; Fig. 4).

No patient had any complications related to DISE, and only two patients (6.67%) presented intra-operative epistaxis that required nasal swab during TD.

Discussion

The results of the present study confirm that TD alone can improve sleepiness, QOL and nasal symptoms. We used well-known and validated questionnaires, which implies subjective assessment by patients. However, subjective data can be considered reliable, and the clinical correlation between SNOT score, ESS index and OSAS has already been described [19 20].

Sleepiness score can be considered the better index of OSAS outcomes. In the present study, it was reduced by 68.9%, with an absolute score of 1.87 points. The importance of TD in the amelioration of disability OSAS-related is also evident. In agreement with literature data, we can confirm that resolution of nasal obstruction generally alleviates daytime sleepiness in OSAS patients [20 21], even if it has little effect on AHI [22].

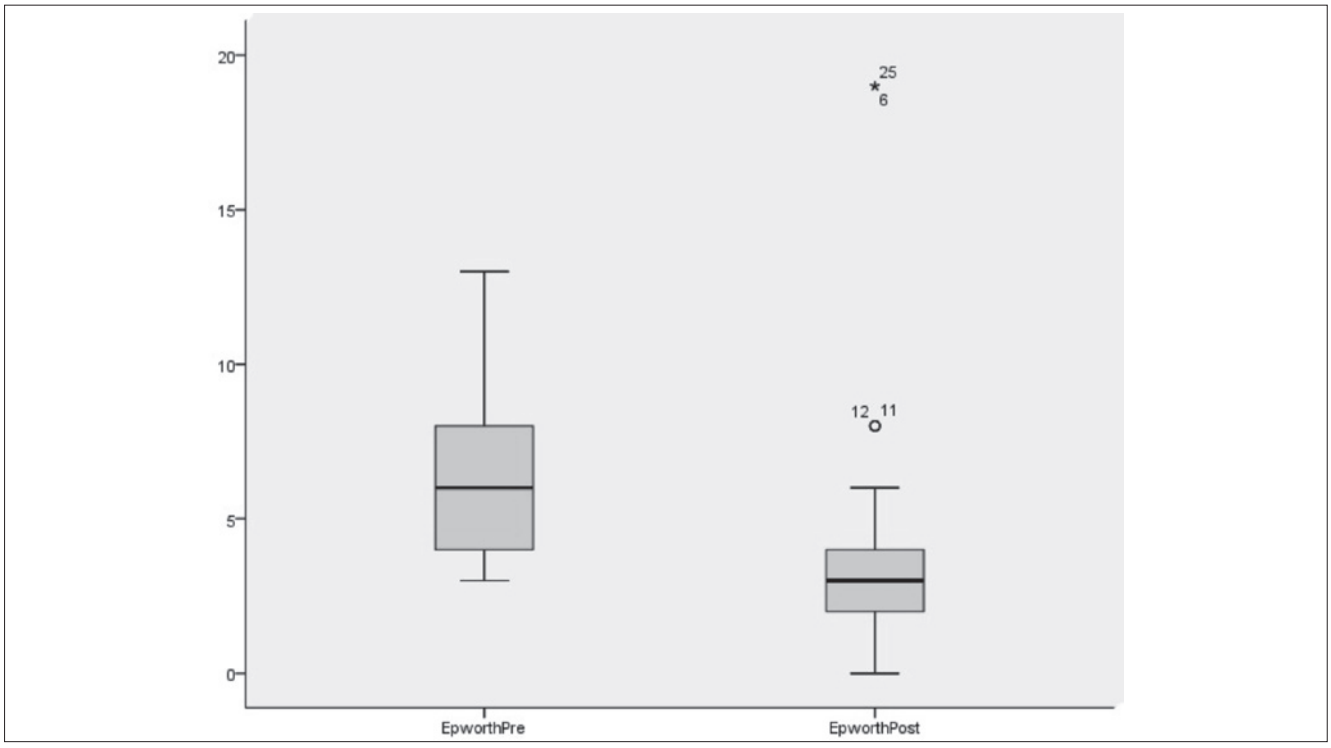

Fig. 3. Epworth score distribution at baseline and after six months after turbinate decongestion. The box plots show the median and inter-quartile range and the error bars show the 5th and 95th percentiles.

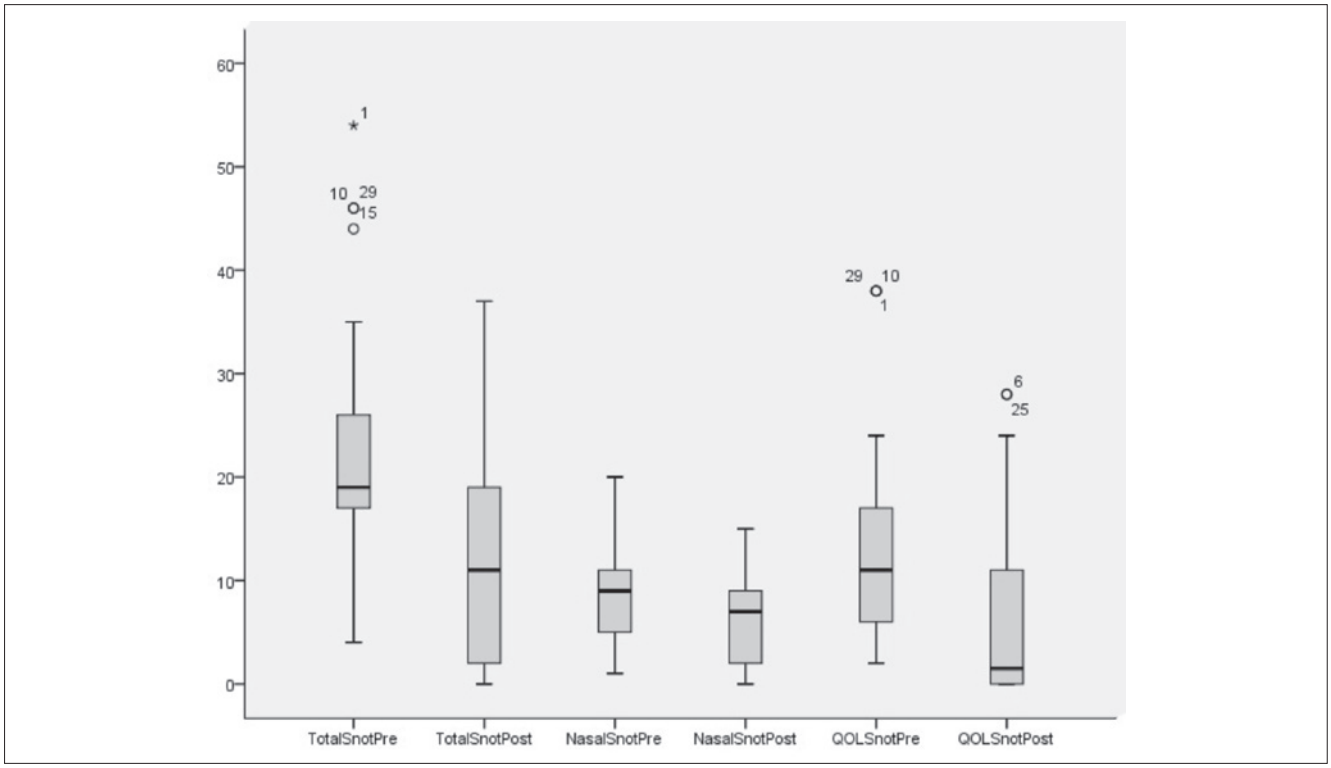

Fig. 4. SNOT-20 score distribution at baseline and after six months from turbinates' decongestion. From left to right: Total Score, Question 1-10 Score, Questions 11-20 Score. The box plots show the median and inter-quartile range and the error bars show the 5th and 95th percentiles.

We showed a significant modification of total SNOT score, which was reduced by 9.3 (58.7%). Moreover, evaluation of the partial SNOT score also showed a significant improvement. In particular, questions 11-20 investigate nasal obstruction related symptoms such as fatigue, sleepiness, lack of concentration and emotional burden; we highlight that the score decreased by 6.2 (53.3%). Questions 1-10 examine nasal symptoms, and showed were reduced by 3 (67.3%). This high percentage confirms that nasal symptoms ameliorate after TD, as stated by the American Academy of Otolaryngology-Head And Neck Surgery [23].
Moreover, the QOL can be considered to be a very significant aspect in management of OSAS patients, and comprises symptoms such as daytime sleepiness, fatigue, reduction in concentration and productivity, irritability and snoring that could cast away sleeping partners. Excessive daytime sleepiness is one of the principal symptoms in patients with OSAS causing reduced productivity, accidents, and errors in professional life are a serious social concern; worsening QOL thus deserves attention by the physician. Our results further confirm the efficacy of TD in improving the QOL.
Assuming that nasal obstruction could be due to turbinate hypertrophy, septal deviation, nasal polyps, rhinosinusitis, neoplasm of nasal cavity and nasal valve collapse, we can argue that it is responsible not only for nasal symptoms, but also for extra nasal symptoms such as fatigue, sleep disturbances, headache and daytime sleepiness, and for this reason it causes a decline in QOL [9 23]. In OSAS patients, nasal resistance is frequently high and varies with body position: it increases in the supine position, compared with the upright position, and is due to an increase in nasal blood flow and reduction of the retropalatal space [11].
Finally, considering the aetiology of chronic nasal obstruction, it cannot be resolved by medical therapy alone, often requiring surgical treatment.
We conclude that given the relative safety [24] of turbinate decongestion, its acknowledged good cost/efficacy ratio in supporting CPAP therapy and the beneficial effects on QOL and sleepiness we found, TD can be considered too important in the management of all patients with OSAS and nasal obstruction.
Another final matter that supports our recommendation is the good cost/efficacy ratio of TD and the short timespan (5 years) for good effects, compared to septal correction that performs the best results in 10-15 years, and improvement of CPAP compliance [10].

Conclusions

Our results suggest that in the absence of a National Health System recognition for DISE, the association of this procedure with TD can be useful not only for diagnostic purposes, but also for suitable management of OSAS, improving the CPAP compliance and adherence, reducing the sleepiness, ameliorating the nasal symptoms, and therefore QOL.
Moreover, at the cost of only one day surgery and one anaesthesiological procedure, we associated an endoscopic diagnostic procedure with surgical treatment.
Finally, teams managing patients with snoring and sleep apnoea should continue their investigations in order to determine whether DISE is fundamental for guiding good medical practice, and to render DISE a more widespread procedure that is useful in OSAS beyond economic considerations alone.

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Rhinology

Implementing strategies for data collection in chronic rhinosinusitis

Strategie integrative nella raccolta dati del paziente affetto da rinosinusite cronica

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SUMMARY

Chronic rhinosinusitis (CRS) is a debated topic in the international rhinologic literature because of its high prevalence, heterogeneity of clinical manifestations and unpredictability of disease course. Recently, the focus in CRS research has moved to identify biological subtypes that might explain its aetiology and clinical variability. However, these analyses are still expensive and limited to scientific purposes, so that they cannot be used on a large scale in daily practice. For this reason, we wondered if it was possible to define a risk stratification for CRS patients based only on first level investigations. The heterogeneity of the disease has given us a large amount of data compelling to find an additional storage system. Herein, we present the results of our work, the RhinoBank, as we believe that it is an easy-to-use tool for those professionals dealing with CRS and an effective system to exploit in clinical research.

KEY WORDS: Chronic rhinosinusitis • Phenotypes • Endotypes • Database • Clinical trials

RIASSUNTO

La rinosinusite cronica (CRS) rappresenta un argomento dibattuto nella letteratura rinologica internazionale a causa della sua alta prevalenza, dell'eterogeneità delle manifestazioni cliniche e della difficoltà a predire l'andamento della malattia. Recentemente l'attenzione della ricerca nella CRS si è spostata verso l'identificazione di sottotipi biologici che possano giustificare l'eziologia e la variabilità clinica. Tuttavia, queste analisi risultano ancora costose e limitate nell'impiego per scopi di ricerca, per cui non applicabili su larga scala e nella pratica clinica quotidiana. Per questo motivo ci siamo domandati se fosse possibile ottenere una stratificazione del paziente rinosinusitico solo sulla base di indagini di primo livello. L'eterogeneità intrinseca della malattia ci ha messo di fronte ad una vasta quantità di dati obbligandoci a trovare strategie di archiviazione addizionali. Presentiamo quindi il frutto del nostro lavoro, il RhinoBank, principalmente per due motivi. Crediamo che sia uno strumento di facile impiego a disposizione di chiunque tratti questa patologia ed un sistema efficace da sfruttare nella ricerca clinica.

PAROLE CHIAVE: *Rinosinusite cronica • Fenotipi • Endotipi • Banche dati • Studi clinici*

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Chronic rhinosinusitis (CRS) is a frequent disease. Its true prevalence is challenging to be accurately estimated because it depends on the epidemiological methodology employed. However, according to studies based on large-scale questionnaires, it ranges from around 10 to 12% in Europe and the US. Moreover, CRS represents a burden both to individuals and society [1].

In recent years, with the demand to justify therapeutic failures, the scientific community has began to critically review the diagnostic criteria for CRS and realised that they were not sufficient to explain the heterogeneity of the disease. There is, in fact, a broad spectrum of rhinosinusitis manifestations, ranging from simple paranasal sinus dysventilation to frank nasal polyposis, which is not adequately taken into account by the phenotypic classification based on guidelines. The classic dichotomy between CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) is too simplistic to explain a disorder that is actually considered as a complex multifactorial disease grounded on the interplay between gene-susceptibility and the exposome (microbiota, immunity, epigenetics, nutrition) [2-4].

In the attempt to overcome this limit, we gradually shifted to a different perspective for which the clinical phenotype

is in reality nothing but the emerging part of a massive iceberg.

All these considerations were inherited from the pulmonology field. Studies on asthma endotyping have been mentioned since 2008, when the literature began to put a new focus on pathogenetic mechanisms, recognising the complexity and variability of chronic inflammatory disorders of the airways [5]. All these efforts have been made to correlate the clinical phenotype to the course of the disease and its response to therapies [6]. In 2013, the concept of endotyping in CRS first appeared [7]. This consensus is the expression of the consciousness that CRS heterogeneity is supported by multiple biological subtypes (endotypes), each of which is defined by a distinct pathophysiological mechanism, determined equally by a well-defined genetic-environmental interaction. Each endotype should be in a theoretical line identified by a biomarker, to be intended both as diagnostic marker and as prognostic and therapeutic indicator. To find a highly predictive biomarker, a long series of key requirements for reproducibility, accessibility and stability must be met. In truth, we are still facing with the lack of an ideal biomarker that identifies CRS endotypes, allows a precise estimation of the severity of inflammation and predicts possible therapeutic responses. Therefore, it is likely that only a combination of biomarkers will be adequate to characterise each specific CRS subtype [8].

It is intrinsic to the concept of a multifactorial disease, as CRS, the existence not only of multiple predisposing factors (risk factors), but also of other concomitant pathological conditions (pre and comorbidities) that contribute in shaping the phenotype. Differentiation of pre- and comorbid and risk factors is not easy, because of the variability in disease definitions, the lack of longitudinal studies that establish temporal relationship between exposure and disease onset and the difficulty of assessing the dose-effect size on disease severity.

Furthermore, the opportunity to attest the effectiveness of "standard" therapies is limited by the wide variability of treatment types, patient selection and outcome assessments.

A non-negligible number of prior studies, which reported high proportions of patients improving following medical and/or surgical treatments, were, however, retrospective analyses, which deduced subjective parameters or collected results though unverified surveys [9]. There was no standard for categorising preoperative status, extent of disease or surgical outcomes, and many of these studies were unable to interpret the clinical relevance of a specific treatment or further delineate subgroups of patients who did or did not experience improvement. In addition, single institution studies have been criticised for the potential lack of generalisability to patients population, an issue at least partially addressed by incorporating a multi-institutional study design [10 11]. In the last years, the introduction of validated disease-specific quality of life (QoL) and general health-related QoL outcomes instruments allowed building a standard assessment of CRS patients.

Notwithstanding, in our opinion, the exclusive evaluation of outcomes based on symptomatic and objective scores (endoscopic, radiological) may be limitative. A previous prospective-designed publication showed that other clinical factors (such as asthma, ASA intolerance, prior sinus surgery etc.) were found to be important predictors of outcomes [10]. Our idea is that patients affected by CRS should be framed as a whole, going beyond a sole rhinological point of view in a multidisciplinary perspective [12].

Consequently, a very frequent disease associated with multiple variables generates a large amount of data that should be collected. It clearly emerges that there is a need to establish a systematic approach for data collection and evaluation of outcomes.

Our tertiary care institution is working toward this direction and has created a CRS online database, called RhinoBank. Its advantages are many. First of all, it allows storing data in a single solution with the possibility of easily retrieving previously stored data. In addition, it provides the physician all the information at a glance, allowing location of missing data in a very simple way. Lastly, it enables data sharing with other work centres.

The aim of this letter is to present the efforts of our work in search of active collaboration. We are aware that the database can be further upgraded thanks to suggestions or implementations from other experts in the field. The database now contains only basic clinical information that can be routinely obtained in any hospital. It is not envisaged to store third level parameters such as genetic or biomolecular markers. This will be the next step, dictated by the possibility to perform a more detailed analysis in our institute.

The proposal is to spread this data collection system to other national centres to obtain large and uniform cohorts of patients. The goal is to overcome that lack of constant parameters, that is a critical element inside systematic reviews that hinders the possibility to draw conclusions on clinical practice [9]. The database is at disposal for consultation at: https://www.rhinobank.eu/demo/admLoginWin.asp. (Account access: Username, Admin; Password, demo000.)

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Rhinology

Bromelain's penetration into the blood and sinonasal mucosa in patients with chronic rhinosinusitis

Farmacocinetica della Bromelina nel sangue e nella mucosa rinosinusale nei pazienti con rinosinusite cronica

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SUMMARY

The aim of this research is to investigate penetration of Bromelain into sinonasal mucosa in patients with chronic rhinosinusitis (CRS) versus a control group. Bromelain is derived from pineapple (*Ananas comosus*) and has various pharmacological effects. 40 patients (20 patients and 20 controls) were enrolled in the study. Bromelain 500 mg tablet twice daily was administered for 30 days. We scored bromelain presence in turbinate and ethmoid mucosas and in the serum of both the groups. Bromelain has an excellent distribution from blood to rhinosinusal mucosa. Its diffusion ability may allow the use of bromelain as an anti-inflammatory agent in paranasal sinus pathologies.

KEY WORDS: Bromelain • Immunohistochemistry • Chronic rhinosinusitis

RIASSUNTO

Lo scopo di questa ricerca è quello di accertare la farmacocinetica di Bromelina nella mucosa rinosinusale e nel sangue in pazienti con rinosinusite cronica (CRS) ed in un gruppo di controllo. La Bromelina è un derivato dell'ananas (Ananas comosus) e dispone di vari effetti farmacologici. In questo studio sono stati arruolati 40 pazienti (20 pazienti e 20 controlli). Abbiamo somministrato Bromelina 500 mg in compresse due volte al giorno per 30 giorni. Abbiamo valutato la presenza di Bromelina nella mucosa dei turbinati e dell'etmoide e nel siero. La Bromelina risulta avere un'ottima distribuzione dal sangue alle mucose rinosinusali. In conclusione la capacità di diffusione può permettere l'uso di bromelina come farmaco anti-infiammatorio nelle patologie rinosinusali.

PAROLE CHIAVE: *Bromelina • Immunoistochimica • Rinosinusite cronica*

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Introduction

We studied the penetration of bromelain into sinonasal mucosa in patients with CRS (group A) versus a control group (group B). Bromelain is derived from pineapple (*Ananas comosus*) and is a mixture of different substances, above all, proteolytic enzymes [1]. It is used in therapy for many problems and has various pharmacological effects, but its mechanism of action is still not completely clear. Several studies, carried out to determine its activity and effectiveness, have identified antithrombin, anti-oedema and fibrinolytic activity.

Clinical trials have shown that bromelain is useful for the therapy of several disorders such as chronic inflammation and autoimmune diseases [2], particularly osteoarthritis and rheumatoid arthritis [3-5]. In vitro, it has demonstrated the ability to modulate the immune response to reduce the allergic reaction and to modulate macrophages, NK cells and T cells. It also increases the secretion of IL-1β, IL-6 and TNFα [6].

In vitro and in vivo research suggests that bromelain may interfere with pathogens such as *Vibrio cholera* and *Escherichia coli,* whose toxins cause diarrhoea [7].

Recent studies show that its administration before dental extraction can reduce the intensity of pain [8 9].

Finally, in vitro studies highlight that bromelain has anti-tumoural activity, through an increase of concentration-dependent inhibition of cancer cell proliferation [10].

Materials and methods

We enrolled 20 patients (13 males, 7 females; 22-77 years) with CRS diagnosed according to the "International Consensus Statement on Allergy and Rhinology" [11] and EPOS criteria [12 13] as group A and 20 patients (9 males, 11 females; 26-64 years) without sinonasal problems as group B.

We defined CRS as rhinosinusal inflammation lasting for more than 12 weeks and characterised by nasal obstruction, nasal drip (anterior and/or posterior), facial pain and alteration of smell. These symptoms had to be associated with other findings including positive nasal endoscopy for presence of purulent secretions or positivity for sinus inflammation on CT scan. Sinus mucosal inflammation is staged, according to Lund-Mackay score system [14], as 0 (complete lucency), 1 (partial lucency) or 2 (complete opacity). The patients enrolled in group A belonged to Lund-Mackay Score grade 2.

This research was carried out with the ENT Dept. of Carol Davila University of Bucharest. All the patients had taken 1 tablet of bromelain 500 mg twice a day by mouth for 1 month.

All patients signed informed consent. The Bucharest Local Health Unit Ethics Committee approved our research. Guidelines and requirements of the Declaration of Helsinki have been respected.

Exclusion criteria were: any other concomitant systemic/topic drug treatment, presence of infections of other sites during all trials, hypersensitivity to any of the constituents of medication, pregnancy or lactation, hepatic or renal insufficiency [15].

For group A, ethmoid and middle turbinate mucosa were taken during functional endoscopic sinus surgery. For group B, samples were taken during transnasal pituitary surgery.

Immunohistochemistry analysis was carried on according to procedures present in the international literature, considered as the most valid and reliable [16].

Samples were washed for 30 seconds in 0.9% sodium chloride solution to minimise blood contamination, and then embedded in paraffin.

Paraffin sections were deparaffinised, rehydrated and rinsed in PBS, pH 7.4. Retrieval with cooking in specific buffer was raised in a microwave oven (Samsung) at 800 W for 5 minutes, and then 440 W for 10 minutes. The immunohistochemical method was an indirect two-stage technique performed with a polymer based detection system (Max Polymer Detection System–Leica RE 7280-k) according to the manufacturer's instructions. All specimens were counterstained with Meyer's haematoxylin, examined and photographed with a Nikon E 200 microscope. Tissue sections were tested by immunohistochemistry using monoclonal antibodies against bromelain (Agrisera AB/AS09 552). The dilution used was 1:2000, cooked overnight at 60º C, 3% H_2O_2.

We scored the quantity of bromelain present in samples of turbinate and ethmoid mucosa taken as grade 0, 1, 2, 3 and 4 based on the following:

- grade 0: no reaction;
- grade 1: positive reaction in < 25% of cells:
- grade 2: positive reaction in 25-50% of cells (Fig. 1);
- grade 3: positive reaction in 50-75% of cells (Fig. 2);
- grade 4: positive reaction > 75% of cells.

A blood sample was taken and bromelain in blood was determined using the Western-blotting method. We scored the presence of bromelain in serum in:

- 0: no Bromelain;
- 1: optical density (OD) values of the migration gel < 60,000;
- 2: OD values 60,000-80,000;
- 3: OD values 80,000-90,000;
- 4: OD values > 90,000.

Results

The data collected are summarised in Table I. In Table I, for almost all enrolled subjects there was a good distribution of bromelain, from serum to nasal and sinus tissues. It is interesting to note that for 3 controls (group B), there was no distribution of bromelain either in serum or in rhinosinusal mucosa.

For patients, the distribution of bromelain between serum and turbinate tissue and between serum and ethmoid tissue was statistically significant (P value of both: 0.0004). For control group, the P values were also significant (P value of bromelain distribution between serum and turbinate tissue: 0.0356; P value of Bromelain distribution between serum and ethmoid tissue: 0.0207). Results of immunohistochemistry are shown in Figures 1 and 2.

Discussion

First of all, we can underline that the distribution of bromelain to serum and from serum to rhinosinusal tissues was higher in patients than in control.

Apart from the empirical results, there was important statistical significance, particularly for patients in group A. Thus, it can safely be stated that bromelain has an excellent distribution from serum to sino-nasal tissues, especially in patients with paranasal diseases. The results of controls (group B) were also statistically significant, even if this significance was less than in group A.

According to the International Rhinosinusitis Advisory

Table I. Data collected.

