ACTA Otorhinolaryngologica Italica

Official Journal of the Italian Society of Otorhinolaryngology Head and Neck Surgery

Organo Ufficiale della Società Italiana di Otorinolaringologia e Chirurgia Cervico-Facciale

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Review

Nitinol *versus* non-Nitinol prostheses in otosclerosis surgery: a meta-analysis

Protesi Nitinol vs non-Nitinol nella chirurgia dell'otosclerosi: meta-analisi

L. ROQUE REIS, M. DONATO, G. ALMEIDA, L. CASTELHANO, P. ESCADA

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SUMMARY

The aim of this study is to perform a systematic review and meta-analysis of observational studies in which hearing outcomes after primary stapes surgery have been reported. After the surgical procedure, the effectiveness of stapes surgery using nickel titanium (Nitinol) or other prostheses were systematically compared and evaluated using a meta-analytic method. A systematic search for articles before January 2017 in Embase, Medline and Cochrane Library databases was conducted. Only articles in English were included. Inclusion criteria for qualitative synthesis consisted of a population of otosclerosis patients, intervention with primary stapes surgery using the Nitinol heat-crimping prosthesis compared with other type of stapes stapedotomy prostheses, and hearing outcome. Inclusion criteria for quantitative analysis consisted of application of audiometry guidelines of the American Academy of Otolaryngology Head and Neck Surgery Committee on Hearing and Equilibrium for evaluation of conductive hearing loss. A postoperative air-bone gap (ABG) ≤ 10 dB was considered effective. A bias assessment tool was developed according to Cochrane guidelines. To evaluate the mean age of the samples we used the chi-square test. Of the 4926 papers identified through the electronic database search (3695 in Pubmed/Cochrane and 1231 in Embase), 540 studies matched the selection criteria (436 in Pubmed/Cochrane and 104 in Embase) after application of filters and elimination of duplicate articles. After analysis of the title and abstract, 459 were excluded (396 in Pubmed/Cochrane and 63 in Embase). Of the remaining 81 papers, 74 were excluded according to the study selection criteria. A total of seven eligible studies with 1385 subjects, consisting of 637 in the Nitinol group and 748 in the non-Nitinol group, were included in our study. There were statistically significant differences in the effectiveness of stapes surgery between the Nitinol and non-Nitinol prostheses; the data showed a combined odds ratio (OR) of 2.56 (95% CI 1.38-4.76, p = 0.003). There were no statistically significant differences in the mean pre-operative age between Nitinol and non-Nitinol prostheses (p = 0.931). Our results suggest that the effectiveness of Nitinol was higher than non-Nitinol prostheses, with superiority of the number of patients with ABG ≤ 10 dB.

KEY WORDS: Stapedotomy • Stapedectomy • Stapes surgery • Otosclerosis • Prostheses

RIASSUNTO

Scopo di questo studio è stato quello di fare una revisione sistematica ed una meta-analisi di studi osservazionali in cui venivano riportati risultati audiologici dopo chirurgia stapediale. Dopo la procedura chirurgica, sono stati sistematicamente analizzati con metodo meta analitico i risultati di efficacia della chirurgia stapediale usando protesi Nitinol o altre protesi. È stata fatta una ricerca sistematica dei lavori sui database Embase, Medline e Cochrane Library prima del Gennaio 2017. Sono stati considerati solo articoli in lingua inglese. Il criterio di inclusione per una sintesi qualitativa era una popolazione di pazienti otosclerotici, sottoposti a chirurgia stapediale primitiva usando la protesi Nitinol, confrontati con altri tipi di protesi, paragonandone gli outcome funzionali. I criteri di inclusione per un'analisi quantitativa consistevano nell' applicazione delle linee guida dell'American Academy of Otolaryngology Head and Neck Surgery Committee on Hearing and Equilibrium per la valutazione della perdita trasmissiva. Il gap post-operatorio aria-osso ≤ 10 Db è stato considerato efficace. Uno strumento di evidenza dei bias è stato sviluppato in accordo con le linee guida Cochrane. Per valutare l'età media del campione abbiamo usato il test chi-quadro. Dei 4926 lavori identificati attraverso la ricerca elettronica (3695 in Pubmed/Cochrane e 1231 in Embase), 540 lavori rispondevano ai criteri di selezione (436 in Pubmed/Cochrane and 104 in Embase) dopo l'applicazione dei filtri e l'eliminazione di articoli doppi. Dopo l'analisi di titolo ed abstract, 459 sono stati esclusi (396 in Pubmed/Cochrane e 63 in Embase). Dei rimanenti 81, 74 sono stati esclusi in base ai criteri di selezione dello studio. Un totale quindi di sette studi con 1385 pazienti, di cui 637 nel gruppo Nitinol e 748 nel gruppo non Nitinol, sono stati inclusi nel nostro lavoro. Vi erano differenze statisticamente significative sull'efficacia della chirurgia stapediale fra le protesi Nitinol e non Nitinol; i dati hanno dimostrato un odds ratio (OR) di 2,56 (95% IC 1,38-4,6, p = 0,003). Non vi sono state differenze statisticamente significative nell'età media preoperatoria fra le protesi Nitinol e non Nitinol (p = 0,931). I nostri risultati suggeriscono che l' efficacia delle protesi Nitinol è maggiore di quelle non Nitinol.

PAROLE CHIAVE: *Stapedotomia • Stapedectomia • Chirurgia stapediale • Otosclerosi • Protesi*

Introduction

Rehabilitation of conductive hearing loss is one of the major challenges in ear surgery. Since the 1950s, when stapes surgery for otosclerosis was introduced, there have been many changes in prosthetic design and materials. The success of stapes surgery may depend on the characteristics of the prosthetic material.

An ideal stapes piston should have good biocompatibility and adequate sound transmission. A variety of materials have been used as a piston between the incus and the stapes such as Teflon (fluoroplastic), titanium, stainless steel, platinum, and Nitinol. The new Nitinol piston was first used in stapes surgery in 2004 [1]. It is reasonable to summarise all other traditional prostheses as the non-Nitinol group.

The unique characteristic of the Nitinol piston is the auto-crimping process of the loop placed over the long process of the incus, allowing firm attachment of the piston in contrast with manual-crimping of the non-Nitinol group [2-5]. This technique may produce better functional results and reduce the risk of damage to the middle and inner ears during the crimping process [5-8].

The present study, using a meta-analytical method, is designed to examine whether the new Nitinol prosthesis for otosclerosis surgery is superior to other previously mentioned non-Nitinol prostheses in terms of rehabilitation of conductive hearing loss and stability.

Materials and methods

Search strategy

A systematic literature review was carried out using the Medline, Embase, and Cochrane electronic databases. Using a combination of keywords including stapedotomy, stapedectomy and stapes surgery, a literature review was performed for studies in which the outcomes of stapedectomy and stapedotomy prostheses published from January 1970 to December 2016 were compared. All relevant papers or abstracts that were published in English were selected for the current investigation. The filters are shown in Table I.

Articles and data were independently extracted and evaluated for quantitative analysis by two coauthors from the included trials. If there was disagreement, a third reviewer was included and the issue was resolved by discussion.

Study selection criteria

Studies that included randomised control trials, retrospective and/or prospective ones were acceptable, and there was no limitation in age, sex, or follow-up periods. A postoperative air-bone gap (ABG) ≤ 10 dB was considered effective. Depending on the available data, the postoperative gap of the ear that underwent surgery was calculated using pure tone audiometry (Table II) according to the guidelines of the Committee on Hearing and Equilibrium from the American Academy of Otolaryngology-Head and Neck Surgery criteria (0.5, 1, 2, 3, and 4 kHz) [9]. Data on pre- and post-operative pure-tone average and ABG were compiled, and the mean thresholds were determined at 0.5, 1, 2 and 3 kHz. When the threshold at 3 kHz was not available, the average of the thresholds at 2 kHz and 4 kHz was estimated according to the new and revised reporting guidelines from the Committee on Hearing and Equilibrium. Studies that followed other quantitative standards were excluded from the current study in addition to duplicate studies (determined by examining the author lists, patient institutions, sample sizes and results). Investigations that included revision surgery, residency training, animal trials, and those that were classified as comments, editorials, or reviews were also excluded.

Only the articles comparing postoperative effectiveness between Nitinol and non-Nitinol prostheses in primary otosclerosis surgery were used in our analysis. Moreover, we used the maximum follow-up date in cases of different follow-up times within the same article. To assess the potential influence by different surgery types, prostheses materials, follow-up periods and surgery procedures, we compared the effectiveness of Nitinol and non-Nitinol prostheses in several subgroups: (1) Nitinol or non-Nitinol material; (2) short-term (≤ 3 months) follow-up period; (3) middle-term follow-up period (3

Table I. Filters activated.

Filters	Characteristics
Articles type	Books and Documents, Classical Article, Clinical Conference, Clinical Study, Clinical Trial, Comparative Study, Congresses, Consensus Development Conference, Controlled Clinical Trial, Evaluation Studies, Lectures, Meta-Analysis, Multicentre Study, Pragmatic Clinical Trial, Randomised Controlled Trial, Review, Scientific Integrity Review, Systematic Reviews
Text availability	Abstract
Publication dates	From 1970/01/01 to 2016/12/31
Languages	English

Table II. The average of the ABG was calculated on different respective frequency, from 500 to 4,000 Hz. We used the mean threshold of four frequencies at least within this range; and assumed that a better value is used if the authors did not supply their calculation.

Frequencies (kHz)	ABG average
0.5, 1, 2, 3	4ABG
0.5, 1, 2, 4	4ABG
0.5, 1, 2, (2 + 4):2	5ABG

months to 3 years); or (4) long-term follow-up period (≥ 3 years).

The quantitative data covered the number of subjects and of those who had reached a postoperative ABG of ≤ 10 dB, mean of pre- and post-operative ABGs, mean of follow-up times and number of excluded prostheses.

Statistical analysis

We performed a DerSimonian and Laird [10] random-effects meta-analysis to pool effect sizes estimates across studies. The results were express in odds ratio (OR), with a 95% confidence interval (CI) calculated by Review Manager (RevMan), V.5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) software. Evidence of heterogeneity was tested using p value (with a $p \leq 0.05$ indicating statistically significant heterogeneity) and I^2 statistic [11] (with an $I^2 \leq 25\%$ indicating slight heterogeneity, an I^2 between 25% and 50% indicating moderate heterogeneity, and an $I^2 \geq 50\%$ indicating high heterogeneity) [12]. We used a random-effects model when $I^2 \geq 50$ % and/or $p \leq 0.05$ because moderate heterogeneity was chosen. In the forest plot, the proportions are depicted with 95% CIs according to Clopper and Pearson with the surface of the squares (point estimates) being proportional to the case number of the study. Funnel plots were tested for asymmetry. Egger's test was performed to evaluate potential asymmetry and publication bias.

Results

Literature search and characteristics

Of the 4926 papers identified through the electronic database search (3695 in Pubmed/Cochrane and 1231 in Embase), 540 investigations matched the selection criteria search (436 in Pubmed/Cochrane and 104 in Embase) after application of filters and elimination of duplicate articles (Fig. 1). However, 459 were excluded after analysis of the title and abstract (396 in Pubmed/Cochrane and 63 in Embase). Of the remaining 81 papers, 74 were excluded according to the study selection criteria. There was unanimity between the researchers regarding the selection of the relevant papers.

The seven trials covered 1385 subjects; 637 were subclassified as the Nitinol group and 748 as the non-Nitinol grou [8 13-19]. Of the seven investigations, two were found to be prospective reports, four were retrospective reports, and one was both a retrospective and prospective report. The post-operative hearing results were measured based on different frequencies. One study used 0.5, 1, 2 and 4 kHz, four studies used 0.5, 1, 2 and 3 kHz, and two studies used 0.5, 1 and 2, and the average of the thresholds at 2 kHz and 4 kHz. The characteristics of all the included trials are summarised in Table III.

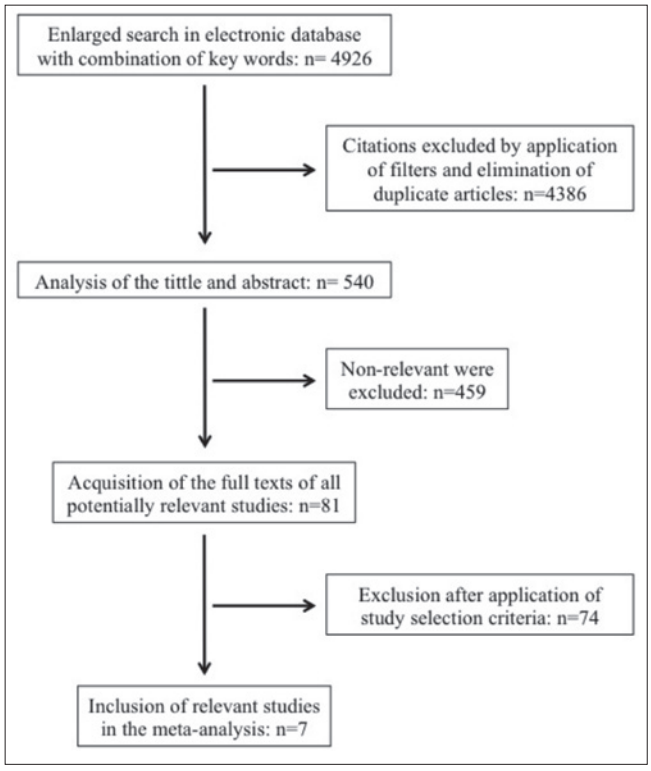

Fig. 1. Flow diagram showing the methodology of the study.

Meta-analysis

The comparisons of prosthetic effectiveness and effectiveness with mean follow-up time between the Nitinol and non-Nitinol groups were performed using a meta-analysis (Fig. 2). For the analysis, subgroups were formed according to the follow-up periods (Figs. 3, 4).

In terms of the effectiveness of the prostheses, the test of heterogeneity was high ($I^2 = 60\%$, $p = 0.02$), thus leading to a random-effects model. It was found that there were significant differences in the post-operative effectiveness of the prostheses between the Nitinol and non-Nitinol groups with a combined OR of 2.56 (95% CI 1.38-4.76, $p = 0.003$). Different results were found when the follow-up period was considered. Of the two studies with short-term results

Table III. Characteristics of the trials included in the meta-analysis.

Reference	Year	Study type	N	Mean age (years)	Prosthesis type	Pre-op. ABG (dB)	Post-op. ABG (dB)	ABG improve-ment	ABG ≤ 10 dB (%)	Follow-up (months)	Complications (%)
Rajan et al. [13]	2007	prospective	90 270	45.3 42.5	Nitinol titanium	38.24 37.15	5.15 12,37	32.4 dB 31.1 dB	90 75	24	0.01
Huber AM et al. [8]	2008	prospective retrospective	75 75	44 46	Nitinol conventional	- -	8 11.6	- -	71 43	12.8 13.1	1
Fayad JN et al. [14]	2009	retrospective	306 110	47.9 48.3	Smart (Nitinol) non-smart (other)	26.1 25.7	7.6 6.0	18.7 dB 19.9 dB	9.6 11.3	5.6 6.9	7.15 10.1
Kuo CL et al. [15]	2010	retrospective	16 21	42.8 45.5	Nitinol manual-crimping	26.79 26.19	7.92 13.09	14.53 dB 9.04 dB	75.0 33.3	2.98 3.27	-
Cho JJ et al. [16]	2011	retrospective	80 21	46 45	Nitinol titanium (Fisch-type)	25.1 28.1	8.2 9.0	-	92.5 95.2	12	-
Brar T et al. [17]	2012	prospective	20 20	(range 18-45)	Nitinol teflon	36.1 34,3	7.6 8.1	78.95% 76.3%	100 90	6	12.5
Canu G et al. [19]	2016	retrospective	50 50 131 50	45 43 45 46	Nitinol teflon first titanium last titanium	22 22 25 21	6 10 10 5	16 dB 12 dB 15 dB 16 dB	84 36 44 92	1 3 3 1	-

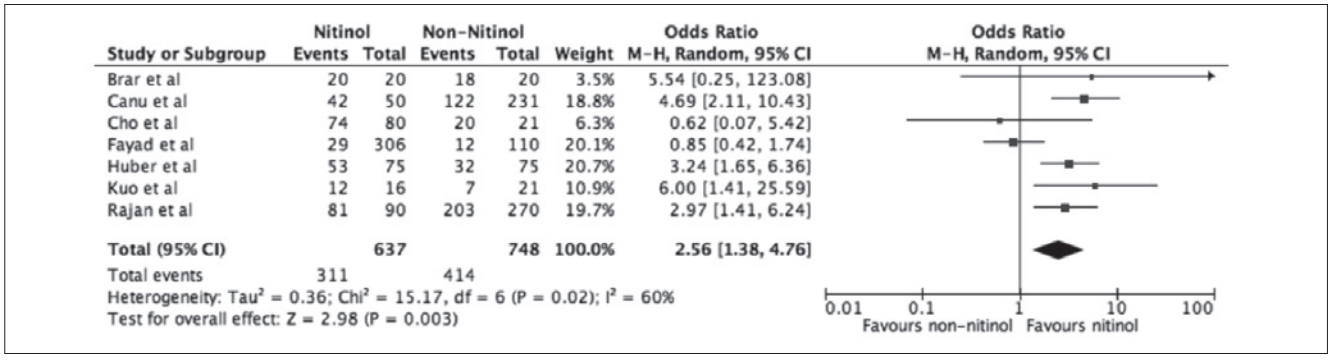

Fig. 2. Forest plot of the odds ratio (OR) for the number of patients achieving a postoperative ABG ≤ 10 in the Nitinol group vs non-Nitinol group.

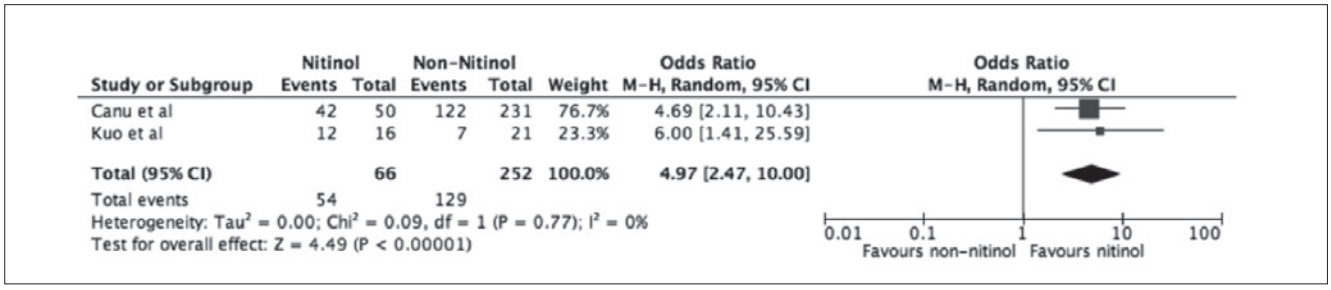

Fig. 3. Forest plot of the risk ratio (RR) for the number of patients achieving a short-term postoperative ABG ≤ 10 in the Nitinol group vs non-Nitinol group.

(≤ 3 months), the postoperative effectiveness between the Nitinol and non-Nitinol groups had a combined OR of 4.97 (95% CI: 2.47-10.00, p = 0.00001), with a slight heterogeneity (I^2 = 0%, p = 0.77). In the five studies with middle-term results (3 months to 3 years), the postoperative effectiveness between the Nitinol and non-Nitinol groups had a combined OR of 1.91 (95% CI: 0.91-3.99, p = 0.08) with high heterogeneity (I^2 = 60%, p = 0.04). There were no studies with long-term results (≥ 3 years). There were no statistically significant differences in the mean age between the Nitinol and non-Nitinol groups with the adjusted chi-square test [χ^2 (11) = 5,000; p = 0.931].

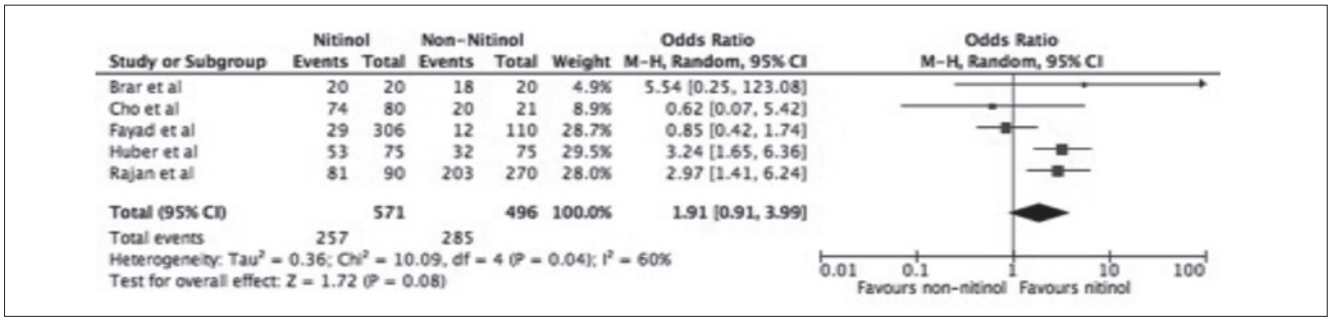

Fig. 4. Forest plot of the risk ratio (RR) for the number of patients achieving a middle-term postoperative ABG ≤ 10 in the Nitinol group vs Non-nitinol group.

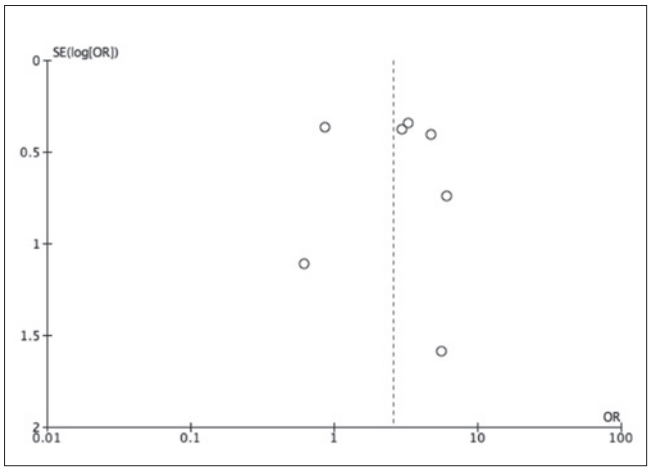

Fig. 5. Funnel plot for the included trials.

Sensitivity analysis and publication bias

The visual assessment of the funnel plot revealed no evidence of obvious publication bias (Fig. 5), nor did the formal evaluation using Egger test (intercept was 0.25, 95% CI -3.82 to 4.33, p = 0.879).

Discussion

Teflon was the first material to be used in a stapedectomy by John Shea in 1956 [20]. Since then there have been many changes in the design and materials used for stapes surgery. Nowadays, Teflon and Titanium (introduced by Kurz Medical Inc. in 1996) are probably the two most common prosthetic materials in use. We chose to study the Nitinol prosthesis because of the ongoing debate about its usefulness and superiority.

Nitinol is an alloy composed of titanium and nickel that has the properties of a shape-memory metal. By heating this metal above a certain temperature, its shape transforms into a predefined form [21]. Thereby, the loop closes on its own, and manual crimping is avoided. It has been proposed that this technique may produce better functional results because of improved sound transmission between the incus and the prosthesis with less variability and a diminished risk for the middle and inner ears during the crimping process [13].

Van Rompaey et al. estimated that a sample size of at least 413 patients is needed in both the intervention and the control groups, in order to detect the smallest difference that is clinically important [22]. In our study, we used data from 1385 patients. To collect the meta-analysis data, we analysed the literature that compared the differences between Nitinol and non-Nitinol prostheses. The value of an ossicular prosthesis depends mainly on rehabilitation of conductive hearing loss and rate of prosthesis exclusion. Thus, we compared the number of patients achieving post-operative ABGs of ≤ 10 dB in the Nitinol and non-Nitinol groups. We found that there were significant differences between the two groups in conductive hearing loss rehabilitation.

When we divided our data into short-and middle-term follow-up periods, we found significant differences in short-term period between these two groups, but we did not find the same significant differences in middle-term follow-up periods. We cannot make a clear judgment about the influence of the follow-up periods and hearing results.

The major concern in using Nitinol prosthesis is the long-term stability of the incus. The pathogenesis of incus erosion and necrosis appears to be controversial and may be affected by having the wire tightly wrapped around it and the heat applied to the prosthesis for the crimping [23,24]. All of the studies in our review mentioned the use of the surgical technique without exclusion. However, other studies refer lateral displacement of the prosthesis out of the vestibule and/or incus, between 8.7 to 11% [25,26]. Long-term data are presently lacking, both concerning hearing outcomes and risks of necrosis to the long process of the incus.

No major complications were reported. Three of the stud-

ies did not even mention complications [15 16 19]. Some transient and minor complications (tinnitus, vomiting, and vertigo) were described [14 17]. Three cases of fixation of the malleus underwent revision surgery [13 27]. No evidence of incus erosion due to the prosthesis was reported.