Group A	Serum	Turbinate	Ethmoid	Group B	Serum	Turbinate	Ethmoid
1	2	1	1	1	0	0	0
2	1	2	0	2	2	1	1
3	1	1	0	3	0	0	0
4	3	1	1	4	0	0	0
5	2	0	0	5	2	0	0
6	3	2	2	6	1	1	1
7	4	1	2	7	1	2	0
8	4	2	1	8	2	1	1
9	3	2	2	9	1	1	0
10	2	2	2	10	2	2	2
11	4	3	2	11	1	2	1
12	2	1	0	12	1	0	1
13	3	0	1	13	3	1	0
14	2	2	3	14	1	0	1
15	3	1	2	15	2	1	2
16	4	2	3	16	2	2	1
17	1	2	1	17	3	0	2
18	3	3	2	18	2	1	2
19	2	1	2	19	1	2	1
20	3	0	1	20	3	1	1

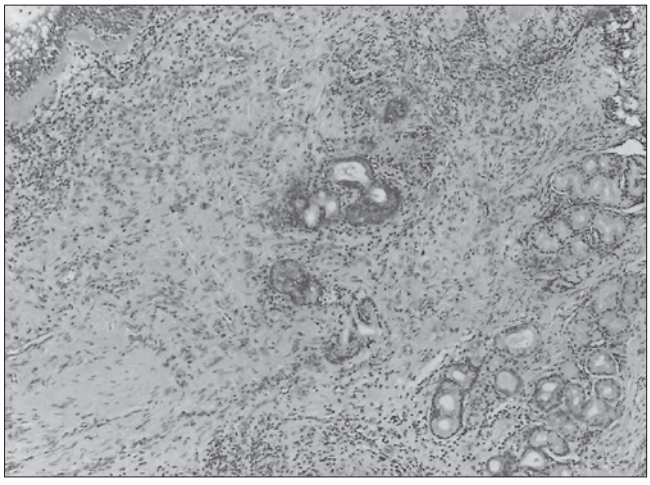

Fig. 1. Immunohistochemistry grade 1 positivity.

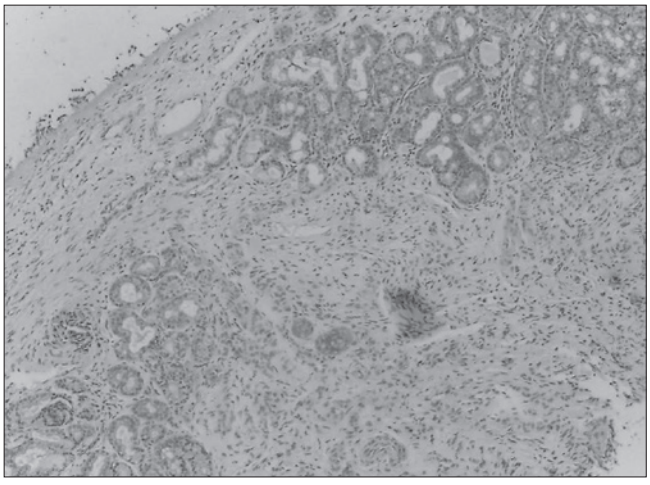

Fig. 2. Immunohistochemistry grade 2 positivity.

Board [17], the goals of rhinosinusitis therapy are to treat the infection, shorten the disease and prevent recurrences; in order to achieve these goals, many different pharmacological approaches have been tested by several study groups. Acute rhinosinusitis (ARS) resolves without antibiotic treatment in most cases, symptomatic treatment and reassurance is the preferred initial management strategy for patients with mild symptoms. Antibiotic therapy should be reserved for patients with severe ARS, especially in presence of high fever or severe (unilateral) facial pain. Clinicians should weigh the moderate benefits of antibiotic treatment against the potential for adverse effects. The aim of a pharmacological approach in ARS is represented by the opening of the ostia, while in chronic inflammatory processes of the nose and paranasal sinuses the goal is to restore healthy respiratory mucosa.

As already stated, bromelain has well-known effects such as antithrombotic, anti-oedema and fibrinolytic activity, and it is active as anti-inflammatory drug in chronic inflammation and/or autoimmune diseases [3-5], reduction of allergic reaction, modulation of macrophages, NK cells and T cells and increase IL-1β, IL-6, and TNFα secretion [6]. Considering these effects, the high concentration in CRS nasal cells we reported is good evidence of its strong potential in upper airways

These findings of the penetration of bromeline strongly call for confirmatory clinical trials in patients with CRS. In fact, the presence in tissue of a particular molecule, with known pharmacological effects, is the first step needed to continue with clinical studies.

Conclusions

Following our results, in the future it will be interesting to understand how distribution from serum to tissues is achieved, how it may be vary among patients and between patients and controls, and whether other modes of administration (for example intranasal administration) are equally effective or not.

Bromelain's ability of diffusion as an anti-inflammatory drug could be exploited in the treatment of nasal and sinus pathologies. The pharmacokinetics and pharmacodynamics characteristics of Bromelain and its safety profile could make it an option to achieve therapeutic results in CRS, thanks to its good tolerability and safety with no specific restrictions.

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Audiology

Effect of loading of the central part of the tympanic membrane on pure tone audiometry

Applicazione di pesi a livello della parte centrale della membrana timpanica: effetti sull'audiometria tonale

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SUMMARY

This study was conducted to determine the effects of loading of the central part of the tympanic membrane by different weights on pure tone audiometry of healthy ears. Sixty patients with normal otoscopic view, normal pure tone audiometry and wide external auditory canal to allow direct and endoscopic visualization of TM, but without any history of ear surgeries, were selected and divided equally and randomly into two groups. Loading of the central part of the TM was carried out using weights ranging from [(1 λ) 13.6 mg] to [(40 λ) 544 mg]; (λ) is a symbol for the weight of 1 microliter of mercury. The study was carried out in two steps assisted by direct oto-endoscopy, and pure tone audiometry was used to measure the effects of loading on both air and bone conduction hearing. Air conduction hearing thresholds increased in a statistically significant pattern at low frequencies, 500 Hz, 1000 Hz and 2000 Hz, when the TM was loaded by 340 mg (25 λ). The maximal effect was recorded at 544 mg (40 λ), which affected air conduction hearing at all tested frequencies (500 Hz, 1000 Hz, 2000 Hz and 4000 Hz). However, no statistically significant effect was detected on bone conduction hearing thresholds throughout the study. In conclusion, loading of the tympanic membrane by different masses affects the air conduction hearing threshold by only 340 mg (25 λ), which is very large in comparison to the mass of ossicles, without any significant effects on bone conduction hearing.

KEY WORDS: Loading • The central part of the Tympanic Membrane • Compressed aluminum pellets • Pure tone audiometry • Hearing threshold

RIASSUNTO

Questo studio è stato condotto al fine di determinare gli effetti sull'audiometria tonale dell'applicazione di masse di peso differente a livello della porzione centrale della membrana timpanica di un orecchio sano. Sono stati selezionati sessanta pazienti con anamnesi negativa per pregressa chirurgia dell'orecchio, aventi otoscopia nella norma, soglia audiometrica tonale nella norma, e un canale uditivo esterno sufficientemente ampio da permettere la visione endoscopica diretta della membrana timpanica. I pazienti sono stati equamente suddivisi in maniera randomizzata in due gruppi, Gruppo I e Gruppo II. Sono state utilizzate masse di differente peso, da 13,6 mg (1 λ) a 544 mg (40 λ); λ è pari al peso di un microlitro di mercurio. Lo studio è stato condotto in due steps, sotto visione otoendoscopica; l'audiometria tonale è stata utilizzata per valutare l'effetto dell'applicazioni dei pesi, sia sulla via aerea sia sulla via ossea. Con l'applicazione di un peso pari a 340 mg (25 λ), la soglia per via aerea è aumentata in maniera statisticamente significativa alle frequenze di 500, 1000 e 2000 Hz. Il massimo risultato è stato ottenuto con un peso pari a 544 mg (40 λ), che ha avuto effetto su tutte le frequenze testate (500, 1000, 2000 e 4000 Hz). Tuttavia, non è stato registrato alcun effetto statisticamente significativo sulla conduzione per via ossea. In conclusione, l'applicazione di pesi a livello della membrana timpanica, utilizzando però masse di peso notevole (340 mg), modifica la conduzione e la soglia per via aerea, ma non ha alcun effetto statisticamente significativo sulla conduzione per via ossea.

PAROLE CHIAVE: *Porzione centrale della membrane timpanica • Audiometria tonale • Soglia audiometrica*

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Introduction

Despite its clinical and surgical importance, mass loading of the central part of the tympanic membrane (TM), particularly point wise version, is a rarely discussed topic in the literature.

Basically, a stroboscopic holographic interferometer, which is used to measure vibration of the human TM, reveals that when it is stimulated by 500 Hz and 1000 Hz, its entire surface moves in points with the major indications occurring in posterior half. However, at 2000 and 4000 kHz, the TM vibrates with multiple (4 to 10) lo-

cal maxima, dispersed throughout the surface of the TM. Many of these maxima occur at the same phase of stimulation, while some occur at the opposite phase, and others show signs of graded phase with position (maxima means the point of maximal vibration) [1 2].

Vibrations of TM are affected by its mass; when it increases, volume velocity generated by the acoustic stimulus is expected to decrease [3]. Moreover, suppression of such vibration leads to variable degrees of hearing loss, if they are suppressed in a selective and focused way (pointwise), which will produce degrees of hearing loss that are essentially different from hearing loss produced from surface loading (covering), in turn inhibiting all TM vibration [4].

The main two studies in this field revealed that a 0.13 cm^3 mercury drop, which weighs about 176 mg, causes a loss of almost 20 to 40 dB, while a water drop of volume 0.2 cm^3 produces a loss of only about 3-27 dB**,** while oil occupies an intermediate position [1 3]; both studies investigated the surface covering effect. This means that mass loading on a specific part of the TM has not been tested on humans. In this study, the 'pointwise' method was used not only because it is not studied previously, but also because if the effect is known clearly it will provide practical, scientific and non-statistical evidence why heavy and light grafts such as cartilage and fascia respectively have similar hearing results as is well known in the current literature [5].

Materials and methods

The study involved 60 patients who attended the outpatient clinic of the Otolaryngology-Head and Neck Surgery Department, Alexandria Main University Hospital seeking treatment for non-otological conditions. They were prospectively recruited to participate in the study which was approved and conducted by the guidelines of the local institutional review board. Moreover, all participants signed informed detailed consent prior to the study.

All participants had normal otoscopic view, normal pure tone audiometry and wide external auditory canal to allow direct and otoscopic visualization of the TM; they did not have any history of ear surgeries.

The 60 participants were divided randomly and equally in two groups, Group I and Group II, each with 30 participants.

The study was done in two steps:

The first step was a preliminary study to detect the load that might affect hearing; it was conducted for group 'I' (60 TMs) according to the following steps:

1. Full audiological assessment of all participants in the form of air and bone conduction pure tone audiometer to make sure that they had normal air and bone conduction hearing thresholds, and imminence audiometer, including tympanometry and static imminence to ensure a normal middle ear condition.

2. Loading of the tympanic membrane:

I. Preparation of loads:

A)Weight of loads:

The basic weight was 13.6 and its multiples because Tonndorf, J. (1964) used a one microliter mercury drop, which weighs 13.6 mg (λ), as the smallest load to measure the effect of mass loading in animals and five multiples of this weight to measure further effects [6]; it is also the weight of the tympanic membrane. Thus, this weight and 10 multiples were used to improve the accuracy of the results:

[(1 λ) 13.6 mg], [(2 λ) 27.2 mg], [(5 λ) 68 mg], [(10 λ) 136 mg], [(15 λ) 205 mg], [(20 λ) 272 mg], [(25 λ) 340 mg], [(30 λ) 408 mg], [(35 λ) 476 mg] and [(40 λ) 544 mg]

B) Shape and surface area:

Because it was extremely difficult to reach the heavy weights in a small surface area to ensure pointwise loading principle and all loads are expected to have standard measures, square shaped aluminum plates with a surface area of 16 mm^2 (Fig. 1) were also used because it is the resting surface area of a mercury drop (13 ± 3.58 mm^2) used by prior researchers.

C) Composition of load:

We selected aluminum loads because it was easy to shape it and easy to reach the target weight with the standardised surface area. The smaller loads, up to 340 mg, were prepared from aluminum foil that was folded to give the

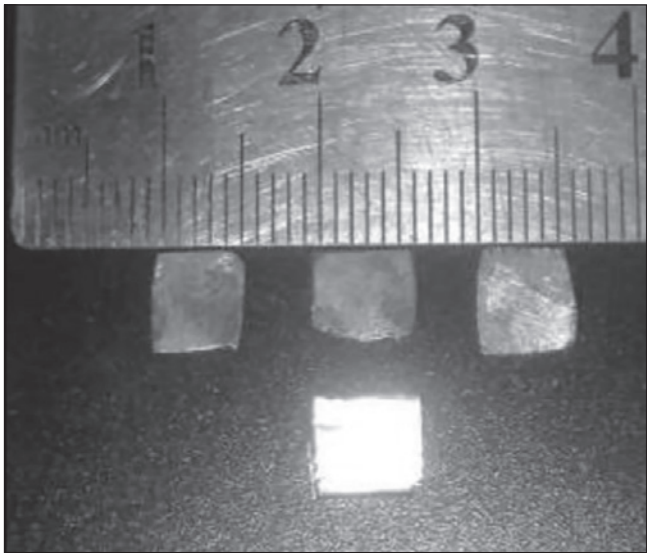

Fig. 1. Different weights with different thicknesses and surface area.

target weight and surface area. However, the larger loads were prepared from thick aluminum pellets that were compressed to the standard measures because it was difficult to reach the target weight using the thin aluminum foil (Fig. 1).

D) Total loads: all 10 selected weights had six further loads each, for a total of 60 loads.

II. Application of loads:

Each ear in group I was loaded by one of the prepared loads as the following:

An otoendoscope of 2.0 mm diameter, 58 mm length and 0° angles, was used to help good visualisation of all circumferences of the TM (umbo, malleus, annulus anterior and posterior malleolar folds), under this clear vision 0.05 ml of greasy and high viscosity petroleum jelly was applied to the umbo to prevent fall of the load.

Then, a plate of unknown weight, for both the patient and audiologist, was adjusted to the TM at the region of the umbo without any contact with the walls of the external auditory canal (Fig. 2).

3) Audiological assessment.

This step was double-blinded as neither the audiologist nor patient had any information about the weight of the plates.

Pure tone audiometry was done immediately after application of the loads to assess the effect of each load on air and bone conduction hearing thresholds at frequencies of (500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz).

4) Plates were removed a few hours after they fell from the TM with oto-endoscopic assistance.

After the results of this stage had been calculated, the second stage was conducted to confirm them, and included the same steps but with some differences:

1. Five weights were selected from the weights of the first step, [(10 λ) 136 mg], [(20 λ) 272 mg], [(25 λ) 340 mg], [(30 λ) 408 mg], [(40 λ) 544 m], to confirm positive and negative results of the first step.

2. Number of loads:

Twelve plates for each of the five selected weights were prepared; 60 loads of above mentioned measures and compositions were prepared. Each ear in group II was loaded by one of the prepared loads using the same method in the first step.

Statistical analysis of loading effects

At the beginning, we calculated the means and standard deviations of pre- and post-loading air and bone conduction hearing thresholds for each load at 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz. Subsequently, the mean differences between pre- and post-loading means were calculated.

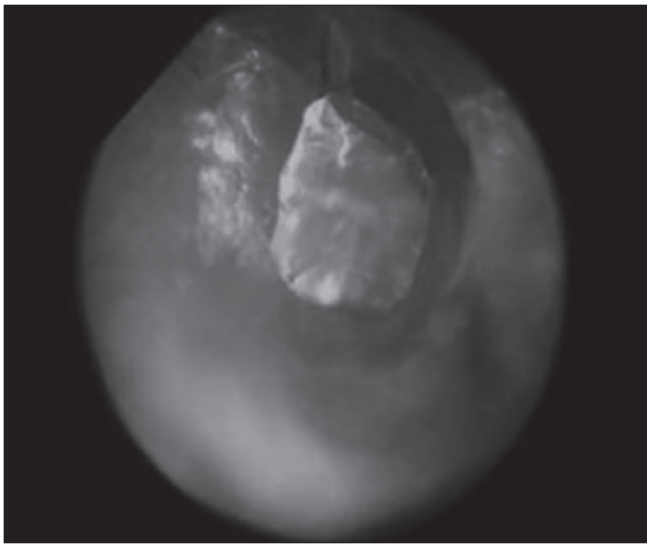

Fig. 2. The weight loaded on the TM.

After that, a t-test was used to compare the mean of the preloading air and bone conduction with post-loading counterparts for every load at 500 Hz, 1000 Hz, 2000 Hz and 4000 Hz. A two-tailed p-value was used to measure the statistical significance of measured differences.

Lastly, 95% confidence interval and standard error of difference was used to improve the accuracy of results for step two results.

Results

The study included 48 males and 12 females, with an age ranging from 20-58 years and a mean of 33.9 ± 10.04 years. The mean weight of the ointment pieces used to hold the plate in place was 3 ± 0.58 mg in vitro. This weight was neglected not only because it was impossible to measure the real weight of the ointment on the TM (in vivo) due to the wasted amount on the tip of the cotton applicator, but also because the same volume (0.05 ml) was used in all cases.

Results of the first step

Small loads (13.6 mg, 27.2 mg, 68 mg, 136 mg, 205 mg, and 272 mg) did not have any significant effect on either type of hearing. The two-tailed p-value was ≥ 0.05.

A statistically significant effect of mass loading was detected when the TM was loaded by 340 mg (25 λ). The mean air conduction hearing losses were 10±3.55 dB, 10 ± 2.35 dB and 10 ± 3.69 dB at 500, 1000 and 2000 Hz, respectively, with a p-value < 0.05. However, some differences were measured at 4000 Hz, although these differences were not statistically significant.

The effect increased gradually as the weight of the load was increased; at 476 mg (35 λ) the mean differences between the mean pre- and post-loading air conduction hearing thresholds were -5 ± 5.22 dB, -10 ± 3.98 dB, -15 ± 4.22 dB, and -15 ± 2.35 dB at 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz, respectively, with a two-tailed p-value < 0.05.

Similarly, at the maximal weight used in this study, 544 mg (40 λ), the mean air conduction hearing loss was 15 ± 4.32 dB at 500 Hz and 10 ± 3.47 dB, 10 ± 4.29 dB and 10 ± 3.22 dB at 1000, 2000 and 4000 Hz, respectively. Lastly, none of the loads had a statistically significant effect on bone conduction hearing thresholds.

Results of the second step

When the TM was loaded by 136 mg (10 λ), the mean of differences between pre- and post-loading air hearing thresholds were 0±0.36 dB at 500 Hz, 1 ± 0.58 dB at 1000 Hz, 0 ± 0.98 dB at 2000 Hz and 1 ± 1.36 dB at 4000 Hz, with no significant differences. However, there were similar differences in bone conduction hearing thresholds (2 ± 0.25 dB at 500 Hz, 0±0.89 dB at 1000 Hz, 1 ± 1.89 dB at 2000 Hz and 1±0.36 dB at 4000 Hz), none of which were statistically significant (p > 0.05 for all; Table I).

Similarly, at 272 mg, there were several differences at air and bone conduction hearing, but none with any statistical significance (Table II). Line graph of pre- and post-loading thresholds were identical without any gaps (Fig. 3).

A statistically significant effect of mass loading was detected when the TM had a central load of 340 mg (25 λ). At this weight, the mean differences at 500 Hz, 1000 Hz and 2000 Hz were statistically significant with paired p-value < 0.05; at 500 Hz the mean difference was -10 ± 2.35 dB, at 1000 Hz the ear lost 10 ± 4.45 dB and at 2000 Hz air conduction hearing loss was 10 ± 2.18 dB. However, the effect of this mass was not significant at 4000 Hz (Table III) (Fig. 4), although with no statistically significant effect on bone conduction hearing (Table III).

At 408 mg (30 λ), the mean air conduction hearing losses (mean differences) were 17±3.38 dB at 500 Hz, 16 ± 4.87 dB at1000 Hz and 10 ± 2.60 dB at 2000 Hz. Nonetheless, this had no significant effect on 4000 Hz (Table IV). There were several differences in bone conduction hearing thresholds, but none with statistical significance (p > 0.05 for all; Table IV).

The above-mentioned effect increased in intensity when TM had a load of 544 mg (40 λ). The mean air conduction hearing loss (mean difference) was 20 ± 3.44 dB at 500 Hz, which continued at other frequencies but to a lesser degree, (14 ± 5.67 dB at 1000 Hz, 10 ± 3.58 dB at 2000 Hz and 10 ± 3.98 dB at 4000 Hz; Table V). Larger gaps between pre- and post-loading air conduction hearing threshold depicted this phenomenon (Fig. 5). Similar

Table I. Changes in hearing thresholds at 136 mg.

Loads Step 2		Changes of air conduction hearing thresholds				Changes of bone conduction hearing thresholds			
		500 Hz	1000 Hz	2000 Hz	4000 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz
136 mg (10 λ)	Mean pre-loading thresholds ± SD	10 ± 2.2 dB	10 ± 1.52dB	10 ± 3.5 dB	12 ± 2.5 dB	11 ± 3.85 dB	10 ± 3.98 dB	12 ± 4.96 dB	10 ± 3.58 dB
	Mean post loading threshold ± SD	10 ± 3.58 dB	12 ± 2.15 dB	10±2.56 dB	14 ± 2.36 dB	10 ± 2.86 dB	10 ± 3.58 dB	13 ± 3.5 dB	11± 2.52 dB
	Mean difference ± SD	0 ± 0.36 dB	1 ± 0.58 dB	0±0.98 dB	1 ± 1.36 dB	2 ± 0.25 dB	0 ± 0.89 dB	1 ± 1.89 dB	1 ± 0.36 dB
	95% CI	± 2.51561	± 2.57634	± 2.5960	-4.0582 dB to 0.0582 dB	-1.87128 to 3.87128	± 3.20483	-4.63429 to 2.63429	-3.62100 to 1.62100
	Standard error of difference	1.213 dB	0.760 dB	1.252 dB	0.992 dB	1.385 dB	1.545 dB	1.752 dB	1.264 dB
	two-tailed P value	1.0000	0.2018	1.0000	0.0563	0.4777	1.0000	0.5740	0.4372

Table II. Changes in hearing thresholds at 272 mg.

Loads Step 2		Changes of air conduction hearing thresholds				Changes of bone conduction hearing thresholds			
		500 Hz	1000 Hz	2000 Hz	4000 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz
272 mg (20 λ)	Pre-loading thresholds ± SD	10 ± 3.65 dB	12 ± 2.36 dB	10 ± 3.78 dB	10 ± 2.87 dB	10 ± 2. 36 dB	14 ± 2.69 dB	12 ± 3.5 dB	10 ± 1.25 dB
	Post-loading threshold ± SD	10 ± 3.25 dB	11 ± 3.25 dB	11 ± 2.63 dB	10 ± 4.52 dB	11 ± 3.25 dB	12 ± 3.58 dB	12 ± 2.85 dB	10 ± 2.87 dB
	Mean difference ± SD	0 ± 0.63 dB	1 ± 0.97 dB	1 ± 0.12 dB	0 ± 0.36	1 ± 0.58 dB	2 ±1.85 dB	0 ± 0.36 dB	0 ± 0.89 dB
	95% CI	± 2.92586	-1.40457 to 3.40457	-3.75685 to 1.75685	± 2.71902	-3.40457 to 1.40457	-0.68087 to 4.68087	± 2.70218	± 1.87409
	Standard error of difference	1.411dB	1.159 dB	1.329 dB	1.311 dB	1.159	1.293	1.303	0.904
	two-tailed P value	1.0000	0.3977	0.4599	1.0000	0.3977	0.1361	1.0000	1.0000

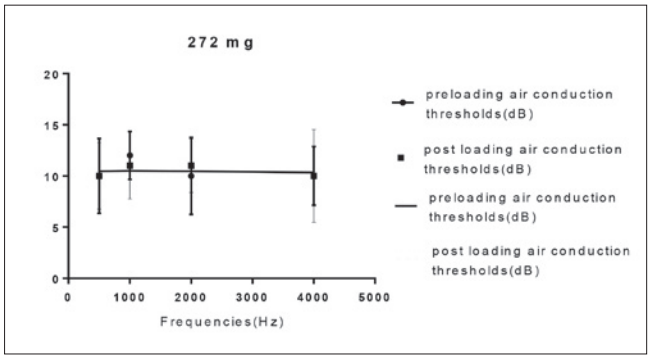

Fig. 3. Changes in air conduction hearing thresholds at 272 mg.

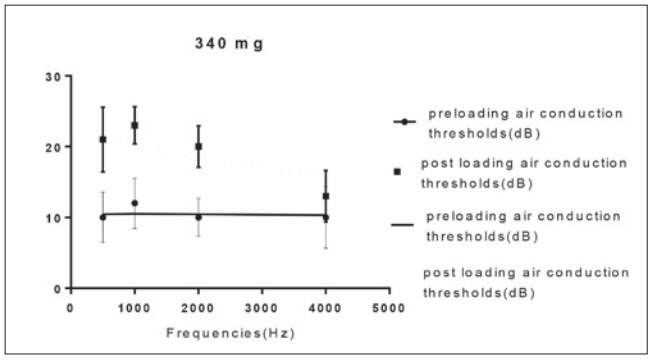

Fig. 4. Changes in air conduction hearing thresholds at 340 mg.

to previous bone conduction results, there was no significant effect.

Discussion

This topic is one of the least discussed issues in the literature despite its great relevance. Its importance can be observed by the fact that the greater the understanding of TM biomechanics, the greater the potential to monitor future advances in medical technology related to its surgical repair (myringoplasty) [7].

There are only a very limited number of studies in the literature about this issue, which also involved animal experiments cats, dogs, rats and guinea pig [6]. Lüscher E. (1945) concluded that pointwise loading of the umbo or the manubrium in cats causes predominantly deafness towards low frequencies, whereas surface loading (covering) of the pars tensa, principally, causes deafness towards high frequencies [4].

According to repeated results of our study, the human hearing system is resistant to pointwise mass loading except at very large masses (340 mg;25 λ). This mass is very large compared to the mean weight of the ossicles and the TM; the mean weights of human ossicles are 23 mg for the malleus, 27 mg for the incus and 4 mg for the stapes; the average weight of the TM is 14 mg [8]. It is also larger than the mean weight of the cartilage graft, which is 20 ± 4.36 mg according to our experiments [5].

Masses from 13.6 mg (λ) to 272 mg (20 λ) did not have any significant impact on air or bone conduction hearing thresholds. The only significant effect was seen when the TM had a load of 340 mg. The air conduction hearing thresholds at 500 Hz, 1000 Hz and 2000 Hz increased by 10 ± 3.69 dB, 10 ± 2.35 dB, and 10 ± 3.55 dB, respectively, without any evident effect at the frequency of 4000 Hz.

When the TM had a load of 408 mg (30 λ), the air conduction hearing threshold was increased by 17 ± 3.38 dB at 500 Hz. This effect decreased towards higher frequencies: at 1000 Hz air conduction hearing threshold increased by16 ± 4.87 dB and at 2000 Hz it increased by 10 ± 2.60 dB, but there was no effect at 4000 Hz which was affected only when the TM had plates of 476 mg (35 λ) and 544 mg (40 λ). At 544mg (40 λ), the mean air conduction hearing loss was 10 ± 3.98 dB at (4000 Hz). However, several experiments revealed an increase in bone conduction responses at low frequencies accompanied by a decrease at high frequencies and both of these changes, which is consistent in some proportion to the applied load [9]. In our study, there was no statistically significant difference in bone conduction hearing thresholds.

These results demonstrates why a heavy and stiff cartilage graft does not affect hearing results and changes some concepts regarding the mechanics of hearing loss in middle ear effusion; it is evident in the literature that the mass of fluid on the TM may reduce middle-ear input admittance [10]; however, the actual effect of the mass of effusion is minor according to our pointwise loading experiments.

Conclusions

The hearing system is very resistant to mass loading except at very large weights (340 mg). Loading of the TM by different masses affects the air conduction hearing thresholds, especially low frequencies, but does not affect bone conduction hearing. Thus, it is now very clear why hearing results of the heavy and light grafts are statistically non-significant. Lastly, there is an obvious practical and experimental demonstration that the mass of the effusion of the middle ear does not have any role in hearing loss.

Table III. Changes in hearing thresholds at 340 mg.

Loads Step 2		Changes in air conduction hearing thresholds				Changes of bone conduction hearing thresholds			
		500 Hz	1000 Hz	2000 Hz	4000 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz
340 mg (25 λ)	Pre-loading thresholds ± SD	10 ± 3.56 dB	12 ± 3.56dB	10 ± 2.69 dB	10 ± 4.36 dB	13 ± 3.69 dB	10 ± 5.36 dB	14 ± 2.65 dB	12 ± 3.35 dB
	Post-loading threshold ± SD	21 ± 4.56 dB	23 ± 2.63dB	20 ± 2.95 dB	13 ± 3.65 dB	14 ± 2.31 dB	12 ± 2.46 dB	13 ± 2.36 dB	12 ± 2.84 dB
	Mean difference ± SD	10 ± 2.35 dBHL	10 ± 4.45 dBHL	10 ± 2.18 dBHL	4 ± 2.36 dBHL	0 ±. 25 dBHL	2 ± .85 dBHL	1 ± 1.63 dBHL	0 ± 0.21 dBHL
	95% CI of difference	-13.36981 to -6.63019	-13.64981 to -8.35019	-12.39010 to -7.60990	-6.40415 to 0.40415	-3.6063 to 1.6063	-5.53072 to 1.53072	-1.12442 to 3.12442	± 2.62928
	Standard error of difference	1.625	1.278	1.152	1.641	1.257	1.702	1.024	1.268
	two-tailed P value	<0.0001*	<0.0001*	<0.0001*	0.0812	0.4347	0.2527	0.3396	1.0000

Table IV. Changes in hearing thresholds at 408 mg.

Loads Step 2		Changes in air conduction hearing thresholds				Changes of bone conduction hearing thresholds			
		500 Hz	1000 Hz	2000 Hz	4000 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz
408 mg (30 λ)	Pre-loading thresholds ± SD	10 ± 2.35 dB	12 ± 3.56 dB	14 ± 4.36 dB	14 ± 2.56 dB	10 ± 2.36 dB	10 ± 3.56 dB	12 ± 2.47 dB	11 ± 3.25 dB
	Post-loading threshold ± SD	27 ± 2.36 dB	28 ± 3.56 dB	24 ± 2.36 dB	16 ± 2.36 dB	10 ± 3.65 dB	12 ± 3.26 dB	10 ± 4.35 dB	10 ± 3.89dB
	Mean difference ± SD	17 ± 3.38 dB HL	16 ± 4.87 dBHL	10 ± 2.60 dBHL	2 ± 1.56 dBHL	0 ± 0.25 dBHL	0 ± .59 dBHL	2 ± 1.36 dBHL	1 ± 0.29 dBHL
	95% CI of difference	-18.99388 to -15.00612	-19.0141 to -12.9859	-12.96808 to -7.03192	-4.08449 to 0.08449	± 2.60215	-4.88989 to 0.88989	-0.99478 to 4.99478	-2.03468 to 4.03468
	Standard error of difference	0.961	1.453	1.431	1.005	1.255	1.393	1.444	1.463
	two-tailed P value	<0.0001*	<0.0001*	<0.0001*	0.0592	1.0000	0.1653	0.1799	0.5015

Table V. Changes in hearing thresholds at 544 mg.

Loads Step 2		Changes in air conduction hearing thresholds				Changes of bone conduction hearing thresholds			
		500 Hz	1000 Hz	2000 Hz	4000 Hz	500 Hz	1000 Hz	2000 Hz	4000 Hz
544 mg (40 λ)	Mean pre-loading thresholds ± SD	10 ± 3.25dB	10 ± 2.89 dB	13 ± 5.85 dB	10 ± 5.85 dB	12 ± 3.36 dB	10 ± 1.56 dB	10 ± 2.47 dB	13 ± 2.25 dB
	Mean post-loading threshold ± SD	30 ± 3.25dB	25 ± 3.65 dB	24 ± 3.69 dB	20 ± 2.36 dB	11 ± 3.21 dB	10 ± 3.24 dB	12 ± 4.25 dB	12 ± 2.89 dB
	Mean difference ± SD	20 ± 3.44 dBHL	14 ± 5.67 dBHL	10 ± 3.58dBHL	10 ± 3.98 dBHL	1 ± 0.25 dBHL	0 ± .89 dBHL	1 ± 1.36 dBHL	1 ± 0.99 dBHL
	95% CI	-22.86821 to -17.13179	-17.78719 to -12.21281	-14.14077 to -5.85923	-13.77650 to -6.22350	-1.78199 to 3.78199	± 2.15284	-4.94287 to 0.94287	-1.19271 to 3.19271
	Standard error of difference	1.383	1.344	1.997	1.821	1.341	1.038	1.419	1.057
	two-tailed P value	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.4639	1.0000	0.1727	0.3545

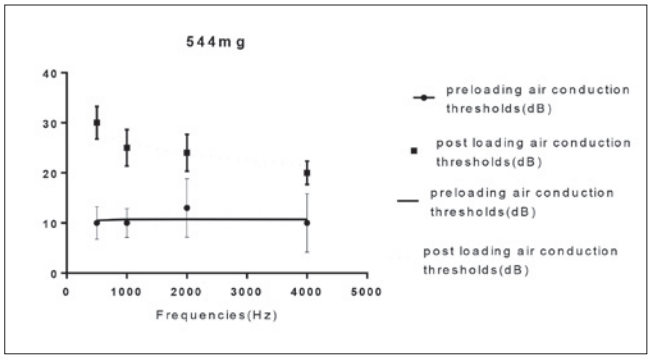

Fig. 5. Changes in air conduction hear at 544 mg.

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only work to produce a positive impact in the literature that could change some concepts in my specialty.

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Audiology

Transtympanic Hearing Aid: exploratory study on a new device

Transtympanic Hearing Aid: studio preliminare con nuova protesi

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SUMMARY

In this paper, we present the preliminary results achieved with a transtympanic hearing aid (THA). This is a modified digital, open-fit external hearing aid (HA) designed for acute study only, which allows coupling with a pre-implanted ventilation tube. The THA conveys amplified sound directly onto the round window, bypassing the ossicular chain, in contrast with traditional HAs that convey sound onto the second or third portion of the external auditory canal. The THA has been developed as an alternative to standard HAs and active middle ear implants for patients who are unsatisfied with traditional HA outcomes and want to avoid middle-ear implantation. The results achieved using the THA were compared to those obtained with an equivalent device, the Latitude 8 Moxi 13 (Moxi), uncoupled from the ventilation tube, and placed onto the outer ear. For this purpose, 12 patients with conductive (1/12), sensorineural (3/12), or mixed (8/12) hearing loss from moderate to severe, with a pre-implanted ventilation tube, underwent audiological evaluation with both the THA and the Latitude 8 Moxi 13 (Moxi). Our initial results showed that the THA provided significant improvement in the warble tone results in comparison to the Moxi. Moreover, patients with a PTA between 41 and 90 also achieved better results in terms of speech recognition using the THA in comparison to the Moxi. In conclusion, these outcomes provide the first evidence of the potential benefits of the THA over standard open-fit HAs. Nevertheless, these preliminary outcomes require further confirmation.

KEY WORDS: Transtympanic hearing aid • Sound conveying • Hearing loss • Round window

RIASSUNTO

Nel presente articolo vengono riportati i risultati preliminari ottenuti con l'apparecchio acustico Transtympanic Hearing Aid (THA). Il THA è una protesi acustica digitale, open-fit modificata, sviluppata per studi clinici in acuto e ideata per essere accoppiata a un drenaggio trans-timpanico, precedentemente posizionato, con lo scopo di inviare il suono amplificato direttamente all'orecchio medio e alla finestra rotonda, bypassando la catena ossiculare. Il dispositivo THA rappresenta una possibile alternativa sia alle protesi acustiche tradizionali, sia alle protesi attive di orecchio medio, per pazienti con risultati insoddisfacenti con protesi acustiche tradizionali, evitando la chirurgia di impianto di protesi di orecchio medio. I risultati ottenuti con la THA sono stati confrontati con quelli ottenuti con un device equivalente, una protesi acustica tradizionale, open-fit, non accoppiata al drenaggio trans-timpanico, la Latitude 8 Moxi 13 (Moxi). Con questo scopo, 12 pazienti affetti da ipoacusia trasmissiva (1/12), neurosensoriale (3/12) o mista (8/12), di entità da moderata a grave, nei quali era stato precedentemente posizionato un drenaggio trans-timpanico, sono stati sottoposti ad una valutazione audiologica sia con il dispositivo THA che con il Moxi. I risultati preliminari hanno dimostrato che la protesi acustica THA ha consentito di raggiungere risultati, in termini di soglia audiometrica in campo libero con device in funzione, significativamente superiori a quelli conseguiti con la protesi Moxi; inoltre usando la protesi THA anche i risultati in termini di percezione verbale sono stati migliori, soprattutto nei pazienti con PTA tra 41 e 90 dB. I risultati preliminari presentati, offrono una iniziale evidenza dei benefici potenzialmente offerti dall'uso del dispositivo THA, rispetto alle protesi acustiche tradizionali. Tuttavia questi dati devono essere confermati da studi ulteriori su un più ampio numero di pazienti.

PAROLE CHIAVE: *Transtympanic hearing aid • Trasmissione sonora • Perdita di udito • Finesra rotonda*

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Introduction

Hearing aids (HAs) improve verbal discrimination and thus interpersonal communication in patients suffering from mild to moderate hearing loss (HL) [1]. Nonetheless, up to 40% of patients with HA indication report that they can either not use the prescribed HA or gain sufficient benefit from it [2].

This reduced acceptance is mostly linked to typical HA drawbacks, such as feedback, signal distortion, occlusion

effect, inadequate amplification of the high frequencies and limited compatibility with outdoor activities. Furthermore, diseases such as chronic otitis media, infections of the external auditory canal (EAC) or a stenotic EAC may limit the effectiveness of the HAs [3]. Also, invasive ear surgeries, such as radical mastoidectomy or ossiculoplasty severely limit the potential benefits of HAs. The current alternative to HAs in these cases is surgical placement of an active middle ear implant (AMEI), which is however far more expensive and invasive than a HA [4]. Accordingly, this is generally not an option for patients without financial support.

The transtympanic hearing aid (THA) described in this paper has been developed as an alternative to standard HAs and AMEIs if the patient is unsatisfied with HA outcomes, but who does not want to undergo middle ear implantation. The THA is a modified digital open-fit HA designed to allow coupling with a pre-implanted ventilation tube. The THA conveys the amplified sound directly onto the round window bypassing the ossicular chain, in contrast with traditional HAs that convey sound onto the second or third portion of the EAC. This configuration is expected to reduce distortion and improve the gain in a wider frequency range in comparison with standard HAs. The study described herein is the first attempt to evaluate this device in patients with different types of HL considering sound perception in the audible frequency range and in terms of speech recognition.

Table I. Characteristics of the tested population.

Enrolment characteristics	No.	%
Subjects enrolled in the study	15	100
Subjects who completed the study	12	80
Withdrawals	3	20
Mean age: 63 years		
Mean HL duration: 29 years		
Gender		
Female	7	58.3
Male	5	41.7
Tested ear		
Right	6	50
Left	6	50
Aetiology		
COM	11	73.3
Ménière's disease	1	6.7
Tympanosclerosis + COM	2	13.3
Stapes ossification + COM	1	6.7
Type of HL		
Mixed	10	66.7
Sensorineural	4	26.6
Conductive	1	6.7
HA experience		
None	10	83.4
1 year	1	8.3
5 years	1	8.3

Materials and methods

The aim of the study is to provide initial assessment on the performance of a new THA (investigational medical device): the results obtained by patients while using the THA, connected to the ventilation tube, were compared with those obtained by the same patients using the Latitude 8 Moxi 13 HA (Moxi), which is considered as a comparator in the present study.

The study group was composed of 15 subjects between 39 and 74 years diagnosed with mild to severe post-lingual HL of conductive (1/15), sensorineural (4/15), or mixed (10/15) origin. Subjects were eligible only if they had not been wearing a HA in the last 2 years and have been previously implanted with a ventilation tube in the ear to be tested for medical purposes. In 7/12 patients, the ventilation tube was placed in the posterior inferior quadrant and in 5/12 patients in the anterior inferior quadrant of the tympanic membrane. All enrolled patients were fluent in Italian. Detailed information about the study population is provided in Table I.

This was an exploratory, two-arm, randomised, cross-over, cross-sectional, monocentric (Pisa, Italy), comparative study. The subjects did not know if they were wearing the THA or the Moxi, and the statistician performing data analysis did not receive information about the type of HA was under evaluation. According to the cross-over design of the study, subjects were randomised with the freeware "Research Randomizer" (https://www.randomizer.org/) in Arm 1, where they were tested first with the THA and then with the Moxi; and in Arm 2, where they were tested first with the Moxi and then with the THA. The wash-out period between the two testings with either device was 1 hour.

The study included a one-off visit to the clinic with 3 consecutive sessions. In the first session (screening), the subject's medical and audiological history were recorded. In addition, a general physical examination was performed as well as otomicroscopy to assess middle ear status and verify the position of the ventilation tube. This session also included pure tone audiometry (PTA; Interacoustics Clinical Audiometer AC40) in a sound-treated room at 0.25-0.5-1-2-3-4-8 KHz and assessment of air conduction thresholds at the same frequencies. Bone conduction thresholds were assessed at 0.25-0.5-1-2-4 KHz. The second and third sessions (testing) were used to evaluate either the THA and

then the Moxi or vice-versa, according to the cross-over design described above. At the beginning of each session evaluating the THA, the latter was connected to the pre-implanted ventilation tube under otomicroscopy and the patient was fitted with BEM-ONE FIT-BOSS-PRO (CRAI S.p.A., Italy). At the beginning of each session evaluating the Moxi, the latter was placed behind the ear (BTE) and the pre-implanted ventilation tube temporally closed under otomicroscopy. At this point, the patient was fitted with Auto Pro 2 software (Unitron, U.S.). Upon fitting with either HA, the patient was given a 40 min adaptation period before being tested. Each subject underwent PTA in free field with warble tone and was asked to perform a disyllabic word and an adult sentence test depending on the study arm they had been assigned to, according to the randomisation matrix. According to the cross-over design of the study, subjects were randomised with the freeware "Research Randomizer" (https://www.randomizer.org/) in Arm 1, where they were tested first with the THA and then with the Moxi; and in Arm 2, where they were tested first with the Moxi and then with the THA.

Warble tone thresholds were obtained at 0.25, 0.5, 1, 2, 3 and 4 kHz in sound field. The subject was placed at 0° azimuth to the speaker. The disyllabic word test was conducted in sound field at 65 dB SPL, with the subject positioned at 0° azimuth to the speaker. Lists of 50 words extracted from "Audiometria Vocale, Volume II, CD 1, GN ReSound Italy S.r.l." were presented. Track 12 was used in the first and Track 13 in the second of the 2 subsequent tests in Arm 1 and Arm 2. The adult sentence test was conducted in a sound field at 65 dB SPL, with the subject positioned at 0° azimuth to the speaker. Lists of 20 sentences extracted from "Audiometria Vocale, Volume II, CD 2, GN ReSound Italy S.r.l." were presented. Track 12 was used in the first and Track 13 in the second of the 2 subsequent tests in Arm 1 and Arm 2. Positive score was counted only when the entire sentence was repeated correctly. In all the tests performed in free field, supra-aural earphones (TDH 39P CA50252 Telephonics 296D000-1) were used to mask the non-tested ear.

Evaluated devices

The THA is manufactured by CRAI S.p.A. and consists of an open-fit external HA modified to be coupled, via a connection tube, with the pre-implanted ventilation tube. The Moxi (comparator) is manufactured by Unitron and distributed in Italy by CRAI S.p.A. It is a standard digital open-fit HA. The two HAs have the same technical characteristics (Fig. 1).

Statistical analysis

Subject characteristics and outcome variables were analysed with IBM SPSS statistics software (Version 21, IBM, NY, USA). Paired T-test for independent variables was used to compare the results with the THA vs. the Moxi. Pearson correlation coefficient was used to evaluate correlation between independent variables. All p-values are results of two-sided tests, and a level of significance was set at $p < 0.05$.

Ethical considerations

Informed consent and approval by the Ethics Committee (Approval n. 3429) was obtained prior to initiation. The study complied with applicable good clinical practice (GCP) guidelines and the Declaration of Helsinki.

Results

12/15 (80%) subjects completed the study. One subject was withdrawn because the ventilation tube was not correctly placed, one subject was withdrawn as unable to undergo the testing because of a suppurating ear and one subject was withdrawn as unable to wear the supra-aural earphone without discomfort.

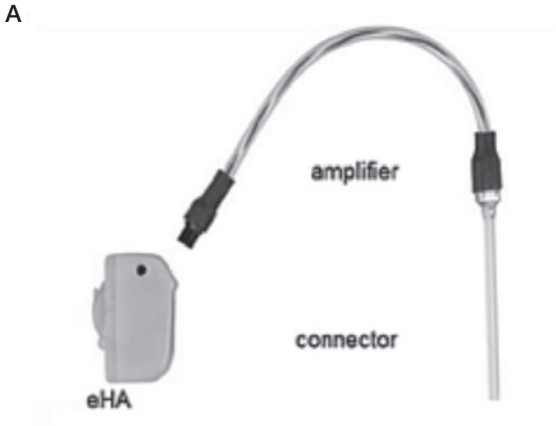
PTA was performed during the screening session and the results are shown in Table II. The PTA at 0.5, 1, and 2 kHz air conduction thresholds rounded to the nearest integer was 63 dB.

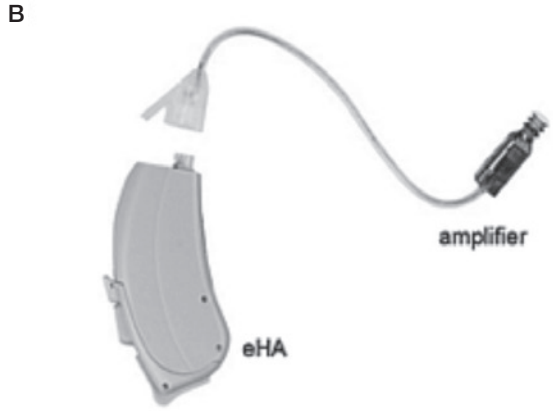
Warble tone analysis showed a statistically significant improvement ($p < 0.05$) with the THA vs. the Moxi only at 0.25, 0.5, 1 and 4 kHz. A graphical representation of the results is depicted in Figure 2.

The analysis of the disyllabic word recognition results showed no statistically significant difference ($p = 0.16$) in performance with the THA and Moxi (Fig. 3). However, when the disyllabic word recognition results were scattered against subjects' PTA, it was observed that in 8/9 (88.9%) of subjects with a PTA between 41 and 90 dB word recognition was between 80% and 100% when wearing the THA, while only 5/9 (55.6%) obtained the same results with the Moxi (Table III). The analysis of the sentence comprehension test showed a trend towards a significant improvement ($p = 0.06$) when the THA was compared with the Moxi (Fig. 3).

Discussion

This paper describes the first testing of the THA in humans, which has been designed to bypass the ossicular chain and directly stimulate the round window. Our initial results showed that the use of the THA provided a significant improvement





C

Technical specifications	THA	Moxi
Configuration	8-channel WDRC	8-channel WDRC
Software	BEM-ONE FIT-BOSS-PRO	Auto Pro 2
Auto-fitting	Performed	Performed
Microphone option	Omnidirectional	Omnidirectional

Fig. 1. A) THA components: external HA, amplifier and connector to which the ventilation tube is coupled; **B)** Moxi components: external HA and amplifier; **C)** Summary of technical specifications of the THA and Moxi.

in the warble tone results in almost all of the frequencies tested - an outcome that could not be achieved with the Moxi. This improvement was supported by the finding that when wearing the THA, 88.9% of the patients with a PTA between 41 and 90 understood between 80% and 100% of the words presented, while only 55.6% of the same patients obtained the same results when wearing the Moxi. Accordingly, these outcomes provide the first evidence of the potential benefits of the THA over standard open-fit HAs.

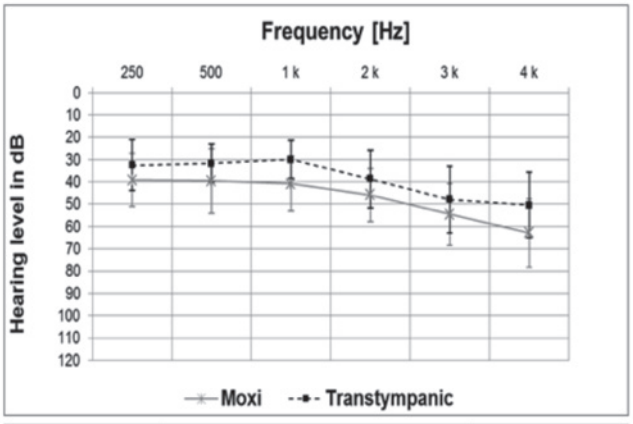
To our knowledge, no other HA similar to the THA is currently being investigated in clinical trials or available on the market. However, the principle behind the development of the THA, i.e. the idea of bypassing the ossicular chain and directly stimulating the round window is not new and has been thoroughly evaluated in the context of implantable and semi-implantable HAs [5-7]. In particular, Colletti et al., in 2006, underlined the possibility to directly stimulate the round window by placing the floating mass transducer (FMT) of the Vibrant Soundbridge (VSB) implant on the round window niche, bypassing the transmission apparatus of the middle ear. The authors reported good results both in terms of audiometric threshold and word discrimination in 7 patients with severe conductive and mixed hearing loss, demonstrating that direct stimulation of the round window is an effective treatment for patients with those characteristics [6]. In 2010, a European multicentre study concluded that direct stimulation of the round window by the FMT of the VSB device represents a safe and effective treatment for adults with conductive and mixed hearing losses [8]. More recently, Colletti et al. (2013) reported on the long-term safety and efficacy of this procedure in a larger series (50 patients) with moderate to severe mixed hearing loss [7]. The potential benefits of the THA over the abovementioned implantable and semi-implantable HAs are its reduced invasiveness, ease of the medical procedure and short time it requires as well as the limited costs for patients.

The study was performed within a limited sample size, including patients with different aetiologies and types of hearing loss. Consequently, it is not possible to generalise the results or consider the hearing loss characteristics as potential predictors for the effectiveness of THA use in comparison with a standard HA.

Another factor that strongly limits the generalisation of the observed results is the THA itself, which is a prototype, was designed exclusively for acute testing rather than for chronic implantation. Accordingly, the testing was "acute" and therefore unable to provide long-term

Table II. PTA results presented as mean values with standard deviation. N/A = not applicable.