As to assessment of prostheses efficacy for sound transmission, the heterogeneity test of I^2 demonstrated that there was significant heterogeneity among the enrolled studies, which probably could be ascribed to the different types of studies, follow-up periods, measurement frequencies, pre-operative hearing conditions, participating surgeons, prostheses and surgical techniques. From the additional sensitivity analysis, we found no evidence of obvious publication bias.

Meta-analysis is the pooling of data from several different investigations and objectively re-analysing the resulting data set to provide a more reliable reference for a clinical decision. Limitations regarding the surgical technique, various types of prostheses used and different follow-up periods were found. In view of the limitations of the current study, future studies should be based on prospective cohort or randomised studies with standardised unbiased methods, larger sample sizes, and longer follow-up periods in order to pursue more reliable implications.

Conclusions

Our meta-analysis indicated that the Nitinol prosthesis showed significant superiority to the non-Nitinol prostheses in terms of effectiveness and stability. Even though the user-friendliness of non-Nitinol prostheses has been confirmed, the disadvantage of expense should also be considered. Therefore, we recommend that a Nitinol prosthesis be chosen for the patient with otosclerosis with consideration of the budget, surgical difficulty and surgeon's proficiency in handling different prostheses.

Acknowledgements

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Review

New frontiers and emerging applications of 3D printing in ENT surgery: a systematic review of the literature

Nuove frontiere e applicazioni emergenti della stampa 3D in ORL: revisione sistematica della letteratura

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SUMMARY

3D printing systems have revolutionised prototyping in the industrial field by lowering production time from days to hours and costs from thousands to just a few dollars. Today, 3D printers are no more confined to prototyping, but are increasingly employed in medical disciplines with fascinating results, even in many aspects of otorhinolaryngology. All publications on ENT surgery, sourced through updated electronic databases (PubMed, MEDLINE, EMBASE) and published up to March 2017, were examined according to PRISMA guidelines. Overall, 121 studies fulfilled specific inclusion criteria and were included in our systematic review. Studies were classified according to the specific field of application (otologic, rhinologic, head and neck) and area of interest (surgical and preclinical education, customised surgical planning, tissue engineering and implantable prosthesis). Technological aspects, clinical implications and limits of 3D printing processes are discussed focusing on current benefits and future perspectives.

KEY WORDS: 3D printing • Additive manufacturing • Rapid prototyping • Otorhinolaryngology • ENT • Systematic review

RIASSUNTO

Le tecnologie di stampa 3D hanno rivoluzionato la realizzazione di prototipi in ambito industriale, riducendo i tempi ed i costi di produzione rispettivamente da giorni ad ore, da migliaia a pochi dollari. Ad oggi, i sistemi di stampa 3D non sono solamente confinati alla creazione di prototipi, ma hanno trovato un crescente impiego in medicina con risultati affascinanti anche nel campo dell'Otorinolaringoiatria. Applicando le linee guida "PRISMA", abbiamo svolto una revisione sistematica della letteratura al fine di esaminare tutti gli articoli inerenti l'Otorinolaringoiatria, che sono stati riportati sui database elettronici (PubMed, MEDLINE, EMBASE) aggiornati fino a Marzo 2017. Complessivamente, 121 studi scientifici hanno soddisfatto specifici criteri di inclusione e sono stati sottoposti alla nostra revisione sistematica. Le pubblicazioni sono state classificate in relazione al campo di applicazione specifico (otologico, rinologico, testa-collo) e all'area di interesse (formazione chirurgica e preclinica, pianificazione prechirurgica personalizzata, ingegneria tissutale e protesi impiantabile). Gli aspetti tecnologici, le implicazioni cliniche ed i limiti delle tecnologie di stampa 3D sono stati ampiamente discussi in riferimento agli effettivi vantaggi attuali ed alle prospettive future.

PAROLE CHIAVE: *Stampa 3D • Prototipizzazione rapida • Produzione additiva • Otorinolaringoiatria • ORL • Revisione sistematica*

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Introduction

Around 1450, Gutenberg developed a printing system that became a stepping-stone in the timeline of communication technology, and considered as one of the most influential events in the sharing of scientific and medical knowledge. Since its first introduction in the early 1980s, 3D printing (3DP) technology has rapidly caught the interest of the industry, healthcare and media with an overall business of $700 million [1-4]. The nature of all 3D printers is the creation of a wide range of 3D objects obtained from digital data of easy management and available in open-access digital databas-

es, allowing a unique opportunity for information exchange (e.g. 3dprint.nih.gov). Almost anything can be produced by 3DP systems: fuel injectors for rockets, jewels and hearing aid shells [5,6]. One of the most fascinating aspects of this technology concerns the employment of imaging studies. Today, radiology plays a pivotal role in diagnostic and therapeutic decision making. However, scans are still displayed on flat screens, resulting in a 2D representation of reality. Surgeons' experience the difficult task of figuring out a three-dimensional image on a daily basis, by analysing CT or MRI-slices in separate two-dimensional axial, coronal and sagittal projections [7]. 3DP systems allow to restore the third dimension that is lacking during visualisation of radiological image data. Along with the production of anatomical models addressed to customised surgical planning, medical teaching and surgical training, research in 3DP has explored the pioneering world of biologic tissue engineering, patient-specific implantation and ultimately of personalised pharmacoprinting. The increasing impact of 3DP processes in the scientific literature has recently involved many aspects of otorhinolaryngology, often followed by great expectations regarding patient care. Up to now, what are the applications of 3DP technologies in ENT surgery? Does this tool provide any substantial benefits in the ENT field? And what about future perspectives? The present work aims to answer these questions by carrying out a systematic review of the literature on the topic, a task that, to the best of our knowledge, has not undertaken previously.

The technology of 3DP systems

3DP is a subset of additive manufacturing (AM) or rapid prototyping in which objects are achieved by gradually layering material, rather than by subtraction from the raw material as is in the case of conventional technologies [8]. The main advantages of AM are its flexibility, precision and relative quickness in creating customised physical structures of almost any complex shape in a myriad of materials. Historically, 3DP processes were employed by the manufacturing industry to rapidly produce a representation of a system or a part before final release or commercialisation [9]. The 3DP was first conceived by C. Hull in 1986 as an "apparatus for production of three-dimensional objects by stereolithography" [3]. During the same year, he also developed the "Standard Triangulation Language" (.STL) file format, which makes it possible to deconstruct the surface of a three-dimensional object in a series of triangles. The .STL file can be obtained from a 3D "Computer-Aided Design" (CAD) software, a medical scan data (e.g. CT scan, MRI), or from existing objects by using point or laser scanners. This virtual model is subsequently sliced into thin 2D layers, which are then sent to the 3D printer. 3DP methodologies differ from one another in the way that materials are deployed and cured [8]. Recently, the ASTM International Committee F42 classified 3DP technologies in 7 different working process categories [10] (Fig. 1).

I. *Vat photopolymerisation:* in this technique a container gets filled with photopolymeric resin. This resin is then hardened by an UV light source.
II. *Material jetting*: this process resembles inkjet paper printing, since the material is dropped through small diameter nozzles. In this case, the base material is a photopolymeric resin subsequently hardened by a UV lamp.
III. *Binder jetting:* this method employs a powder base material and a liquid binder. In the build chamber, the powder is spread in equal layers and binder is applied through jet nozzles that "glue" the powder particles together in the shape of a programmed 3D object.
IV. *Material extrusion:* the most widespread and popular 3DP technology on the market. These printers are fed a thermo-plastic filament that gets pushed through a heating chamber: the fused material is moulded and then solidified through cooling, allowing the deposition of successive layers.
V. *Powder bed fusion*: this technology uses a high-power laser source to fuse small particles of plastic, metal, ceramic or glass powders into a mass that has the desired three-dimensional shape. The laser selectively fuses the powdered material by scanning the cross-sections generated by the 3D modelling program on the surface of a powder bed.
VI. *Sheet lamination*: in this technique sheets of material are bound together through external force. These processes can be further categorised based on the mechanism employed to achieve bonding between layers: gluing or adhesive bonding, thermal bonding, clamping, or ultrasonic welding.
VII. *Direct energy deposition*: this process, mostly used in the high-tech metal industry, enables the creation of parts by melting material as it is being deposited. The 3DP is usually attached to a multi-axis robotic arm composed of a nozzle that deposits metal powder or wire on a surface and an energy source (laser, electron beam or plasma arc) that melts it, forming a solid object.

Materials and methods

All existing articles sourced through updated electronic databases (PubMed, MEDLINE, EMBASE) and published up to March 2017 were examined according to the "Preferred Reporting Items for Systematic Reviews and Meta-analyses" (PRISMA) guidelines [11]. The research was conducted using the following keywords: "3D printing OR three di-

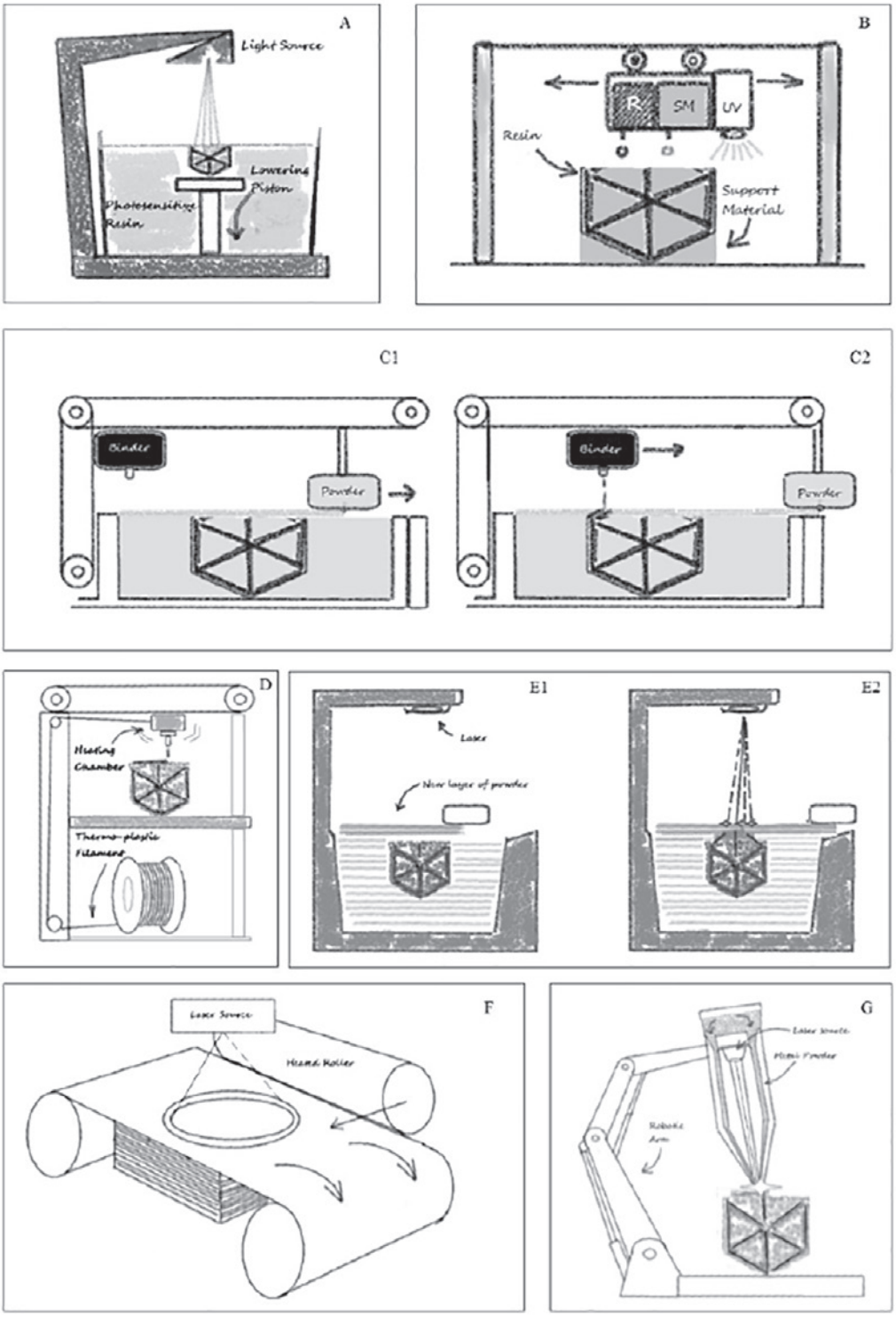

Fig. 1. Schematic representation of AM technologies: (A) vat photopolymerisation, (B) material jetting, (C1, C2) binder jetting (R: resin, SM: supporting material, UV: UV lamp), (D) material extrusion, (E1, E2) powder bed fusion, (F) sheet lamination, (G) direct energy deposition.

mensional printing AND otorhinolaryngology NOT plastic surgery", "3D printing OR three dimensional printing AND ENT NOT plastic surgery", "3D printing OR three dimensional printing AND otology NOT plastic surgery", "3D printing OR three dimensional printing AND rhinology NOT plastic surgery", "3D printing OR three dimensional printing AND head neck NOT plastic surgery", "3D Printing OR three-dimensional printing AND mandible NOT plastic surgery". Other sources analysed for additional relevant trials were reference lists of previous systematic reviews and evaluated works, journal homepages and publications citing included trials. Furthermore, experts in the field of 3D printing and engineering were contacted to ensure that all relevant studies had been included. Searches were done at all stages, from the initial drafting of the paper to submission of the revised and final version. Works lacking clinical or surgical relevance, such as engineering and bio-engineering publications and those regarding the evaluation of accuracy of the 3DP models were excluded since these are out of the expertise of ENT surgeons. Moreover, papers primarily addressing maxillofacial surgery, plastic surgery, thoracic surgery, neurosurgery and dentistry were also excluded. Exclusion criteria also applied to animal research and studies with ambiguous information regarding the modalities of production and employment of the 3DP methodology. Articles not written in English, review articles, letters, editorials and congress abstracts were omitted as well. All the considered studies were classified according to the specific field of application (otologic, rhinologic, head and neck). Each field was furthermore categorised into three distinct areas of interest: surgical and preclinical education, customised surgical planning and tissue engineering and implantable prostheses.

Results

The electronic database search yielded 258 citations and a further 123 articles were identified from additional sources, but after removing duplicates the total number of articles decreased to 278. A total of 157 records were removed as they did not fulfil inclusion criteria. Overall, 121 studies were included in the systematic review (Fig. 2). Figure 3 shows the studies according to the spe-

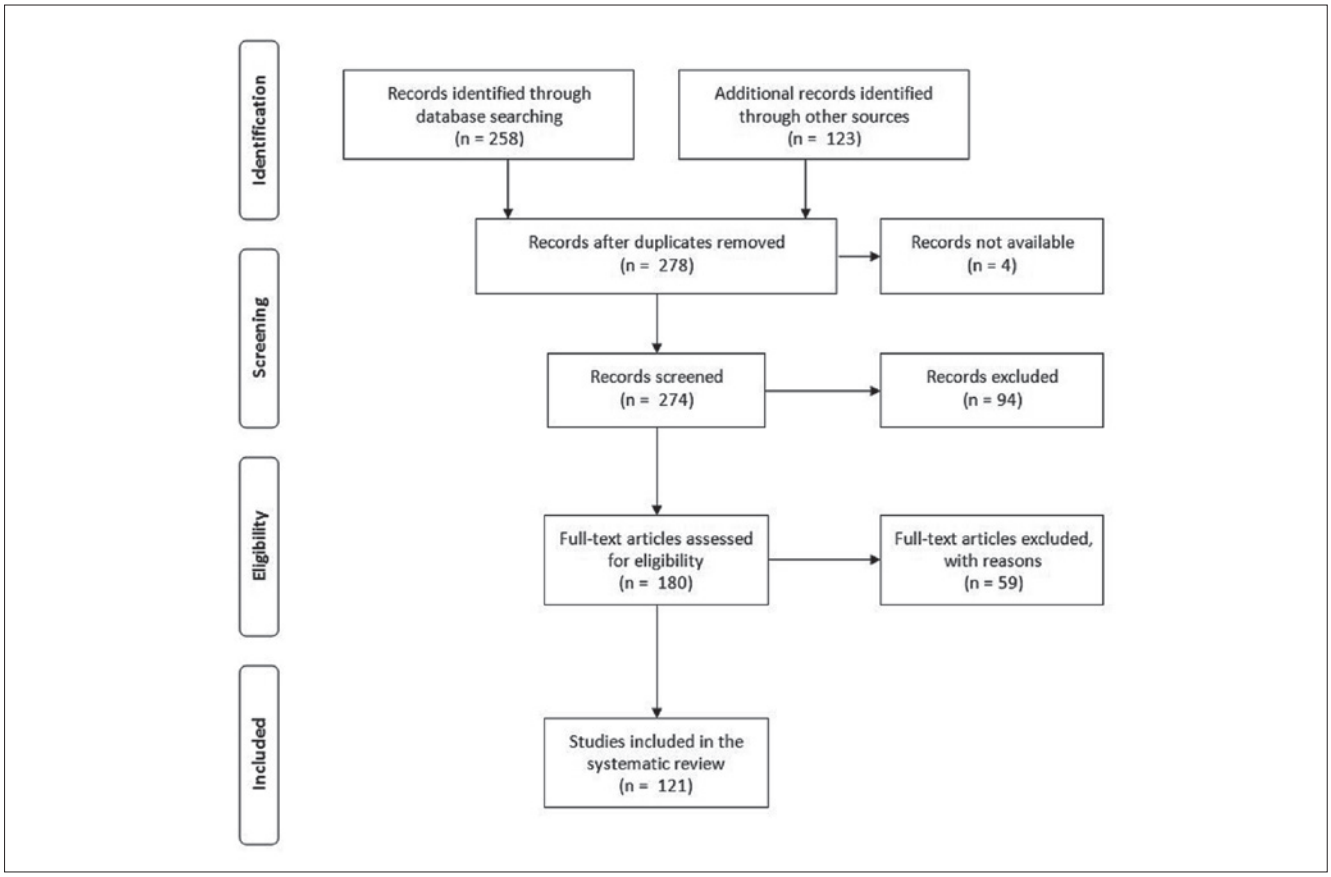

Fig. 2. PRISMA flowchart showing the study selection process.

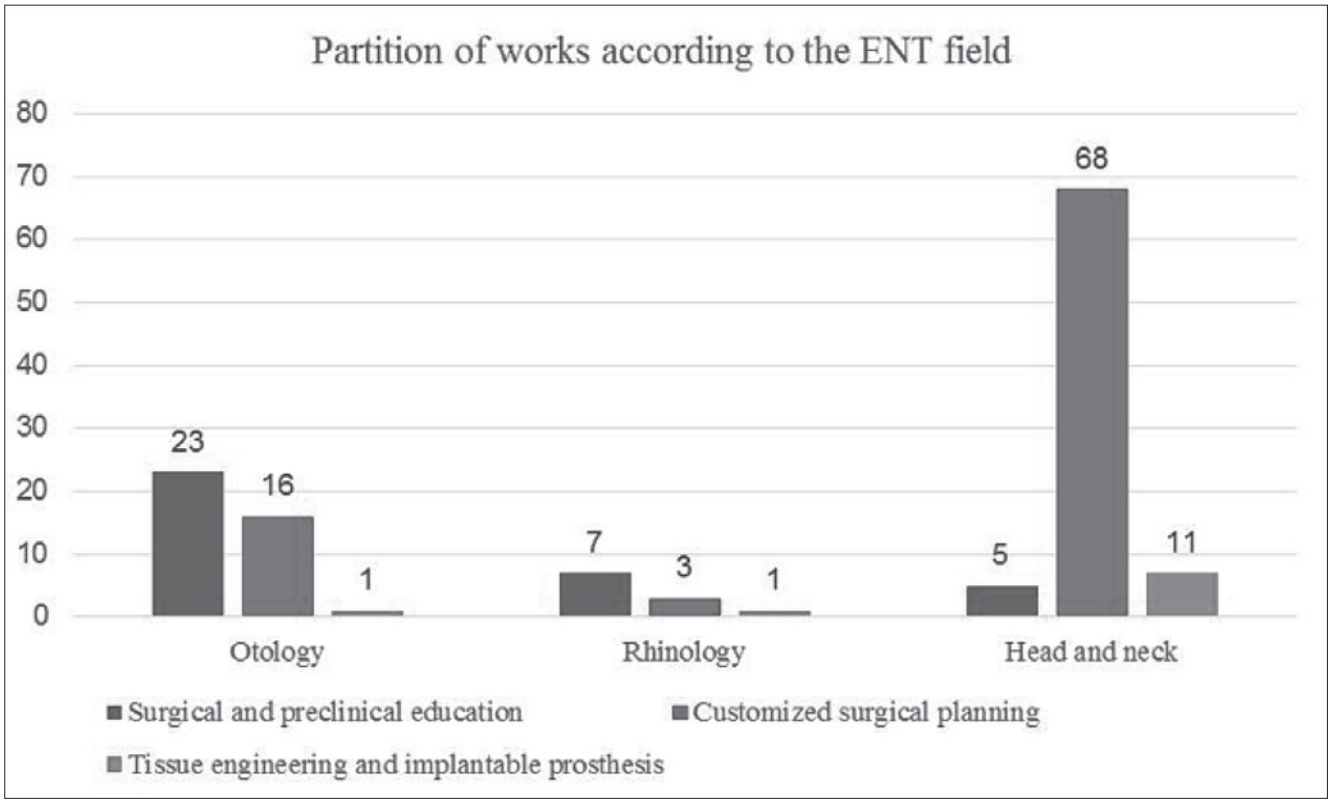

Fig. 3. Number of studies according to ENT field.

cific field of application (otologic, rhinologic, head and neck) and area of interest (surgical and preclinical education, customised surgical planning, tissue engineering and implantable prostheses). The total number of articles in Figure 3 is 135, and not 121, since 14 articles belong to more than one field of application and/or area of interest. Employed AM technology is summarised in Figure 4 considering the three areas of interest.

Otologic applications (Table I)

Surgical and preclinical education [12–34]

Twenty-three studies of the otologic ones (n = 39) involved the surgical and preclinical education area (59.0%) and mostly concerned the field of temporal bone dissection. Since the first report in 1998[31], technological efforts aimed to overcome the restrictions of the initial 3DP models. These first models, which employed a sole material and a single colour, allowed acceptable anatomical results, but limited haptic and drilling features. The evolution of 3DP systems (e.g. binder jetting) led to greater anatomical fidelity thanks to the employment of multiple colours and materials that are able to reproduce the mechanical properties of trabecular mastoid bone with realistic drilling experience. Moreover, the development of printed models coupled with electronic simulators provided a real-time alert in case of injury to vital structures during dissecting practice [28].

Customised surgical planning [29 35-49]

The production of patient-specific 3DP temporal bones based on preoperative CT was considered suitable for surgical planning and simulation in five cases of challenging anatomy (e.g. congenital aural atresia, acquired subverted anatomy) and in one case of cochlear implant surgery [29, 35-38]. Four papers dealt with the creation of 3DP operative templates to assist surgical positioning of a transcutaneous bone-conduction hearing device [39-42]. Finally, six studies were on the combined use of surgical navigation and 3DP technology [43-48]. In particular, a Japanese publication described the development of a registration method based on bone-anchored fiducial markers using 3DP templates without requiring a preoperative invasive marking process or additional CT. Since its first publication, this process has been simplified and further improved.

Tissue engineering and implantable prosthetics [50]

Kozin et al. tested a customised 3DP prosthesis for repair of bony superior canal defects on cadaveric temporal bones, even if clinical uses were not yet reported [50].

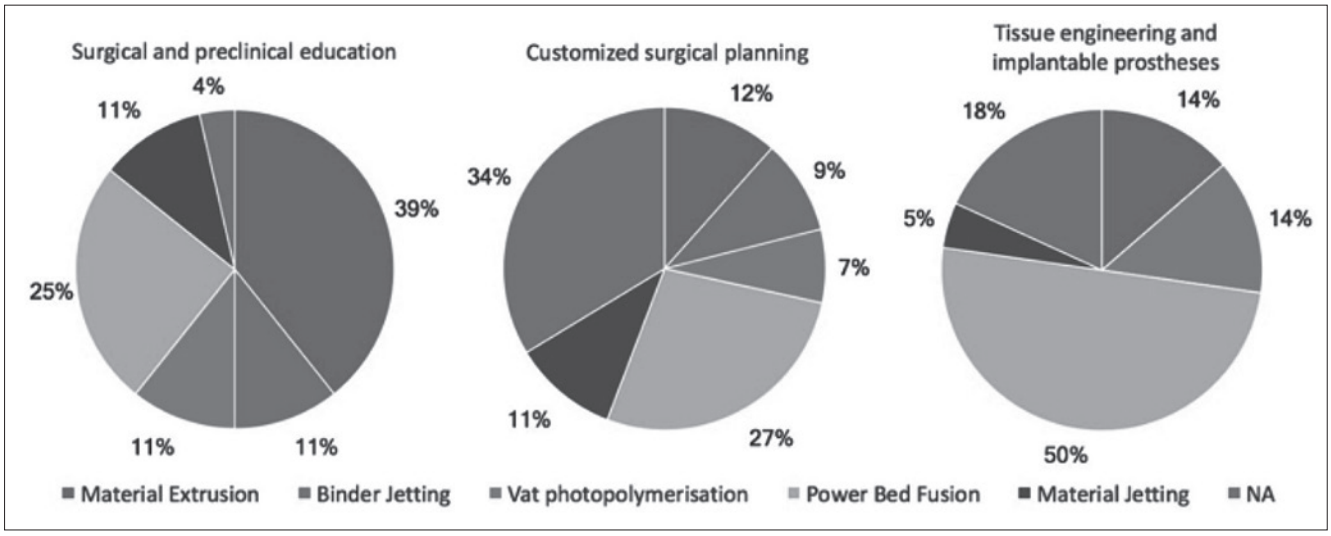

Fig. 4. Employed AM technology considering the area of interest.