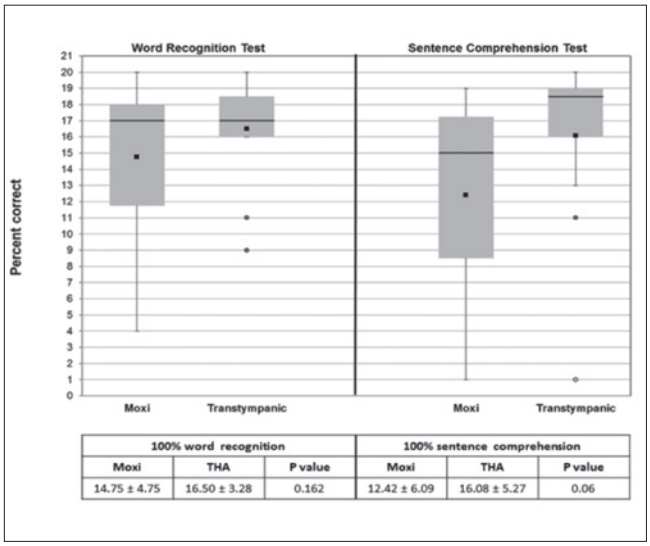
Tested frequency (in kHz)	Bone conduction (in dB)	Air conduction (in dB)
0.25	17.72 ± 10.74	57.50 ± 21.65
0.5	31.67 ± 15.59	56.67 ± 23.48
1	35.83 ± 16.93	65.42 ± 23.13
2	48.33 ± 14.62	67.50 ± 19.09
3	N/A	72.33 ± 16.58
4	45.45 ± 16.44	79.17 ± 16.18
8	N/A	90.50 ± 11.50



Tested frequency (in kHz)	Warble tone (in dB)		P value
	THA	Moxi	
0.25	32.50 ± 10.90	39.17 ± 11.33	0.003*
0.5	31.67 ± 8.25	39.58 ± 13.91	0.003*
1	30.00 ± 8.16	40.83 ± 11.87	0.009*
2	38.75 ± 12.27	45.83 ± 11.33	0.116
3	47.92 ± 14.36	54.58 ± 13.14	0.197
4	50.42 ± 14.06	62.92 ± 14.78	0.044*

** = Paired t-test detected a significant improvement with the THA compared with the Moxi.*

Fig. 2. Above: Mean scores of the warble tone thresholds (in dB) ± standard deviation obtained with the THA and the Moxi. **Below:** All warble tone results presented as mean values with standard deviation. Paired t-test for independent variables was used to test comparisons between the two hearing aid performances.



100% word recognition			100% sentence comprehension		
Moxi	THA	P value	Moxi	THA	P value
14.75 ± 4.75	16.50 ± 3.28	0.162	12.42 ± 6.09	16.08 ± 5.27	0.06

Fig. 3. Above: Word recognition and sentence comprehension test results. The data are displayed as "Box and Whisker" plots, with the mean depicted as black quadrats and the median as horizontal lines. The black circles represent outliers. Only words and sentences repeated by the subjects 100% correctly were considered as positive results. **Below:** Results of the word recognition and sentence comprehension tests with the THA and with the Moxi. All results are presented as mean values with standard deviation. Paired t-test for independent variables was used to test comparisons between the THA and Moxi performance.

results that could confirm the benefits of wearing a THA in real life conditions. Based on these considerations, confirmation of our results should be assessed in long-term studies with more homogeneous and larger cohorts of patients. Both THA and MOXI were fitted based on the respective default parameters, which were minimally different from each other. Thus, we believe they may have had a reduced impact, if any, on the comparison between the results obtained with the two hearing aids.

Considering the promising results of this study, the next steps regarding the THA project include the development of a device for chronic implantation that should be tested to determine whether it is able to guarantee clinically significant acoustic gain, e.g. beneficial modification of the high frequencies (≥ 2 kHz) spectrum. In this respect, the clinical testing of the THA will be performed in larger cohorts of patients with more homogeneous characteristics in terms of aetiology and type of hearing loss.

Conclusions

In agreement with the literature, the results of our study indicate that the direct stimulation of the middle ear and round window may offer advantages over traditional acoustic stimulation. However, the true significance of our results is not yet clear. Although the results of the warble tone are encouraging and to some extent supported by the word and sentence recognition test, the design of our study does not allow drawing generalised conclusions, both because of the limited and heterogeneous study population and the development stage of the THA. Moreover, we can speculate that benefits may be in relation to the

Table III. Scattering of word recognition results in percentage (x-axis) vs. PTA3 (y-axis). The numbers are the subjects' ID (T=word recognition percentage with the subject's wearing the THA; M=word recognition percentage with the subject's wearing the Moxi).

PTA3 (dB)	Word Recognition Percentage									
	100-90	89-80	79-70	69-60	59-50	49-40	39-30	29-20	19-10	09-0
0-10										
11-20										
21-30										
31-40	7T 7M	1T 1M								
41-50	11T 11M 13M	13T								
51-60	10T		10M							
61-70	8M	5T 8T		5M	6T	6M				
71-80	2T	2M 3T						3M		
81-90	9M 9T									
> 91					14M	14T				

type of hearing loss and that the greatest benefits can be achieved in cases with mixed hearing loss, due to conductive deficits (e.g. tympanosclerosis). As a consequence, it seems that possible candidates for the THA device may be patients with chronic otitis media or tympanosclerosis, with moderate-to-severe mixed hearing loss, who can only to a limited extend benefit from traditional hearing aids, including open-fit.

Further, larger studies with more homogeneous populations may help to determine the true effectiveness of the THA and the best target population for its future clinical use.

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Audiology

Genes important for otoneurological diagnostic purposes - current status and future prospects

Geni importanti per la diagnostica otoneurologica: stato dell'arte e prospettive future

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SUMMARY

This review focuses on the current knowledge of the genes responsible for non-syndromic hearing loss that can be useful for otoneurological diagnostic purposes. From among a large number of genes that have been associated with non-syndromic hearing impairment, we selected several best-known genes, including the COCH gene, GJB2, GJB6 and SLC26A4, and we describe their role and effects of mutations and prevalence of mutations in various populations. Next, we focus on genes associated with tinnitus. Important areas for further research include assessment of genes potentially involved in pathophysiology of tinnitus and vertigo, which have traditionally been considered as being of otological aetiology, while advances in neuroimaging techniques have increasingly shifted studies toward neurological correlations.

KEY WORDS: Genetics • Tinnitus • Vertigo • Hearing impairment • Epilepsy

RIASSUNTO

La presente review tratta lo stato dell'arte nella conoscenza dei geni responsabili delle ipoacusie non sindromiche, che potrebbero risultare utili a scopo diagnostico nell'ambito della otoneurologia. Tra i diversi geni identificati in associazione ad un calo dell'udito di tipo non sindromico, ne sono stati selezionati alcuni tra i più noti, come il gene COCH, GJB2, GJB6 e SLC26A4. Di questi vengono descritti il ruolo, l'effetto delle mutazioni a carico e la prevalenza delle stesse mutazioni in diverse popolazioni. Successivamente ci si focalizza sui geni associati al tinnito. Una valida area di ricerca per il futuro è infatti data dall'identificazione di geni potenzialmente coinvolti nella fisiopatologia del tinnito e della vertigine, condizioni tradizionalmente considerate di eziologia otologica, ma che, a seguito di un avanzamento delle tecniche di neuroimaging, si è sempre più propensi a correlare con la sfera neurologica.

PAROLE CHIAVE: *Genetica • Tinnito • Vertigine • Ipoacusia • Epilessia*

Acta Otorhinolaryngol Ital 2018;38:242-250

Introduction

The inner ear consists of two organs that are evolutionarily related and have a similar physiology: the cochlea and the peripheral vestibular system (labyrinth). The cochlea is involved in sound perception. The vestibular labirynth consists of the sacculus and utriculus, which predominantly register linear accelerations, including gravity, and the semicircular canals, which register rotary motions [1]. Due to the remarkable resemblance between both parts of the inner ear, it seems logical that a number of inner ear-specific genes will have both cochlear and vestibular functions, and consequently, mutations in these genes would be expected to lead to both auditory and vestibular dysfunction. Of the many described in the literature, we selected genes which may be the starting point for rapid otoneurological diagnosis. We considered both the impact of mutation on phenotype and differences in the frequency of mutations between populations. For brevity, proposed genes are summarised in Table I. In the first part of this review, we focus on the genes considered responsible for non-syndromic hearing loss that can be useful for otoneurological diagnostic purposes. Next, we discuss the genes potentially involved in the pathophysiology of vertigo and tinnitus, which have traditionally been considered as being of otological aetiology, while advances in neuroimaging techniques have increasingly shifted studies toward its neurological correlation.

Table I. Genes proposed for otoneurological diagnostic purposes.

Gene	Gene function	Disorder	References
COCH	Cochlin	Non-syndromic hearing loss with/without vestibular dysfunction	Chen et al., 2013 Kim et al., 2016 Usami et al., 2003 Fransen et al., 2001 Kamarinos et al., 2001 Collin et al., 2006 de Kok et al., 1999 Street et al., 2005 Gallant et al., 2013
GJB2	Gap junction protein beta-2	Non-syndromic hearing loss	Zheng et al., 2015
GJB6	Gap junction protein beta-6	Non-syndromic hearing impairment	del Castillo et al., 2002 Seeman et al., 2005
SLC26A4	Pendrin	Pendred syndrome, non-syndromic hearing loss with/without EVA	Miyagawa et al., 2014 Tsukada et al., 2015 Yang et al., 2009
KCNE1	Potassium voltage-gated channel subfamily E member 1	Chronic tinnitus	Sand et al., 2010 Pawełczyk et al., 2012
KCNE3	Potassium voltage-gated channel subfamily E member 3	Chronic tinnitus	Sand et al., 2011
KCNQ4	Potassium voltage-gated channel subfamily Q member 4	Non-syndromic hearing loss	Kim et al., 2011 Uehara et al., 2015
KCTD12	Potassium channel tetramerisation domain containing 12	Chronic tinnitus	Sand et al., 2012a
GDNF	Glial cel derived neurotrophic factor	Chronic tinnitus	Sand et al., 2012b
BDNF	Brain derived neurotrophic factor	Chronic tinnitus	Sand et al., 2012b

Genes associated with hereditary hearing loss

Hearing impairment is the most common human communication disorder and hereditary causes play an important role in its aetiology. Genetic hearing loss can be classified into non-syndromic and syndromic hearing loss. Non-syndromic hearing loss (NSHL) constitutes approximately 75% of cases of genetic hearing loss. To date, more than 90 genes and 140 loci have been associated with non-syndromic hearing impairment (Hereditary Hearing Loss homepage, http://hereditaryhearingloss.org/main.aspx?c=.HHH&n=86162). Non-syndromic deafness can be autosomal dominant (DFNA), autosomal recessive (DFNB), or X-linked (DFNX). Hearing loss can also be caused by mutations in mitochondrial DNA (mtDNA) [2]. Balance problems are also relatively frequent, but considerably less is known about the causes. However, it is commonly known that many hearing-impaired people also suffer from vertigo. Moreover, it is now recognised that many syndromes with genetic hearing impairment also show dysfunction of the vestibular system [3].

COCH

One of the most common forms of DNFA hearing loss, which is clinically characterised by late onset progressive sensorineural hearing loss accompanied by vestibular dysfunction, are mutations at the DFNA9 locus, localised to chromosome 14q12-q13. Mutations at this locus result in alterations in the cochlin protein, encoded by the COCH gene [4]. Cochlin is a 550-amino acid extracellular protein that consists of a signal peptide, LCCL domain and two vWFA domains, and has been reported to be expressed most abundantly in the inner ear [5]. Mutated forms of cochlin have been found to interfere with disulphide bonds and correct protein folding, resulting in disturbances affecting substantial cochlear functions [6]. The first three nucleotide changes cause missense mutations in COCH, resulting in amino acid substitutions of Val to Gly at codon 66 (p.V66G), Gly to Glu at codon 88 (p.G88E) and Trp to Arg at codon 117 (p.W117R) were found in hearing-impaired family by Robertson et al. [7]. These residues are localised in LCCL domain, in close proximity to a cluster of cysteines, near the amino terminus of COCH [7]. The mutation p.Ala119Thr located in close spatial proximity to W117 was found to be responsible for autosomal dominantly inherited hearing loss accompanied by vestibular symptoms in a Japanese population [8]. The substitution of conserved proline for a serine at position 51 of the protein (p.P51S) has been found in Dutch, Belgium and The

Netherlands DFNA9 families [1 9]. Kamarinos et al. found a mutation changing the isoleucine 109 to an asparagine (I109N) in a family with DFNA9 deafness and vestibular disorder from Australia [10]. The highly conserved amino acid glycine at codon 87 was found to be changed as two separate mutations, p.G87W in the Dutch DFNA9 family and p.G87V in a Chinese DFNA9 family [5 11]. The first mutations found outside of the LCCL domain was p.C542F in exon 12, which is in the von Willbrand factor A2 (vWFA2) domain [12]. Another mutation in vWFA2 domain is 18 base pair deletion, resulting in an in-frame deletion of 6 amino acids, p.Ile399_Ala404del found in family with sensorineural hearing impairment and tinnitus [13]. The mutations in COCH gene are a frequent cause of late onset hearing impairment, and would imply COCH as a major gene for cochleovestibular impairment. Coch–/– mice do not develop the degeneration and hearing loss as observed in DFNA9 patients, suggesting that DFNA9-associated COCH mutations may be gain of function in nature [14]. Yao et al. proposed that misfolding of mutant and WT cochlins in dimers and oligomers leads to a toxic gain of function that is responsible for the cellular degeneration and hearing loss in DFNA9 patients [6]. Recently, a genotype-phenotype correlation was proposed that individuals with vWFA domain mutations predominantly exhibit hearing loss, while individuals with LCCL domain mutations have hearing loss accompanied by vestibular dysfunction [15 16].

GJB2

The GJB2 gene encodes a protein called connexin 26 (Cx26), which is member of the connexin protein family. Connexin proteins form channels called gap junctions, which allow communication between adjacent cells. Unlike typical ion channels, gap junction channels possess a relatively large pore size and can allow passage of ions, cell signaling molecules and small molecules up to ~ 1.5 kDa. This direct intercellular communication pathway plays an important role in embryonic and postembryonic development, cancer suppression and many physiological and pathological functions. Connexin mutations can cause severe deafness, indicating that connexin gap junctions play a critical role in hearing. The pathogenesis and deafness mechanisms underlying connexin mutation-induced hearing loss – a common hereditary deafness have been extensively summarised by previous reviews e.g. [17] –. More than 150 different GJB2 variants have been identified, including missense, nonsense and frameshift mutation (the Connexin-deafness homepage, http://davinci.crg.es/deafness). Moreover, the spectrum of GJB2 mutations vary among different ethnic groups. For example, the GJB2 c.35delG mutation is the most common cause of hearing loss worldwide and is identified frequently in European, North African, Middle East Asian and American populations, but rarely in South Asian, Southeast Asian, or East Asian countries. The GJB2 c.235delC mutation is one of the most common genetic causes of hearing loss in East Asian countries (Japan, Korea, and China). In South Asian countries (India, Pakistan, and Bangladesh) the most common cause of hearing loss is GJB2 p.W24X mutation, which is also found in Middle Eastern and East European populations at a moderate frequency and at a low frequency in Southeast Asian populations, but it was not observed in African or East Asian populations. The GJB2 p.V37I mutation is the most common cause of hearing loss in Southeast Asian and Oceanic countries (Thailand, Malaysia, Indonesia and Taiwan). The GJB2 c.167delT mutation is the most frequent variant in Ashkenazi Jewish families [18 19]. Thus, knowledge of ethnic and regional differences in the GJB2 mutation spectrum is very important and could help guide genetic testing and assist in clinical decision making.

GJB6

In addition to GJB2 (Cx26), multiple connexin genes, GJB6 (Cx30), GJB3 (Cx31), GJC3 (Cx29) and GJA1 (Cx43), have been identified in the cochlea, but compared to the extensive expression of Cx26 and Cx30, the expression of other connexins in the cochlea is limited. Mutations of these connexin genes can also cause hearing loss. Del Castillo et al. (2002) identified a 342-kb deletion in the GJB6 gene which is the second most frequent (after the 35delG mutation in GJB2) genetic cause of non-syndromic prelingual hearing impairment in the Spanish population [20]. A multicentre study, involving 9 countries, showed that the GJB6 deletion was present in most of the screening populations, with higher frequencies in France, Spain and Israel [21]. In the Czech population, the 342-kb deletion in the GJB6 gene was identified in 1 of 13 patients with prelingual nonsyndromic sensorineural deafness who also carried 1 pathogenic mutation in the GJB2 gene and was not detected in 600 control chromosomes from individuals with normal hearing [22]. Seeman et al. (2005) concluded that the 342-kb deletion is very rare in central Europe compared to reports from Spain, France and Israel.

In contrast to the results of European and Asian populations mutations, neither GJB2 nor GJB6 are associated with non-syndromic deafness in Africans [23-25]. The data indicate that the high frequency of GJB2 and GJB6 mutations in non-syndromic hearing loss have evolved in Eurasian populations after their migration out of Africa, and

spread with population migrations due to founder effects, and not to a putative ''protective'' variant in the genomic structure in Africans [26]. Therefore, both GJB2 and GJB6 should not be investigated routinely in clinical testing in patients of African ancestry.

SLC26A4

The SLC26A4 mutations are the second genetic cause of NSHL after GJB2 mutations. The SLC26A4 gene, encodes a transmembrane protein (pendrin) expressed in thyroid gland, inner ear and kidney, where it is involved in the transport of anions such as iodide, chloride and bicarbonate. Mutations in the SLC26A4 gene are known to be responsible for a broad phenotypic spectrum, from typical Pendred syndrome, characterised by hearing loss, goiter and eventually hypothyroidism, with/without enlarged vestibular aqueduct (EVA) or other inner ear malformations to non-syndromic hearing loss, called DFNB4, characterised by hearing loss with/without EVA [27]. The prevalent of SLC26A4 mutations (90% in Pendred syndrome and 78.1% in non-syndromic hearing loss associated with EVA) indicates the importance of this gene in the pathophysiology of this category of hearing impairment [28]. More than 170 mutations have been found in SLC26A4 gene (Pendred/BOR homepage, http://www.healthcare.uiowa.edu/labs/pendredandbor/), and as in the case of GJB2 different mutational spectrums among different ethnic groups have been reported. Studies on NSHL have revealed biallelic SLC26A4 mutations in 2% to 3.5% of Caucasian patients, but in 5.5% to 12.6% of East Asian patients. Moreover, the most common SLC26A4 mutations found in the Asian population were quite different from that found in the Caucasian population. The most common mutations of SLC26A4 gene observed in the East Asian, South Asian and Southeast Asian populations were p.H723R, c.919-2A>G and p.V239D. These mutations were not detected in the Caucasian population. The c.1001+1G>A, p.V138F, p.T416P, p.L236P and p.G209V mutations are prevalent in the Caucasian population [18].

Genetics of vestibular disorders

The most representative otoneurological vestibular disease located in the labyrinth is Meniere's disease. Numerous hypotheses have attempted to explain the aetiology of the disease. However, the anamnesis usually confirms the familiar history of Meniere's disease. A genetic basis, involving DTNA and FAM136A genes, is believed to be responsible for familiar incidents of the disease [29]. The situation is complicated because Meniere's disease is variously expressed through pronounced cochlear or dominated vestibular syndromes. Finally, 83 genes are suspected to be altered when balance and hearing symptoms are present. Some of them, such as CRYM and OTOG, are more specific for the vestibule, while others, such as AQP1, COL11A2 and OTOS, are expressed much higher in the cochlea; MYO6 and USH1C are associated with both hearing and vestibular defects [30]. Li et al. reported on 15 genes that are strongly inferred with Meniere's disease. The most important are CD4, NOTCH2 and IL6 [31]. Eppsteiner and Smith suggested a special phenotype for Meniere's patients associated with DFNB1 [32]. Looking at a biochemical cause of Meniere's disease, a hypothesis of disturbed ionic transport following rs3746951 and rs487119 mutations has been taken into consideration [33].

The most common vestibular disease according to otoneurological literature is benign paroxysmal positional vertigo (BPPV). Its aetiology is correlated with head injury and sense organ degeneration in an advanced age. The only treatment is canalith repositioning procedure, but its success is varies individually. Tsai at al. reported on the influence of miR-34a and Sirtuin 1 (SIRT1) gene expression on the effect of therapy [34]. Canalith repositioning decreased PPAR-γ, PGC-1 and FoxO gene expressions just after the procedure. After complete cure of BPPV, the previously decreased expression increased together with he ROS concentrations, pro-inflammatory cytokine concentrations and p53 levels [34].

Apart from vertigo originating from the labyrinth, the central nervous system disorders are followed by dizziness or vertigo. The migraine represents them. Vestibular migraine is clinically difficult to confirm – it is not clear if it is real vestibular migraine or if vestibular symptoms belong to aura of regular migraine –. Nevertheless, the family penetrance and interaction of genes are observed in vestibular migraine or vestibular component of typical migraine. Until now, no specific gene has been identified. Because migraine can be associated with ataxia, the GBA2, TGM6, ANO10, SYT14, KCNA1 and CACNA1A genes are destined as possible targets [35]. Typical vestibular migraine as a subtype of migraine seems to be connected with GWAS and endophenotypes of MTDH, TRPM8 and LRP1 genes [36].

Up to the present moment, numerous dizziness and vertigo patients are still not identified as suffering from special vestibular disease. For these idiopathic cases, the name of ''recurrent vertigo'' is accepted. For the future genetic investigations, this group is the most interesting. Initially, the regions 22q12 and 5p15 are designated as potentially responsible for vestibular pathology [37].

Tinnitus associated genes

Tinnitus, which affects approximately 40 million people worldwide chronically, is currently the biggest challenge

of otoneurological diagnostics. It is believed that every individual over 20 has experienced tinnitus at least once. The aetiology of tinnitus, defined as hearing sounds with no audible sources in the environment is unknown, but an established association with various forms of sensorineural hearing impairment and frequent precipitation by noise exposure suggest substantial overlap with pathologies of the inner ear, and related disorders of auditory information processing. The genetics of tinnitus and data that suggests a complex or multifactorial genetic aetiology has been discussed in recently published reviews [38].
While the underlying biological mechanisms are still incompletely understood, research into the pharmacological treatment of tinnitus has emphasised the role of cellular ion regulation and transport [39].

Annel genes
Voltage-gated ion channels directly control neural transmission of auditory input. In the inner ear, sensory neurons are surrounded by endolymph rich in KCl and constantly recycle potassium for the generation of endocochlear potentials. KCNE1, a potassium channel subunit gene has been implicated in maturation defects of central vestibular neurons, in Menière's disease, and in noise-induced hearing loss [40]. Sand et al. (2010) screened 201 Caucasian outpatients with a diagnosis of chronic tinnitus for mutations in the KCNE1 gene. Although no significant differences in KCNE1 coding allele frequencies of control subjects and tinnitus patients were found, Sand et al. (2010) hypothesised that primary chronic tinnitus could be a part of the phenotypic spectrum associated with KCNE1 [40]. Pawełczyk et al. (2012) found that mutations in the KCNE1 gene contributed to tinnitus that developed independently of hearing loss in a Polish population [41]. A prominent role of the KCNE1 subunit in auditory perception is underscored by degeneration of sensory hair cells and deafness in KCNE1 knock-out animals [42], and deleterious effects of a spontaneous KCNE1 null mutation on hearing in mice [43]. Like KCNE1, KCNE3 is expressed in the mammalian inner ear and brain. Both proteins interact to regulate trafficking, surface expression and activation of another potassium channel, KCNH3, in the cortex and in other parts of the central nervous system. So far, a limited number of investigations has addressed sequence variations in KCNE3, a linkage hotspot for autosomal recessive, non-syndromic hearing impairment. In an association study involving 288 individuals, no significant effect of KCNE3 on the risk for developing chronic tinnitus or predicting the severity of tinnitus was noted. It is likely that mutations in KCNE3 alone may not be indicative of tinnitus, but when mutations are present in either KCNE3 or the channel with which it is interacting (e.g. KCNQ1) the biophysical properties of the channel complex are significantly altered [39]. An example of coexistence are mutations in other K+ channel gene, KCNJ10 that are associated with non-syndromic hearing loss in carriers of SLC26A4 mutations with an EVA/PS phenotype [44]. Dysfunctional channels and mutations in the gene encoding the KCNQ4 subunit are a hallmark of autosomal dominant deafness 2A [45]. KCNQ4 is expressed in the outer sensory hair cells of the cochlea and is responsible for recycling K+ ions after stimulation of the hair cel [46]. KNCQ4 mutations were identified in European, American, Japanese, Taiwanese and Brazilian families with non-syndromic hearing loss [47 48]. So far, no association has been found between mutations in KNCQ4 gene and tinnitus.
A well-established theoretical framework for tinnitus has been provided by the disruption of GABAB receptor signaling in animal models [49]. A key role for GABAB receptors has been confirmed by the effects of receptor agonists on tinnitus symptomatology. An auxiliary subunit that is closely associated with the carboxy terminus of GABAB2 receptors is KCTD12, a potassium channel tetramerisation domain-containing protein. Co-assembly of KCTD12 and GABAB2 changes the properties of the GABAB [1 2] core receptor by increasing agonist potency, by altering G-protein signaling, and by promoting desensitisation [50]. A study involving 95 Caucasian outpatients with a diagnosis of chronic tinnitus screened for mutations in the KCTD12 gene revealed weak association with tinnitus but KCTD12 genotype did not predict tinnitus severity [51].

GDNF and BDNF
Glial cell-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) play key roles in the early development of the central auditory pathway and the inner ear. Both have been successfully employed to treat experimental forms of hearing loss and are likely to operate in a broad spectrum of auditory phenotypes, including phantom perceptions of sound. Although previous research has suggested a role of variant BDNF in modulating the genetic susceptibility to chronic tinnitus with hearing impairment [52], a later study failed to observe any major effects of individual BDNF or GDNF variants on susceptibility to the phenotype [53]. The findings are compatible with minor, additive effects, which would appear to be gender-sensitive according to subjective ratings of symptoms, offering a possible explanation for distinct patterns of central nervous activity in tinnitus [53].