Table I. Otologic studies classified according to each area of interest.

SURGICAL AND PRECLINICAL EDUCATION				
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**
Temporal bone dissection training model	Cohen J et al., 2015 [12]	Material extrusion	Dimensions SST 1200es	Abs + resin (support material)
	Da Cruz MJ et al., 2015 [13]	Binder jetting	Spectrum Z510	Chalk-like powder + binder + colors
	Hochman JB et al., 2015 (1) [14]	Binder jetting	ZPrinter 650	Chalk-like powder + binder + colors
	Hochman JB et al., 2015 (2) [15]	Binder jetting	ZPrinter 650	Chalk-like powder + binder + colors
	Longfield EA et al., 2015 [16]	Binder jetting	Spectrum Z510	Chalk-like powder + binder + colors
	Mowry SE et al., 2015 [17]	Material extrusion	MakerBot 2x	ABS + HIPS
	Rose AS et al., 2015 [18]	Vat photopolymerisation	Objet Connex 350	Photo-polymer resins with different mechanical properties
	Hochman JB et al., 2014 [19]	Binder jetting	ZPrinter 650	Chalk-like powder + binder + colors
	Unger BJ et al., 2014 [20]	Binder jetting	ZPrinter 650	Chalk-like powder + binder + colors
	Mick PT et al., 2013 [21]	Binder jetting	ZPrinter 650	Zp®131 powder binder(Zb®7) + colors
	Roosli C et al., 2013 [22]	Binder jetting	Spectrum Z510	Chalk-like powder + binder + colors
	Bakhos D et al., 2010 [23]	Vat photopolymerisation	SLA® 5000	Somos® 14120
	Mori K, 2009 [24]	Powder bed fusion	NA (commercial available prototype)	Polyamide nylon and glass beads
	Mori K et al., 2009 [25]	Powder bed fusion	NA (commercial available prototype)	Polyamide nylon and glass beads
	Mori K et al., 2008 [26]	Powder bed fusion	NA (commercial available prototype)	Polyamide nylon and glass beads
	Suzuki M et al., 2007 [27]	Powder bed fusion	NA	Polyamide nylon and glass beads
	Grunert S et al., 2006 [28]	Binder jetting	Spectrum Z510	Plaster + post-processing with polyurethane and acetone
	Suzuki M et al., 2004 (1) [29]	Powder bed fusion	NA	Polyamide nylon and glass beads
	Suzuki M et al., 2004 (2) [30]	Powder bed fusion	NA	Polyamide nylon and glass beads
	Begall K et al., 1998 [31]	Vat photopolymerisation	Laser Model stereolithographic System by Fockele & Schwarze GmbH	Photosensitive; expoxy resins

continues

Table I. follows

SURGICAL AND PRECLINICAL EDUCATION					
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**	
Surgical middle ear training model	Monfared A et al., 2012 [32]	Material jetting	Objet Polyjet printer	Combination of 2 photosensitive resins	
Endoscopic ear surgery training model	Barber SR et al., 2016 [33]	Binder jetting	ZPrinter 650	Zp® 151 composite material + binder (ColorBond zbond® 90) + colors	
Functioning anatomical middle ear model	Kuru I et al., 2016 [34]	Powder bed fusion	EOS Formiga P100	Polyamide powder PA2200	
CUSTOMISED SURGICAL PLANNING					
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**	
Temporal bone surgical simulation	Rose AS et al., 2015 [35]	Material jetting	Objet Connex 350	Photo-polymers with different mechanical properties	
	Suzuki M et al., 2005 [36]	Powder bed fusion	NA	Polyamide nylon and glass beads	
	Suzuki M et al., 2004 (1) [29]	Powder bed fusion	NA	Polyamide nylon and glass beads	
	Lopponen H et al., 1997 [37]	Vat photopolymerisation	NA	Acrylic solution	
	Andrews JC et al., 1994 [38]	Vat photopolymerisation	3D Systems SLA 250	Liquid plastic	
Template-guided surgery	Pai I et al., 2016[39]	Material jetting	Objet Eden 250	Transparent photo-polymer	
	Matsumoto N et al., 2015[40]	Vat photopolymerisation	NA	Transparent photo-polymer	
	Cho B et al., 2014[41]"	Material jetting	Objet Connex 500	Transparent photo-polymer	
	Takumi Y et al., 2014[42]	Vat photopolymerisation	NA	Transparent photo-polymer	
Navigation for otoneurosurgery	Yamashita M et al., 2016 [43]	Material jetting	Objet Connex 500	Phantom	TangoPlus FLX930, VeroWhitePlus RGD835
				Template	VeroWhitePlus RGD835
	Ritacco LE et al., 2015 [44]	NA	NA	NA	
	Oka M et al., 2014 [45]	NA	NA	NA	
	Cho B et al., 2013 [46]	Powder bed fusion	NA	NA	
	Matsumoto N et al., 2012 [47]	Powder bed fusion	NA	NA	
	Matsumoto N et al., 2009 [48]	Powder bed fusion	NA	NA	
Lateral skull base approaches	Muelleman TJ et al., 2016 [49]	Material extrusion	uPrint SE Plus	Thermo-plastic material	
TISSUE ENGINEERING AND IMPLANTABLE PROSTHESIS					
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**	
Prosthesis for superior canal dehiscence	Kozin ED et al., 2015 [50]	Vat photopolymerisation	FormLabs Form 1+	Photo-polymer	
		Powder bed fusion	EOS Formiga	Plastic-based material; Aluminium-based material	

ABS: Acrylonitrile Butadiene Styrene; PLA: PolyLactic Acid; HIPS: High Impact PolyStyrene; NA: not available.

Rhinologic applications (Table II)

Surgical and preclinical education [51-57]

Four studies focused on the development of 3DP training models for endoscopic sinonasal and skull base surgery [51-54]. Medium-high fidelity simulators allowed developing surgical skills in the main endoscopic procedures, including drilling techniques and skull base exposure. Low-cost models were primary limited by the materials employed to mimic human bone as much as possible.

Customised surgical planning [58–60]

Two studies took advantage of the versatility of 3DP systems to fabricate operative templates tailored on the patient's anatomy. Daniel et al. produced 3DP cutting guides to design an osteoplastic flap during frontal surgery [59]; Onerci Altunay et al. used 3DP templates to fashion septal prosthesis for large irregular septal perforations [58]. 3DP endoscopic sinus surgery simulation was carried out in two patients with chronic rhinosinusitis to obtain safer and faster procedures [60].

Table II. Rhinologic studies classified according to each area of interest.

SURGICAL AND PRECLINICAL EDUCATION					
Field of work	**Authors, year**		**AM category**	**3D printer**	**3DP material**
Endoscopic sinonasal and skull base training models	Chang DR et al., 2017 [51]		Material extrusion	Airwolf 3D HD2X	ABS + molding with Aquasil Ultra XLV silicone
	Tai BL et al., 2016 [52]		Material extrusion	NA	Thermo-plastic material
	Narayanan V et al., 2015 [53]		Material jetting	Objet Connex 500	Photo-polymers with different mechanical properties
	Chan HHL et al., 2015 [54]	Paranasal sinus phantom	Material extrusion	Vantage - Stratasys	ABS
		Skull base phantom	Binder jetting	ZPrinter 310 - ZCorp	ZP-130 plaster powder + CA101 cyanoacrylate; ZP-15 plaster powder + infiltrant elastomeric
		Mandible templates	Material extrusion	Vantage - Stratasys	Polycarbonate
Septoplasty training model	AlReefi MA et al., 2017 [55]		Material jetting	Objet Connex 500	VeroWhitePlus, Tango-Plus and their combination to simulate different mechanical properties
Nosebleed training model	Estomba C et al., 2016 [56]		NA	NA	PLA + Polyurethane
Anatomical models	Sander IM et al., 2017 [57]		Material extrusion	LulzBot TAZ 5	PLA
CUSTOMISED SURGICAL PLANNING					
Field of work	**Authors, year**		**AM category**	**3D printer**	**3DP material**
Template-guided surgery	Onerci Altunay Z et al., 2016 [58]		Binder jetting	Spectrum Z510	Z131 powder
	Daniel M et al., 2011 [59]		Binder jetting	ZPrinter 310 plus	NA
Endoscopic sinus surgery simulation	Raos P et al., 2015 [60]		Binder jetting	ZPrint 310	NA
TISSUE ENGINEERING AND IMPLANTABLE PROSTHESIS					
Field of work	**Authors, year**		**AM category**	**3D printer**	**3DP material**
Customised prosthesis	Nahumi N et al., 2015 [61]		NA	NA	PolyEtherEtherKetone

ABS: Acrylonitrile Butadiene Styrene; PLA: PolyLactic Acid; HIPS: High Impact PolyStyrene; NA: not available.

Tissue engineering and implantable prosthetics [61]

One child with a craniofacial fibrous dysplasia was submitted to resection and reconstruction of the fronto-orbital region by means of a custom 3DP polyetheretherketone implant resulting in good aesthetical and safe outcomes.

Head and neck applications (Table III)

Surgical and preclinical education [62–66]:

Two studies focused on resident training for laryngeal surgical procedures. In 2014, Ainsworth et al. created a laryngeal model, including the extra-laryngeal soft tissues, to simulate trans-cervical injection of vocal folds [64]. More recently, Kavanagh et al. developed a 3DP paediatric laryngeal model reproducing several challenging surgical conditions (e.g. subglottic cysts, laryngomalacia, subglottic stenosis and laryngeal clefts) [62].

Customised surgical planning [54 65 67-132]

This was the most frequent ENT application of 3DP technology and mentioned in 68 of the 121 papers (56.2%). Among these, 95.6% of studies (65 out of 68) [54 67-130] concerned surgical management of head and neck tumours requiring mandibular resection and/or reconstruction. The first date to the ’90s and dealt with creation of 3DP mandibles to allow a direct handling of the neoplastic lesion, leading to the early surgical resection simulators. However, the most relevant contribution concerned the reconstructive aspects of oncologic surgery, guiding the employment of plates or autografts. Patient-specific 3DP mandibles were developed to “pre-bent” plates preoperatively. More recently, the introduction of image-guide systems used to plan the harvest and positioning of autografts (e.g. fibula flap, iliac crest bone flap) has led to the production of self-fabricated

Table III. Head and neck studies classified according to each area of interest.

SURGICAL AND PRECLINICAL EDUCATION				
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**
Laryngeal model	Kavanagh KR et al., 2017 [62]	Material extrusion	MakerBot	ABS, PLA, HIPS
	Johnson CM et al., 2016 [63]	Material extrusion	MakerBot 2XL	ABS (best performance), HIPS, PLA; Dragon Skin Fast silicon casting in a 3D printed mold
	Ainsworth TA et al., 2014 [64]	Material extrusion	Dimension Elite - Stratasys	ABSplus + silicone casting
Carotid artery model	Govsa F et al., 2017 [65]	Material extrusion	MakerBot	PLA
Tracheostoma model	Grolman W et al., 1995 [66]	Vat photopolimerisation	NA	Synthetic liquid resin
CUSTOMISED SURGICAL PLANNING				
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**
Guided surgery for oro-mandibular resection and reconstruction	Bosc R et al., 2017 [67]	Material jetting Material extrusion	Objet 30Pro – Stratasys Zortrax M200 - Zortrax SARL	Biocompatible photopolymer ABS
	Rachmiel A et al., 2017 [68] Skull	Material jetting	Objet260 Dental - Stratasys	Photopolimer resin
	Template	Powder bed fusion	EOS	Titanium
	Shah S et al., 2017 [69]	Binder jetting	ZPrinter 310 plus	Gypsum-based material
	Lee UL et al., 2016 [70]	Powder bed fusion	Arcam A1 (Electron Beam Melting)	Ti-6Al-4 V-ELI medical grade powder
	Lim SH et al., 2016 [71] Mandible	Binder jetting	ProJet 360 - 3D Systems	NA
	Cutting/ positioning guides	Material jetting	ProJet 3500 HDMax - 3D Systems	Biocompatible materials
	Numajiri T et al., 2016 [72]	Material extrusion	MakerBot	PLA
	Yamada H et al., 2016 [73]	NA	NA	NA
	Chan HHL et al., 2015 [54] Paranasal sinus phantom	Material extrusion	Vantage - Stratasys	ABS
	Skull base phantom	Binder jetting	ZPrinter 310 - ZCorp	ZP-130 plaster powder + CA101 cyanoacrylate; ZP-15 plaster powder + infiltrant elastomeric
	Mandible templates	Material extrusion	Vantage - Stratasys	Polycarbonate
	Man QW et al., 2015 [74]	NA	NA	NA
	Modabber A et al., 2015 [75]	Powder bed fusion	NA	Polyamide Powder
	Reiser V et al., 2015 [76]	Material jetting	A Objet – Stratasys machine (Model NA)	Biocompatible plastic polymers
	Schepers RH et al., 2015 [77]	NA	NA	Polyamide (for the cutting guides)
	Shan XF et al., 2015 [78] Residual skull	Material extrusion	Stratasys FDM 400-mc	NA
	Mesh	NA	NA	Titanium
	Steinbacher DM et al., 2015 [79]	NA		
	Succo G et al., 2015 [80]	NA	NA	NA
	Wilde F et al., 2015 [81]	Powder bed fusion	NA	Polyamide
	Ayoub N et al., 2014 [82]	Powder bed fusion	NA	NA
	Azuma M et al., 2014 [83]	Binder jetting	ZPrinter 310 plus	NA

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Table III. follows

CUSTOMISED SURGICAL PLANNING				
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**
	de Farias TP et al., 2014 [84]	Binder jetting	Z-Corp Spectrum Z510	Gypsum, cyanoacrylate, and ZP150
	Liu YF et al., 2014 [85]	Powder bed fusion	Sinterstation HiQ +HiSTM - 3D Systems	DuraForm - biocompatible nylon
	Modabber A et al., 2014 [86]	Powder bed fusion	NA	Polyamide
	Tsai MJ et al., 2014 [87]	NA	NA	NA
	Watson J et al., 2014 [88]	Powder bed fusion	Direct metal Powder bed fusion (Model NA)	Medical-grade titanium alloy Ti6AL4V - 3TRPD
	Wilde F et al., 2014 [89]	Powder bed fusion	NA	Biocompatible Polyamide
	Yamada H et al., 2014 [90]	NA	NA	NA
	Coppen C et al., 2013 [91]	Powder bed fusion	NA	DuraForm PA - 3DWorknet
	Foley BD et al., 2013 [92]	NA	NA	NA
	Hanasono MM et al., 2013 [93]	NA	NA	NA
	Mazzoni S et al., 2013 [94] Plate	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Titanium Ti64
	Guide	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Cobalt-Chrome MP1
	Mandible	NA	Stratasys machine	Resin
	Zheng GS et al., 2013 [95]	Vat photopolymerisation	SLA-3500 3D Systems	NA
	Ciocca L et al., 2012 (1) [96] Plate	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Titanium Ti64
	Guide	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Cobalt-Chrome MP1
	Mandible	Material extrusion	Stratasys machine	ABS
	Ciocca L et al., 2012 (2) [97] Plate	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Titanium Ti64
	Guide	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Cobalt-Chrome MP1
	Mandible	Material extrusion	Stratasys machine	ABS
	Dérand P et al., 2012 [98]	Powder bed fusion	ARCAM EBM A2	Ti6Al64V ELI powder
	Hou JS et al., 2012 [99]	NA	NA	Photopolymer
	Lethaus B et al., 2012 [100]	Material extrusion	Maastricht Instruments	NA
	Modabber A et al., 2012 (1) [101] Guide	Powder bed fusion	NA	Polyamide
	Skull	NA	NA	Acrylic Resin
	Modabber A et al., 2012 (2) [102] Guide	Powder bed fusion	NA	Polyamide
	Skull	NA	NA	NA
	Patel A et al., 2012 [103]	NA	NA	NA
	Sink J et al., 2012 [104]	NA	NA	NA
	Wilde F et al., 2012 [105]	Binder jetting	ZTM 510 - 4D Concepts	NA
	Zheng GS et al., 2012 [106]	Vat photopolymerisation	SLA-3500 3D Systems	NA
	Abou-ElFetouh A et al., 2011 [107]	Vat photopolymerisation Binder jetting	3D Systems InVision Si2 3D Systems VisiJet SR 200	NA NA
	Antony AK et al., 2011 [108]	NA	NA	NA
	Bell RB et al., 2011 [109]	NA	NA	Acrylic resin
	Hou JS et al., 2011 [110]	NA	NA	Polybutadiene-styrene resin
	Mehra Pet al., 2011 [111]	Vat photopolymerisation Material extrusion	NA	Acrylic, Epoxy Starch

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Table III. follows

CUSTOMISED SURGICAL PLANNING				
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**
	Yamanaka Y et al., 2010 [112]	NA	NA	Acrylic plastic
	Zhou LB et al., 2010 [113]	Vat photopolymerisation	LPS 600 laser prototyping	Resin
	Cohen A et al., 2009 [114]	Material jetting	Eden 500 V	Photo-polymer
	Farina R et al., 2009 [115]	Vat photopolymerisation Binder jetting	3D Systems SLA-250/30 Z-Corporation Z406	8110 resin (DSM Somos) Starch-cellulose material
	Juergens P et al., 2009 [116]	NA	NA	NA
	Leiggener C et al., 2009 [117]	Powder bed fusion	NA	Medical grade polyamide
	Liu XJ et al., 2009 [118]	NA	NA	Resin
	Chow LK et al., 2007 [119]	NA	NA	Starch, epoxy resin, acrylic
	Lee JW et al., 2007 [120]	NA	NA	NA
	Ro EY et al., 2007 [121]	NA	NA	Epoxy
	Toro C et al., 2007 [122]	Vat photopolymerisation	SLA 3500 – 3D Systems	Epoxy resin Watershed 11120
	Yeung RWK et al,. 2007 [123]	NA	NA	NA
	Hallermann W et al., 2006 [124]	Powder bed fusion	NA	Duraform PA12 - 3D Systems
	Hannen EJM et al., 2006 [125]	NA	NA	Resin
	Cunningham LL et al., 2005 [126]	Vat photopolymerisation Binder jetting	3D Systems SLA-250/30 Z-Corporation Z406	8110 resin (DSM Somos) Starch-cellulose material
	Wong TY et al., 2005 [127]	NA	NA	NA
	Singare S et al., 2004 [128]	Vat photopolymerisation	LPS 600	Photo-polymer
	Kernan BT et al., 2000 [129]	NA	NA	NA
	Komori T et al., 1994 [130]	Vat photopolymerisation	Solid Creation System (D-MEC Ltd, Tokyo, Japan),	Desolight SCR- 100, D-MEC Ltd)
Guided surgery for cranio-cervicofacial teratoma	Wiedermann JP et al., 2017 [131]	NA	NA	NA
Carotid artery model	Govsa F et al., 2017 [65]	Material extrusion	MakerBot	PLA
MRI compatible laryngoscope	Paydarfar JA et al., 2016 [132]	Material jetting	Objet Eden250 - Stratasys	MED610 (Stratasys) biocompatible photopolymer
TISSUE ENGINEERING AND IMPLANTABLE PROSTHESIS				
Field of work	**Authors, year**	**AM category**	**3D printer**	**3DP material**
Customised prosthesis for mandibular reconstruction	Rachmiel A et al., 2017 [68] Skull	Material jetting	Objet260 Dental - Stratasys	Photopolymer resin
	Template	Powder bed fusion	EOS	Titanium
	Lee UL et al., 2016 [70]	Powder bed fusion	Arcam A1 (Electron Beam Melting)	Ti-6Al-4 V-ELI medical grade powder
	Schepers RH et al., 2015 [77]	NA	NA	Polyamide (for the cutting guides)
	Shan XF et al., 2015 [78] Residual Skull	Material extrusion	Stratasys FDM 400-mc	NA
	Mesh	NA	NA	Titanium
	Watson J et al., 2014 [88]	Powder bed fusion	Direct metal Powder bed fusion (Model NA)	Medical-grade titanium alloy Ti6AL4V - 3TRPD
	Mazzoni S et al., 2013 [94] Plate	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Titanium Ti64
	Guide	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Cobalt-Chrome MP1
	Mandible	NA	Stratasys machine	Resin

continues

Table III. follows

TISSUE ENGINEERING AND IMPLANTABLE PROSTHESIS					
Field of work	**Authors, year**		**AM category**	**3D printer**	**3DP material**
	Ciocca L et al., 2012 (1) [96]	Plate	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Titanium Ti64
		Guide	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Cobalt-Chrome MP1
		Mandible	Material Extrusion	Stratasys machine	ABS
	Ciocca L et al., 2012 (2) [97]	Plate	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Titanium Ti64
		Guide	Powder bed fusion	EOSINT M270 - Electro-Optical Systems	EOS Cobalt-Chrome MP1
		Mandible	Material extrusion	Stratasys machine	ABS
	Dérand P et al., 2012 [98]		Powder bed fusion	ARCAM EBM A2	Ti6Al64V ELI powder
	Zhou LB et al., 2010 [113]		Vat photopolymerisation	LPS 600 laser prototyping	Resin
	Singare S et al., 2004 [128]		Vat photopolymerisation	LPS 600	Photopolymer

ABS: Acrylonitrile Butadiene Styrene; PLA: PolyLactic Acid; HIPS: High Impact PolyStyrene; NA: not available.

customised 3DP cutting guides. Many authors experienced a decrease in surgical time and the risk of undesirable events during reconstructive approaches, which resulted in a proper mandibular function. Concerning AM technology, in 38.2% of the studies (26 of 68) the AM category was not specified, mainly due to the outsourcing of all 3D printing operations to external services, which are becoming more common in recent years.

Tissue engineering and implantable prosthetics [68 70 77 78 88 94 96-98 113 128]

This area included 9.1% of all studies (11 of 121). All these investigations dealt with mandibular reconstruction following tumour resection in a total of 33 patients. The authors employed 3DP technology to develop patient-specific reconstruction plates, trays, meshes and mandibular implants. Titanium alloys (e.g. Ti6Al4V) were used in all cases due to their suitable physical and mechanical properties: low specific weight, corrosion resistance and good biocompatibility [96]. 3DP reconstruction plates, tray and meshes were associated with a bone autograft in 9 studies: 66.6% opted for a fibula free flap [77 78 94 96-98] and 33.3% for an iliac crest free flap [68 113 128]. Differently, Lee et al. made use of a mandibular implant without the support of a bone autograft, proving an acceptable alternative in cases of unsuitable free flap surgery [70]. A total of 27 patients (81.9%) showed good aesthetical and occlusion outcomes and thus correct oral rehabilitation [68 70 77 78 88 94 96 97 113 128]. Complications were observed in 2 subjects (6%): one patient experienced bone resorption and infection, while the other had flap necrosis [77 113]. The authors reported a reduction of the operating time between 30 [98] and 120 minutes [94], enabling economic benefits at the expense of the additional cost of the 3DP prosthesis.

Discussion

Personalised medicine, minimally-invasive surgery, tissue engineering and regenerative medicine are the watchwords of third millennium healthcare. The arising popularity around the world of 3DP systems may be explained through the opportunities offered by this new technology to support new trends in modern medicine. Since its first applications in the early 1990s, researchers have explored the advantages of 3D printers, publishing 121 studies in otorhinolaryngology (Fig. 2). Customised surgical planning was evaluated in 71.9% of studies, proving to be the main direction of investigation (Fig. 3). The manufacture of anatomical models before surgery allowed both the understanding of specific anomalies and guidance for the operative strategy. The first and most frequently explored clinical application was resection and reconstruction of oro-mandibular tumours due to their easier medical image processing in comparison with other fields. The development of 3DP operative templates for cutting and/or reconstruction guides minimised the surgeon's fatigue and complication rates, and optimised the operating room time, which led to lower morbidity. Similar approaches have been employed for complex cases of temporal bone and sinonasal surgery.

Clinical benefits were advocated by the authors to justify the main limitations of AM technology: costs, necessity for technical skills and technological availability. Cost-effectiveness was widely debated in literature: the decreased surgical time and employment of self-fabricated 3DP models or guides (instead of outsourced manufacturing) appeared to counter balance the price of the starting technological investments and the technical skills required

for pre- and postprocessing printing activity [94]. Interestingly, for 34% of studies on customised surgical planning, a specific description of the technology adopted was not available (Fig. 4): this arises from the choice of externalization of the 3D printing process, as often declared by authors themselves [45 77 80 93 110 121]. To date, the rapid expansion of AM machines and materials has significantly lowered costs, making this technology more accessible. The most employed technology in this field of application was power bed fusion (27%), which offers medical grade materials (like titanium, or biocompatible polyamide) to be used as intra-operative templates, followed by material extrusion (12%), which also offers biocompatible materials, even if with lower printing resolution. Surgical and preclinical education represents the second most studied 3DP application. Surgical training traditionally made use of physical models, animals, or human cadavers. The adoption of both fixed and fresh human specimens in labs has long been and still is a core component in training for ENT surgery, but it has certain limitations such as transmission of infectious agents, exposure to potentially carcinogenic formaldehyde and excessive costs. More recently, 3DP models were used in the teaching of complex anatomy and to simulate critical surgical procedures with particular regard to temporal bone and skull base dissection. The most employed AM technology for this application (Fig. 4) was material extrusion (39%): this is not surprising, since this is the most affordable technology, especially in terms of printing materials. Material extrusion is actually the most suited to apply for teaching and training, where models are usually subjected to damage and need to be produced in high numbers. 25% of studies used power bed fusion machines, thanks to the availability of materials (e.g. polyamide) with mechanical properties that are suitable for drilling and dissection operations. The complexity of temporal bone anatomy and related surgical procedures, essentially based on bone drilling and removal, explain the extensive research on this issue.

The evolution of 3DP systems and materials has enabled the reproduction of even the finest chromatic details and mechanical properties of the object resulting in highly representative 3DP simulators. These solutions are unfortunately still expensive, and consequently less employed for the production of didactic devices, as confirmed by the limited use of technologies with high chromatic resolution (binder jetting, 11%) and with tuneable mechanical properties (material jetting, 11%).

Tissue engineering and implantable prostheses is discussed in fewer reports since it represents the most recent 3DP application, but it also entails more exciting future perspectives. The current literature reported the application of 3DP customised titanium alloy prostheses in 33 cases of mandibular reconstruction after tumour resection. Power bed fusion is confirmed as the most widely employed technology in the field, used in 50% of studies: the most common materials are titanium and cobalt-chrome, which are also widely employed in implant standard manufacturing. Preliminary data have provided encouraging results in terms of safety and effectiveness, opening new frontiers of investigation.

Nowadays, AM technology has been involved in the production of biocompatible matrices aimed to be cellularised (scaffold), hence forming a new functional tissue. ENT scaffold research is at present confined to a preclinical stage (in vitro and animal testing), with relevant applications in the reconstruction of the upper aerodigestive tract [133 134], replacement of tympanic membrane [135] and plastic rebuilding of auricular and nasal cartilages [136 137]. Even though scaffold research is in its infancy, it represents a future direction of high interest. New perspectives will concern the microstructure of 3DP scaffolds to overcome many currently unsolved questions as well as proper vascularisation to avoid cell degeneration and adequate stem cell proliferation/specialisation. The final goal would entail functional aspects to produce functional tissues and organs by involvement of multiple types of cells and biomaterials.

Moreover, in the foreseeable future, technical advancements will possibly provide a better solution to issues involving biocompatibility and sterilisation protocols of 3DP materials.

Conclusions

3DP systems have revolutionised prototyping in the industrial field by lowering production time from days to hours and costs from thousands to only a few dollars. Today, 3D printers are no longer confined to prototyping, but are increasingly employed in the medical discipline with fascinating results, even in many aspects of otorhinolaryngology. Nevertheless, current reports are still limited to small case-series of patients and lack of comparative objective data to validate 3DP technology in daily clinical practice. 3DP bioengineering is at the beginning of an exciting research field, and the positive results to date are far from what it will be possible to achieve in forthcoming clinical applications.

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HEAD AND NECK

Pilot study on microvascular anastomosis: performance and future educational prospects

Studio pilota sulle microanastomosi vascolari: risultati a confronto e prospettive didattiche future

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SUMMARY

The introduction of microvascular free flaps has revolutionised modern reconstructive surgery. Unfortunately, access to training opportunities at standardised training courses is limited and expensive. We designed a pilot study on microvascular anastomoses with the aim of verifying if a short course, easily reproducible, could transmit microvascular skills to participants; if the chosen pre-test was predictive of final performance; and if age could influence the outcome. A total of 30 participants (10 students, 10 residents and 10 surgeons) without any previous microvascular experience were instructed and tested during a single 3 to 5 hour course. The two microanastomoses evaluated were the first ever performed by each participant. More than the half of the cohort was able to produce both patent microanastomoses in less than 2 hours; two-thirds of the attempted microanastomoses were patent. The pretest predicted decent scores from poor performances with a sensitivity of 61.5%, specificity of 100%, positive predictive value of 100% and negative predictive value of 40%. Students and residents obtained significantly higher scores than surgeons. Since our course model is short, cost-effective and highly reproducible, it could be introduced and implemented anywhere as an educational prospect for preselecting young residents showing talent and natural predisposition and having ambitions towards microvascular reconstructive surgery.

KEY WORDS: Microvascular anastomosis • Free flap • Microvascular training • Surgical skills • Education

RIASSUNTO

L'introduzione dei lembi liberi microvascolari ha rivoluzionato la moderna chirurgia ricostruttiva. Purtroppo, l'accesso a corsi specifici e intensivi è attualmente limitato e costoso. Abbiamo organizzato uno studio pilota sulle microanastomosi vascolari con lo scopo di verificare: se un corso economico e facilmente riproducibile potesse trasmettere ai partecipanti delle abilità microchirurgiche di base; se il test preliminare scelto fosse predittivo dei risultati finali; e se l'età potesse essere un fattore in grado di influenzare la performance. Sono stati selezionati un numero complessivo di 30 partecipanti (10 studenti, 10 specializzandi e 10 chirurghi), senza nessuna precedente esperienza microchirurgica, ai quali è stato proposto un corso della durata di 3-5 ore. Le anastomosi conclusive, sottoposte a valutazione, sono state le prime realizzate da ciascun partecipante. Più della metà degli individui testati è stata in grado di confezionare entrambe le microanasromosi funzionanti e in un tempo inferiore alle 2 ore; nel complesso due terzi delle microanastomisi realizzate erano pervie. Il test preliminare è stato in grado di predire risultati finali buoni rispetto a risultati finali scarsi con una sensibilità del 61%, una specificità del 100%, un valore predittivo positivo pari al 100% ed un valore predittivo negativo del 40%. Studenti e specializzandi hanno ottenuto punteggi significativamente migliori rispetto ai chirurghi. Dato che il corso da noi proposto è breve, dai costi contenuti e facilmente riproducibile, riteniamo possa essere facilmente implementato in altre strutture al fine di selezionare giovani specializzandi dotati di talento con una naturale predisposizione per la microchirurgia, e che dimostrino interesse ed ambizione nel campo della chirurgia ricostruttiva.

PAROLE CHIAVE: *Anastomosi microvascolari • Lembi liberi • Esercitazioni microvascolari • Abilità chirurgiche • Apprendimento*

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Introduction

In the early years of the 20th century, Carrel codified the triangulation technique obtaining functioning vascular anastomosis [1-3] to investigate the feasibility of organ transplantation.

Otolaryngologists were the first physicians to use microsurgical techniques. In 1921, the microscope was used for

the first time in ear surgery by a Swedish otologist, Carl Olof Nylen [3 4], the next year Gunnar Holmgren replaced the first monocular microscope with a binocular one [4]. The application of the microscope to vascular anastomosis allowed the first successful microvascular transfer of a jejunal interposition flap in 1959 [5].

The introduction of microvascular free flaps revolutionised the surgical treatment of head and neck tumours and represents one of the major advancements in the management of these neoplasms. Microsurgery requires considerable practice before any attempt is made at clinical application. Live animals represent the ideal training medium; however, unlimited access to the animal laboratory is not always possible [6]. In such situations, segments of fresh human placenta or other surrogates such as pig coronaries or chicken wing vessels can provide adequate specimens for microsurgical practice.

At our tertiary referral academic hospital, to date, no specific microvascular training program is available for students, residents or surgeons, and no specific financial support is provided for those who intend to build up these skills attending national and/or international microvascular courses. This situation is diffuse all over the country, and this aspect penalises even more youngest surgeons with access to limited economic resources, despite the fact that it seems that age in itself plays an important role in the abrupt of the learning process. In fact, a study comparing the results of microsurgical education after the completion of a comprehensive microsurgical course program between students and surgeons demonstrated that the students achieved higher scores, with a significant better performance in tissue handling [7].

We designed a pilot study on microvascular anastomoses with the aim of verifying if even a simple short course, easily reproducible, could transmit microvascular skills to participants; if the chosen pre-test was predictive of the final performance; and if age could influence the outcome.

Materials and methods

From April to September 2016, 30 voluntary participants were enrolled and tested in our pilot study.

Population

Inclusion criteria were: motivation and willingness to participate, complete lack of previous microvascular experience, age in concordance with the professional position.

The cohort was formed of 3 groups;

- Group 1 (G1): 10 students attending the sixth (last) year of medical school, all aged under 28 years (mean 24.8), with no surgical experience; 7 expressed ambition to a career in a surgical specialty.
- Group 2 (G2): 10 residents in surgical disciplines (5 in otolaryngology, 3 in general surgery, 1 in vascular surgery, and 1 in orthopaedic surgery), aged between 28 and 35 years (mean 29.8), usually attending macroscopic surgery as second operator, sometimes as first operator under supervision, but none with specific experience on microvascular anastomosis or microscopic ear surgery.
- Group 3 (G3): 10 surgeons (8 otolaryngologists, 1 vascular surgeon, and 1 maxillofacial surgeon) aged over 35 years (mean 51.5) usually performing macroscopic surgery as first operator. The 8 otolaryngologists were skilled in microscopic laryngeal surgery, but none had any experience in microvascular anastomosis or microscopic ear surgery.

Microanastomosis course

Each participant was instructed and then individually tested in one session that varied between 3 to 5 hours; a schematic representation of the structure of the course is displayed in Figure 1.

A brief theoretical introduction to experimental microsurgery was provided: correct posture, use of the microscope, handling of microinstruments, end to end microsuture technique for artery and vein, exercises on a macroscopic model for microscopic arterial and venous microanastomoses and tasks required.

A preliminary pre-test was initially administrated, namely the so-called 'round-the-clock' training model [8], which proved to be an inexpensive and readily available valid tool to provide instant assessment of the individual microsurgical predisposition (Fig. 2). Participants were asked to pass a 9/0 nylon microsuture needle through the needle

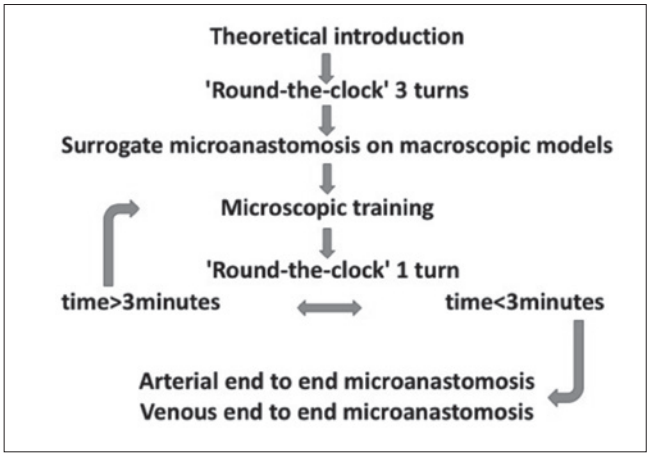

Fig. 1. Schematic representation of the course.

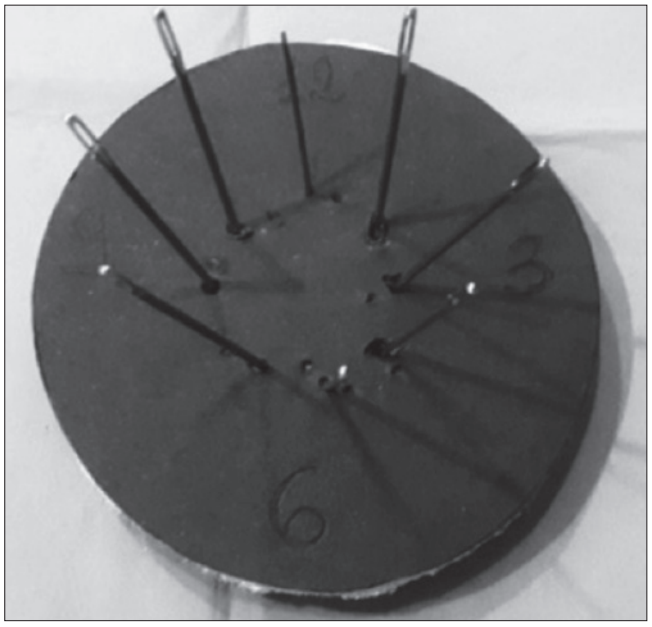

Fig. 2. The 'round-the-clock' test, each run had to be completed clockwise under microscopic magnification at 6X using 9/0 suture.

eyes of nine sewing needles planted on a rigid support, each run had to be completed clockwise; the time of each run for 3 consecutive runs was recorded, without considering steadiness or handling abilities.

Participants were then familiarised with microsuture techniques for end-to-end arterial and venous anastomoses using a macroscopic model: the artery surrogate was a silicon tube, since the lumen is maintained; the vein surrogate was a finger glove, since lumen tend to collapse. When performing surrogate microanastomoses, the same handling rules and same techniques required for true microanastomoses had to be respected, with the only difference of working without the microscope and using a 5/0 suture. For the surrogate arterial microanastomosis, the triangulation technique with interrupted sutures was used; for the surrogate venous anastomosis, after placing the first two stay sutures at 2 o'clock and 10 o'clock, the superior 180° were sutured, and then the inferior 180°. During the surrogate microanastomoses the instructor surveyed the participant, pointing out and explaining all handling errors according to the technical requirements previously explained in the theoretical introduction.

The next step was microscopic training on an animal model; we used one half rooster per participant since it provided good quality vessels, very similar in diameter to those encountered for microvascular head and neck reconstructions, with average diameter of 3 mm (range 2.5 mm - 3.5 mm). Participants were free to familiarise themselves with microsutures using the sciatic nerve or other tissues, but not vessels; no time restrictions were given for this training, while the instructor provided constant feedback on handling and errors, by pointing out: incorrect posture, incorrect use of the microscope, incorrect microinstrument handling, incorrect tissue handling, incorrect needle handling and needle damage, unwanted perforations, asymmetric knots placement, incorrect knots tying. All this followed the requirements listed by the Northwestern Objective Microanastomosis Assessment Tool (NOMAT) [9].

After microscopic training, participants performed a fourth run at the 'round-the-clock' test, if the exercise was completed in less than 3 minutes (this cut-off was chosen considering four times the mean time recorded by the experienced microsurgeon, AD) they could move forward to perform the microanastomoses, otherwise they had to return to the microscopic training before re-attempting another 'round-the-clock' run until the threshold of 3 minutes was satisfied. Times recorded on this fourth run at the 'round-the-clock' test were considered good when below 2 minutes, intermediate when between 2 and 3 minutes, and poor when above 3 minutes.

Microvascular end-to-end arterial anastomosis using 8/0 nylon interrupted sutures was performed first, then the venous end-to-end anastomosis was performed. Participants had one-hour to complete each microanastomosis or to complete at least the minimal-task. During the microanastomoses participants were surveyed, but no feedback on errors and no suggestions were provided by the instructor, who, instead, took note and counted all errors.

All macro-microsurgical instruments were propriety of the senior author (AD), all animal models were bought by the first author (GB) at her own expense; the macro-micro surgical suture materials were outdated and ready to be discarded.

Scoring system

Arterial and venous microanastomoses were separately evaluated. The minimal-task for the artery was considered to be the correct placement of the first 3 stay sutures and this was awarded with 50 points, the minimal-task for the vein was the correct suture of the superior 180°, for which 50 points were awarded. Zero points were scored in case of inability/incorrect placement of the first 3 stay sutures on the arterial microanastomosis, inability/incorrect suture of the superior venous wall, or in case of minimal-task resulting in lumen obliteration. If the minimal-task was met, the participant could move forward to complete the microanastomosis as much as possible within the one-hour time limit. The time to complete each microanastomosis was re-

corded, and microanastomoses were evaluated in terms of patency and continence, 100 points were awarded if the microanastomosis was patent and continent, 95 points if the microanastomosis was patent but leaking if one extra stitch was needed and 90 points if two extra stitches were needed. In case three or more stitches were required to restore continence, or if the microanastomosis was not patent, the score was 50 points considering only achievement of the minimal-task. At the final score 2 points were deducted for each incorrect gesture performed during the microanastomosis.

Global (arterial + venous) scores above 160 were considered high, scores between 100 and 160 were considered intermediate and scores below 100 were considered low.

Times recorded for the fourth run at the 'round-the-clock' test were correlated with the microanastomoses global scores.

Statistical analysis

Differences between groups were tested with ANOVA; the variables, all continuous, were expressed as mean values and standard deviation, and were compared between two different groups with the t-Student test: p values less than 0.05 were considered statistically significant.

Results

Times (minutes' and seconds'') of the first, second, third and fourth run at the 'round-the-clock' are shown in Table I; the analysis showed a significant difference for the fourth round between G1 and G2 vs. G3 (p = 0.025).

Mean arterial and venous microanastomoses scores are shown in Table II.

Comparison of the global (arterial + venous) performance between groups showed a significant difference between G1 vs. G3 (p = 0.0010) and between G2 vs. G3 (p = 0.0013), indicating a better performance for students and residents compared to surgeons, while no differences were seen between students and residents (p = 0.47), Figure 3.

As already stated, all participants had to complete each microanastomosis within a maximum time limit of 1 hour. The mean arterial and venous microanastomoses times were, respectively, 56 and 51 minutes for G1, 48 and 52 minutes for G2 and 52 and 52 minutes for G3.

The 3 minute cut-off time on the fourth run at the 'round-the-clock' test was able to predict good and intermediate microanastomoses global scores from poor performances with a sensitivity of 61.5% and a specificity of 100%; the positive predictive value was 100% and the negative predictive value was 40%.

Table I. Results at 'round-the-clock' test.

	First run	Second run	Third run	Fourth run
G 1	8'38"	5'33"	4'11"	2'41"
G 2	7'	4'59"	4'22"	2'35"
G 3	12'	8'11"	5'5"	3'38"

Table II. Mean scores of arterial and venous microanastomoses.

	ARTERY	VEIN
G 1	82.8 (SD 21.66)	87.5 (SD 15.62)
G 2	78 (SD 31.10)	75.7 (31.68)
G 3	56.9 (SD 42.04)	45.1 (SD 38.45)

SD: standard deviation

Discussion

The surgeon's experience is a critical factor influencing the outcomes of microvascular reconstructions, and the success rate in experienced hands is usually above 95% [10-12]. However, a study on the first year of clinical experience of three microvascular head and neck surgeons yielded a success rate of 97.5%, showing that well-trained junior microvascular surgeons can achieve survival rates comparable with those of experts [12].

Lascar et al. compared the patency rates obtained by residents in plastic surgery with different microsurgical experience, varying from residents of the first year with no microsurgical experience to residents of the sixth year with considerable experimental and clinical microsurgical experience. A proportional narrowing of outcome differences among groups was seen with the increase in number of microanastomoses performed by the less experienced ones, until no outcome differences were recorded after 52 microanastomosis [13], demonstrating that constant intense training is necessary to achieve high standards. In this view, a 3 to 5 hour course is usually considered much too short for formal microsurgery training. However, the principal philosophy of the study was not focused on offering intensive microsurgical education, but rather on implementing a highly accessible and inexpensive microsurgery facility. Our course was effective in transmitting a microvascular starting point, with the aim of identifying the youngest and most talented physicians for future advancements.

The results indicate that even a simple, rapid, highly reproducible and low-cost course can effectively transmit microvascular skills to participants. In fact, more than the half of the cohort (7 students, 7 residents and 3 surgeons) was able to produce, in less than 2 hours, both arterial and venous microanastomoses functioning. Furthermore, two-thirds

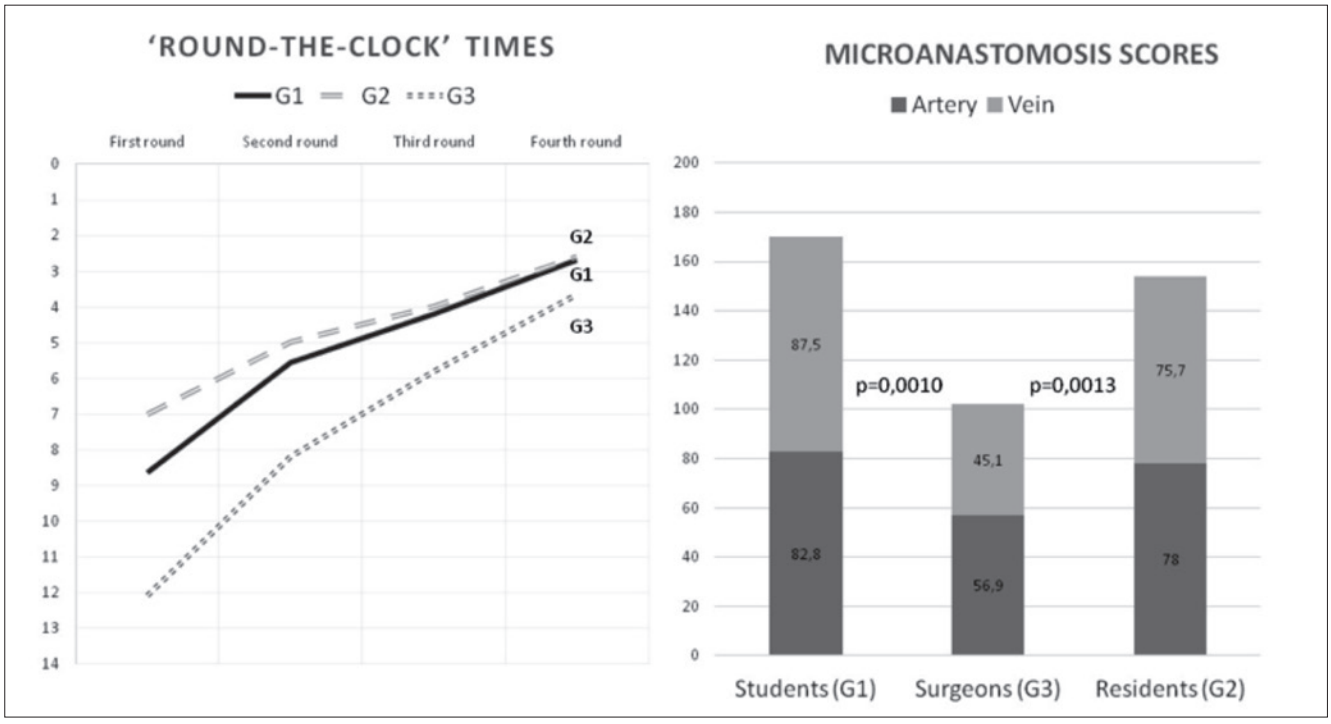

Fig. 3. Performances of the three groups at the pretest and microanastomosis.

(39 of 60) of the attempted microanastomoses were patent, and among these, 20 were also perfectly continent, while in 17 cases one extra stitch was needed to stop leakage, and in 2 cases 2 stitches were needed. The time limit of 1 hour per microanastomosis was set because from a clinical point of view two hours is an acceptable ischaemia time for most fascio-cutaneous and osteo-cutaneous free flaps.

These results are remarkable considering that none of the subjects possessed any microvascular experience, no one had attended other microvascular courses before and the two microanastomoses considered were the first ever performed by all participants. Our evaluation system is not validated, but we preferred to design a scoring system that was more focused on final microanastomosis evaluation rather than homogeneously evaluating the ongoing performance during the microanastomosis. We felt that the NOMAT system was more suitable for monitoring the evolution in building up the global microvascular experience within more structured courses, rather than assessing the results of a single attempt in a mini-course.

The 'round-the-clock' test with a 3 minute cut-off time confirmed to be an effective exercise and a powerful predictive tool with 100% specificity and positive predictive values, which means that it could be effectively used to preselect subjects who almost surely will perform well. In fact, none of the 16 subjects who completed the fourth run in less than 3 minutes had a poor microanastomosis global score: 13 had good performance (5 students, 6 residents, 2 surgeons) and 3 an intermediate performance (2 students and 1 resident).