Epilepsy related genes

Recent study has found that patients suffering from tinnitus have abnormal electrical activity of the cerebral cortex [54 55]. The presence of abnormal electrical activity of the cerebral cortex in patients with tinnitus, where an important role seems to be played by hyperactivity and epilepsy related activity, implies the assumption that tinnitus can be a substitute for epilepsy. Although the aetiology of epilepsy is not yet fully recognised, it is known that genetic factors play an important role. Research on families suffering from epilepsy concerning a mendelian inheritance mechanism have shown that the genetic basis consists mainly of mutations in genes encoding subunits of ion channels and receptors for neurotransmitters. They do not, however, explain all cases. An example can be generalised epilepsy with febrile seizure (GEFs +), which essentially is a set of symptoms of epilepsy with different phenotypes. In a family with GEFs + mutations have been identified in the genes encoding subunits of sodium channels (SCN1B, SCN2 and SCN1A) and GABA receptor subunit (GABRG2 and GABRD), but in general these mutations are responsible for only about 10% of GEFs + [56]. In rare cases, association studies indicated a significant effect of mutations in the genes encoding subunits of the GABA receptor (GABRG2 and GABRA1) and chloride (CLCN2) and calcium channels (CACNA1H), as well as in the gene encoding EFHC1 calcium-binding protein. Nevertheless, most epilepsy cases are determined by multiple genes and the phenotypic effect is a result of coexistence of many common sequence variants [57]. Advances in genomic technology research made in recent years have created new opportunities to identify genetic risk factors. Recent wide-genome association studies and meta-analyses have identified new loci associated with the risk of epilepsy, both in genes encoding ion channels (e.g. rs6732655, rs12987787 and rs11890028 located in the SCN1A gene), as well as genes unrelated to "channelopathies" (e.g. rs1044352 located in PCDH7 gene or rs1939012 located in MMP8 gene) [58 59]. The detection of the links between epilepsy and tinnitus is important to the selection of effective treatment. A recent study has showed that disorders characterised by neuronal hyperexcitability, such as epilepsy and tinnitus are associated with a reduction in KCNQ2/3 channel activity. This reduction is due to a shift in the voltage dependence of KCNQ2/3 channel activation to more positive voltages (60). In vivo studies demonstrated that anti-epileptic drugs that a shift in the voltage dependence of KCNQ to more negative voltages prevent the development of tinnitus [60 61].

It is alleged that epilepsy may also play a role in generating vestibular pathology [62]. However, "vestibular epilepsy" as the cause of vertigo is difficult to prove [63]. Family history of epilepsy, previous head injuries and vestibular symptoms intertwine with other epileptiform symptoms that may indicate this specific form of epilepsy [62]. Since there is no specific test confirming the "vestibular epilepsy", it seems that genetic research may give credence to this diagnosis [64].

There are also many other types of dizziness of which the origin is unclear. Vertigo lasting seconds is often classified as benign paroxysmal positional vertigo. Such recognition seems to be overused, in many cases tests (such as Hallpike's and roll-on) confirming the diagnosis are controversial and the causes of dizziness remain unknown, or at least uncertain. The situation is similar with regard to dizziness that lasts for minutes or hours (without hearing impairment) and often repeats itself. These symptoms require a long diagnostic process involving many specialists. Vestibular hyperactivity observed during caloric stimulation of the labyrinths is sometimes the only pathological vestibular finding, in such case "channelopathy" may be one of or the only suspected cause of dizziness [65]. Vestibular migraine seems to derive from this neuronal conduction pathology [66].

Future prospects

The clinical heterogeneity of tinnitus and its associated comorbidities makes the selection of patients for genomic studies challenging. Improving the diagnosis of tinnitus patients and their categorisation into a homogenous group is essential to investigate the contribution of genetic factors to the development of the disease. New possibilities for better understanding of the tinnitus are provided by TINNET, a European research network created in 2014 (http://tinnet.tinnitusresearch.net/index.php). The strategy of TINNET is to standardise and coordinate clinical, neuroimaging and genetic assessment of tinnitus patients and to aggregate data in a large-scale database in order to identify tinnitus subtypes and their neurobiological underpinnings. The genetics group (WG4) of TINNET is working towards determining the genetic basis of tinnitus subtypes using next generation sequencing technologies and advanced tinnitus phenotyping strategies that will circumvent tinnitus heterogeneity and help to define tinnitus endophenotypes for genetic studies. This will facilitate the development of new therapies and improve the efficacy of currently available treatments [67]. The research aiming at verifying the coincidence of tinnitus and vertigo with genetic predisposition to epilepsy opens the possibility of preventing people from being exposed to environmental factors triggering tinnitus, such as noise

or ototoxic drugs. In the case of vertigo, this would make it possible to eliminate or thoroughly monitor candidates or professionals whose performance requires outstanding sense of balance.

Additionally, this knowledge could help conduct genetic testing and assist in clinical decision making. Genetic confirmation of the epileptic etiology of tinnitus and vertigo will enable the implementation of anti-epileptic treatments for tinnitus and vestibular disturbances, which is currently not a standard.

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Otology

From CT scanning to 3D printing technology: a new method for the preoperative planning of a transcutaneous bone-conduction hearing device

Dalla TC alla stampante 3D: un metodo innovativo per la pianificazione chirurgica di una protesi a conduzione ossea transcutanea

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SUMMARY

The aim of the present study was to assess the feasibility and utility of 3D printing technology in surgical planning of a transcutaneous bone-conduction hearing device (Bonebridge®) (BB), focusing on the identification of the proper location and placement of the transducer. 3D printed (3DP) models of three human cadaveric temporal bones, previously submitted to CT scan, were created with the representation of a topographic bone thickness map and the sinus pathway on the outer surface. The 3DP model was used to detect the most suitable location for the BB. A 3DP transparent mask that faithfully reproduced the surface of both the temporal bone and the 3DP model was also developed to correctly transfer the designated BB area. The accuracy of the procedure was verified by CT scan: a radiological marker was used to evaluate the degree of correspondence of the transducer site between the 3DP model and the human temporal bone. The BB positioning was successfully performed on all human temporal bones, with no difficulties in finding the proper location of the transducer. A mean error of 0.13 mm was found when the transducer site of the 3DP model was compared to that of the human temporal bone. The employment of 3D printing technology in surgical planning of BB positioning showed feasible results. Further studies will be required to evaluate its clinical applicability.

KEY WORDS: 3D printing• Bonebridge • Implantable hearing aid • Bone conduction implant • Surgical planning

RIASSUNTO

Il presente studio ha lo scopo di descrivere un'innovativa metodica che mediante l'utilizzo della stampante 3D permetta una più agevole pianificazione preoperatoria del posizionamento di una protesi a conduzione ossea transcutanea (Bonebridge®) (BB). Tre ossa temporali umane conservate in formalina sono state sottoposte a studio radiologico TC ad alta risoluzione al fine di consentire la realizzazione dei corrispettivi modelli 3D, utili all'identificazione della corretta ubicazione del BB. Ciascun modello 3D di temporale umano era caratterizzato dalla rappresentazione sulla sua superficie sia di una mappa topografica a colori rappresentativa dello spessore dell'osso temporale, sia del seno sigmoideo nel suo decorso. Un secondo modello 3D trasparente ("template") è stato stampato con lo scopo di riprodurre fedelmente la superficie dell'osso temporale, in modo da trasferire l'area designata al posizionamento del BB dal modello 3D all'osso temporale. L'accuratezza della procedura è stata quindi verificata attraverso un successivo studio TC mirato alla comparazione del sito di posizionamento del device tra modello 3D e osso temporale umano. In tutti e tre i casi è stato possibile posizionare correttamente la protesi, senza riscontrare difficoltà nell'identificazione del sito di impianto. Nella valutazione della corrispondenza del sito designato tra modello 3D e osso temporale è stato riscontrato un errore medio di 0.13 mm. L'utilizzo della tecnologia di stampa 3D nella pianificazione chirurgica del posizionamento del BB ha mostrato risultati incoraggianti; ulteriori studi saranno necessari per appurarne l'applicabilità clinica.

PAROLE CHIAVE: *Stampante 3D • Bonebridge • Protesi acustica impiantabile • Protesi a conduzione ossea • Pianificazione chirurgica*

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Introduction

During the last years, the continuous technological progress of 3D printing has given new and fascinating applications in the medical and surgical fields, with interesting applications in the otologic surgery as well. 3D printing refers to the process of creating 3D objects starting from a virtual 3D model. The growing interest in the medical field regarding this new kind of technology is linked to the possibility of producing anatomical models that are useful to surgical training and operative planning [1-6]. The Bonebridge® (BB, Med-El, Innsbruck, Austria) is a semi-implantable transcutaneous bone-conduction implant, whose effective element ("Bone Conduction Floating Mass Transducer" or BC-FMT) is implanted subcutaneously, without any continuity with the outside [7]. Compared to percutaneous bone-conduction implants, the BB has a decreased risk of skin adverse events without the audiological limitations that characterise passive transcutaneous hearing systems due to dampening effects of the skin [7 8]. The surgical procedure required for placement of the BC-FMT is challenging because of the physical dimensions required: 8.7 mm in thickness, 15.8 mm in diameter and 23.8 mm between anchor holes [7 8]. Surgical positioning requires sufficient bone thickness and a relatively flat surface in order to fix the device into the cortical bone through the anchoring screw, at the extremity of the two lateral rigid wings. Currently, the optimal BB position is chosen by simulating the placement preoperatively through the creation of a 3D image based on the CT of the patient, by manual measurements of the bone thickness in separate two-dimensional axial, coronal or sagittal CT slices. Then the surgeon, according to the anatomic pattern of each patient, identifies the planned position on the temporal bone using as reference specific anatomical landmarks. Based on the most recent literature, different surgical and radiological strategies have been proposed to facilitate positioning of the BB [9-13]. The aim of the present study was to report an innovative technique employing 3D printing technology, with the purpose of making positioning of the BB easier and safer. The execution modalities and results in a cadaveric feasibility study will be discussed.

Materials and methods

Construction of 3DP models

Three temporal bones were submitted to a high-resolution CT scan (slice thickness 0.5 mm). The images allowed printing the corresponding 3D models (Fig. 1) characterised by the following innovations: (1) representation on the 3DP model external surface of a topographic map indicating the thickness of the underlying bone; (2) representation of the sigmoid sinus pathway on the external surface of the 3DP model. The resulting model was 3DP using a Projet 460Plus (3D Systems), which is based on binder jetting technology: the printer works with chalk-like powder cured layer by layer using a binder and ink colours. The printer has a layer thickness resolution of 100 µm and a chromatic resolution of more than 2.8 million of colours, and is thus perfectly suitable for the present application. A second 3DP model ("template") (Figs. 2, 3A) was printed based on the CT images of the cadaveric temporal bone and employing specific algorithms to obtain a transparent "mask" of 1 mm of thickness. The template was specular to the human bone surface, perfectly reproducing all radiological and anatomical landmarks (posterior wall of the external auditory canal with the Henle's spine, mastoid tip, zygomatic process of the temporal bone with the temporal line). Consequently, the template was developed to be univocally lodged on the specimen and allowing only one possible placement. The purpose of the template was to allow the transfer of the area designated for the BC-FMT from the 3DP model to the human temporal bone. The template was 3DP using a Form2 (FormLabs) printer, based on stereo-lithographic technology, using a layer thickness resolution of 25µm. A transparent photopolymer was used for prototyping to ensure visibility of the underlying thickness map.

Surgical planning and surgical procedure

The best BC-FMT position was detected for each 3DP temporal bone model taking into consideration bone thickness (through the topographic map), flatness of the bone surface and surgical landmarks (sigmoid sinus, external acoustic meatus, mastoid tip). After positioning the template on the 3DP model, the site chosen for the BC-FMT was marked on the template using the T-sizer (Fig. 3B). The marked area was drilled using a precision drill press with a 16 mm bit (Fig. 3C), hence the template was suitable for the surgical procedure (Fig. 3D). The drilled template was placed on the human temporal bone to report the BC-FMT site (Fig. 4). BB implant surgery was performed according to the literature in order to place the BC-FMT into the bony well and fix the implant with anchor screws (Fig. 5).

Accuracy of BC-FMT surgical placement

To verify the accuracy of surgical placement, a radiological marker (a lead ball of a 0.2 mm diameter) was placed on the centre of the BC-FMT site of both the 3DP model and human temporal bone (Fig. 6), using the 3DP template for a precise positioning. Both 3DP model and human temporal bone were submitted to CT scan and the

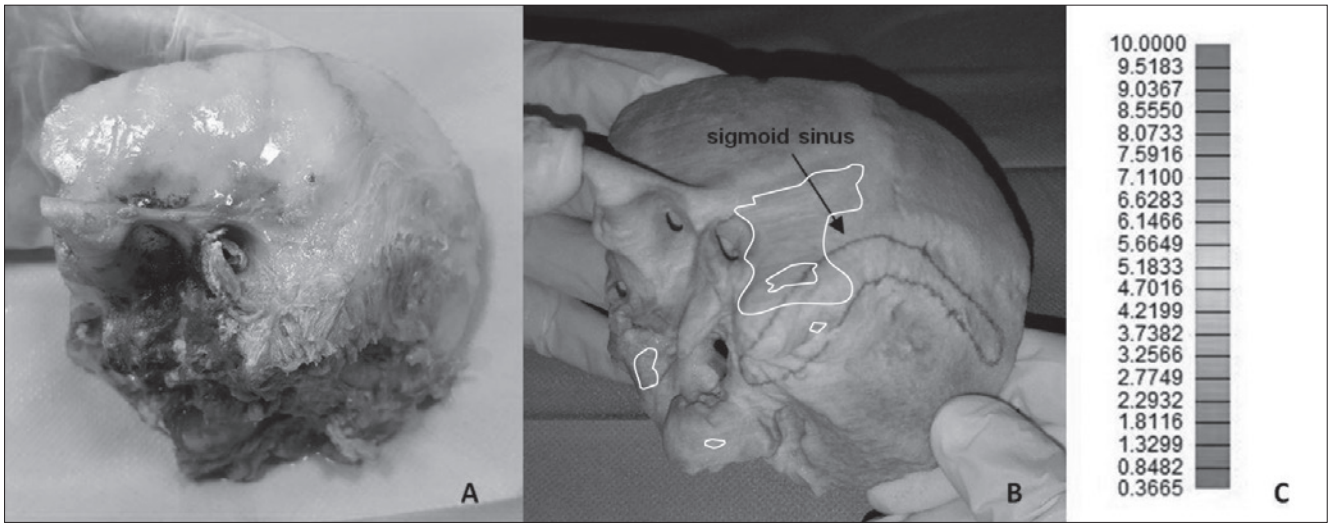

Fig. 1. Human temporal bone (A). 3DP temporal model with topographic bone thickness map and sigmoid sinus pathway representation (B). Legend of the topographic bone thickness map (example: white line area represents a 10 mm bone thickness) (C).

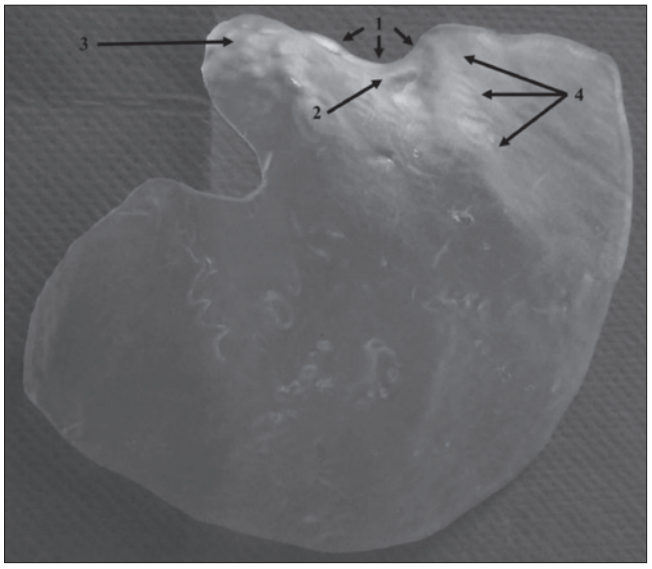

Fig. 2. 3DP template with the anatomical landmarks that allow its proper lodging on the temporal bone. 1: posterior wall of the external auditory canal; 2: Henle's spine; 3: mastoid tip; 4: temporal line.

distance of the radiological marker to specific anatomical landmarks was measured and compared to evaluate the degree of correspondence (Table I).

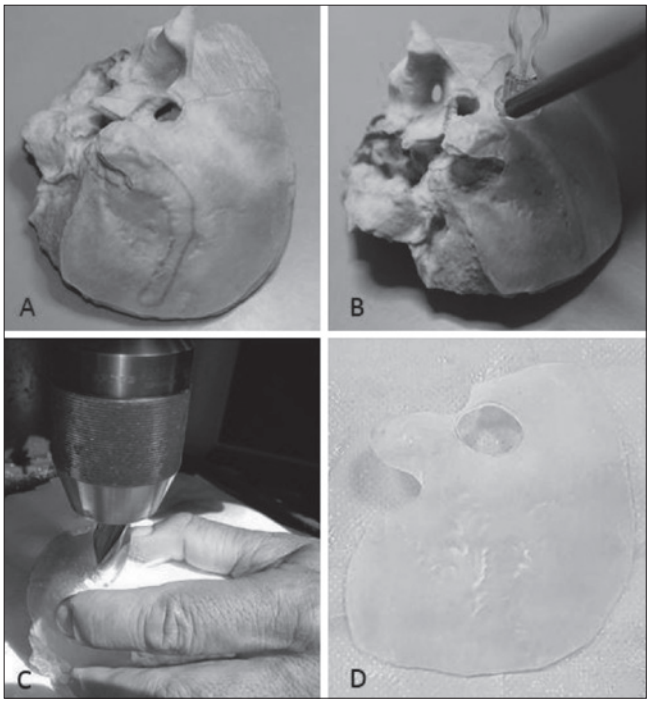

Fig. 3. The transparent template positioned over the 3DP model (A). Marking the BC-FMT area with the T-sizer on the template (B). 3DP template drilling in correspondence of the BC-FMT location (C). Template with surgical guide for BC-FMT positioning (D).

Results

In all the three cases, BC-FMT positioning was successfully performed. The optimal placement was always identified at the sino-dural angle. Dural decompression was not needed in any case to allow BC-FMT positioning. The sigmoid sinus was never subjected to surgical decompression. The procedure was performed with a mean time of 21 minutes, without difficulties in finding the proper BC-FMT site. A mean error of 0.13 mm was found when the BC-FMT site of the 3DP model was compared to that of the human temporal bone.

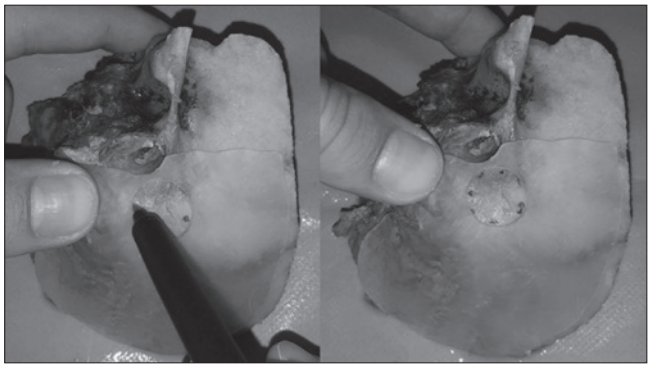

Fig. 4. Marking of the BC-FMT positioning area on the human temporal bone with the drilled template.

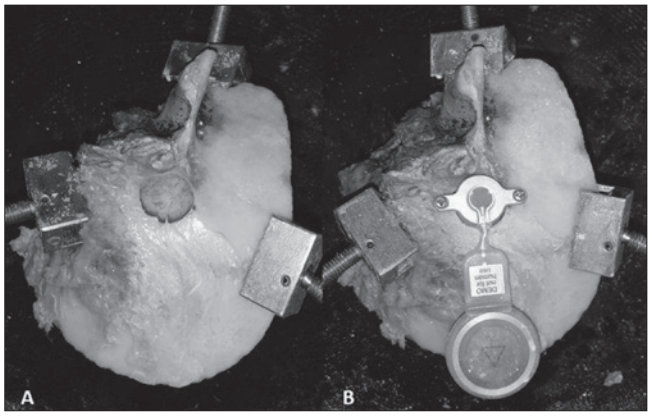

Fig. 5. Bony well for BC-FMT placement on human temporal bone (A). BC-FMT placement and fixation with both anchoring screws (B).

Discussion

Current selection criteria for BB include conductive bilateral hearing loss, mixed bilateral hearing loss and single side deafness with normal hearing in the opposite ear [8]. Nowadays, only two surgical approaches are still utilised: the retrosigmoid approach and the presigmoid one. Most commonly reported in literature, the presigmoid technique concerns BC-FMT placement in the sino-dural angle considering its anatomical limits: the sigmoid sinus, the posterior wall of the external auditory canal and the dura [8]. BB implantation is a demanding procedure requiring general anaesthesia and a skilled surgeon. Surgical technical difficulties mainly derive from pre-operative planning: choosing the most suitable site to place the BC-FMT (sufficient bone thickness and relatively flat surface), avoiding lesions of nearby anatomical structures and then finding the defined location on the temporal bone of the patient [8]. Up to now, BC-FMT placement is a difficult cognitive task for the surgeon in terms of three-dimensional spatial reconstruction of the preoperatively planned position on CT. In fact, the three-dimensional information of a CT scan is usually displayed as series of two-dimensional images, and therefore a comparison between the three radiological planes is required to conceive a three-dimensional evaluation of the temporal bone. Furthermore, the two-dimensional information provided by the radiological scans is not adequate to understand whether the surface of the bone is regular or flatter. The identification of the proper location of the BC-FMT, relying only on the radiological images, could be challenging because the best surgical positioning requires a three-dimensional study of the temporal bone considering its thickness, surface and all anatomical landmarks.

Another laborious issue is localisation of the planned position on the temporal bone, without losing precision [8]. Newly acquired scientific studies have been reported in literature, aimed at overcoming the aforementioned surgical procedure limits. In 2015, Wimmer et al. [9] first applied a topographic bone thickness map in the preoperative planning for the BB positioning in seven patients, although the thickness map was only radiologically elaborated and required the identification of anatomical landmarks to detect the BB location on the patient. In 2014, the Japanese Kyushu and Shinshu Universities [10-12] and later the St. Thomas Hospital in London [13], developed a 3DP template to surgically identify the BC-FMT site previously planned on CT. According to the authors, the template fulfilled the issue of how to find a defined anatomical area detected in CT on the temporal bone, while the remaining restrictions were still unresolved.

In our experience, a 3DP temporal bone model was developed allowing intuitive preoperative assessment of the BB placement. In particular, the choice of the proper location of the device was much more intuitive and practical in comparison to radiological preoperative planning due to the employment of a three-dimensional model that is able to define all anatomical landmarks, regularity of the bone surface and thickness of the underlying bone. In all three cases, quick identification of the appropriate BC-FMT position was accomplished. Compared with the study of Wimmer et al. [9], the 3DP model of the temporal bone added the advantage to define where the bone surface was the flattest and thus the most suitable for BC-FMT screw anchoring, a procedure that is not intuitive if based on a bi-dimensional CT image. Finally, in all cases the transparent template enabled safe identification of the planned BC-FMT position on the temporal bone, previously localised on the 3DP model, with a mean error of 0.13 mm. A critical analysis of our study, supports the need of an optimal

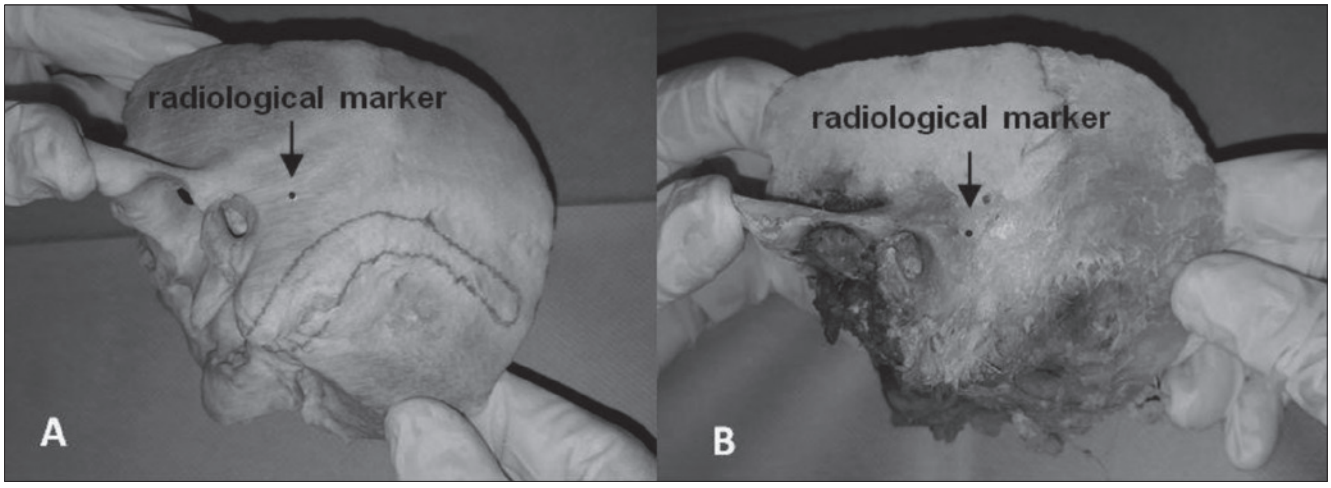

Fig. 6. Radiological marker positioned on the 3DP model (A) and on the human cadaveric bone (B).

Table I. Distance of the radiological marker to specific anatomical landmarks in the three radiological planes on the 3DP model and the human temporal bone.

	3D model	Human model	Radiological plane
Specimen 1	46.84 mm	46.51 mm	Axial
	19.41 mm	19.19 mm	Coronal
	31.38 mm	31.26 mm	Parasagittal
Specimen 2	43.51 mm	43.41 mm	Axial
	22.61 mm	22.92 mm	Coronal
	47.85 mm	47.93 mm	Parasagittal
Specimen 3	58.66 mm	58.66 mm	Axial
	29.15 mm	29.18 mm	Coronal
	31.13 mm	31.16 mm	Parasagittal

decrease of template dimensions to allow its feasibility during the clinical phase of our work. A minimally invasive approach considering soft tissue management represents a key point that should be clarified for the application of our 3D printing-guided method on the patient.