Students and residents obtained significantly higher scores than surgeons. This seems to confirm that age is a real and crucial factor for acquisition of microvascular skills: 3 surgeons were unable to complete the minimal task and only 3 produced both functioning microanastomoses. Therefore, our data indicate that age seems to be more determinant than surgical background experience for the acquisition of microvascular skills, and this is paralleled by the finding that surgeons were more prone to errors than the younger participants.

For all participants, the arterial microanastomosis was performed first and the venous anastomosis was performed subsequently. Venous microanastomosis is recognised to be technically more difficult than an arterial one, mainly because the vessel lumen tend to collapse, and beginners are expected to perform better with less demanding duties; G1, instead, was the only group with venous scores higher than the arterial ones. This data is interesting: students demonstrated the ability to keep on learning and perfecting the technique as 'on the job' training. When performing a venous anastomosis, students were already

familiarised with the microscope and with microinstruments during the previous microanastomosis.

Conclusions

Our mini-course aroused substantial enthusiasm among all participants. In our country, surgery is unfortunately started late and practiced in autonomy mostly by aged surgeons after the achievement of permanent working positions. Residents are often frustrated by the lack of sufficient hands-on experience during training, and our data confirm the importance of investing in young, talented and motivated surgeons, since they can produce excellent performances. Since this course model is short-timed, cost-effective and highly reproducible, it could be introduced and implemented anywhere as a future educational prospect for preselecting young residents showing talent and natural predisposition and having ambitions towards microvascular reconstructive surgery.

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HEAD AND NECK

Association between the increase in incidence of papillary thyroid carcinoma in Crete and exposure to radioactive agents

Associazione tra l'aumento dell'incidenza del carcinoma papillare della tiroide e l'esposizione ad agenti radioattivi

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SUMMARY

The mean gamma-ray distribution in Crete during the years after the nuclear accident at Chernobyl and its correlation with the Papillary Thyroid Cancer (PTC) distribution was identified. A total of 4285 patients underwent total thyroidectomy in our centre between 1990 and 2012. Data of gamma-ray (nSv/h) distribution were selected from the Greek Statistical Authorisation. A geo-spatial statistical model was used to estimate the expected number of patients with PTC and Kriging interpolation prediction model to estimate their distribution. Geographical weighted regression was performed to estimate the risk of PTC in relation to gamma ray distribution. All factors that were examined were found to be statistically significant for PTC distribution in Crete. Gamma-ray was determined as a significant risk factor (OR = 2.89; 95% CI = 1.682-4.989; p value = 0.03). There is a significant correlation between gamma-ray exposure and the increased prevalence of the PTC suggesting that the former may have been a significant risk factor.

KEY WORDS: Papillary thyroid carcinoma • Crete • Gamma-ray

RIASSUNTO

In questo studio è stata valutata la distribuzione media di raggi gamma a Creta durante gli anni successivi al disastro nucleare di Cernobyl e la correlazione esistente con distribuzione di Carcinomi Papillari della Tiroide (PTC). Abbiamo valutato 4285 pazienti sottoposti a tiroidectomia presso il nostro centro tra il 1990 e il 2012. I dati riguardanti la distribuzione di raggi gamma (nSv/h) sono stati selezionati dall'Autorizzazione Statistica Greca. Per stimare il numero atteso di pazienti con PTC e la loro distribuzione sono stati utilizzati rispettivamente un metodo statistico geo-spaziale e il metodo di interpolazione di Kriging. I raggi gamma sono stati identificati come un fattore di rischio significativo (OR = 2,89; 95%CI = 1,682-4,989; p value = 0,03). C'è pertanto una forte correlazione tra esposizione a raggi gamma e aumento della prevalenza di PTC.

PAROLE CHIAVE: *Carcinoma papillare della tiroide • Creta • Raggi gamma*

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Introduction

An increased incidence of papillary thyroid carcinoma (PTC) in Crete, with a synchronous stability in the other carcinoma subtypes, is reported. Among the risk factors for developing thyroid cancer, the most established one is ionising radiation [1-3]. Data obtained from studies involving patients subjected to external radiation and more recently the Chernobyl accident, show that radiation plays an important role in thyroid carcinogenesis and principally in PTC [1-5].

The island of Crete is the southernmost part of the European continent and is located in the eastern Mediterranean Sea. It spreads over an area of 8261 km^2 and according to the latest census has 800,000 inhabitants.

We investigated the demographics of PTC as found in specimens of total thyroidectomies performed in an academic tertiary referral medical centre during the last 22 years, and estimated the risk of disease in relation to the gamma ray distribution in the years since 1990.

Methods

Sample and study design

This study was conducted by three departments (Department of Surgery, Department of Surgical Oncology, and Department of Otorhinolaryngology, Head and Neck Surgery) of an academic tertiary referral medical centre (University Hospital of Crete, Heraklion, Crete, Greece), among 4285 patients who underwent total thyroidectomy between 1990 and 2012. Patient records were placed in a database, which included demographics, history and biopsy results. Histopathological slides were reviewed thoroughly in all cases, with a special focus on PTC, since this type is the most frequent type. Patients who had previously undergone a partial thyroidectomy, or who were previously diagnosed with another primary carcinoma elsewhere, were excluded from the study. All patients included in the study were born before 1986.

A geo-spatial statistical model was used to estimate the anticipated number of PTC cases in the whole region of Crete. Additionally, the mean gamma-ray distribution in Crete and its correlation to patient distribution was identified. The data were collected from the Greek Atomic Energy Commission (GAEC) [6] Gamma-ray records. Data of gamma-ray (nSv/h) distribution were calculated as mean values for one year period per station in Crete. The analysis was performed in the Arcmap 10 Geographical Information Systems (GIS) [7].

Geo-spatial analysis

Spatial distribution of the numbers of thyroidectomies performed per prefecture, as well as the number of thyroidectomies performed per 100,000 people, together with the basic demographic characteristics (age and gender) was performed using the Arc map's toolbox construction, in a thematic map. The island of Crete is divided in four prefectures, as is shown in Figure 1. Since patient records could probably be biased due to the fact that these were patients treated in a single medical centre on the island, a prediction map (Kriging interpolation) was constructed. The same procedure was also done for the gamma-ray records, as shown in Figure 2.

Kriging interpolation prediction model was applied using both patient and gamma-ray data to estimate their distribution throughout the island [8 9]. This was distributed in a prediction map after having verified the reliability of the prediction model through its semi-variogram and cross-validation process. The Kriging interpolation model is a technique to interpolate the value of a random field (e.g. patients, gamma-ray values) at an unobserved location from observations of its value at nearby locations, using mathematical polynomials. Specifically, it estimates the number of cases in areas where there are no records from the database, based on known records and their spatial trends [7-10]. Through this procedure two sets of data were examined both as independent and dependent phenomena and the prediction was measured as the estimated number of patients per 50 km^2 (randomly selected).

Finally, geographical weighted regression (GWR) was performed to estimate the risk of disease in relation to gamma ray distribution, as well as gender and age at a confidence level of 95%. The GWR is a local version of spatial regression that generates parameters disaggregated by the spatial units of analysis. Therefore, it allows assessment of the spatial heterogeneity in the estimated

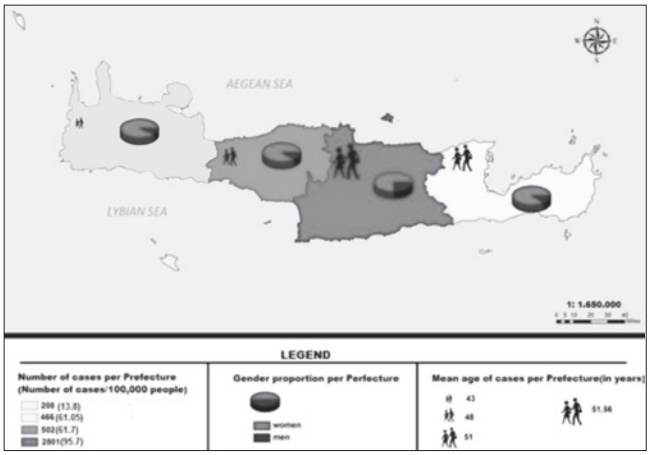

Fig. 1. Spatial distribution of thyroidectomy cases performed, age and gender. The island of Crete is divided in four prefectures, light grey = Chania, grey = Rethymno, dark grey = Heraklion, white = Lasithi.

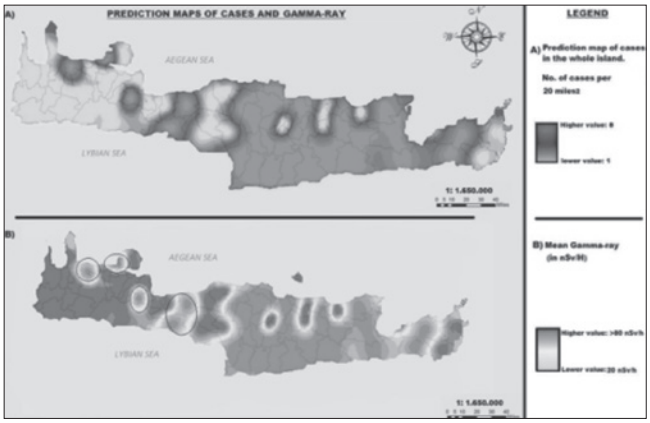

Fig. 2. (A) Prediction map of cases of expected number of thyroidectomies per 50 km^2; **(B)** Prediction map of expected numbers of gamma-ray in nSv/h, in the island of Crete.

relationships between the independent and dependent variables [9 11 12]. The ethics committee for research of the University Hospital of Crete approved the study, based on the anonymous processing of personal health information.

Results

Our series consisted of 4285 patients; 308 patients were from out of Crete, for example islands close to Crete (Santorini, Rhodes), and were were excluded from the study. The percentage of PTC per prefecture was almost standard, about 25%. That means 52 patients in prefecture of Chania, 117 patients in prefecture of Lasithi, 126 patients in the prefecture of Rethymno and 701 patients in prefecture of Heraklion. According to the population its prefecture as it seen in Figure 1, the distribution is heterogeneous among the different prefectures of the island of Crete, ranging from 13.8 patients per 100,000 people in Chania to 95.7 patients per 100,000 people in Heraklion. Rethymnon and Lasithi follow with 61.7 and 61.05 patients per 100,000 people respectively (P < 0.001). Similarly, the age distribution of these patients varies significantly (P = 0.02) among the different prefectures. The mean age of the patients is calculated at the year of 2013 from the database. The mean age of patients in Heraklion is much higher than in the other prefectures (mean age = 51.66). Conversely, the lowest mean age appears in Chania (mean age = 43). The mean age of patients at the time of the Chernobyl accident falls within the range of 18 to 25 years old prospectively. On the other hand, gender distribution is almost homogeneous among the prefectures (P = 0.63), with the proportion of men/women to be 1 (men) in 3 (women).

In order to deal with possible data bias (due to the fact that all patient records originate from a single hospital in Heraklion), the prediction map was produced and estimated the expected number of cases with PTC, in the overall region of Crete (Fig. 2A). Values range from 1 to 8 patients per 50 km^2. A higher number of cases (incidents) were expected in all municipalities of Heraklion and in most of the municipalities of Lasithi (ranging from 4.5 to 8 cases per 50 km^2), whereas lower numbers of patients were expected in Chania (almost 1 to 2 patients per 50 km^2) and in Rethymnon (1.5 to 4 patients per 50 km^2).

Using the same model, a prediction map was created for the mean gamma-ray distribution (Fig. 2B) with values ranging from 20 to > 80 nSv/h. The gamma-ray distribution seems to follow a similar spatial pattern with the case distribution (prediction). Higher values appear in Heraklion and Lasithi (reaching even 60-80 nSv/h), in contrast to Rethymnon and Chania, where lower values are expected although there are regions (hot spots) with extremely high values (70-80 nSv/h). Such regions are marked with red colour and are seen inside the administrative borders of the municipalities of Foinika and Nikiforou Foka in the prefecture of Rethymnon, and Chania, Acrotiriou, Platania and Krioneridas in the prefecture of Chania (outlined with a black circle in Figure 2). Several patients with PTC are observed all over the island identifying the regions where higher values of gamma-ray are distributed, highlighting a strong spatial variation.

Finally, in Table I and Figure 3, the results of the GWR are presented identifying both the risk factors and the high risk areas for PTC at a given time. As observed in Table I, all three factors examined were found to be statistically significant for the distribution of PTC in Crete. Women presented higher risk (ExpB = 2.34; 95% CI = 1.359-3.028; $p < 0.001$) than men, that is compatible to the current literature [13 14], while, for every year of increase in age, the risk for PTC increases almost 2.19 times (95% CI = 1.092-4.517; $p = 0.04$). Finally, the gamma-ray was

Table I. Gamma-ray, gender and age as possible risk factors of the disease in Crete according to the GWR model.

Factors	Exp B (95% CI)	P value
Gender	-	< 0.001
Men	1	-
Women	2.34 (1.359-3.028)	-
Age	2.19 (1.092-4.517)	0.04
Gamma-ray (nSv/h)	-	0.03
< 60	1	-
> 60	2.89 (1.682-4.989)	-

CI = Confidence Interval, Exp B = Exp B = exponentiation of the B coefficient.

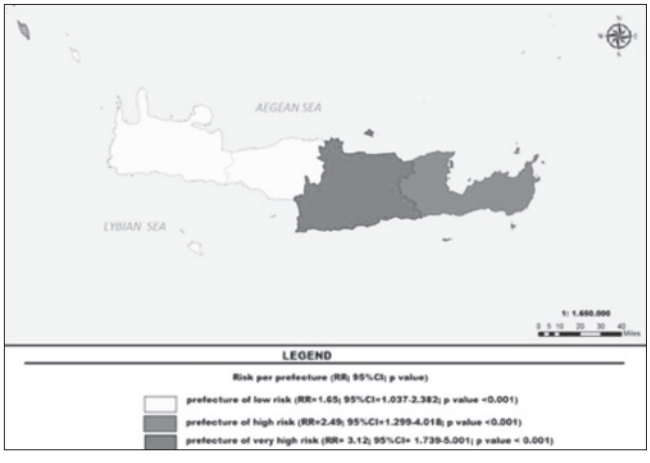

Fig. 3. Map of risk areas per prefecture, in relation to the risk factors, the observed and expected number of PTC, according to the GWR model (RR = relative risk, Exp B = exponentiation of the B coefficient, CI = Confidence Interval).

determined as a significant risk factor that has a proportional correlation with the case distribution (p = 0.03). This is particularly true for the high values of gamma-ray (> 60 nSv/h), which present a higher risk for the disease (OR = 2.89; 95% CI = 1.682-4.989; p = 0.03).

Furthermore, the interaction of these risk factors in relation to the observed and estimated number of patients is demonstrated in a risk map (Fig. 3), which identifies the high risk areas for PTC. Moreover, it outlines the prefectures that call for potential intervention and further research. Thus, the prefectures were clustered in three groups according to the degree of estimated risk (yellow: low risk, orange: high risk, red: very high risk). Even though the prefectures of Chania and Rethymnon were found to present 1.65 times greater risk for PTC in comparison with areas with normal levels of gamma-ray exposure, they were characterised as relatively low risk (95% CI = 1.037-2.383; $p < 0.001$). Additionally, the prefecture of Lasithi presented higher risk (RR = 2.49; 95% CI = 1.299-4.018; $p < 0.001$), while the prefecture of Heraklion presented the highest risk (RR = 3.12; 95% CI = 1.739-5.001; $p < 0.001$). All estimations were found to be statistically significant, identifying areas of high risk with reliability.

Discussion

The increased incidence of thyroid cancer in the island of Crete corresponds to an increase in PTC form of thyroid cancer [13]. It should be mentioned that an increased incidence of PTC was also noticed in the mainland of Greece, but has been correlated with the increased incidence of papillary microcarcinoma detection [14], as well as changes in the diagnostic approach boosted by more careful histopathological examination [15]. Among the risk factors for thyroid cancer, the most established is ionising radiation [16]. Twenty-five years ago, on the 26th of April 1986, a nuclear power plant catastrophe took place in Chernobyl, near Kiev, in the Ukraine leading to the worst nuclear power plant accident in history. Among the residents of Belarus, the Russian Federation and Ukraine, there had been, up to 2002, about 4000 cases of thyroid cancer reported in children and adolescents that were exposed to radiation at the time of the accident [17 18]. Other data obtained from studies involving patients subjected to external radiation show that radiation plays an important role in thyroid carcinogenesis and principally in PTC [18-20]. Although in Greece there is no heavy industry and no use of radioactive agents, there was a remarkable geographical variability of radiation contamination. The radioactive agents should have been delivered to the island of Crete because of the changes of the climatic phenomena in accordance with the climate changes that were occurred the last decades worldwide. For example, the increased radiation contamination in Crete may be correlated with the increased rainfall volume noticed from period after Chernobyl accident. It is noticeable that rainfall volume in May 1986 (33.5 mm) was 2.54 times higher than average (13.16) of the last 50 years, according to Hellenic National Meteorological service [21 22]. The radiation contamination is certainly linked to the "coloured rain phenomenon", as in rain falling from clouds that contain dust from the Sahara Desert and North Africa region, almost covering each year at certain time the entire mainland of Greece [22 23].

Another possible mechanism of transmission is via underground water flow and seawater flow. According to recent research, increased concentrations of radioactive agents such as caesium-137 (Cs-137) and plutonium-239 were detected in the water column along the Algerian costs. The Cs-137 activity concentration in surface water increased from the west to the east, documenting a presence of modified Atlantic water in the region. Higher concentrations observed in deep waters may be due to an intrusion of Levantine intermediate water, which carries higher levels of Cs-137. These agents should be also transported by advection to maintain the observed ratios in deep waters [24].

In the past, dietary patterns have been associated with the aetiology and increased incidence of thyroid cancer. Nowadays, Greek dietary patterns are rich in raw vegetables, thus protective against thyroid cancer, whereas no association with PTC is found [25 26]. It should also be mentioned that the available salt in Greece is iodine enriched; therefore, an association between nutrition and an increase in thyroid cancer cannot be easily established.

Additionally, overtreatment or overdiagnosis was ruled out in a previous study, and our study is based on true evidence of thyroid cancer and consist of patients who were definitely diagnosed with PTC after thyroidectomy. A silent subtype case of PTC represents an accidental finding with no clinical suspicion and sometimes possibly reflects an overdiagnosis. An upward trends of thyroid cancer worldwide has also been associated with increased diagnostic activity because of more sensitive diagnostic tests [27]. The use of more sensitive tests is associated with more accurate diagnosis of early stages of the disease. However, it may not explain the apparent increase in the incidence of larger lesions e.g. > 1 cm. During the last three years in our hospital, we include elastography as a prerequisite test prior to thyroidectomy [28]. Thus, due to the extensive use of elastography, the need for preoperative FNA has been decreased [29] and been used after performing elastography by unclear imaging results.

This study has a high level of significance mainly due to its large sample size and the means of data analysis. Spatial statistics and mathematical polynomials are methods of high scientific significance that provide the ability to test for possible statistical errors and correct the final prediction model.

Conversely, there are also some limitations in this study, initially the fact that all the patient data were obtained only from one hospital in the Prefecture of Heraklion. Even though this is the main referral medical centre in Crete, there is still the General Hospital of Chania in which thyroidectomies are also performed. This is the main reason for the significantly lower number of thyroidectomies in the prefecture of Chania (Fig. 1). Therefore, we tried to overcome this limitation by estimation of the expected number of thyroidectomies (Fig. 2A). Additionally, these data were derived from different periods of time and were not analysed as contemporal data in this study. Thus, a spatio-temporal analysis of these data is suggested in a future study. At the same time, even though the data about gamma-ray were obtained from two reliable, official sources, the estimations were made from specific stations on the island covering most of its surface but not all of it. Although the interval radiation level was not higher than the international recommendation standards for radiation, which is 1 mSV/y, in this study it is shown that there is increase of incidence of the PTA which corresponds to higher radiation levels. The methods of analyses selected for this study aimed to stay within with these limitations and provide data smoothing and estimation of expected values where no data were available.

The spatial regression model helps to identify the risk for disease with statistical confidence, while it offers an interesting result when it was compared with the distribution map (Fig. 1). It also should be mentioned that our hospital is the major institution in the island which refers from all other minor hospitals. In Figure 3, the prefectures of the lowest risk are those of Chania and Rethymnon. This is an important variation since the prefecture of Rethymnon had the second highest rates per 100,000 people (Fig. 1). This indicates a strong predisposition to the future number of patients expected to develop PTC, and to the influence of gamma-ray exposure as a risk factor.

Conclusions

By correlation of geographical distribution of PTC cases in the island with the geographical distribution of radiation, it is obvious that the gamma-ray distribution follows the spatial pattern of the disease and its estimated rates. This is verified not only by observing the two maps (Fig. 2), but also by the results of the GWR model that mathematically proves their significant correlation (Table I).

On the 11th of March 2011 another nuclear accident took place, in Fukushima, Japan. In Japan, a number of prospective epidemiological studies on human health risks from low-dose radiation exposure and comprehensive health protection from radiation have been organised [30]. The public concerns about the long-term health effects of radioactive environmental contamination have increased based on the lessons learnt from the Chernobyl nuclear power plant accident. Our estimates are expected to be verified in the near future and if used by clinicians with the help of a field epidemiologist, could be the basis for future interventions, prevention, screening, or more aggressive treatments.

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Laryngology

Differential chemokine expression patterns in tonsillar disease

Differenti pattern di espressione delle chemochine nella patologia tonsillare

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SUMMARY

Expression profiles of CXC- and CC-chemokines in various forms of tonsillar disease were studied to evaluate whether certain chemokines play a predominant role in a specific subset of tonsillar disease. Total RNA was isolated from 89 biopsies (21 hyperplastic palatine tonsils, 25 adenoids, 16 chronic inflammatory palatine tonsils and 27 chronic inflammatory palatine tonsils with histological prove of acute inflammation), reverse transcribed and subjected to PCR amplifying IL-8, Gro-alpha, eotaxin-1, eotaxin-2, MCP-3, MCP-4 and RANTES. 2% agarose gel electrophoresis revealed a predominance of IL-8 in the chronic inflammatory palatine tonsil group compared to tonsillar hyperplasia. Furthermore, eotaxin-2 was strongly overexpressed in adenoid samples compared to chronic inflammatory specimens. Our data suggest that the majority of diseases related to adenoid formation are mediated via an eotaxin-2 expression, whereas chronic inflammatory tonsillitis is associated with IL-8 upregulation. These data imply that adenoids are related to a Th-2, and chronic inflammatory tonsillitis to a Th-1 based immune response.

KEY WORDS: Chemokines• Tonsillar disease • Eotaxin-2 • Interleukin-8

RIASSUNTO

Sono stati valutati i profili di espressione delle chemochine CXC e CC in varie patologie tonsillari allo scopo di valutare quali specifiche chemochine abbiano un ruolo predominante nel determinare specifiche patologie tonsillari. L'RNA è stato isolato da 89 biopsie (21 tonsille palatine iperplastiche; 25 adenoidi; 16 tonsille con infiammazione cronica e 27 tonsille con infiammazione cronica e diagnosi istologica di infiammazione acuta), inversamente trascritto, e sottoposto ad amplificazione per IL-8, Gro-alpha, eotaxin-1, eotaxin-2, MCP-3, MCP-4 e RANTES. L'elettroforesi su gel di agarosio al 2% ha rivelato una predominanza di IL8 nel gruppo delle tonsille con infiammazione cronica rispetto al gruppo delle tonsille con iperplasia. Al contrario l'eotaxina 2 era fortemente iperespressa nel gruppo delle adenoidi, se comparata al gruppo con infiammazione cronica. I nostri dati suggeriscono che la maggior parte delle patologie correlate con la formazione delle adenoidi sono mediate dall' espressione di eotaxina 2, mentre la tonsillite cronica infiammatoria è associata all' iperespressione di IL8. Questi dati implicano che le adenoidi sono correlate ad una risposta immunitaria Th2, mentre la tonsillite infiammatoria cronica ad una risposta Th1.

PAROLE CHIAVE: *Chemochine • Patologie tonsillari • Eotaxina-2 • Interleukina-8*

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Introduction

In 1884, Waldeyer first described an annular arrangement of lymphatic tissue at the entrance of the aerodigestive tract, which he suggested plays an important part in the host immune system, including the palatine tonsils, pharyngeal tonsil, lingual tonsils and tubal tonsils [1]. Especially the palatine as well as pharyngeal tonsils are known to play a key role in the development of the human host defense due to their location, histologic anatomy and functional capacities [2]. These structures harbour numerous different types of tissues, including epithelium, lymphoid follicles, blood vessels and connective tissues [3 4]. The combination

of these different tissues as well as their anatomic arrangement in crypts facilitates optimised exposure and processing of antigens, which aid in evoking effective immune responses [5 6]. The palatine as well as the pharyngeal tonsils are secondary lymphoid organs and an integral part of the mucosa associated lymphatic tissue complex [7]. Multiplication, differentiation and stimulation of B-lymphocytes represent one of their major functions and are essential in processing the specific immune response [8]. B-lymphocytes constitute more than 50% of the tonsillar lymphocyte population, in contrast to the blood and other lymphoid organs [9 10]. The induction of the tonsillar-specific immune responses involves several distinct processes where chemokines play an integral role [11-14]. Chemokines are chemotactic cytokines, which can be classified into four subgroups depending on their arrangement of amino acids, namely CXC-, CC-, C- and CX_3C-chemokines [15 16]. CXC-chemokines, like interleukin (IL)-8 and growth-regulated peptide – alpha (GRO-alpha), are responsible for chemotaxis of neutrophils, whereas CC-chemokines, like eotaxin-1, eotaxin-2, monocyte chemotactic protein (MCP)-3, MCP-4, and RANTES (*R*egulated upon *A*ctivation, *N*ormal *T*-cell *E*xpressed and *S*ecreted), promote the attraction of lymphocytes, monocytes, eosinophils and basophils [17].

The presented work investigated the importance of different chemokines, i.e. IL-8, GRO-alpha, eotaxin-1, eotaxin-2, MCP-3, MCP-4 and RANTES in tonsillar inflammatory disease and tonsil hyperplasia.

Materials and methods

Tissue samples

This study included 89 patients who underwent surgery at the Department of Otorhinolaryngology, Head and Neck Surgery at the University of Schleswig-Holstein, Campus Kiel, Germany, for hyperplasia of either the palatine or pharyngeal tonsil, or chronic inflammation of the palatine tonsil, defined as recurrent acute tonsillitis over a period of > 6 months. Samples were retrieved during surgery after written patient consent was obtained, in accordance with the ethical commission of the Christian-Albrechts-University of Kiel, subjected to the 1975 Helsinki Declaration. Tissue samples were divided into four subgroups, based on origin, patient past medical history, clinical presentation and histologic diagnosis established by certified pathologists of the University of Schleswig-Holstein, Campus Kiel. The subgroups included 21 hyperplastic palatine tonsils, 25 adenoids, 16 chronic inflammatory palatine tonsils and 27 chronic inflammatory palatine tonsils with histologic signs of acute inflammation as per presence of cryptal ulcerations and leukocyte infiltration. At the time of procedure, the median of age for each subgroup was 6, 5, 26, and 7, respectively. Immediately after collection, samples were frozen in liquid nitrogen, and stored at -80°C for further processing.

RNA isolation

Frozen tissue samples were ground by mortar, and 1 ml of TRIzol™ reagent (Gibco, Ingolstadt, Germany) was applied upon 200 mg of tissue. Total RNA was isolated following the manufacturer's instructions. After determination of the RNA content using the UVICON-931 UV-spectralphotometer (Kontron, Hamburg, Germany), samples of total RNA were adjusted to 1.0 µg for first strand cDNA synthesis. Quality assessment of the RNA was conducted using a 1% agarose ethidium-bromide stained gel electrophoresis.

Reverse transcription

1.0 µg RNA was heat-denatured (65°C, 10 min), chilled on ice, and subjected to random hexadeoxynucleotide primed reverse transcription using the first strand cDNA synthesis kit (Pharmacia, Freiburg, Germany). Reverse transcription (final volume 15 µl) was conducted at 37°C for 60 min in the presence of 0.2 µM of random hexanucleotide primer and 40 U RNase inhibitor (RNAsin, Gibco, Germany). Following synthesis of the completed first strand cDNA the resulting RNA-cDNA double-stranded helix was heat-denatured (95°C, 5 min) to provide cDNA as a template for polymerisation.

Primers

We used the following oligonucleotides for high-stringency PCR reaction as listed below. Glutaraldehyde-3-phosphate-dehydrogenase (G3PDH) was used to compare expression of the genes mentioned below:

RANTES sense: 5'-CAT CCT CATT GCT ACT GCC CTC TG-3', *RANTES* antisense: 5'-TAA CTG CTG CTC GTC GTG GTC-3'; *Eotaxin-1* sense: 5'-CAT CCT CAT TGC TAC TGC CCT CTG-3', *Eotaxin-1* antisense: 5'-CGG GTT CAC GCC ATT CTC CT-3'; *Eotaxin-2* sense: 5'-CAC ATC ATC CCT ACG GGC TCT-3'; *Eotaxin-2* antisense: 5'-GGT TGC CAG GAT ATC TCT GGA CAG GG-3'; *MCP-3* sense: 5'-GAG CTA CAG AAG GAC CAC CAG T-3', *MCP-3* antisense: 5'-AAG TCC TGG ACC CAC TTC TG-3'; *MCP-4* sense: 5'-TCA TCT TTC CAC AAT AAC ATA TTT A-3', *MCP-4* antisense: 5'-GTT TAT TTG AGT ATT GCT GAT CTT T-3'; *IL-8* sense: 5'-CTT TCA GAG ACA GCA GAG CAC-3', *IL-8* antisense: 5'-ACT GTG AGG TAA GAT GGT GGC-3'; *GRO-alpha* sense: 5'-TGA ACT GCG CTG CCA GTG C-3', *GRO-alpha* antisense; *G3PDH* sense: 5'-CATC-CTCATTGCTACTGCCCTCTG-3', *G3PDH* antisense: 5'-ATGAGCCCCAGCCTTCTCCAT-3'.

Polymerase chain reaction (PCR)

Reverse transcribed cDNA products (0.2 5µl) were incubated in 50 µl reaction mixture containing 0.2 µM 5'-3' sequence specific sense oligonucleotide primers, 0.2 µM of 3'-5' corresponding antisense oligonucleotide primers, 200 µM dNTP's, 1.5 mM $MgCl_2$, 5.0 µl 10x PCR-buffer, and 2.5 U Taq-polymerase (Gibco, Ingolstadt, Germany). The reaction mixture was covered with a mineral oil layer (Applied Biosystem, Weiterstadt, Germany) to prevent evaporation. The PCR was conducted in a Biometra T_3 thermocycler. Following initial denaturation (3 min at 95°C), high stringency PCR was run for 34 cycles (94°C for 75 sec, 60°C for 30 sec, and 72°C for 2 min) with an increased annealing temperature of 67°C in the first two cycles, to amplify the *RANTES*, *Eotaxin-1*, *Eotaxin-2* and *G3PDH* cDNA. In case of the other chemokines, the PCR parameters were modified to a 40 cycles of 95°C for 60 sec, 60°C for 30 sec, and 72°C for 2 min, with an increased annealing temperature of 68° to 60° over the first 8 cycles. After PCR, all samples were subjected to ethidium-bromide stained 1.5% agarose gel electrophoresis.

Densitometry

The amplicons were evaluated in quantity using Herolab E.A.S.Y. Win32 software (Herolab, Wiesloch, Germany). At first, G3PDH bands were compared among each other, in order to assess relative sample signal strength. Subsequently, all other signals of the chemokine bands were adjusted to the relative strength by division through the G3PDH band signal.

Statistical analysis

All densitometric data obtained from the SQRT-PCR were analysed using SPSS 9.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). All data assembled in this study were tested for normal distribution using the Kolmogoroff-Smirnov test. Expression profiles of each chemokine were analysed among each group using simple block variance analysis and Kruskal-Wallis test for normally and non-normally distributed data, respectively. A p-value < 0.05 was considered significant, and a p-value < 0.01 was considered highly significant.

Results

Quality assessment of the isolated RNA was made by agarose gel electrophoresis exhibiting non-fragmented RNA with sufficient quantity for reverse transcription and subsequent processing. After RT-PCR procedures, samples were processed by agarose gel electrophoresis, and the amplicons of the chemokines were measured and adjusted to the relative G3PDH signal strength, comparative analysis was initiated. All data followed normal distribution and are displayed as mean ± SD in the following.

Four cohorts were examined: patients with tonsillar hyperplasia (n = 21), adenoids (n = 25), chronic tonsillitis (n = 16) and chronic tonsillitis with histological proof of acute inflammation (further in the text referred to as "acute tonsillitis") (n = 27).

RANTES and Eotaxin

Relative RANTES total-mRNA expression showed a median of 1.37 for tonsil hyperplasia 1.58 for adenoids, 1.32 for chronic tonsillitis and 1.56 for acute tonsillitis (Table I, Fig. 1A). As shown in Table II and Fig. 1B, relative mRNA expression for eotaxin-2 was 1.02 in tonsillar hyperplasia (median value), 1.34 for adenoids, 1.16 for chronic tonsillitis and 1.16 for acute tonsillitis. A significant overexpression of eotaxin-2 was observed in adenoids compared to patients with chronic tonsillitis ($p < 0.05$).

IL-8 and GRO-alpha

Relative expression of IL-8 are shown in Table III and Figure 2A. A relative IL-8-mRNA expression was found to be a median of 1.12 in tonsillar hyperplasia, 1.97 for adenoids, 1.82 for chronic tonsillitis and 1.41 for acute tonsillitis. IL-8 was significantly overexpressed in patients with chronic inflammatory tonsillar disease (with and without acute inflammation) compared to tonsil hyperplasia. Relative GRO-alpha mRNA expression levels are shown in Table IV and Figure 2B. Median values were 0.84 for tonsillar hyperplasia, 1.07 for adenoids, 0.60 for chronic tonsillitis and 0.55 for acute tonsillits.

All other examined cytokines were not significantly expressed in the groups analysed.

Discussion

Waldeyer's tonsillar ring acts as the first line of immune defence against microbes, entering the body nasally or orally. Especially in children, the immunogenic properties of the palatine tonsils are of particular importance. Chemokines are small signalling proteins, whose expression in various tissues is variably regulated during immune responses as well as acute and chronic infection. Previous studies have suggested a functional role for chemokines in hepatitis, colitis, pancreatitis, asthma and various malignancies as well as acute and chronic infections of the upper aerodigestive tract [18-23]. Most chemokines are only produced and secreted upon appropriate stimulation of cells by bacterial or viral products [24]. In cases of acute and chronically infected tonsils, accumulation of certain subsets of chemokines and neutrophilic dynamics has been observed [25].

Table I. Relative expression of total RNA for RANTES. A total of 89 tissue samples were analysed.

	Tonsillar hyperplasia (n = 21)	Adenoids (n = 25)	Chronic tonsillitis (n = 16)	Acute tonsillitis (n = 27)
Minimum	0,41	0.00	0.54	0.00
1. Quartile	1.07	1.34	0.97	1.15
Median	1.37	1.58	1.32	1.56
3. Quartile	1.79	2.11	1.45	1.97
Maximum	5.57	2.46	1.95	2.46

Table II. Relative expression of total RNA for Eotaxin-2. A total of 89 tissue samples were analysed.

	Tonsillar Hyperplasia (n = 21)	Adenoids (n = 25)	Chronic tonsillitis (n = 16)	Acute tonsillitis (n = 27)
Minimum	0.55	0.40	0.63	0.30
1. Quartile	0.89	1.16	0.84	0.80
Median	1.02	1.34	0.95	1.16
3. Quartile	1.31	1.60	1.21	2.14
Maximum	3.83	2.50	1.82	2.86

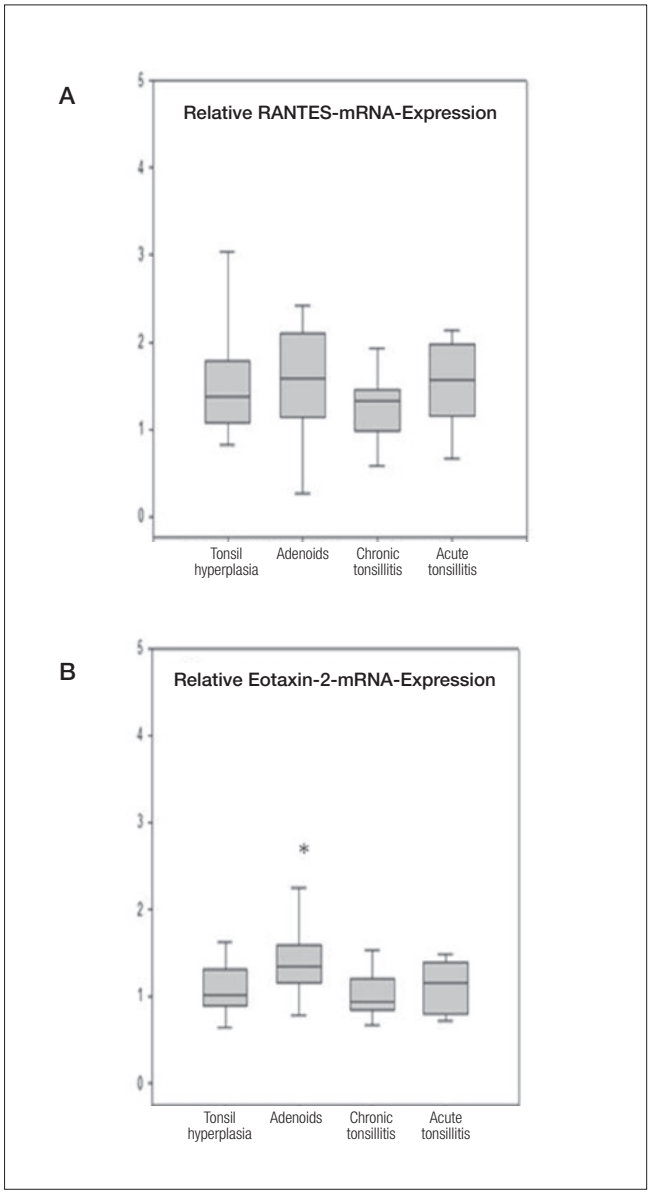

Fig. 1. (A) Relative expression of total RNA for RANTES. **(B)** Relative expression of total RNA for eotaxin-2. Expression was correlated to the expression of the housekeeping gene G3PDH in each sample. The plots represent 89 tissue samples. * $p < 0.05$. ** $p < 0.01$.

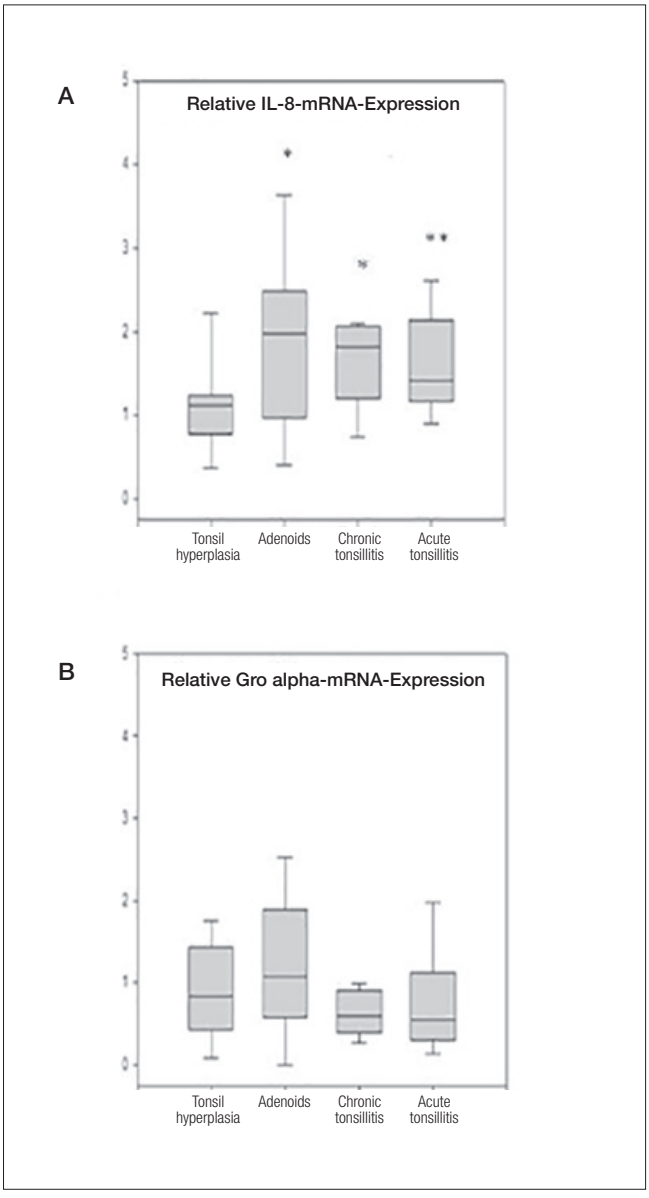

Fig. 2. (A) Relative expression of total RNA for IL-8. **(B)** Relative expression of total RNA for gro-alpha. Expression was correlated to the expression of the housekeeping gene G3PDH in each sample. The plots represent 89 tissue samples. * $p < 0.05$. ** $p < 0.01$.

Table III. Relative expression of total RNA for IL-8. A total of 89 tissue samples were analysed.

	Tonsillar hyperplasia (n = 21)	Adenoids (n = 25)	Chronic tonsillitis (n = 16)	Acute tonsillitis (n = 27)
Minimum	0.13	0.27	0.61	0.52
1. Quartile	0.78	0.98	1.20	1.17
Median	1.12	1.97	1.82	1.41
3. Quartile	1.24	2.50	2.06	2.14
Maximum	2.55	4.98	2.90	2.86

Table IV. Relative expression of total RNA for Gro-alpha. A total of 89 tissue samples were analysed.

	Tonsillar hyperplasia (n = 21)	Adenoids (n = 25)	Chronic tonsillitis (n = 16)	Acute tonsillitis (n = 27)
Minimum	0.00	0.00	0.00	0.00
1. Quartile	0.43	0.59	0.40	0.30
Median	0.84	1.07	0.60	0.55
3. Quartile	1.43	1.90	0.91	1.12
Maximum	2.44	3.33	1.28	2.97

IL-8 has been described to be very potent in neutrophil activation and migration [26 27]. Our data shows significant overexpression of IL-8 in chronic inflammatory tonsillar disease. Since IL-8 expression has been reported to be effectively stimulated by TNF-α und IL-1ß for neutrophil chemotaxis as well as activity, our data suggests that a Th1 response is predominatly involved in the pathogenesis of chronic tonsillitis.

Both IL-8 and GRO-α are known to be synthesised by neutrophils and fibroblasts in response to various stimuli [28 29]. In vitro, IL-1β and TNF-α seem to be potent stimulators of chemokine production, whereas IFN-γ inhibits their production [18]. GRO-alpha is further produced by endothelial cells, fibroblasts and monocytes after stimulation with lipopolysaccharide, IL-1 or TNF-α *in vitro*. In addition, it induces neutrophil accumulation and chemotaxis [29]. In our data however, GRO-alpha was not significantly overexpressed in chronic tonsillar diseases.

Tonsillar hyperplasia appears to be a result of increased proliferation of lymphoid tissue predominantly triggered by bacterial infections. Previously, tonsil size has been shown to be directly proportional to the mean bacterial load [30]. The kind of bacteria found in hyperplastic tonsils does not seem to greatly differ from those in recurrently active infected tonsils. However, Haemophilus infection, besides Staphylococcus aureus and Streptococcus pyogenes appears to be more common in tonsillar hyperplasia [30]. Our investigations demonstrate that hyperplastic tonsillitis is characterised by an acute inflammatory chemokine pattern as IL-8 expression on mRNA levels correlates with the presence of actively infected tissue. IL-8 expression was significantly elevated in acutely infected tissue compared to hyperplastic tonsils ($p < 0.01$), and in adenoids and chronic tonsillitis compared to hyperplastic tonsils ($p < 0.05$). Therefore, as IL-8 is an acute phase chemokine expressed in chronic tonsillitis, this suggests an inflammatory process in the pathogenesis of chronic tonsillitis. An elevation of IL-8 in acute infections has been described previously [31 32], and therefore anticipated for tonsillar disease. However there have been studies showing an equal expression of IL-8 in hyperplastic tonsils and chronic tonsillitis [33]. Another reason for a high expression of this chemokine in chronic and hyperplastic disease could be its additional extensive effect on cell proliferation [34].

A similar result would have been expected for the expression of GRO-alpha, since this chemokine also plays an important role in host immune defence by conveying chemotaxis and activation of neutrophils, similar to IL-8. However, in our data, mRNA expression levels of GRO-alpha were not significantly elevated in acute nor chronic tonsillitis compared to tonsillar hyperplasia.

MCP 1-4, RANTES, eotaxin, eotaxin-2 und eotaxin-3 are CC-chemokines. RANTES is a selective attractant for T-cell and monocytes migration [35]. Proinflammatory cytokines, such as TNF-α or IL-1ß, have been described to be some of the most potent stimulators of RANTES expression. Furthermore, a combination of TNF-α and INF-χ strongly stimulate production of RANTES [36]. Another important function of RANTES is its ability to enhance the mucosal as well as systemic humoral production of antibodies, via an elevation of the production of IFN-χ-, IL-2-, IL-5-and IL-6, and further an induction of co-stimulatory molecules as well as expression of cytokine receptors for CD 4^+T cells [37].

In our data, RANTES expression was evident in all subgroups analysed with no significant differences in relative mRNA expression levels within these groups.

Eotaxin-2 is also described to be a potent chemoattractor for eosinophils *in vitro* and *in vivo* [38 39]. In eosinophils, eotaxin-2 causes a dose-dependent increase of the production of free radicals, mobilisation of intracellular calcium and upregulation of CD11b [40].

Eotaxin-2 expression was evident in all analysed subgroups with a significant upregulation in adenoids, suggesting an involvement of Th2- immune responses as eotaxin-2 which is known to play a crucial role in the pathogenesis of atopic diseases involving Th 2- cell activation [41]. Significant upregulation of eotaxin-2 has been

observed in bronchial asthma [42] and allergic rhinitis [43]. However, the significance of eotaxin-2 expression in lymphatic tissue of the Waldeyer's tonsillar ring still largely remains uncertain, like their role in the maturation of the adaptive immune system in the mucosa of the upper aerodigestive tract. However, it is remarkable that eotaxin-2 as well as RANTES are constitutively expressed in the mucosa of the gastrointestinal tract, an organ with distinctive antigen contact, such as Waldeyer`s tonsilar ring [44 45]. To further evaluate the role of the chemokines analysed in the function of the lymphatic tissue of the Waldeyer's tonsillar ring, it is essential to evaluate their expression on protein level to examine alterations through potential post-transcriptional splicing. Also, immunohistochemical studies would be of interest to evaluate their expression in relation to certain cell populations. These studies would greatly aid in understanding the pathophysiology of tonsillar disease, from which patients could benefit in the future.

Conclusions

The presented data suggest that the majority of diseases related to adenoid formation are mediated via an eotaxin-2 expression, whereas chronic inflammatory tonsillitis is associated with IL-8 upregulation. Thus, these data imply that adenoids are related to a Th-2 response, and chronic inflammatory tonsillitis to a Th-1 based immune response.

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Rhinology

Effectiveness of endoscopic septoplasty in different types of nasal septal deformities: our experience with NOSE evaluation

Efficacia della settoplastica endoscopica nei vari tipi di deformità settale: la nostra esperienza con il questionario NOSE

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SUMMARY

Septal deviations are the most frequent cause of nasal obstruction, and represent a common complaint in rhinologic practice. Since the first description of Lanza et al. in 1991, the use of the endoscope for the correction of septal deformities is increasingly more frequent. The purpose of this study is to evaluate the effectivenes of the endoscopic septoplasty for the correction of each of the 7 types of septal deformities according to the Mladina's classification. A retrospective chart review was performed in 59 consecutive patients presenting to our Department for Endoscopic Septoplasty from February 2012 to August 2014. For each deviation, descriptive statistics (mean and standard deviation, significant increase/decrease) was used to asses the corrective capacity and time-dependent effects at follow-up. This study shows that the corrective power of endoscopic septoplasty is different according to the type of deviation. To our knowledge this is the first study that evaluates the corrective capacity of this technique for each deviation by analysing pre- and postoperative objective outcomes as well as subjective outcomes gathered from the validated NOSE questionnaire. Even if endoscopic septoplasty may now be considered a reliable alternative to the classic technique, it is essential to identify the right deformity preoperatively in order to provide the correct therapeutic choice.