Conclusions

According to the most recent literature, there is a growing interest in the use of 3D printing for ENT surgical decision-making. In our preliminary experience, the employment of 3D printing technology in the surgical planning of BB positioning showed feasible results and was a practical and intuitive alternative to the current methods described in literature. Further studies will be required to evaluate its clinical applicability, and in particular in overcoming the limits related to soft tissues.

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Otology

Bone-anchored hearing implant surgery: our experience with linear incision and punch techniques

La chirurgia delle protesi acustiche ancorate all'osso: la nostra esperienza con le tecniche con incisione lineare e con punch

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SUMMARY

In recent years, bone-anchored hearing implants (BAHIs) have found wider application in the treatment of conductive and mixed hearing loss. Several surgical techniques have been developed to reduce complications, enhance healing and improve audiological and aesthetic results. We report our experience on the use of three BAHI surgery techniques: Group 1, linear incision with thinning of the subcutaneous tissue; Group 2, linear incision without thinning of the subcutaneous tissue; Group 3, punch technique (Minimally Invasive Ponto Surgery, MIPS). We retrospectively analysed patients undergoing BAHI surgery; results were evaluated on the basis of any intra-operative complication, duration of surgery and occurrence of adverse effects at the implantation site over 1 year of follow-up. We collected a total of 30 implantations (12 for Group 1, 8 for Group 2, 10 for Group 3) with an intra-operative complication rate of 25%, 0% and 10%, respectively. The average surgical time was 62.08 minutes, 34.37 minutes and 18.7 minutes respectively. During follow-up, we reported the occurrence of adverse effects in 10.63% of observations in Group 1, 3.12% in Group 2 and 2.5% in Group 3. This study confirms the low rate of intra and postoperative complications during BAHI surgery and documents the simplicity of execution of the novel MIPS technique, with a significant reduction in surgical time compared to the other two techniques, and positive effects in terms of health care costs.

KEY WORDS: Bone-anchored hearing implants • Punch technique • Linear incision • Minimally Invasive Ponto Surgery

RIASSUNTO

Negli ultimi anni, le protesi acustiche ancorate all'osso (BAHIs) hanno trovato larga applicazione nel trattamento delle ipoacusie trasmissive e miste. Sono state sviluppate diverse tecniche chirurgiche per ridurre le complicanze, accelerare la guarigione, migliorare i risultati audiologici ed estetici. Nel presente studio riportiamo la nostra esperienza nell'uso di tre tecniche chirurgiche per l'impianto delle protesi acustiche ancorate all'osso: Gruppo 1, incisione lineare con assottigliamento del tessuto sottocutaneo; Gruppo 2, incisione lineare senza assottigliamento del tessuto sottocutaneo; Gruppo 3, tecnica a punch (Minimally Invasive Ponto Surgery, MIPS). I pazienti sottoposti a questo tipo di chirurgia sono stati studiati in via retrospettiva e i risultati valutati sulla base dell'eventuale comparsa di complicanze intraoperatorie, durata dell'intervento e comparsa di reazioni locali nel sito di impianto durante un follow-up di 1 anno. Abbiamo raccolto un totale di 30 impianti (12 per il Gruppo 1, 8 per il Gruppo 2, 10 per il Gruppo 3), con un tasso di complicanze intraoperatorie del 25%, 0% e 10% rispettivamente. La durata media dell'intervento è stata di 62,08 minuti, 34,37 minuti e 18,7 minuti rispettivamente. Durante il follow-up abbiamo osservato la comparsa di reazioni locali nel 10,63% delle osservazioni nel Gruppo 1, 3,12% nel Gruppo 2, 2,5% nel Gruppo 3. Questo studio conferma il basso tasso di complicanze intra e postoperatorie nella chirurgia delle protesi acustiche ancorate all'osso e mostra la semplicità di esecuzione della nuova tecnica MIPS, con una riduzione significativa dei tempi chirurgici rispetto alle altre due tecniche utilizzate ed effetti positivi in termini di costi sanitari.

PAROLE CHIAVE: *Protesi acustiche ancorate all'osso • Tecnica a punch • Incisione lineare • Minimally Invasive Ponto Surgery*

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Introduction

In recent years, hearing aids have seen a remarkable development in terms of sound quality and potential for application. In the treatment of conductive and mixed hearing loss, or single-sided deafness, bone-anchored hearing implants (BAHIs) have found wide application, overcoming some important disadvantages that may oc-

cur with traditional bone conduction devices (BCDs). The latter, in fact, need good contact between the transducer and temporal bone through a high pressure on the skin, with the possibility of developing inflammation, pain and lesions. Their prolonged use is often associated with onset of headache. Another important disadvantage in this kind of device is due to the filtering effect on some frequencies by the interposed skin layer, with dissipation of a certain amount of energy and deterioration in the quality of sound. Furthermore, the correction of severe hearing losses requires the application of an important amount of sound energy, which results in distortion phenomena and further loss of quality. In addition, the aesthetic problem related to this kind of device, usually placed in the glasses temple tips, should not be overlooked [1]. Regarding this latter aspect, more comfortable BCDs have been developed in recent years, such as Bruckhoff hearing systems, that elegantly combine glasses with a hearing module inserted in malleable temple tips; the result is a small, discreet and comfortable system that allows considerable improvement in aesthetic appearance [2]. With reference to the other aspects, percutaneous implants outdo these difficulties through a titanium abutment surgically inserted in the temporal bone, which after osseo-integration allows bone transmission of sound from an external receiver directly to the inner ear, with an improvement of the auditory gain of at least 10-25 dB compared to conventional bone conduction devices [3]. However, the installation of such devices is not free of complications. In fact, the standard surgical technique described by Tjellström et al. in 1977, used for decades, requires the creation of a pedicle skin flap with extensive thinning of the subcutaneous tissue until the periosteum [3 4]; this can lead to the appearance of an area of alopecia around the device and can compromise revascularization of the skin flap with risk of dysesthesia, infections and impaired wound healing, with possible aesthetic implications [5]. Recently, to enhance healing and aesthetic results, less invasive techniques have been adopted that use a linear incision of the skin with thinning of the subcutaneous tissue [6 7]; the introduction of longer abutments has allowed to avoid the thinning of the subcutaneous tissue. This technique has many benefits: it shortens both surgical and healing time, brings no significant dysesthesia in long-term follow-up, and reduces local infections with a better appearance of the surgical area [8 9]. An additional evolution of the technique has further reduced surgical trauma through the use of a biopsy punch and removal of a piece of skin, subcutaneous tissue and periosteum through which the housing for the abutment in the temporal bone is achieved. This technique shortens surgical times, accelerates healing, improves aesthetic appearance and reduces the risk of skin overgrowth around the abutment [3 10].

In recent years, a non-skin-penetrating bone conduction hearing implant has also been developed that uses a magnetic coupling through the skin. A passive magnet is implanted under the soft tissue of the scalp and anchored to the skull; an external active sound processor is attached to a second magnet and positioned over the implanted anchor. The magnetic attraction allows to hold the processor in place and to transmit acoustic energy. The limits of this kind of device are represented by energy loss through the skin layer and possible discomfort and complications due to soft tissue pressure [11 12]. Unfortunately, our experience with magnetic BAHIs is still insufficient for a definite conclusion.

In this paper, we report our experience on the use of three bone-anchored hearing implants surgery techniques: 1) linear incision with thinning of the subcutaneous tissue; 2) linear incision without thinning of the subcutaneous tissue; 3) punch technique (Minimally Invasive Ponto Surgery).

Materials and methods

This retrospective study was carried out on patients with middle/inner ear disorders causing conductive or mixed hearing loss who underwent application of a bone-anchored hearing implant. In all our patients, the decision to resort to this kind of solution was taken after an unsuccessful trial with conventional BCDs.

The results were evaluated on the basis of any intra-operative complication, duration of surgery and occurrence of adverse effects at the implantation site. The latter was assessed by applying Holgers classification (Table I) during 4 postoperative control visits (T1, 7 days; T2, 30 days; T3, 60 days; T4, 1 year). Patients were divided into 3 groups based on the used surgical technique and the implanted processor. Patients in Group 1 were implanted with a Baha® System (Cochlear Bone Anchored Solutions, Mölnlycke, Sweden) and those in Groups 2 and 3 with a Ponto™ System (Oticon Medical AB, Askim, Sweden). The allocation of patients in the three groups was determined by surgical technique

Table I. Holgers classification of skin reaction.

Grade 0	No skin reaction
Grade 1	Redness with slight swelling
Grade 2	Redness, moistness and moderate swelling
Grade 3	Redness, moistness and moderate swelling, with tissue granulation
Grade 4	Profound signs of infection, resulting in removal of the implant

evolution over the years and the availability of newer hearing devices at our clinic.
All patients signed an informed consent form.
Data were collected and processed using the Epi Info™ 7.2.0.1 (CDC, USA).

Group 1. Linear incision with thinning of the subcutaneous tissue

After performing a trichotomy on the surgical area, the site of implant was identified 65 mm posterior-superiorly from tragus. Under local anaesthesia (Mepivacaine 20 mg/ml with adrenaline 1:100.000), a linear vertical incision down to the periosteum was made; retractors were positioned, and a cross-cut was made on the periosteum, which was then centrifugally moved away from the site of the implant, where a 3 mm hole was practiced, then deepened to 4 mm and widened. Subsequently, the fixture with the abutment was inserted and the subcutaneous tissue adjacent to the cut was thinned. After suturing the incision with Vicryl, the healing cap was applied and gauze with antibiotic ointment was delicately wrapped beneath it to exert a slight pressure on the surgical cut. Finally, a compression dressing was applied.

Group 2. Linear incision without thinning of the subcutaneous tissue

The surgical procedure is analogous to that in Group 1, but differed in the need to measure skin thickness with a needle before infiltration of local anaesthetics (to use an abutment with the right length) and for the lack of thinning of the subcutaneous tissue.

Group 3. Punch technique (MIPS)

This technique was performed according to the new procedure developed by Oticon Medical AB, Askim, Sweden (MIPS) for the application of their implants. After trichotomy, the site of implantation is determined by the intersection of two lines, the first starting from the external corner of the homolateral eye and going posteriorly tangentially to the helix and the other starting from the external auditory meatus and going posterior-superiorly for 55-65 mm. The skin thickness was measured before the infiltration of local anaesthetics as for Group 2. A circular incision up to the bone surface was then practiced with a 5 mm diameter biopsy punch. Through the incision, the periosteum was carefully removed from the implant site using a raspatorium and after positioning a guide cannula, a guide drill was first used with a spacer in place to realise a guide hole of 3 mm. Once confirmed the presence of bone at the implant site with a probe, the spacer was removed and the guide hole deepened to 4 mm. Next, a widening drill was used to widen the hole. Prior to all steps, the cannula was filled with saline solution and copious irrigation was used during and after drilling to facilitate cooling and removal of bone debris. Inserted the abutment through the incision, the healing cap, the gauze with antibiotic ointment and an external dressing were applied.

Results

We collected data on 29 patients with a mean age 60.41 years (range 24-87), 16 of which were women (55.17%) and 13 men (44.83%). We performed bilateral implantation on one patient, for a total of 30 recorded implants, 14 to the right (46.7%) and 16 to the left (53.3%). All surgeries were performed by the same experienced surgeon (PV) except for 2 cases in Group 3, which were performed by a young surgeon (RP).
In Table II we report the adverse effects of the three groups during follow-up, as assessed by Holgers classification.

Group 1

This technique was used in 12 cases (40%). During surgery, there were no complications in 9 cases (75%); in 1 case (8.33%), while drilling, the Dura Mater was exposed with important bleeding thus requiring the displacement of the site of implant; in 2 cases (16.67%), while drilling the hole, there was temporary bone bleeding, which slightly increased surgical times, but did not require displacement of the implant site. Average surgical time was 62.08 min (range 50-75, standard deviation 9.40, median 62.5). At T1 in all cases we registered a grade 0 in Holgers classification (100%). At T2, we registered only 1 case (8.33%) of hyperaemia and swelling of the skin around the abutment, to which Holgers 1 was scored. All others classified as grade 0. At T3 in 2 cases (16.67%), because of local inflammation, Holgers 2 was assigned; in 1 case (8.33%) the patient showed significant signs of infection (Holgers 4) which required removal of the implant; in the remaining 9 cases (75%) there was no local skin reaction (Holgers 0). At T4, of the 11 still implanted patients, another (9.09%) presented significant signs of infection (Holgers 4) requiring the removal of the implant. All others classified as grade 0. Thus, after 47 observations in total, we recorded the presence of postoperative complications in 5 cases (10.63%).

Group 2

This technique was used in 8 cases (26.7%). In this group, we never recorded intra-operative complications. Average surgical time was 34.37 min (range 25-40, standard

Table II. Holgers classification of skin reaction in the three groups.

	Holgers 0	Holgers 1	Holgers 2	Holgers 3	Holgers 4	Mean score
T1						
Group 1	12	0	0	0	0	0
Group 2	8	0	0	0	0	0
Group 3	9	0	1	0	0	0.2
T2						
Group 1	11	1	0	0	0	0.08
Group 2	8	0	0	0	0	0
Group 3	10	0	0	0	0	0
T3						
Group 1	9	0	2	0	1	0.41
Group 2	8	0	0	0	0	0
Group 3	10	0	0	0	0	0
T4						
Group 1	10	0	0	0	1	0.36
Group 2	7	1	0	0	0	0.12
Group 3	10	0	0	0	0	0

T1: 7 days; T2: 30 days; T3: 60 days; T4: 1 year.

deviation 5.63, median 35). During the first three postoperative controls (T1, T2, T3), none of the patients (100%) showed local skin reactions around the abutment (Holgers 0). At T4, we registered 1 case (12.5%) of hyperaemia and swelling of the skin around the abutment (Holgers 1), which was treated with local medications. Therefore, in a total of 32 observations, we reported postoperative complications in only 1 case (3.12%).

Group 3

The new technique was used in 10 cases (33.3%). During surgery, in one case (10%) we recorded exposure of the Dura Mater after drilling at 3 mm with important bleeding as a complication; after stopping the bleeding, the abutment was directly applied without deepening the hole. The average surgical time was 18.7 min (range 9-40, standard deviation 9.59, median 17.5). On the first postoperative control (T1), 1 patient (10%) showed signs of inflammation of the skin around the abutment (Holgers 2). The remaining 9 patients (90%) did not show signs of skin inflammation (Holgers 0). In subsequent inspections (T2, T3, T4), we never observed local reactions (Holgers 0, 100% of cases). In this group, out of a total of 40 observations we recorded only 1 postoperative complication (2.5%).

Discussion

The advent of BAHIs has resulted in a considerable improvement in overall quality compared to conventional bone conduction devices, as well as a significant reduction of tedious local and aesthetic complications related to the use of older devices. However, the application of bone-anchored implants can also be accompanied by complications, both intra-operative (bleeding, exposure of dura mater, cerebrospinal fluid leakage) and postoperative (necrosis of skin flaps, alopecia, local infections, implant extrusion, dysaesthesia, abnormal scarring with aesthetic consequences).

Since Tjellström's first description of the surgical technique, this has been modified in order to reduce the onset of these complications [4]. The original technique, which involved the creation of a skin flap with thinning of the subcutaneous tissue, was burdened with high rates of complications. The same Tjellström, in an initial review of the first 100 implants, reported implant extrusions in 10% of cases [13]. Reyes, instead, after 8 years of follow-up, recorded adverse implant skin reactions in 30% of cases [14]. In a large series of more than 1000 implants by modified technique (linear incision and thinning of the subcutaneous tissue), Dun reported adverse events during follow-up in 4.5% of cases [15]. Finally, in a series of 149 patients, House reported an incidence of local complications in 12.8% of cases (skin overgrowth, implant extrusion, wound infection, flap necrosis) [16]. In our experience, using this surgical technique (Group 1), we observed the development of complications in the postoperative period in 10.63% of cases. In particular, we recorded 2 cases of

important infection at the implant site that required removal of the device at 60 days and 1 year after surgery, respectively.

The introduction of longer abutment has allowed a further evolution of the surgical technique, i.e. a linear incision without the need for thinning of the subcutaneous tissue. This has undoubtedly led to benefits in terms of local inflammatory reactions, time for wound healing and development of dysaesthesia, in addition to a better aesthetic result. Hultcrantz using this technique reported local infection in 14% of cases, compared to 43% of surgeries with thinning of the subcutaneous tissue [9]. In a recent paper, Den Besten, using the tissue preservation technique, scored a higher percentage of local reactions (28%) compared to a control group subjected to linear incision with thinning of the subcutaneous tissue (4%); these local reactions were managed with 1-2 local medications [17]. Even Martinez et al. compared these two techniques, observing a significant reduction of local reactions of a certain entity (Holgers 3) after 1 week after surgery in the case of the subcutaneous tissue preservation (preservation 7% *vs* thinning 28%); however, the authors reported a higher percentage of minor local reactions (Holgers 1-2: 93% *vs* 72%), but a faster healing rate at 1 month after surgery (Holgers 0-1: 86% *vs* 75%) [18]. In a recent paper, Caruso et al., using the linear cut without thinning of the subcutaneous tissue on 49 patients, observed mild skin reactions (Holgers 0 or 1) in 96% of the visits (n = 116); the authors reported five adverse skin reactions (Holgers ≥ 2) across all visits (4%) [19]. During follow-up of the tissue preservation technique, in our series (Group 2) we observed local reactions in 3.12% of cases, thereby confirming a significant reduction in surgical trauma that is typically found in patients who underwent the technique with thinning of the subcutaneous tissue.

Finally, the latest evolution of the technique has permitted to further limit trauma, reducing duration of surgery at the same time. The use of a biopsy punch, and the drilling and placement of the implant through the incision allows for less manipulation of surrounding tissues, therefore reducing the risk of complications. The disadvantages of this technique consist in the lower visibility of the surgical area, which is in part obviated by using the operating microscope. Goldman in 2013 described the use of this technique on 15 patients, employing a 12 mm diameter biopsy punch; in the postoperative period, the author observed mild hyperaemia of the surgical area in all cases, but no complications of Holgers 2 or higher [10]. Later, Wilson et al. used a 4 mm diameter biopsy punch with enlargement of the surgical area, removing the surrounding subcutaneous tissue and periosteum in a conical shape from the scalp to the cranial surface, to improve the visualisation of the site of implant; out of 11 patients, the authors reported 2 cases of local reaction with Holgers 2 or higher in the postoperative follow-up that were treated with local medications [20]. In 2015, Gordon and Coelho compared the punch technique with the linear incision plus thinning technique; they used a 6-mm diameter biopsy punch, removing skin, subcutaneous tissue and periosteum in a single step. Two cases of local reaction (Holgers ≥ 2) were observed at the first and at the last postoperative control and 1 case of implant extrusion (despite a Holgers 0 during the first control) [3]. The latest evolution of the punch technique was introduced by Oticon Medical AB (Askim, Sweden) and called "MIPS"; the aim was to optimise preservation of tissues, minimise trauma and provide a standardised procedure and surgical instruments, thus eliminating surgical variability.

Using this surgical technique (Group 3), we recorded only 1 case (10%) of inflammatory reaction around the abutment during the first postoperative control, that was easily treated with local medication; at subsequent controls, we did not observe local reactions (postoperative complication rate, 2.5%).

When comparing the local reactions in the 3 groups, we did not record significant differences during follow-up (T1, $p = 0.38$; T2, $p = 0.48$; T3, $p = 0.11$; T4, $p = 0.55$, one-way ANOVA test); therefore, in our experience, the use of less invasive surgical techniques does not affect the rate of postoperative complications, which is already low compared to previous techniques.

In our opinion, a very interesting aspect of this study consists in the significant reduction of surgical time that shows a decreasing tendency from the linear incision technique with thinning of the subcutaneous tissue, to the linear incision technique and preservation of the subcutaneous tissue, to the MIPS technique. In Group 1, in fact, the average intervention duration was 62.08 min (range 50-75, standard deviation 9.40, median 62.5) *vs* 34.37 min (range 25-40, standard deviation 5.63, median 35) in Group 2 and 18.7 min (range 9-40, standard deviation 9.59, median 17.5) in Group 3, with statistically significant differences ($p < 0.00001$, one-way ANOVA test). In the 2011 Hultcrantz study, the average duration of interventions with and without subcutaneous tissue thinning (linear incision for both groups) was, respectively, 44.6 and 28.1 min [9]; using the same techniques, Martinez in 2015 reported similar durations (42 and 27 min, respectively), while Den Besten recorded shorter surgeries (31.9 and 24.6 min, respectively) [17 18]. Caruso et al., using the technique with linear incision and preservation of the subcutaneous tissue, showed a mean surgical time

of 20.3 min [19]. Using the punch techniques, Goldman reported a mean duration of the surgery of 15.2 min, against 32.3 min reported by Wilson and the 13.4 min reported by Gordon and Coelho [3 10 20]. The MIPS technique, at the current time, has only been described in the recent multicentre study by Johansson et al., which reported an average duration of the surgery of 16 min [21].

It is therefore evident how the shift to less invasive techniques involves a significant reduction in surgical time; in our experience, we recorded slightly longer times in the use of the two techniques with linear incision compared to the literature, while using the MIPS technique we observed a similar duration to that of other experiences with punch and to the only existing study with MIPS. In our opinion, the reduction of surgical time that we observed in our series in the transition from the more invasive to less invasive techniques should not be attributed to a learning curve, since all surgeries (except for 2 MIPS) were performed by the same experienced surgeon (PV). Moreover, we noticed that both 2 interventions with MIPS technique performed by the young surgeon (RP) had a duration of 20 min, which is very close to the average value of the other 8 procedures performed with the same technique by the experienced surgeon (18.37 min), with no statistically significant difference ($p = 0.84$, unpaired t-test); therefore, we believe that the shorter duration of the operations carried out with MIPS technique is related to the surgical technique and not the surgeon's experience. It is also important to note that in the same group (Group 3) we registered a case of lengthening of surgical time (40 min) because of an intra-operative complication (dura mater exposure with important bleeding).

The present study, being a retrospective study, lacks statistical power. The case study, nevertheless, is definitely limited by the low number of patients eligible for this kind of prosthetic application or who accept this solution. Another limit is represented by the short duration of follow-up, given that local complications, especially of aesthetic nature, may also appear at a later time. In this regard, it is important to not underestimate possible variability among the patients in daily care for the appropriate cleaning of skin-abutment interface; indeed, poor hygiene of the implant site can determine the appearance of late complications. However, we think that this risk was the same for all three study groups, because is independent of the surgery technique, and so it does not represent a possible bias. Adequate counseling with regards to the absolute need of a daily cleaning of the skin-abutment interface was similarly given to all patients in the three groups. Moreover, the importance of a correct hygiene was stressed during each control visit in the outpatient clinic. Finally, a further limitation could be not having performed a blinded follow-up. We believe, however, that comparing three different surgical techniques for the application of BAHIs allows us to obtain interesting data about the benefits of the new MIPS technique, especially in relation to the duration of the intervention and its simplicity of execution.

In the present study, we did not consider the time required for osseointegration and stability of the system, in addition to various audiological parameters. In this regard, there is an ongoing randomised controlled trial by Calon et al. to compare the MIPS and linear incision techniques with preservation of subcutaneous tissue, by assessing various parameters that may help in the prediction of outcomes and complications [22]. The study is still ongoing and the authors expect to complete it in August 2018.

Conclusions

The surgical techniques for the application of percutaneous bone-anchored implants have been refined over the years, with the aim of reducing the healing time, local complications and aesthetic consequences. This study confirms the low rate of intra- and postoperative complications of the described techniques and attests the simplicity of execution of the MIPS technique, with a significant reduction of surgical time compared to the other two techniques, and positive effects in terms of health care costs.

To the best of our knowledge, this is the first study that compares the MIPS technique to linear incision techniques, with or without preservation of subcutaneous tissue.

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Otology

The short- and long-term adverse effects of FGF-2 on tympanic membrane perforations

Gli effetti avversi di FGF-2 nelle perforazioni della membrana timpanica

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SUMMARY

The objective of this study was to investigate the short- and long-term adverse effects of fibroblast growth factor-2 treatment of tympanic membrane perforations. A total of 134 patients with traumatic tympanic membrane perforations were randomly divided into two groups: an observational group and a fibroblast growth factor-2 treatment group. The closure rate, closure time and principal side-effects were compared between the groups at 6 and 12 months. At 6 months, 131 patients were examined to determine healing outcomes and short-term side-effects. The total closure rate differed significantly between the fibroblast growth factor-2 and observational groups (95.5% *vs* 73.4, $p < 0.01$). The fibroblast growth factor-treated group exhibited a significantly shorter closure time than the observational group (11.9±3.1 days *vs* 52.6 ± 18.1 days, $p = 0.00$). Three patients with secondary otitis media with effusion, and three with reperforations, were noted in the fibroblast growth factor-2 group. We additionally performed long-term follow-up on 89.1% of the patients in the observational group and 92.5% of the patients in the fibroblast growth factor-2 group; follow-up was performed 16-42 months after perforation closure. Only a small perforation of the pars flaccida developed in the fibroblast growth factor-2 group. No middle ear cholesteatoma was noted in either group. This study suggests that the topical application of fibroblast growth factor-2 to human traumatic tympanic membranes is safe. Otorrhoea was the most common short-term side-effect; other less common side-effects included otitis media with effusion and reperforation. No serious long-term side-effects were found.