KEY WORDS: Endoscopic septoplasty • Septal deviation • NOSE scale • Septal deformities • Cottle's area

RIASSUNTO

Le deviazioni del setto sono la causa più frequente di ostruzione nasale e rappresentano un problema comune nella pratica rinologica. L'uso dell'endoscopio per la correzione delle deformità del setto dalla prima descrizione di Lanza et al. nel 1991 ad oggi è sempre più frequente. Lo scopo di questo studio è quello di valutare l'efficacia della settoplastica endoscopica per la correzione di ciascuno dei 7 tipi di deformità del setto secondo la classificazione di Mladina. Una revisione retrospettiva è stata eseguita in 59 pazienti che si sono presentati presso il nostro Dipartimento per essere sottoposti a settoplastica endoscopica da febbraio 2012 ad agosto 2014. Per ogni deviazione, è stata effettuata un'analisi statistica descrittiva (media e deviazione standard, aumento/ diminuzione significativa) al fine di valutare la capacità correttiva e gli effetti al follow-up. Questo studio ha dimostrato che il potere correttivo della settoplastica endoscopica è diverso a seconda del tipo di deviazione. Questo è il primo studio che valuta la capacità di correzione di questa tecnica per ogni deviazione, analizzando i risultati oggettivi pre e post-operatori, nonché gli esiti soggettivi raccolti dal questionario NOSE. Anche se la settoplastica endoscopica può essere considerato ora una alternativa affidabile alla la tecnica classica è essenziale identificare preoperatoriamente il tipo di deformità al fine di fornire la corretta scelta terapeutica.

PAROLE CHIAVE: *Settoplastica endoscopica • Deviazione settale • NOSE scale • Deformità settale • Area di Cottle*

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Introduction

Septal deviations are the most common cause of nasal obstruction, representing a common compliant in rhinologic practice. Since its introduction, procedures for correction of nasal septal deformities have undergone several modifications, from radical septal resection, to possible preservation of septal framework and nasal mucosa. Frequently, septal deformities can be associated with lateral wall diseases or may be the cause of them. A significantly deviated nasal septum has been implicated in epistaxis, sinusitis, obstructive sleep apnoea and headaches attributable to contact point with structures of lateral nasal wall [1].

For this reason, correction of septal deformities cannot be separated from treatment of disorders of the lateral wall when present. Thus, endoscopic septoplasty is a useful technique for treating symptomatic deformities, but also for improving intraoperative surgical access to lateral nasal wall surgeries (e.g. dacryocystorhinostomy, functional endoscopic sinus surgery) [2,3].

Since the first description by Lanza et al. in 1991, the use of the endoscope for the correction of septal deformities is increasingly more frequent [4].

In the literature there is an increase of the consensus in favour of endoscopic septoplasty compared to a conventional approach. However, to date, no author has focused attention on the effectivenes of endoscopic correction, considering all types of septal deformities. More than 20 years ago, Mladina published a systematic classification of septal deformities, precisely defining clinical findings at the nasal septum, and proposing seven different types of deformity [5,6].

The purpose of this study is to evaluate the effectivenes of the endoscopic septoplasty for the correction of each of the 7 types of septal deformities according to Mladina's classification.

Materials and methods

A retrospective chart review was performed in 184 consecutive patients presenting to our Department for endoscopic septoplasty during a 30-month period (February 2012 to August 2014). The patients were 22 females and 37 males with a mean age of 34.9 years, ranging from 18 to 69. Inclusion criteria were as follows: at least 17 years old, septal deformity with nasal obstruction, persistent symptoms after at least a 4-weeks of therapy including topical nasal steroids in combination or not with antihistamines. Patients with sinonasal malignancy, being in need of nasal surgery other than septoplasty (such as functional endoscopic sinus surgery – FESS –, nasal valve surgery, turbinate surgery etc.), sinonasal infections, sinonasal inflammatory disease, were excluded from the study.

Given the presenting symptoms of patients that may suggest some forms of rhinosinusitis (chronic or acute recurrent forms), all patients were preoperatively evaluated by paranasal sinus computed tomography (CT) (120 kV, 215 mA s, 1 mm slice thickness).

Among the 184 patients studied, 125 were excluded for the presence of radiological signs of chronic rhinosinusitis with some anatomical variants as follows: inferior turbinate hypertrophy in 93% of cases, middle turbinate pneumatisation in 37% of cases, uncinate process pneumatisation in 8% and dysventilated sinuses in 60% of cases.

Therefore, 59 patients (32%) fulfilled the inclusion criteria for the present study. The most frequent symptoms encountered were nasal obstruction in all cases; facial pain in 27 cases and postnasal drip and headache in 7 cases each. All patients were submitted to allergic evaluations with skin prick tests for inhalants. The degree of septal deviation was calculated using OSIRIX® Software (Pixameo SARL, Bernex, Switzerland, 2003-2014). The angle defined by a line passing through the most deviated point and a line perpendicular to the floor of the nose was calculated to determine the degree of the septal deviation (Fig. 1). Moreover, nasal spaces were directly assessed by nasal endoscopy in all cases.

Using these examinations, we were able to stratify the patient cohort into seven groups based on Mladina's classification of nasal septum deviation (Table I).

All patients included in our study underwent endoscopic septoplasty according to the technique described herein.

Table I. Classification proposed by Mladina.

Type 1	Unilateral vertical septal ridge in the valve region that does not reach the valve itself; it does not change the physiologic valve angle (15%) and therefore usually plays just a mild role in the nasal pathophysiology
Type 2	Unilateral vertical septal ridge in the valve region that touches the nasal valve, thus reducing the physiologic valve angle (15%)
Type 3	Unilateral vertical ridge that is located more deeply in the nasal cavity, opposite the head of the middle turbinate
Type 4	Bilateral deformity consisting of type 2 on one side and type 3 on the other
Type 5	Almost horizontal septal spur that sticks laterally and deeply into the nasal cavity. The opposite side of the nasal septum is always flat
Type 6	Massive unilateral intermaxillary bone wing with a "gutter" between it and the rest of the septum on this septal side. On the other septal side, there is an anteriorly positioned basal septal crest.
Type 7	Very variable combination of the previous types

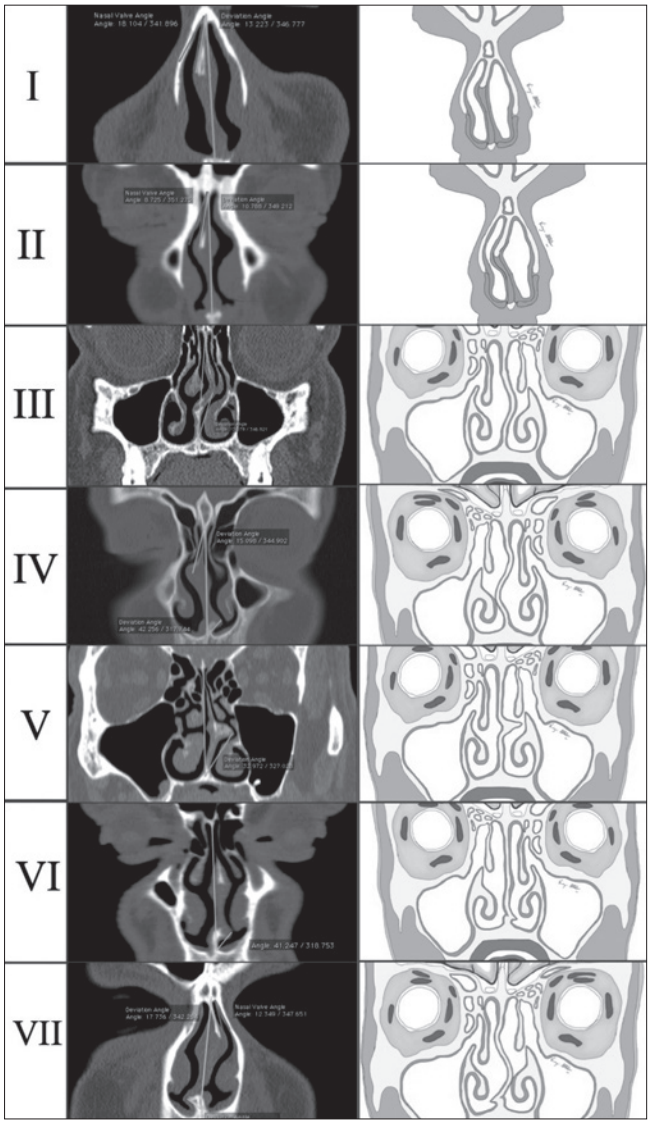

Fig. 1. The seven types of septal deviations proposed by Mladina. In the first column, CT scans processed with OsiriX program are shown; the second column shows schematic illustrations for each deviation.

The procedure was performed under general anaesthesia. The septum was injected with 1% xylocaine in 1:20,000 epinephrine on the convex side of the septum using a 0° rigid 4 mm Hopkins Rod Lens endoscope.

In Mladina's type 5 and 6 (Cottle's area IV and V) deformity, a horizontal hemitransfixation incision was made, parallel to the nasal floor on the apex of the spur to expose the most deviated part (Fig. 2a). A submucoperichondral flap was raised using a Cottle elevator under endoscopic visualisation to expose the underlying bone at the most deviated part. To avoid contralateral mucosal damage, careful submucoperichondral dissection on the opposite side was performed using a Cottle elevator. Flaps were elevated superiorly and inferiorly to expose the underlying bony or cartilaginous spur (Fig. 2b). The bony protrusion was removed using a chisel placed on the base of the spur.

In Mladina types 2, 3 and 4 deformities (Cottle's area I, II, III), we performed an "endoscopic assisted septoplasty". A vertical incision was made on the concave side of the septum to expose the abnormality at the bony cartilaginous junction. The initial mucoperichondrial flap was elevated using Freer's elevator and nasal speculum. Further elevation was done using 0° rigid nasal endoscope (4 mm), held in the left hand, keeping the tip of the endoscope between the mucoperichondrial flap and the septal cartilage (Fig. 2c). The right hand was used for instrumentation. Flap elevation in the correct cleavage plane to minimise bleeding. Exposure was limited to the target area. A subluxated cartilage from the crest was shaved using a No. 15 blade Bard parker knife to resect the excess cartilage inferiorly, without dislocating the vomero-chondral junction (Fig. 2d).

In all cases mucosal flaps were repositioned back in place and is fixed using a silastic stent in order to avoid the mucosal damage during packing removal. Nasal packing was placed in both nasal fossae (Merocel, Medtronic, Mystic, CT, USA) and were removed after 48 hours.

Patients were usually discharged after 48 hours. All pa-

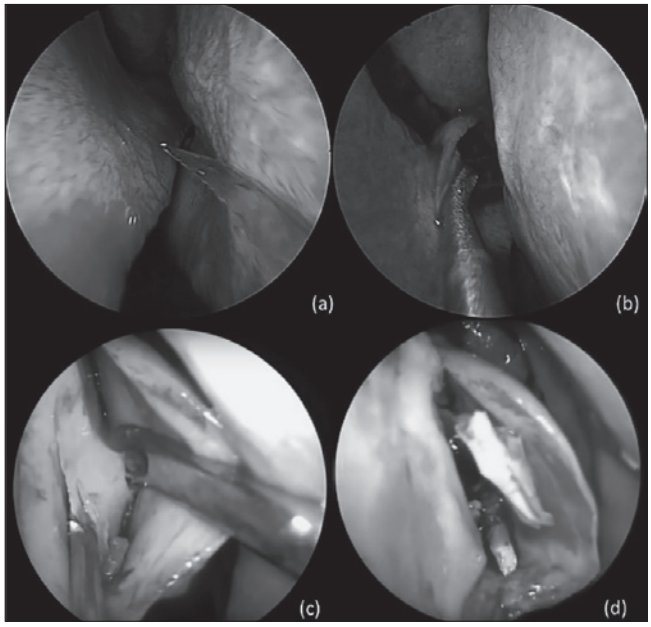

Fig. 2. Intraoperative picture showing: **a)** horizontal hemitransfixation incision parallel to the nasal floor on the apex of the septal spur; **b)** flaps elevation to expose the underlying bony or cartilaginous spur; **c)** 0° rigid nasal endoscope (4 mm), inserted between the mucoperichondrial flap and the septal cartilage; **d)** Cartilage excess resection without dislocating the vomero-chondral junction.

tients received post-operative antibiotic therapy with oral cephalosporin for one week, saline nasal douching and oral steroids with decreasing dosage.

The main outcome measure used in the study was the NOSE scale (Nasal Obstruction Symptom Evaluation) including a grading score from 0 to 5 (Fig. 3). All patients were asked to complete the NOSE scale one week before surgery and then at 3 and 6 months post-operatively. Non-parametric analysis (Wilcoxon signed rank test) was used to compare baseline and follow-up NOSE scores. P values < 0.05 were considered statistically significant. For each deviation, descriptive statistics (mean and standard deviation, significant increase/decrease) were used to assess the possibility to correct each type of deviation.

Nasal endoscopy was performed in all patients at given intervals (15 days, 1 month, 3 months, and 6 months after surgery) to assess possible complications.

Results

Mean follow-up time was 6.3 months (range 3-14 months).

The patient cohort was divided according to the Mladina classification as follows: type 5 was the most frequent deviation observed (23.7%, 14 cases); type 3 and 6 were also relatively frequent (20.3%, 12 cases and 18.6%, 11 cases, respectively); types 2 and 1 were observed in equal frequency (13.5%, 8 cases and 11.8%, 7 cases, respectively); types 4 and 7 were rare (6.7%, 4 cases and 5%, 3 cases, respectively).

Nasal obstruction and Septoplasty Effectiveness Scale (NOSE)

Surname……Name……Date……

For the patient: This test is useful to unterstand better the actual impact of nasal obstruction on the quality of his life.

Considering the last month, quantify the impact of each problem listed on its quality of life.

Indicate with X the most correct answer

	Not a problem	Very mild problem	Moderate problem	Fairly bad problem	Severe problem
1. Nasal obstruction and stuffiness	0	1	2	3	4
2. Nasal obstruction	0	1	2	3	4
3. Trouble breathing through my nose	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Unable to get enough air through my nose during exercise	0	1	2	3	4

The test should be repeated after 3 and 6 months after surgery

Fig. 3. The NOSE questionnaire.

Table II. Our cohort divided according to the Mladina classification. Disease-specific QOL scores (mean and standard deviation) assessed with the NOSE scale at baseline; 3 and 6 months after surgery.

Deviation	Sample rate (%)	Baseline	3 months	6 months
I	11.8%	14.1 ± 1.2	12.3 ± 1.7	12.0 ± 1.9
II	13.5%	16.0 ± 1.0	15.6 ± 1.0	15.5 ± 1.0
III	20.3%	15.0 ± 0.8	14.5 ± 0.9	14.1 ± 0.6
IV	6.7%	17.8 ± 0.5	11.0 ± 3.7	10.2 ± 3.3
V	23.7%	18.1 ± 1.3	0.8 ± 0.4	0.7 ± 0.4
VI	18.6%	17.6 ± 1.4	7.9 ± 7.7	7.7 ± 6.7
VII	5%	16.6 ± 1.2	15.3 ± 0.6	15 ± 1.0

The disease-specific QOL scores assessed with the NOSE scale at different intervals of time are detailed in Table II. Compared to baseline, the scores registered at 3 and 6 months after surgery showed significant improvement in nasal symptoms ($p < 0.05$). The results are shown in Table III.

Significant decreases in nasal obstruction, trouble sleeping, snoring and mouth dryness in the morning were observed between the preoperative period and 3 months after septoplasty. On the other hand, no statistically significant differences between the 3 and 6 month scores were observed.

The analysis of the NOSE scores for each deformity showed a different corrective power depending on the type of deviation treated. More in detail, the greater corrective capacity was found for the deviation types 5 and 6, and then gradually decreased for the septal deviation types 4, 1 and 7, becoming very limited for types 3 and 2. This trend remained unchanged over time (3 month - 6 month follow-up) (Fig. 4).

In our series, 1 septal abscess (Mladina type 4) and 1 saddle nose deformity (Mladina type 2) were reported after endoscopic septoplasty. No haematoma, no synechiae, or perforations were observed.

Table III. Wilcoxon non-parametric test to compare QOL scores registered at baseline, 3 and 6 months after surgery.

Deviation	P-value	
I	$T_0 - T_1$	0.015**
	$T_1 - T_2$	0.172
	$T_0 - T_2$	0.019**
II	$T_0 - T_1$	0.080
	$T_1 - T_2$	0.998
	$T_0 - T_2$	0.080
III	$T_0 - T_1$	0.017**
	$T_1 - T_2$	0.082
	$T_0 - T_2$	0.005***
IV	$T_0 - T_1$	0.046**
	$T_1 - T_2$	0.058
	$T_0 - T_2$	0.027**
V	$T_0 - T_1$	0.000***
	$T_1 - T_2$	0.336
	$T_0 - T_2$	0.000***
VI	$T_0 - T_1$	0.000***
	$T_1 - T_2$	0.167
	$T_0 - T_2$	0.001***
VII	$T_0 - T_1$	0.057
	$T_1 - T_2$	0.423
	$T_0 - T_2$	0.038**

T_0: baseline; T_1: follow up 3 months; T_2: follow up 6 months.
***: < 0,05; ***: < 0,01.*

Discussion

Over the years, many surgical techniques for the correction of septal deformity have become diffuse. The concept of septoplasty was firstly popularised by Killian (1904) [7] and Freer (1902) [8] separately more than 100 years ago. In 1947, Cottle defined surgical septoplasty as a treatment to correct nasal airway obstruction, and standardised the technique [9]. This technique has remained largely unchanged up to now. Recently introduced endoscopic endonasal techniques provide better magnification and illumination of the surgical field and can also be used to assist septal surgery [11].

The application of endoscopic techniques for correction of septal deformities was initially described in 1991 by Stammberger. Since that time, surgeons have performed endoscopic septoplasties not only to treat symptomatic nasal obstruction, but also to improve surgical access to the middle meatus as an adjunct to endoscopic sinus surgery (ESS) [10-16].

Endoscopic septoplasty is now an attractive alternative to traditional headlight approach for septoplasty.

Bothra et al. showed better results and fewer complications with endoscopic septoplasty compared to conventional approaches, as endoscopy gave better illumination and improved access to high deviations and spurs [17].

The same opinion in favour of endoscopic septoplasty was expressed later by several authors who compared the

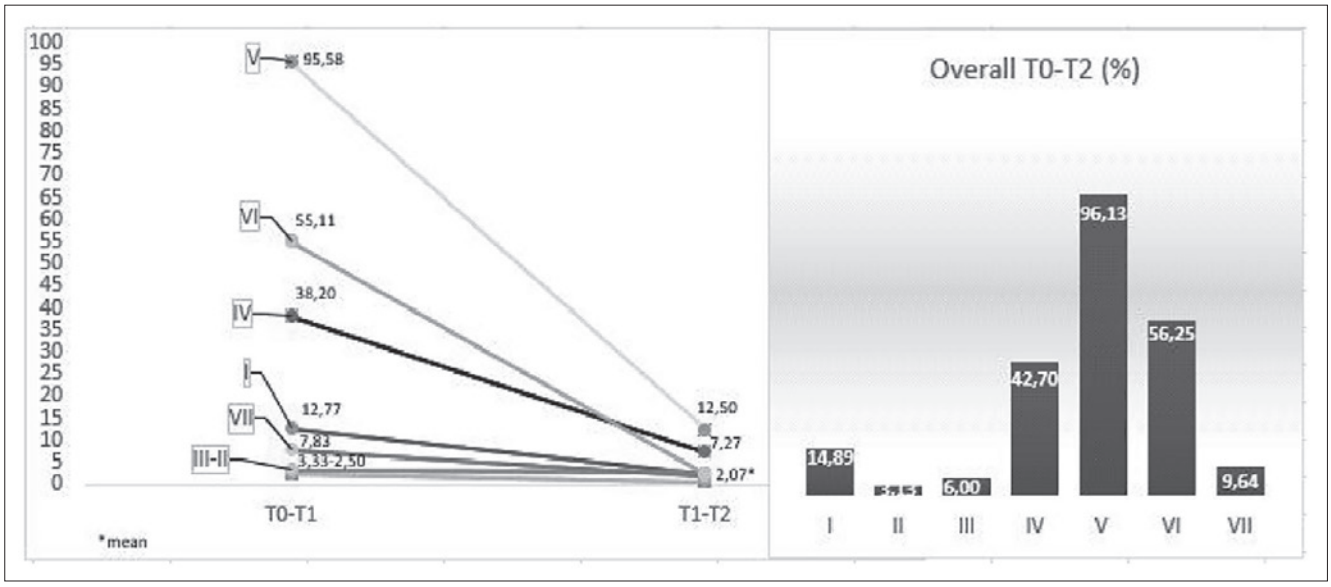

Fig. 4. Percentages decreased for each deviation type at 3 months, 6 months and overall.

two techniques [18]. Gulati et al [19] found that an endoscopic approach to septoplasty simplifies identification of the pathology due to better illumination, improved accessibility to remote areas and magnification, while allowing for limited incision and elevation of flaps without compromising adequate exposure of the pathological site. Paradis et al. [20] compared endoscopic vs classic septoplasty. The authors recruited 63 patients with a septal deviation meeting strict inclusion/exclusion criteria and measures outcomes including surgical time, intraoperative complications and pre- and post-operative data from the Nasal Obstruction Symptom Evaluation (NOSE) questionnaire. There were subjective improvements in nasal obstructive symptoms in both groups, but without significant differences between endoscopic and classic septoplasty. However, objective outcome measures, including operative time and intraoperative complications, were favoured by the endoscopic technique. Therefore, considering these findings and the advantages of endoscopy (e.g., improved visualisation of the surgical field, increased precision and enhanced teaching opportunity), the use of an endoscopic approach for septoplasty is suggested over a traditional technique for correction of septal deviation.

While the majority of authors seem to prefer the endoscopic technique, no one has analysed the effectiveness of this procedure in resolving different septal deformity.

To our knowledge, this is the first study that evaluates the corrective capacity of this technique for each type of deviation by analysing pre- and post-operative objective outcomes, as well as subjective outcomes gathered from the validated NOSE questionnaire [21-23].

Mladina et al. codified a classification for septal deformity based on direct observations of 2589 patients. The authors concluded that almost 90% of subjects showed 1 of the 7 types of septal deformity described [6 24]. We divided our cohort based on this simple and effective classification. By direct endoscopic visualisation and data processing of coronal CT scans, it was easily possible to stratify our sample into each of the seven types described by Mladina et al. [6].

For those who deal with functional nasal surgery, evaluation of the nasal airflow perception is the most difficult parameter to study. Nasal breathing is a complex function of the nose that may be influenced by various conditions such as humidity, nasal resistance and contact of inspiration air with nasal surfaces. Stewart et al. in 2004 completed the validation of a disease-specific instrument to assess nasal obstruction: the NOSE scale [25]. According to Kahveci et al., who found the NOSE scale a very efficient tool to evaluate outcomes of septoplasty, we adopted this tool to assess the effectiveness of endoscopic septoplasty in different types deviations, comparing outcomes observed preoperatively and at 3 and 6 months post-operatively [21].

Generally, turbinate surgery was not accepted as an exclusion criterion when functional outcomes of septoplasty were evaluated [22 25 26]. However, we preferred to include only patients with septal deviation without any other confounding factors (e.g. inferior turbinate hypertrophy) to evaluate the efficiency of septoplasty.

Data analysis from the NOSE score showed a marked improvement in airflow perception in all patients treated. No

significant differences were appreciated by comparing the NOSE score at 3 months and 6 months after surgery. According to Skitarelic et al., these findings showed that endoscopic septoplasty is an effective procedure with stable results over time [19].

What we consider very interesting is that the analysis of the NOSE score for individual septal deformity highlighted a different efficacy of the surgical procedure. In particular, the corrective power seems to be greater for deviation types 5 and 6, gradually decreasing in types 4, 1 and 7 and becoming minimal for types 3 and 2. As already shown by Gupta et al., endoscopic vision allows excellent lighting of the septum in the rear portion (Cottle's area IV, V) and faciltates correction of all deviations in this area. Because deviations type 5 and 6 are located mainly in the posterior areas, this could explain the increased corrective power obtained for these deviations in our sample [27 28].

Nayak et al. reported that about 10% of cases with anterior septal deformity had persistent septal deviation after endoscopic septoplasty. In the same way, we have found greater difficulty in performing endoscopic procedure for deviations in this area (Cottle 's area I, II, III) [11 27].

In these areas, it is difficult to obtain a good endoscopic vision for the lack of support for the endoscope. Moreover, the elastic recoil of the cartilage requires detaching a large portion of the septum and to release it in the caudal portion. Therefore, significant bleeding requiring too frequent cleaning of the endoscope's tip render the procedure difficult in this area. We believe that this may explain the reduced corrective capacity of the endoscopic septoplasty for type 2, 3 and 7 deformities.

Conclusions

This study has shown that the corrective power of endoscopic septoplasty is different according to the type of deviation. Even if endoscopic septoplasty may be considered as a reliable alternative to traditional techniques, it is essential to properly identify the type of deformity preoperatively in order to select the adequate surgical strategy. Long term follow-up and larger series are necessary to more accurately assess the indications and limitations of endoscopic-assisted septoplasty in all types of deviation.