KEY WORDS: Fibroblast growth factor-2 • Tympanic membrane regeneration • Side-effects • Reperforation

RIASSUNTO

L'obiettivo di questo studio è stato quello di valutare gli effetti avversi del trattamento con fattore di crescita dei fibroblasti 2 (FGF-2) delle perforazioni della membrana timpanica. 134 pazienti sono stati suddivisi in maniera randomizzata in due gruppi: un gruppo di controllo, sottoposto semplicemente ad osservazione clinica, ed un gruppo trattato con FGF-2. Sono stati quindi comparati tra i due gruppi i tassi di chiusura, i tempi di chiusura, e gli effetti avversi, a 6 e 12 mesi. A 6 mesi, sono stati valutati 131 pazienti. Il tasso di chiusura totale è stato significativamente diverso tra il gruppo trattato con FGF-2 e il gruppo di controllo (95,5% e 73,4% rispettivamente, $p < 0,01$). Inoltre il gruppo sottoposto a trattamento con FGF-2 ha mostrato dei tempi di chiusura significativamente più brevi rispetto al gruppo di controllo (11,9 ± 3,1 giorni contro 52,6 ± 18,1 giorni, $p = 0,00$. Nel gruppo trattato con FGF-2 sono stati registrati due casi di otite media effusive secondaria, e tre casi di riperforazione. Inoltre, è stato aggiunto un ulteriore follow-up nell'89,1% dei pazienti appartenenti al gruppo di controllo, e nel 92,5% dei pazienti trattati con FGF-2. Tale follow-up è stato eseguito tra 16 e 42 mesi dopo la chiusura della perforazione. Nei pazienti trattati con FGF-2 si è verificata solo una perforazione della pars flaccida della membrane timpanica. In entrambi i gruppi non sono stati segnalati casi di colesteatoma. Questo studio suggerisce che l'applicazione topica di FGF-2 sulle membrane timpaniche danneggiate da un trauma è sicura. L'otorrea è stato il più comune effetto avverso a breve termine. Altri effetti a breve termine, meno comuni, sono stati l'otite media effusive e la riperforazione. Non stati riportati gravi effetti collaterali a lungo termine.

PAROLE CHIAVE: *FGF-2 • Rigenerazione della membrana timpanica • Effetti collaterali • Riperforazione*

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Introduction

Fibroblast growth factor-2 (FGF-2) is a polypeptide mitogen that stimulates the proliferation of epidermal and connective tissue cells [1]. FGF-2 has been used to repair clinical and experimental tympanic membrane perforations (TMPs) [2-6]. Clinical studies have shown that topical application of FGF-2 significantly improves the closure rate and shortens the closure time of human traumatic

TMPs [4 7 8]. The topical application of FGF-2 also showed a higher success rate for the repair of human chronic TMPs, thus providing an alternative to conventional myringoplasty [3 5 9]. However, the side-effects and safety of FGF-2 remain controversial. FGF-2 treatment of TMPs has not been widely adopted worldwide. Although some scholars previously undertook a series of clinical studies, the short- and long-term side-effects of FGF-2 on TMPs have not been not fully addressed. In addition, previous studies focused on traumatic TMPs without a history of middle ear disease and myringosclerosi [4 7 8].

Most studies have suggested that FGF-2 is not ototoxic to the middle or inner ear, and does not cause sensorineural hearing loss [2-6 10 11]. However, a few studies found that topical application of FGF-2 facilitated the proliferation of epithelial keratinocytes, causing short-term hyperplasia of the external auditory canal (EAC) and myringitis, and long-term cholesteatoma of the middle ear [6 12-15]. In addition, Laxarou et al. [16] found that continuous application of FGF-2 inhibited the synthesis of collagen, in fact promoting collagen catabolism. Thus, apart from efficacy, the safety and side-effects of FGF-2 remain important clinical issues. The objective of this study was to investigate the short- and long-term side-effects of FGF-2 on human TMPs.

Materials and methods

The study was reviewed and approved by the institutional ethical review board of the Affiliated YiWu Hospital of Wenzhou Medical University, China, and was conducted in compliance with the Helsinki Declaration. Informed consent was obtained from all participants.

Study subjects were recruited from consecutive patients diagnosed with a traumatic TMP who visited the Department of Otorhinolaryngology of Affiliated YiWu Hospital (Wenzhou Medical University) between February 2010 and June 2013.

The inclusion criteria were as follows: (1) a traumatic TMP caused by a blunt injury, such as a physical blow (e.g., an open-handed slap, a fist strike, or a ball hit), or an explosive noise (e.g., the blast of firecrackers or fireworks); (2) a perforation size of at least one-quarter of the pars tensa; and (3) no middle ear infection, granulation tissue, or damage to the ossicular chain at the time of the hospital visit. A large perforation was defined as one exceeding 25% of the entire TM when viewed using ImageJ software (NIH, Bethesda, MD, USA) [17 18]. The status of the residual tympanic membrane was divided into two categories: normal and pathological. A pathological membrane was defined as a residual membrane with local atrophy or exhibiting deposition of calcified plaques, whereas a normal membrane was defined as a membrane lacking local atrophy or calcified plaques. Short-term side-effects were defined as those developing within 6 months of treatment; long-term side-effects were those developing 12 months after treatment.

The principal investigator, aided by a registered nurse, allocated patients to various treatments using simple random sampling. Specifically, consecutive subjects who met the inclusion criteria and signed the consent form were assigned random numbers generated by the SPSS for Windows software package (version 19.0; SPSS, Inc., Chicago, IL, USA) that allocated them to one of the FGF-2 or observational groups.

Technical methods

Observational group. The residual tympanic membrane of patients in this group received no intervention, but underwent regular follow-up.

FGF-2 group. The external auditory canal (EAC) was cleaned with a cotton bud soaked in povidone/iodine solution at the time of the first visit. The edge of the perforation was not approximated, and no scaffolding material was used. A bottle of recombinant bovine FGF-2 solution (21,000 IU/5 mL; Yi Sheng, Zhuhai City, China) was prescribed by a clinician with instructions for the patient to apply three drops (approximately 0.15 mL) once daily to the eardrum at home. The treatment ear was kept in an upward position for at least 30 min after the application of the eardrops to ensure that the FGF-2 solution moistened the residual eardrum. The FGF-2 application time until complete perforation closure was either confirmed by the physician or taken to be 6 months.

Short-term follow-up

Follow-up was scheduled once weekly for all patients until perforation closure. The tympanic membrane was oto-endoscopically examined at every follow-up to assess the size of the perforation and the otorrhoea status. At 6 months after treatment, all patients (whether their perforations had closed or not) were followed-up to assess the surface morphology of the eardrum (with or without reperforations). Pure-tone audiograms were obtained with the aid of a GSI 10 audiometer (VIASYS Healthcare, Inc., Conshohocken, PA, USA) with the patients in a quiet room. Audiograms were obtained before treatment, at perforation closure, and at the final follow-up after FGF treatment. The Pure Tone Average 4 (PTA4) air conduction threshold at 0.5, 1, 2, and 4 kHz was used to obtain the pure-tone average. Computed tomography (CT) of the temporal bone for perforations secondary to otorrhoea

was performed at perforation closure, while CT was not performed for perforations that failed to close. CT was planned to be repeated at 1-3 months after the first CT scan if it revealed abnormal imaging results in the middle ear.

Long-term follow-up

Follow-up was scheduled at 12 months after perforation closure. We oto-endoscopically evaluated the surface morphology of healed eardrums. A CT scan of the temporal bone was performed to determine the cholesteatoma status of the middle ear in patients exhibiting atrophy of the eardrum or perforation of the pars flaccida.

Statistical analysis

Power Analysis and Sample Size software (version 11.0; NCSS, Kaysville, UT, USA) was used to estimate the sample size required to provide a power of 80% and a type I error of 5% (a = 0.05). A 15% difference in closure rate between the treatment and observation groups was predicted. The closure rate of the observational and FGF-2 drops groups would thus be 80.0% and 95%, respectively. Using these values, it was determined that 56 patients per group were required. Assuming a loss to follow-up of 10%, the number of patients needed per group was increased to 62, giving a total of at least 124 patients. All results are expressed as means ± SDs or as percentages. Statistical analyses were performed using SPSS software (version 11.0; SPSS, Inc.). The paired χ^2 test and the matched-pair t-test were used to compare closure rates and times, respectively. A $p < 0.05$ was deemed to indicate statistical significance.

Results

Short-term healing and auditory outcomes

A total of 134 patients met the inclusion criteria and only 3 (in the observation group) were lost to follow-up. Thus, 131 patients were finally analysed for short-term side-effects. Of these, 64 were in the observational group and 67 in the FGF-2 group (Fig. 1). The demographic data of the two groups are shown in Table I. There was no statistically significant between-group difference in terms of age, sex, cause of injury, duration of injury, status of the residual tympanic membrane, side of ear, or hearing level. The healing outcomes of the two groups are shown in Table II. The total closure rate was significantly different between the FGF-2 and observational groups (95.5% *vs* 73.4, $X^2 = 12.34$, $p < 0.01$). The FGF-treated group had a significantly shorter closure time than the observational group (11.9 ± 3.1 days *vs* 52.6 ± 18.1 days, $p < 0.01$). Of the 64 patients with perforation closure in the FGF-2 group, 58 required a bottle of FGF-2 solution, and two bottles of FGF-2 solution were prescribed for 6 patients. The price of a bottle of FGF-2 solution was approximately $ 3.10.

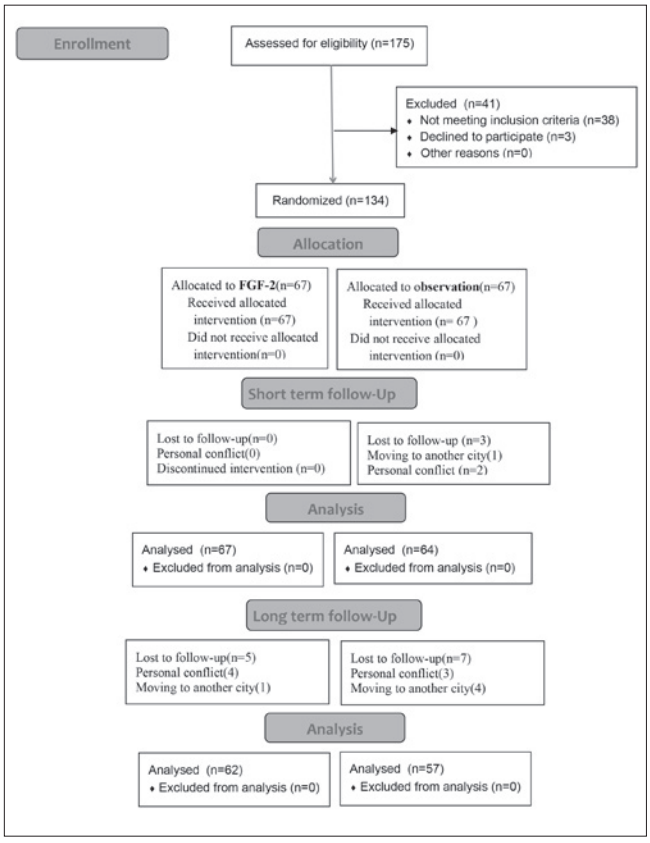

Fig. 1. Enrollment, randomization, and follow-up of the study participants.

Table II shows that the closure rate was not significantly different between normal and pathological residual tympanic membranes in the FGF-2 (98.1% *vs* 86.7%, $X^2 = 1.38$, $p > 0.05$) or observational groups (76.9% *vs* 58.3%, $X^2 = 0.91$, $p > 0.05$). The mean closure times of normal and pathological residual tympanic membranes did differ significantly ($p < 0.01$) between groups. In addition, the closure rate did differ significantly between the FGF-2 and observational groups for either normal residual tympanic membrane (98.1% *vs* 76.9%, $X^2 = 10.64$, $p < 0.01$). However, the closure rate did not differ significantly between the FGF-2 and observational groups for pathological residual tympanic membranes (86.7% *vs* 58.3%, $p = 0.09$). The mean closure times differed significantly between the FGF-2 and observational groups for both the normal and pathological residual tympanic

Table I. Demographic data for patients who underwent short-term follow-up in each group.

	Observation group	FGF-2 group	p-value
	64	67	
Sex (male : female)	29 : 35	25 : 42	0.532 a
Age, years	37.4 ± 5.1	37.4 ± 6.7	0.68 b
Ear (left : right)	42 : 20	40 : 27	0.813 a
The cause of injury (B : E)	5 : 59	3 : 64	0.568 a
The duration of injury, days	3.6 ± 4.1	6.4 ± 2.8	0.482 b
Residual TM (normal : pathology)	52 : 12	52 : 15	0.715 a
Hearing level	28.6 ± 5.3	29.1 ± 4.3	0.818 b

$p < 0.05$ was considered statistically significant; a = χ^2 test; b = t test; B = blunt injury; E = explosion injury.

Table II. The short-term healing outcome and side-effects in each group.

		N	Healing outcome		Side-effects			
			Closure rate, n. (%)	Closure time, days	Purulent otorrhoea	Pseudomembrane	Otitis media effusion	Reperforation
Observation group	Normal residual TM	52	40 (76.9%)	47.1 ± 15.8	4	2	0	0
	Pathologic residual TM	12	7 (58.3%)	56.6 ± 19.2	3	0	0	0
FGF-2 group	Normal residual TM	52	51 (98.1%)	10.1 ± 4.6	11	0	3	0
	Pathologic residual TM	15	13 (86.7%)	19.1 ± 7.3	3	0	0	3

membranes ($p < 0.01$). The hearing improvement rates in the two groups did not differ significantly (13.6 ± 2.6 *vs* 12.7 ± 4.3 dB, $p = 0.72$).

Short-term side-effects

In the FGF-2 group, there were 46 (46/67, 68.7%) patients whose residual tympanic membrane remained moist during treatment. However, 21 patients (21/67, 31.3%) did not comply with the protocol and applied a greater number of eardrops, resulting in a liquid residue in the EAC and middle ear causing otorrhoea. Of these patients, 14 (66.7%) developed secondary purulent otorrhoea. Nevertheless, the rate of purulent otorrhoea among the two groups did not differ significantly (14/67 *vs* 7/64, $p > 0.05$).

None of the seven patients in the observational group with secondary purulent otorrhoea achieved closure. In the FGF-2 group, of the 21 patients with otorrhoea, 7 patients with single otorrhoea and 12 patients (12/14, 85.7%) with purulent otorrhoea achieved complete closure, and the closure rate did not differ (19/21 *vs* 45/46, $p > 0.05$) between perforations with and without otorrhoea. Of the 19 closed perforations with otorrhoea, the CT scans of 13 patients revealed no abnormal imaging results in the middle ear, while the CT scans of 6 patients revealed soft-tissue shadows in the mastoid cavity at first examination. Of the six perforations with soft tissue in the middle ear cavity, the perforation size was more than 50% of the pars tensa in four perforations, and two patients had subtotal perforations. Nevertheless, CT was repeated, and soft-tissue shadows of the mastoid cavity disappeared after 1-2 months for six patients with abnormal imaging results on the first CT scan.

No severe side-effect (pain, severe vertigo, myringitis, or hyperkeratosis) was observed in either group; other side-effects are shown in Table II. A pseudomembrane was found in two patients in the observation group. In the FGF-2 group, of the 21 perforations with otorrhoea, three patients developed secondary otitis media with effusion (Fig. 2) and three reperforations were found. All of the reperforations were in residual tympanic membranes with myringosclerosis (Figs. 3, 4). Because the sample number of reperforations in the two groups was small, statistical analysis could not be performed.

Long-term side-effects

Long-term follow-up was performed on 57 (57/64, 89.1%) patients in the observational group and 62 (62/67, 92.5%) patients in the FGF-2 group at 16-42 months after perforation closure. The hearing improvement rates did not differ significantly between the two groups (12.5 ± 4.1 *vs* 12.7 ± 4.3 dB, $p = 0.86$). All long-term side-effects are shown in Table III.

A small perforation of the pars flaccida was noted in one blast injury patient from the FGF-2 group (Fig. 5).

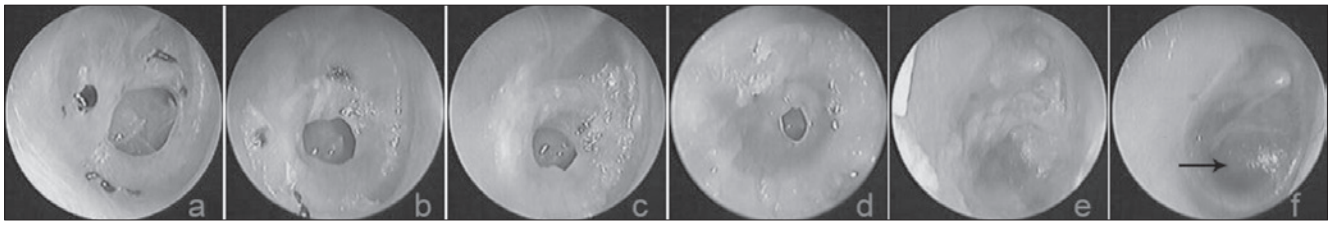

Fig. 2. The follow-up results at various time points after FGF-2 treatment: **a)** 10th day after perforation; **b-f)** 4 days, 8 days, 11 days, 15 days and 22 days after treatment, respectively (→indicates otitis media effusion).

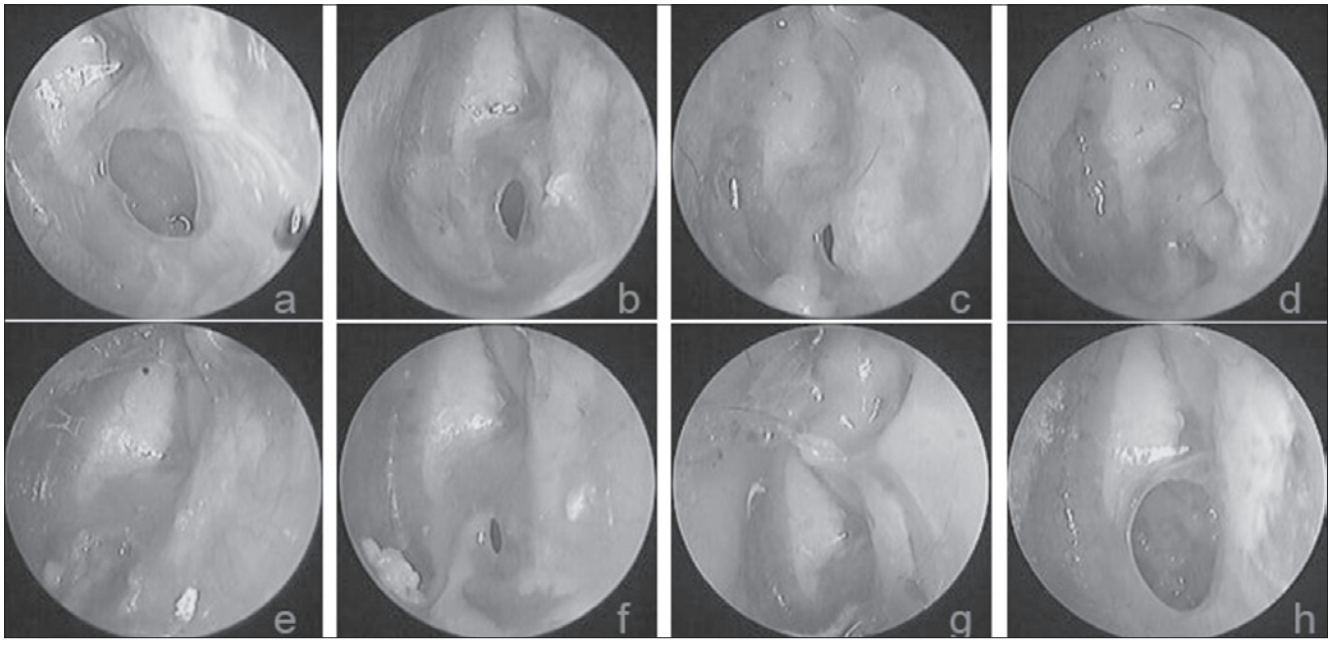

Fig. 3. The follow-up results at various time points after FGF-2 treatment: **a)** 20 days after perforation; **b-g)** 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 8 weeks; and **h)** 26 months after treatment, respectively.

However, reperforation occurred in two patients with a pseudomembrane in the observation group. A pseudomembrane is different from an atrophic eardrum; it is a crust-like substance without the epithelial and fibrous layers of the eardrum (Fig. 6). An atrophic eardrum includes only the epithelial layer, without the fibrous layer of the eardrum. Atrophy of the healed eardrum was evident in 22 (22/57, 38.6%) patients in the observational group and 18 (18/62, 29.0%) patients in the FGF-2 group; this difference was not significant ($X^2 = 1.22$, $p > 0.05$). CT revealed no middle ear cholesteatoma in 40 patients with atrophic eardrums or a small perforation of the pars flaccida in the two groups.

Discussion

The effectiveness of FGF-2 used to treat TMP has been well-demonstrated both clinically and experimentally [1-9]. Application of FGF-2 alone or FGF-2 combined with patching, significantly improved TMP closure rates and shortened closure times [3 4 6 18]. This study verified these findings. However, the side-effects of FGF-2 on human TMPs remain incompletely understood. Otorrhoea was a common short-term side-effect of the FGF-2 treatment of traumatic TMPs in this study. Twenty-one patients (21/67, 31.3%) developed secondary otorrhoea, mainly caused by failure of the patient to abide by the study protocol. These patients applied a greater number of FGF-2 solution eardrops, resulting in an increased liquid environment in the EAC and middle ear. Nevertheless, the rate of purulent otorrhoea between the FGF-2 and observational groups did not differ significantly (14/67 *vs* 9/64, $p > 0.05$). None of the 7 patients in the observational group with secondary purulent otorrhoea was found

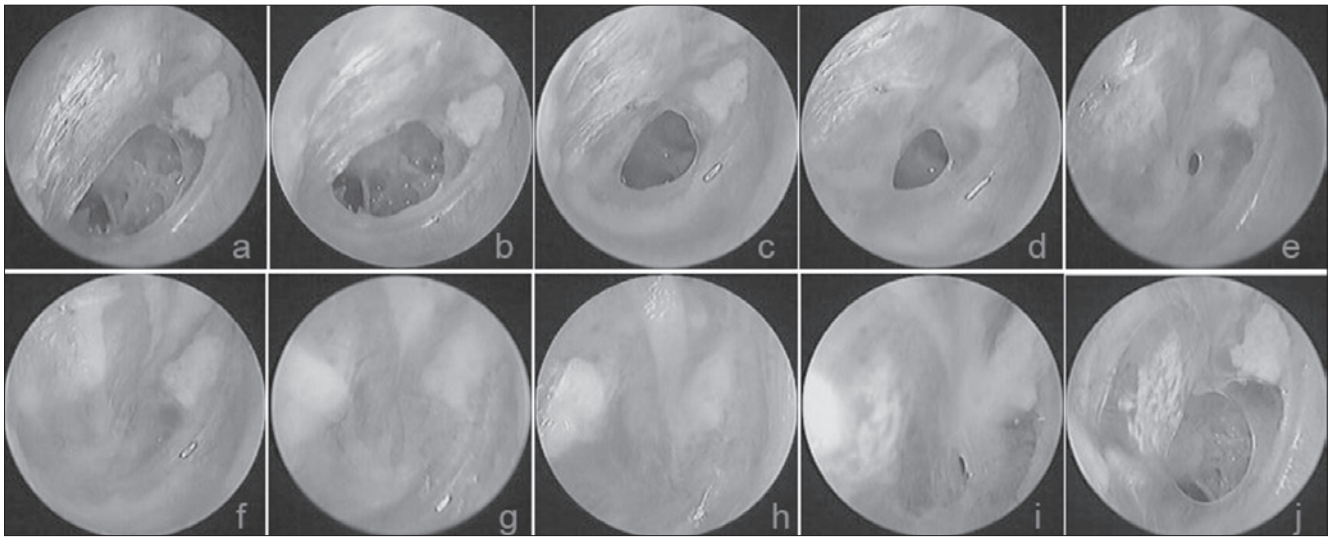

Fig. 4. The follow-up results at various time points after FGF-2 treatment: **a)** 3rd day after perforation; **b-j)** 3 days, 7 days, 10 days, 14 days, 16 days, 20 days, 25 days, 30 days and 6 months after treatment, respectively.

Table III. The long-term side-effects in each group.

		N.	Reperforation	Atrophic eardrum	Cholesteatoma
Observation group	Normal residual TM	48	2	13	0
	Pathologic residual TM	9	0	9	0
FGF-2 group	Normal residual TM	49	1	5	0
	Pathologic residual TM	13	0	13	0

to have achieved closure, whereas 12 of 14 such patients (85.7%) in the FGF-2 group attained complete closure. A previous study showed that infections of the middle ear adversely affected the spontaneous healing of traumatic TMPs; however, the topical application of FGF-2 could overcome the bacteria-induced inhibition of healing [19]. The prescription of FGF-2 to treat inflamed tissue enhanced leukocyte recruitment and expression of the endothelial cell adhesion molecule, indirectly enhancing the antimicrobial activity of the ear [20 21].

In this study, 3 patients developed secondary otitis media with effusion 3-10 days after closure of the perforation among 21 patients with otorrhoea. It is unclear whether the development of otitis media with effusion was associated with FGF-2. However, a large amount of FGF-2 solution in the middle ear may facilitate hyperplasia of the granulation tissue of the mucus membrane of the middle ear and Eustachian tube, thus obstructing the tube and inducing otitis media effusion.

Hakuba et al. [3 9] reported that the rate of reperforation of FGF-2-treated chronic TMPs was 18% after one year. In this study, during 6 months of short-term follow-up, the reperforation rate was found to be much lower. Reperforation occurred in 3 patients (3/67, 4.48%) with myringosclerosis; none of the normal membranes suffered reperforation in the FGF-2 group, and no reperforations were found in the observation group. During long-term follow-up, two patients with a pseudomembrane experienced reperforation in the observation group. A small perforation of the pars flaccida was noted in one blast injury patient from the FGF-2 group. However, the original position of the pars tensa did not show reperforation. Because the sample number of reperforations in the two groups was small, statistical analysis could not be performed. The long-term reperforation in two patients with a pseudomembrane in the observation group could have been due to the fact that the pseudomembrane gradually fell off over time. The cause of reperforation in the short-term is unclear in the FGF-2 group and requires further study. Perforation of the pars flaccida in the long-term in the FGF-2 group could be due to the fact that excessive FGF-2 solution facilitated hyperplasia of the granulation tissue of the mucus membranes of all of the tympanic cavity, tympanic antrum, aditus ad antrum and Eustachian

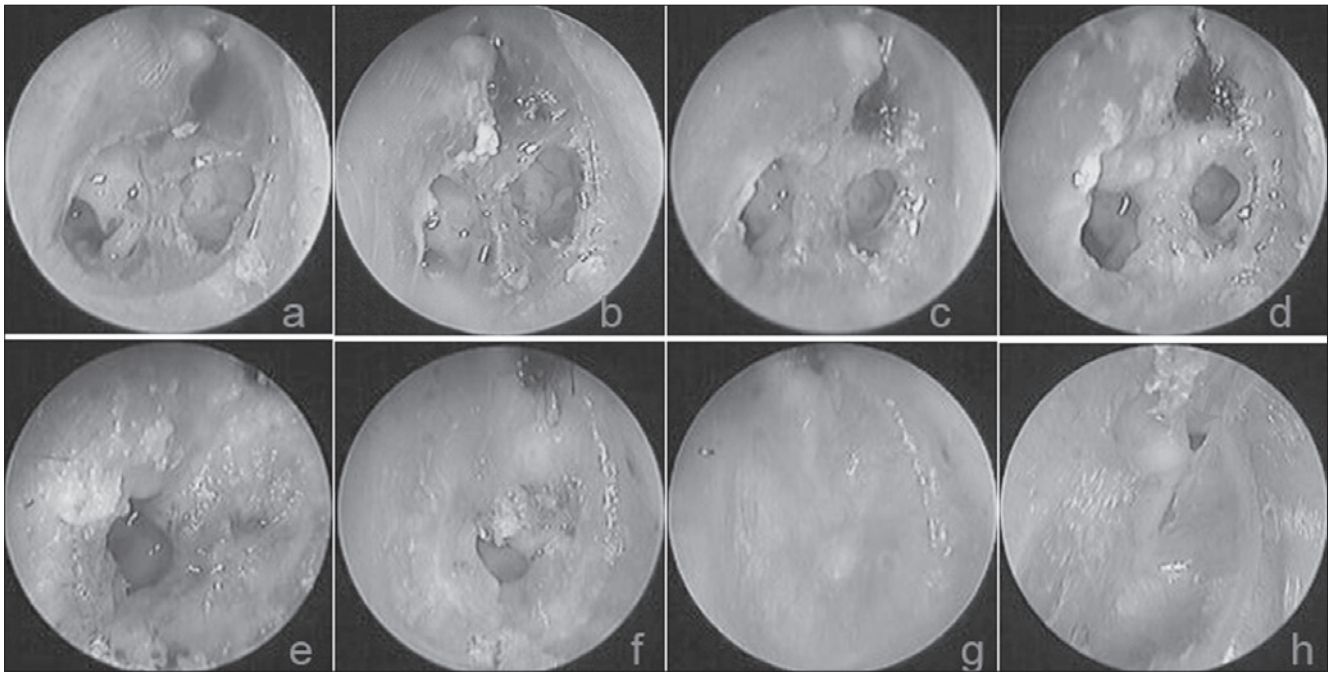

Fig. 5. The follow-up results at various time points after FGF-2 treatment: **a)** 1st day after perforation; **b-h)** 1 day, 3 days, 5 days, 8 days, 11 days, 14 days and 23 months after treatment, respectively (→indicates a small perforation in the pars flaccida).

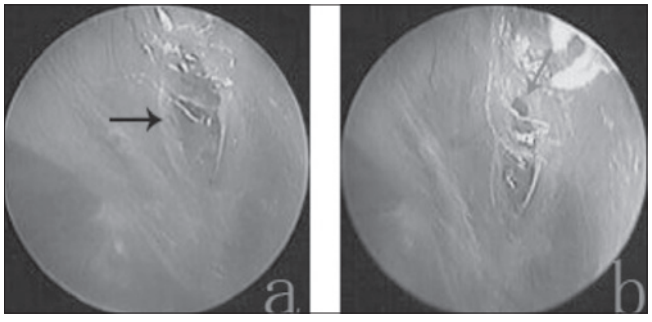

Fig. 6. a) A crust-like substance formed the pseudomembrane and closed the perforation (black arrow indicates the pseudomembrane); **b)** The partial pseudomembrane fell off and formed a reperforation (grey arrow indicates the perforation).

tube, thereby interfering with gas exchange between the Eustachian tube, mastoid sinus and tympanic cavity. Ultimately, a retraction pocket formed and the pars flaccida became reperforated over time.

One previous study found that FGF-2 was unlikely to be ototoxic [10]. The cited work showed that auditory findings did not change either short- or long-term after perforation closure. Kase et al. [10] found that topical FGF-2 applied to the middle ear did not significantly reduce the endocochlear DC potential (EP), damage the stapes, or trigger perilymph leakage. The gradual toxic effect of FGF-2 could affect the high-frequency level. Unfortunately, audiometric data at the high-frequency level were not recorded in this study. Nevertheless, other studies have suggested that topical FGF-2 does not trigger degenerative changes in structures of the organ of Corti and is not ototoxic [8 22]. In contrast, several interesting reports have shown that FGF-2 protects sensory hair cells [23 24].

The functional outcomes of the eardrum must be considered when analysing healing after FGF-2 treatment. The tympanic membrane is normally a three-layered structure featuring an epithelial layer, a fibrous layer and a mucus layer. Absence of the fibrous layer causes atrophy eardrum. Over the long-term follow-up period, atrophic eardrums were found in 18 (18/62, 29.0%) patients in the FGF-2 group and 22 (22/57, 38.6%) patients in the observation group. Thus, atrophy is not unique to patients treated with FGF-2 [16]. A previous study confirmed that spontaneous healing of the tympanic membrane was characterized by healing of only two of the layers; the fibrous layer was lacking [25 26]. Theoretically, topical application of FGF-2 induced fibroblast proliferation and facilitated hyperplasia of the fibrous layer; thick tympanic membranes were formed [3 9 27]. Atrophic eardrums should not occur after FGF-2 treatment. The reason why FGF-2 treatment caused eardrum atrophy thus requires further study. A previous study showed that prolonged application of FGF-2 inhibited collagen synthesis and promoted collagen ca-

tabolism [16]. Inhibition of collagen deposition affords a minimal benefit, and might even be detrimental, causing atrophy of the eardrum and reperforation [16 22]. In addition, the molecular environment of a chronic wound may impair the effectiveness by which growth factors stimulate healing and de novo vascularisation [28 29].

Whether the topical application of FGF-2 may trigger cholesteatoma of the middle ear remains controversial. Some studies have suggested that FGF-2 may induce keratinocyte proliferation in the epithelium of the eardrum. Middle ear cholesteatoma may then develop via ingrowth of the epidermis through the hyperplastic connective tissue of the lamina propria [12 13]. However, Friedman et al.13 found that the risk of cholesteatoma formation after short-term use of FGF-2 to treat acute perforations was minimal in an animal model. In our study, CT revealed no middle ear cholesteatoma formation in patients with an atrophic eardrum and reperforation.

Hakuba et al. [15] reported a 5% incidence of cholesteatoma when FGF-2 was used to treat chronic TMPs. However, cholesteatoma formation is not confined to patients treated with FGF-2. Sridhara et al. [30] reported that the incidence of cholesteatoma was 9% in patients with traumatic TMPs treated by tympanoplasty. Kronenberg et al., in two reports, found that the incidences of cholesteatoma were 7.6% and 4.8% during spontaneous healing of traumatic TMPs [31 32]. Thus, cholesteatoma formation in the middle ear could be not associated with FGF-2 per se, but rather with the nature and wet environment of the middle ear. Previous studies showed that a wet environment in the middle ear may induce cholesteatoma formation [33 34]. Our recent clinical observations confirmed these findings [35]. In the present work, CT performed after perforation closure revealed soft tissue shadows of varying extent in the mastoid cavities in six patients, with subsequent gasification to form normal mastoid processes. We speculate that the soft tissue hyperplasia in the middle ear cavity was related to the size of the perforation rather than a greater number of eardrops. The perforation size was more than 50% of the pars tensa and subtotal in six patients. This fact is important because one may assume that the larger a perforation is, the greater the likelihood that the solution will enter the middle ear cavity and, consequently, the higher the possibility of a reaction in the middle ear mucosa.

A limitation of this study is that it was not a placebo-controlled study using saline. This would have allowed us to better understand the effect of a moist environment on eardrum healing. However, previous experimental studies showed that the topical application of saline did not aid eardrum healing. Second, assessment of the function of the Eustachian tube was not undertaken. This would be beneficial in order to analyse the side-effects of reperforation and atrophic eardrums. In addition, high-frequency audiometric data should be evaluated in future.

Conclusions

This study suggests that the topical application of FGF-2 on human TMPs is safe. FGF-2 is simple, convenient and inexpensive (the price of a bottle of FGF-2 solution is $3.10). However, regular follow-up is required. Clinicians should check and correct the number of FGF-2 solution bottles used by patients at each follow-up visit since the optimum amount of FGF-2 solution should keep the remnant eardrum moist each day and avoid the accumulation of solution in the middle ear. Otorrhoea was the most common short-term side-effect of FGF-2 treatment for a TMP, with other less common side-effects including otitis media with effusion and reperforation. No serious long-term side-effects were found.

Acknowledgements

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CASE SERIES AND REPORTS

Is the team leading surgeon criminally liable for his collaborators' errors? Judges confirm responsibility and condemn an otorhinolaryngologist

Il chirurgo capo-équipe risponde penalmente anche per gli errori dei suoi collaboratori? I giudici confermano la responsabilità e condannano un otorino

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SUMMARY

In current healthcare, delivery of medical and surgical treatment takes place in a multidisciplinary manner. This raises the problem of distinguishing the conditions under which the person who has properly carried out his duties, respecting the related leges artis, can be held responsible for damages materially caused by another member of the medical team. Jurisprudence has developed the so-called "principle of trust" for which every member of the team can rely on the fact that other members are acting in compliance with the leges artis of their specialisation. The Supreme Court has limited the application of this principle. The authors examine the jurisprudence on responsibility of the team in otolaryngology and conclude that individual liability should be limited to the specific expertise of the individual specialist.

KEY WORDS: Team leader responsibility • Principle of trust • Équipe responsibility • Legal medicine

RIASSUNTO

Nella realtà sanitaria contemporanea, la prestazione terapeutica si svolge in forma multidisciplinare. Si pone, quindi, il problema di distinguere a quali condizioni colui che ha espletato correttamente le proprie mansioni rispettando le leges artis a lui richieste, può essere chiamato a rispondere del danno materialmente causato da altro membro dell'équipe medica. La dottrina ha elaborato il "principio di affidamento", approssimativamente traducibile in "principle of trust", ossia ogni membro dell'equipe può fare affidamento sul fatto che gli altri soggetti agiscano nell'osservanza delle leges artis della loro specializzazione. La Suprema Corte ha limitato l'applicazione di tale principio al fine di aumentare la possibilità di evitare eventuali errori dei colleghi. Gli autori esaminano la giurisprudenza che si è formata sulla responsabilità in équipe in casi di interesse otorinolarigoiatrico e concludono che l'ambito della responsabilità dovrebbe essere circoscritto alle specifiche competenze dei singoli.

PAROLE CHIAVE: *Responsabilità capo équipe • Principio di affidamento • Responsabilità d'équipe • Medicina legale*

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Introduction

The medical profession, especially a surgical one, often requires the multidisciplinary collaboration of many professionals, each with their own expertise. This type of activity raises some delicate matters concerning the criteria to determine individual responsibility within the multidisciplinary team. Can a physician, who has correctly performed his duties and followed the guidelines within his own area of expertise and specialisation, be required to answer for the harmful behaviour of another member of the team? Does he have the duty to supervise and verify the correct professional behaviour of the other team members?

Every professional is required to exercise a level of expertise as high as his degree of specialisation [1]. Complicity in any accidental crime can be present when a party knowingly partakes in said crime perpetrated by others. That also happens when the physician is aware of other professionals being tasked with a patient's treatment [2].

It should be clarified that each individual, within the broader medical or surgical team, works to safeguard the patient and not to supervise the work of other physicians or prevent their mistakes. Medical-legal literature [3] and court proceedings [4] concerning professionals' collaboration apply the principle of trust to the colleagues' work; this allows the individual professional of the medical or surgical team to dedicate himself with diligence, prudence and expertise to the specific duties of his own competence, free from the burden of monitoring the work of someone else. Therefore, he must trust that his colleagues correctly fulfil their part of the job and must be held responsible only for his own negligent conduct.

The logic behind this principle is clear: if a member of the team were required to watch over each step of his colleagues' activity, aiming at preventing or solving possible mistakes, he would not be able to concentrate on his work. On the other hand – and the authors do agree on it – it is not realistic that a single professional has all the competences and expertise necessary to perform a complex task, like surgical intervention, especially in a reality – such as the present – in which all disciplines have become increasingly specialised.

However, within the scope of medical activities, the Supreme Court has ascribed a somewhat definite value to the principle of reliance. First of all, such a principle of trust in someone else's conduct cannot be legitimately pointed to when the party who chooses to rely on said conduct is already at fault for having breached given precautionary principles or for having omitted certain conducts, and, despite all that he or she trusts others to nullify said breach or remedy a given omission [5]. Secondly, the team leader has the duty to supervise, monitor and coordinate the work of the other physicians. Therefore, he must carry out his tasks with diligence, but is also required to coordinate the activity of his collaborators and watch over their professional behaviour in all the phases of the operation, including the post-operative course [6]. In particular, the team leader's duties do not end at the moment he exits the operating room: in the subsequent phase, he must always ensure, even through a delegate, that correct assistance is granted to the patient and that the appropriate therapeutic treatment is provided [7]. On this basis, a logical deduction consists in overlapping the role of the team leader with that of the physician responsible for the entire ward: the latter is also responsible for the omissions of nurses; the fact that nursing staff has not informed him about the condition of a patient does not reduce his responsibility, being his duty to inquire about the patient's conditions [8].

Monitoring is essential even when there are indications suggesting that the behaviour of one of the team physicians is incorrect. In this case, the team leader must perform further diagnostic evaluations. Moreover, the team leader has to inform the members of his team about the patient's possible health problems that, if not disclosed, may influence clinical choices of the other physicians. For this reason, judges have condemned a surgeon who had not informed the anaesthesiologist about the patient's cardiac pathology for manslaughter [9]. The team leader also has the responsibility to control and monitor preoperatively. This is why a head physician has been condemned for the mistake of one of his assistants, who had failed in reaching diagnosis. Unfortunately, as an ultrasound investigation had not been performed preoperatively, the surgeon removed a healthy organ instead of the sick one [10]. Therefore, a surgeon should not completely entrust a diagnosis made by a colleague: the surgeon who performs a surgery following an indication of a colleague, even if part of the team, is considered imprudent and responsible for the loss of a healthy organ [11].

The third limit to the principle of trust consists in the following rule: each and every health professional must know and verify the correctness of the activity performed previously or contextually on the same patient by another colleague, even if specialised in a different discipline. If the latter commits mistakes that may have been prevented even by a physician non-specialised in the same field, all the colleagues of the team are held responsible [12]. Therefore, according to the Italian Supreme Court, when a member of the team recognises that the behaviour of a colleague may jeopardise the patient's health, he has the duty to inform both the team leader and that negligent colleague.

If some members of the team are specialised in the same field, it is easier to watch over other colleagues' actions and, therefore, the chances are greater to timely correct mistakes. Conversely, when specialisations are different, it is likely that the judge does not find any responsibility, considering the mistake as not noticeable of specialised competence. In case of a multidisciplinary team, the team leader must coordinate the work of the other physicians in the various phases of the intervention. In that case, he does not have many opportunities to recognise the mistakes of other people, as he does not have the skills necessary to argue about the decisions of the other team members, especially if with different expertise. Anyhow, if a common mistake appears preventable by non-specialised physician, the same principle of responsibility applies to both the team leader and the other members of the team.

Moreover, the principle of trust may not be applied to the physician even when the team leader gives him directives that are not correct and appropriate. The Italian Supreme

Court has recognised a physician responsible for manslaughter who did not manifest his dissent to the team leader's decision, proposing an alternative solution. The collaborator is not a mere executor of orders, but must evaluate critically the work of other physicians, including the team leader [13]. The judges stated that when a member of the team does not agree with his head physician's opinions, his/her dissent should be written down [14].

Case report (The case judged by the Criminal Appeal Court, Section IV, 28 July 2015, n. 33329) [15]

A 16-year-old girl was hospitalised in the hospital of Vibo Valentia for a peritonsillar abscess with oedema. After admission, the otorhinolaryngologist surgeons administered antibiotic (cephalosporin) and corticosteroid therapy; the disease evolution did not show any evidence of exceptionality [16-19]. In the following days, the patient's condition worsened and required the drainage of the peritonsillar abscess; this is a possible evolution of a treatment-resistant condition [20]. The Italian Association of Otolaryngology developed national guidelines on tonsillectomy in 2008, revised in 2011, that also included indications for medical and surgical therapy in case of peritonsillar abscesses. Guidelines recommend treating peritonsillar abscess in children and adults with systemic antibiotics, abscess incision and drainage according to the patient's clinical conditions. In case of complications, guidelines recommend a careful clinical observation with hospitalisation to monitor the airways. The decision to perform a tonsillectomy can be postponed after resolution of the acute phase [21]. The patient was treated according to the above-mentioned guidelines. Following drainage, she developed respiratory difficulties that required tracheotomy. In the operating room, the anaesthesiologist tried twice to induce general anaesthesia with the administration of and relative intubation [22]. However, both the curare muscle relaxant effect and the abscess caused the complete obstruction of the respiratory tract and did not allow endotracheal intubation. Such condition resulted in asphyxia and oedema, worsened by the intubation attempts. The anaesthesiologist tried an emergency tracheostomy, but the scalpel also incised the oesophagus and some vessels. The patient died of cardio-circulatory arrest due to asphyxia induced by the curare treatment utilised during general anaesthesia. Therefore, the girl's death was due to an anaesthesia error. The judges condemned the anaesthesiologist but also the otolaryngologist, even if establishing that: a) ENT therapy was appropriate; b) instrumental exams with laryngoscope and fiberscope had been carried out; c) the decision to perform a tracheostomy was correct. Hence, the judges did not find any element of negligence or imprudence. Even if the problem regarded the anaesthesiology field, the otolaryngologist surgeon should have evaluated the consequences of the anaesthesia with curare which would have paralysed the vocal cords, and then the tissues violently struck by the intubation attempts. The experts agreed that if the patient had not been treated with curare and not subject to wrongly performed intubation attempts, she would have continued to breathe autonomously, maintained the normal oxygen saturation and survived. Actually, the otolaryngologist did oppose verbally to anaesthesia with curare and suggested the anaesthesiologist to perform an optical fibre bronchoscope guided endotracheal intubation. Such a technique would have facilitated intubation, in accordance with the anaesthesiology guidelines [23]. The anaesthesiologist refused because the optical fiber bronchoscope tube was too short. According to the judges, the otolaryngologist, being himself the team leader, should have had impeded the anaesthesiology procedure that caused the fatal event. For these reasons, the judges condemned him since, as a physician, he had the necessary skills to evaluate the risks related to the anaesthesia with curare and, as a team leader, he should have suspended the surgical procedure, which was urgent but not an impelling emergency. According to the judges, as the anaesthesiologist has specialist expertise, in case he makes a mistake, he has to respond personally for his own choices. Anyhow, if the anaesthesiologist mistakes the operative manoeuvre, the team leader, on the basis of his own expertise, must intervene on the anaesthesiologist and propose solutions which he/she considers most appropriate. What if the anaesthesiologist refuses to comply? Again, according to the judges the team leader might as well stop the surgical operation and ask the anaesthetist to leave the operating room.

Discussion

With this judgment, the Supreme Court reaffirms that each member of the team, including the team leader, must recognise and prevent mistakes within his area of competence, even if committed by other members of the team. The judges state such principle in order to safeguard the patients' health; this means that each professional must control and monitor the behaviour of his colleagues. However, in this way the principle of trust is valid only in theory as, in practice, it is not applied. In fact, said principle entails that each and every team member must be able to focus on those tasks in which his or her competencies do apply, without having to oversee his colleagues' work,

and must only answer for the mistakes of his or her making. On the contrary, the above-mentioned Supreme Court rulings entail every professional's duty to watch over his colleagues' behaviour. This complicates the surgical operation and is risky for the patient as, having to monitor the activity of others, the professional might not be adequately focused on his own. To avoid that the physician is sentenced without a real fault, the Supreme Court states that the professional may be condemned for somebody else error only if such error is clearly noticeable and is not of "specialistic" nature. Anyhow, in the otorhinolaryngologist branch, this rule might raise some problems, as both the anaesthesiologist and the otolaryngologist act in the same anatomical district. Yet, this does not necessarily imply that the otolaryngologist is able to recognise the anaesthesiologist's error. Furthermore, when the anaesthesiologist's behavior is inconsistent with the otolaryngologist's knowledge, it should be considered that they have two different specialisations. The diversity of skills should limit the specific responsibility of each individual. It would be, however, advisable to ascribe criminal liability only to those cases where gross negligence is involved [24].

In conclusion, the surgeon should respond only to surgical errors. Similarly, the anaesthesiologist should only be responsible for anaesthesia-related risks. Consequently, it seems excessive to claim that the otolaryngologist team leader has to prevent the anaesthesiologist from performing the anaesthesia and that the team leader is responsible for the errors made by a physician with different expertise.

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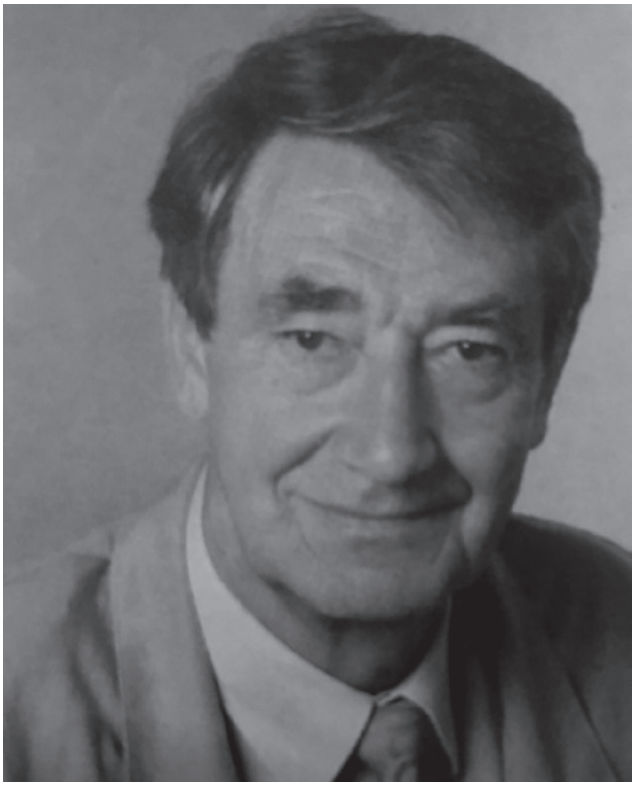
In Memoriam of Mirko Tos



Perhaps it will have been for our common Balkan origin, perhaps for a nice affection that has always approached us, perhaps for the common scientific speculative interests, but our relations have been in the last 30 years really close.

Professor Mirko Tos, light and leader in the Otorhinolaryngological field , born in1931 in Vitomarci (Slovenia) at that time Jugoslavija, left us at the end of last January.

As a young doctor he left Slovenia with his wife Nives for Germany and worked on the Verband station in Wuppertal; later they moved to Veile in Denmark where he found a position as junior physician for two years.

In order to go on as doctor in Denmark, he graduated once again on the University of Copenhagen and was appointed as ENT in the Glostrup County Hospital.

In 1972 was appointed as co-chairman n the ENT department of Gentofte Hospital and in 1980 became Chairman.

For many years his clinic was the destination of hundreds of ENTs, from all over the world, who have always received a warm and productive welcome.

Mirko was truly a great teacher, he deeply loved the discussion with the young specialists who, in every scientific occasion, sought him to have advice and areas of new research.

A clever surgeon, he was also a scrupulous researcher, he organised many clinical researcher groups on Otology and Rhinology, and published hundreds of works on tubo-tympanum, acoustic neuroma, cholesteatoma Secretory Otitis Media and on nasal polyps going deep into the pathophysiology of the middle ear and nasal mucosa with important histological observations.

He was the organiser and chairman of a number of international instructional courses on middle ear and acoustic neuroma surgery, as well as courses on functional endoscopic sinus surgery.

Several times we discussed together with Jacob Sadè, David Lim and Charlie Bluestone the role of the Eustachian tube in otitis and I was honoured and happy to participate and listen to their ideas, sometimes conflicting, but always lively and productive.

In 2001, at the age of 70, he retired, but the next year he was appointed as the first professor of Oto-rhino-laryngology at the new University in Maribor; while maintaining this position until 2011, but still living in Denmark, during spring and fall he enjoyed days in Slovenia, on beautiful lake Bled in the Julian Alps.

Active and a sportsman, during holidays Mirko enjoyed tennis, skiing and windsurfing, especially in the Adriatic sea never without his wife Nives and loved daughters Miriam, Vivian and Tina.

Certainly, we will miss Mirko's smile and leadership, but his memory can never disappear from those who knew him, appreciating his skills of excellent clinician and enthusiastic researcher.

Desiderio Passali