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Rhinology

Extracorporeal septoplasty with internal nasal valve stabilisation

La chirurgia extracorporea del setto nasale con stabilizzazione della valvola nasale interna

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SUMMARY

Among various septoplasty techniques, the extracorporeal one is used for severe deformities of the caudal septum and consists essentially in removal of the nasal septum followed by correction of deformities. Reconstruction of the neo-septum is carried out by repositioning the septal fragments in a straight position. The disadvantages of this surgical technique are the septal haematoma, oedema of the mucosa in the valve area and some types of abnormalities of the middle third of the nose such as saddling of the dorsum. All of these conditions can be associated with various degrees of functional disorders. To prevent these possible complications, we developed a suture technique to fix the caudal portion of the neo-septum and avoid alterations or narrowing of the internal nasal valve. The purpose of this study is to describe extracorporeal septoplasty results with this suture technique in stabilising the internal nasal valve. From January 2011 to December 2013, a retrospective review of adult patients treated with extracorporeal septoplasty was performed at the ENT department of Imola Hospital. Pre- and post-operative evaluations were carried out by rhinomanometry and acoustic rhinometry. Statistical analysis was performed with commercially available software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). 133 cases fulfilled inclusion criteria and were enrolled. A significant improvement was evident after surgery based on the results of rhinomanometry and acoustic rhinology. Extracorporeal septoplasty with stabilisation of the internal nasal valve is an effective and reproducible surgical technique that yields optimal functional results.

KEY WORDS: Extracorporeal septoplasty • Suture • Deviated septum • Nasal valve • Results

RIASSUNTO

Tra le diverse tecniche di settoplastica, quella extracorporea viene utilizzata per le gravi deformità del setto anteriore e consiste essenzialmente nella rimozione del setto nasale, nella correzione delle deformità e nella ricostruzione settale che viene attuata con il riposizionamento dei frammenti settali nella sede corretta. Gli inconvenienti di questa tecnica chirurgica sono l'ematoma settale, l'edema della mucosa nella zona della valvola e la comparsa di alterazioni del terzo medio del naso quali insellamenti del dorso. Tutte queste condizioni si possono associare a vario grado di disturbi funzionali. Per evitare queste possibili complicanze abbiamo sviluppato una particolare tecnica di sutura per fissare la porzione caudale del neosetto ed evitare alterazioni o restringimenti della valvola nasale interna. Lo scopo di questo studio è descrivere i nostri risultati nella settoplastica extracorporea con una tecnica di sutura per la stabilizzazione della valvola nasale interna. Da gennaio 2011 a dicembre 2013 è stata effettuata presso l'Unità Operativa di Otorinolaringoiatria dell'Ospedale di Imola una revisione retrospettiva di pazienti adulti trattati con settoplastica extracorporea. Le valutazioni pre- e post-operatorie sono state eseguite con l'utilizzo della rinomanometria e della rinometria acustica. L'analisi statistica è stata prodotta con il software IBM SPSS Statistics per Windows, versione 21.0 Armonk, NY: IBM Corp. 133 pazienti sono rientrati nei criteri di inclusione e sono stati pertanto reclutati per questo studio. Un miglioramento statisticamente significativo è stato evidente dopo l'intervento chirurgico sulla base dei risultati di rinomanometria e rinometria acustica. La settoplastica extracorporea con stabilizzazione della valvola nasale interna è una tecnica chirurgica efficace e riproducibile che si accompagna a risultati funzionali ottimali.

PAROLE CHIAVE: *Chirurgia extracorporea del setto • Sutura • Deviazione del setto nasale • Valvola nasale • Risultati*

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Introduction

Nasal septal deviation is a common disorder in otolaryngology and one of the major causes of nasal obstruction. In some cases, septal deviation is non-symptomatic, but in a high number of patients it causes functional disturbance. The degree of septal deviation affects the severity of symptoms so that severe nasal obstruction strongly affects the quality of life. In patients with mild or moderate deviation of the septum, traditional techniques of septoplasty are ef-

fective to improve nasal breathing. On the other hand, in severe anterior deformities, usually associated with stenosis of the nasal valve, these methods have unsatisfying results and can sometimes cause functional problems due to over resection or over weakening of the cartilage [1-3]. In case of severe anterior deformities of the septum, removal of the whole septum, followed by extracorporeal reconstruction and reinsertion, is recommended [4-6]. This technique can be carried out using an open or a close approach. Hence, extracorporeal septoplasty has been demonstrated to be effective in correcting obstructive deviation of anterior nasal septum involving the internal nasal valve. The main drawback of this procedure is destabilisation of the junction of the quadrilateral cartilage and nasal bones with consequent alteration of the dorsal contour and functional impairment of the internal nasal valve. To minimise these events, we developed a modified technique. The purpose of this study is to evaluate outcomes of this modified extracorporeal septoplasty in long-term follow up. Additionally, we report the average operative time for extracorporeal septoplasty, which has not yet been investigated in the international literature to date.

Materials and methods

Between January 2011 and December 2013, we retrospectively reviewed the medical records of all patients treated by extracorporeal septoplasty in the ENT department of Imola Hospital, Italy. The Institutional Review Board of the Hospital approved this retrospective study. Written informed consent for the procedure was obtained from all patients. We included cases of adult patients suffering from a structural or mixed nasal patency impairment. Information regarding perioperative data including patient demographics, preoperative data, side of the nasal patency impairment, diagnostic studies, operative details, postoperative outcomes and complications was obtained. The side of the nasal obstruction was determined by anterior rhinoscopy and nasal endoscopy. Nasal patency was assessed using anterior active rhinomanometry (AAR) with a Rhinopocket® rhinomanometer and acoustic rhinometry (AR). We performed both examinations before surgery and after during the follow-up period, based on the Consensus report on acoustic rhinometry and rhinomanometry [7]. According to our clinical protocol, we considered a mean total resistance of 0.24 Pa/cm^3/s with a range of 0.12-0.52 Pa/cm^3/s a normal rhinomanometric result. Resistance is determined at a pressure of 150 Pa. Data was acquired at a flow/pressure display. The AR software provides minimal cross-sectional areas in two separate points: the first minimal cross-sectional area (MCA 1) from 10 mm to 32 mm of the nostril, and the second minimal cross-sectional area (MCA 2) which is located from 32 mm to 64 mm of the nostril. A median MCA 1 value of 0.73 cm^2 (range 0.57-1.45 cm^2) was considered a normal result [7]. Preoperatively, AAR and AR were performed in basal conditions after decongestion and dilatation [8 9]. Surgical outcomes were evaluated by comparing pre- and postoperative baseline results taking into consideration the last follow-up. We carried out rhinomanometric, acoustic rhinometric and clinical controls at 3, 6 and 12 months after surgical intervention. All examinations were done by the same operator after a 15-minute period of acclimatisation.

Surgical technique

All procedures are performed using an endonasal approach under general anaesthesia and oro-tracheal intubation. All patients underwent an extracorporeal septoplasty procedure. In particular, the entire quadrangular cartilage was surgically removed leaving only a small 3 mm strip of cartilage close to the keystone area (Fig. 1). Once the septal bony structures were removed, nasal packing was inserted. Packing keeps the crushed bone and/or cartilage fragments repositioned during posterior reconstruction. Reconstruction prevent from dystrophic sequelae which may in turn lead to a flaccid septum and even to a possible septal perforation or prolapse of the turbinates. The most regular, defect-free area of the quadrangular cartilage (which was previously removed) is trimmed and shaped into a rectangle; in some cases, this may include part of the perpendicular plate. The graft is tethered to the muco-pericondral flap with polyglactin 910 suture 3-0 (Vycril®) (Fig. 2).

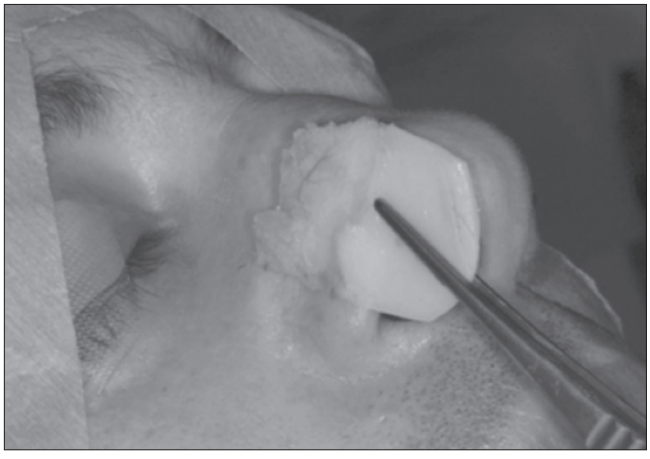

Fig. 1. Quadrangular cartilage with ethmoid bone.

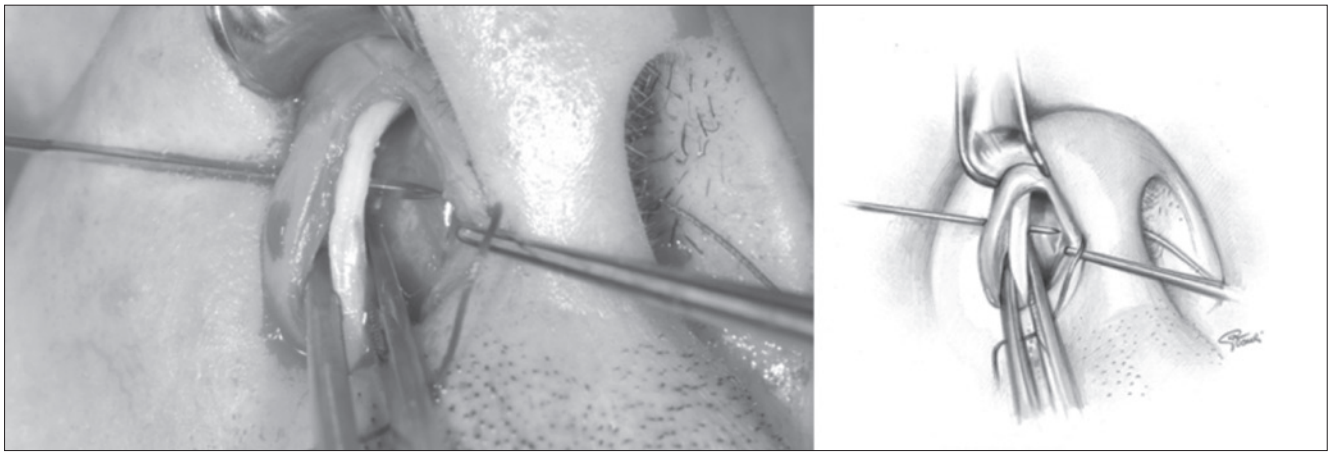

Fig. 2. Graft tethered to the muco-pericondral flap.

Nasal valve stabilisation

A transfixed suture should be performed under the replaced quadrangular cartilage to give support to the inserted graft in order to prevent the development of saddling deformity (Fig. 3). The graft is also secured to the membranous septum and cartilaginous vault to support and stabilise the valve area and to support the naso-labial junction. The hemitransfixion incision is then closed, suturing the septum and columella with transfixed sutures. Closure of the hemitransfixion incision is performed using 3-0 Vicryl suture with a 6 cm straight needle. Synthetic packing is left in the nasal fossae for 2 days to ensure the

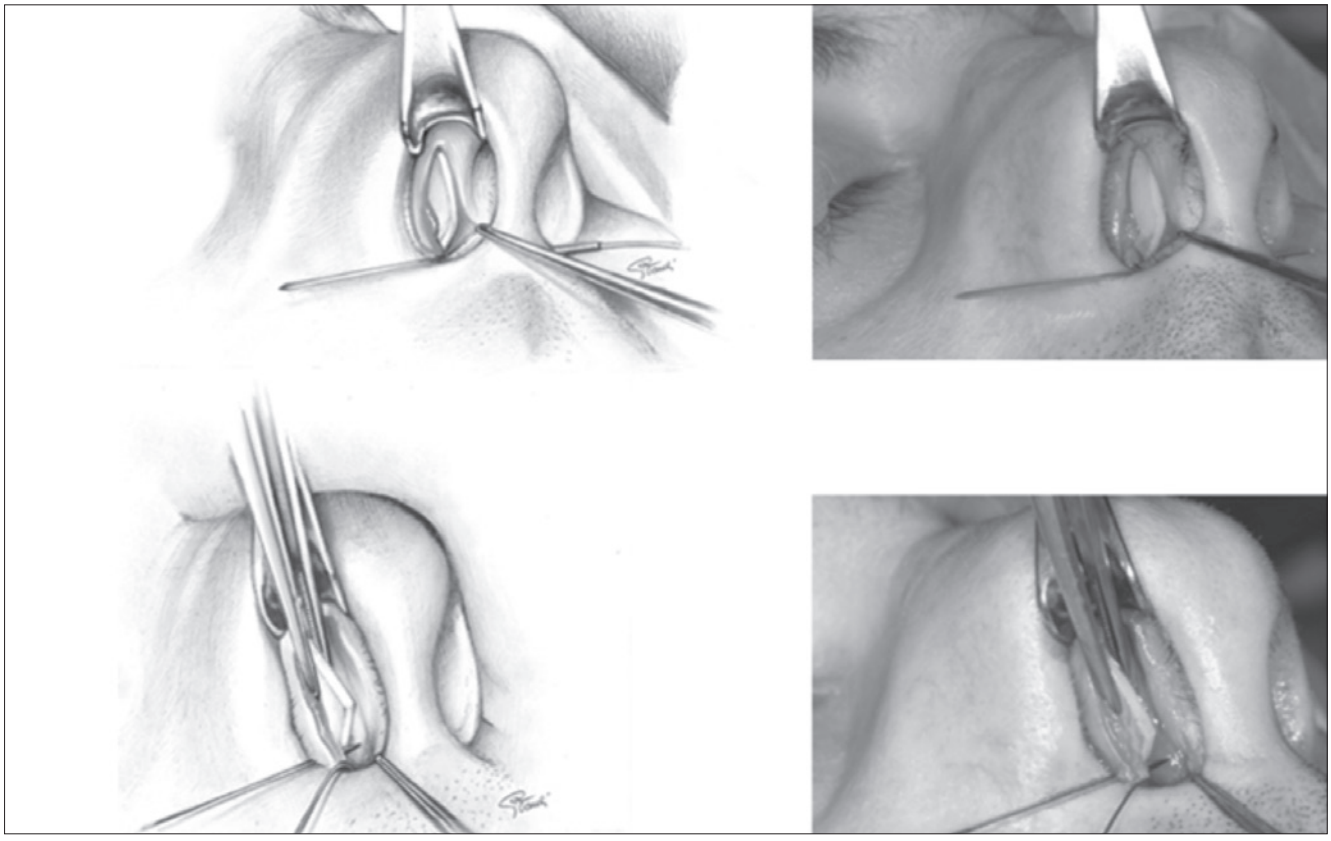

Fig. 3. Suture inserted under the quadrangular cartilage.

flap adheres, and prevent septal haematoma and displacement of the inserted fragments. This is accomplished by an L-inverted shaped caudo-cranial and antero-posterior suture (Figs. 4, 5). If saddling of the dorsum still persists at the end of the reconstruction procedure, the profile should be normalised by inserting crushed septal cartilage into a dorsal tunnel created through the hemitransfixion incision [10].

Statistical analysis

All data were collected using a commercially available database programme (Excel® 2013; Microsoft®, Redmond, WA, US). The unit of analysis was each patient before and after surgery. In the descriptive analysis, quantitative variables with normal distribution were expressed with means and standard deviation and the ones with abnormal distribution with medians and range; qualitative categorical variables were summarised as frequency and percentage. Preoperative nasal resistance obtained by AAR and nasal cross sectional areas obtained by AR were compared with postoperative results using the non-parametric Wilcoxon Sign Rank test. Differences were considered significant at a p value < 0.05. Statistical analysis was performed with commercially available software (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp).

Results

Among adult patients treated with extracorporeal septoplasty from January 2011 to December 2013, 133 cases fulfilled inclusion criteria for the purposes of the study. Patient characteristics are summarised in Table I. Surgeries were done by five different surgeons of the ENT department with a median operating time of 42 minutes (range, 20-58). No intraoperative complications were reported. All patients were hospitalised for 48 hours. Early complications included septal haematoma in one patient (0.75%) that required drainage in the outpatient clinic. Mean follow-up was 12 months (SD, 6.3). Three patients (2.25%) had to be re-operated on due to impaired nasal patency. A significant improvement was evident after surgery based on rhinomanometric and acoustic rhinometric outcomes (Table II).

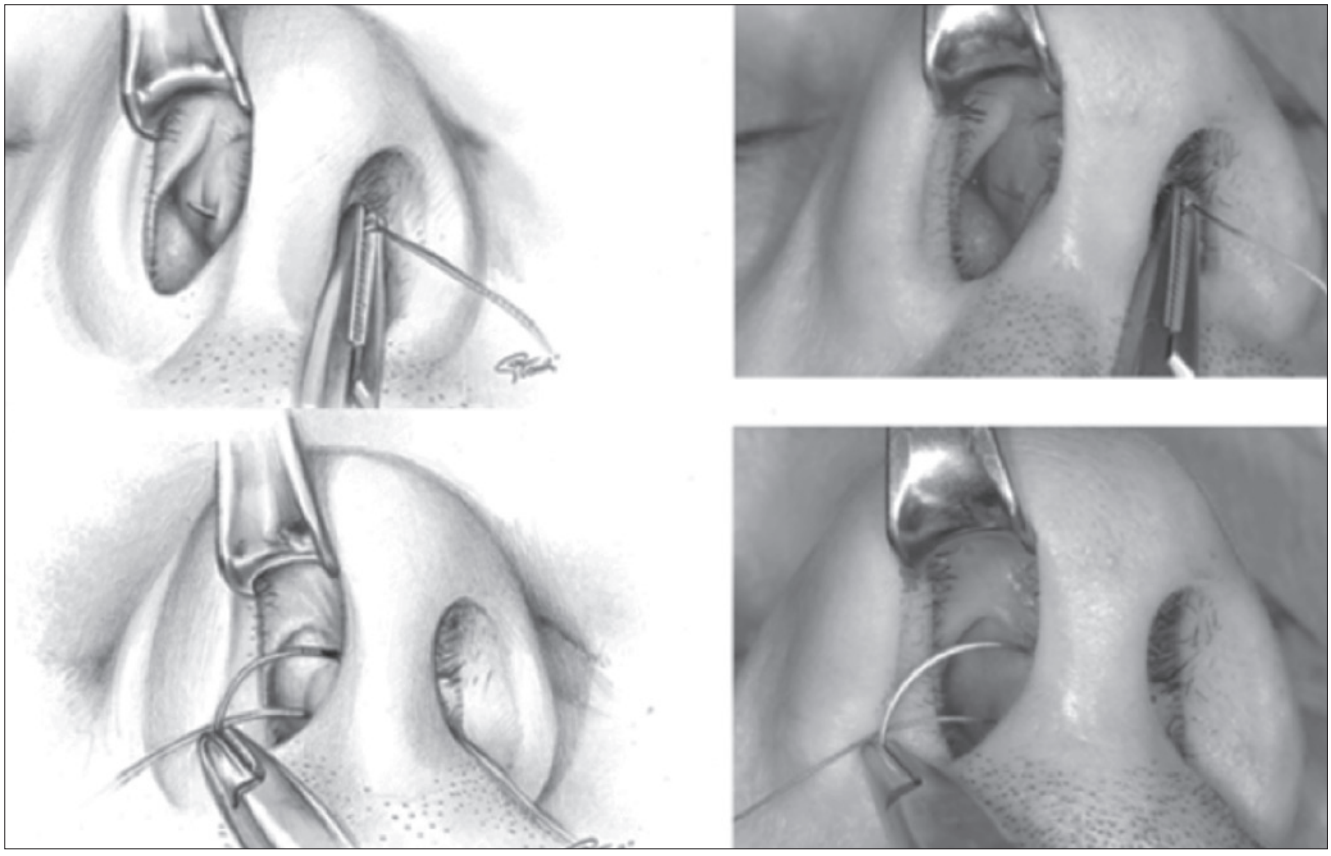

Fig. 4. Vertical mattress suture to stabilise the valve area.

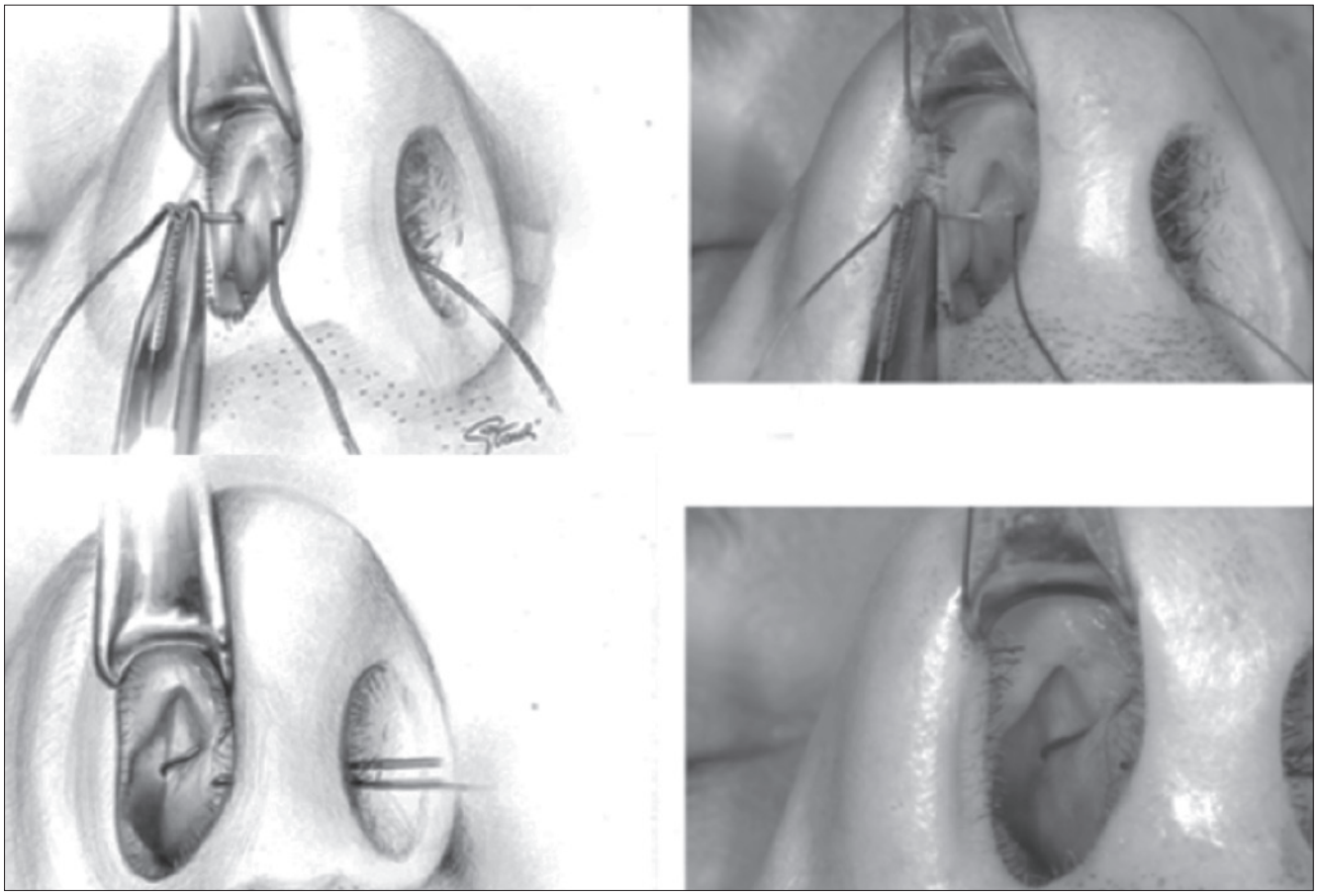

Fig. 5. Horizontal mattress suture to stabilise the valve area.

Discussion

Septoplasty is the most common procedure performed in rhinology. Ever since Killian and Freer introduced the concept of submucous resection, the technique has been gradually developed by many operators sustainably and scientifically. In the last century, there have been significant advances in surgical septal procedures. The methods of approaching the caudal nasal septum are cartilage reshaping procedures and septal reconstruction manoeuvres. Modern septal surgery was developed in the 1950s by Cottle [11]. For decades, the maxilla-premaxilla approach has been the workhorse for nose surgeons; limitations of this technique include extreme anterior or superior septal deviations and complex deviations due to multiple fracture lines or lack of cartilage. Nowadays there is no standard treatment for all types of deformities. Techniques such as suture, swinging door, septal batten, ethmoid bone sandwich graft, tongue-in-groove and extracorporeal septoplasty have been used in managing caudal septal deviation [1-6]. This broad range of approaches illustrates the difficulty in treating caudal septal deviation and this is the reason why we consider that there is no doubt regarding the need to obtain pre- and post-operative rhinometric measures if objective results in septal surgery are to be achieved. Extracorporeal septoplasty with the described suture technique has several advantages over other correc-

Table I. Patient characteristics.

Variable	N = 133
Male, n (%)	109 (82)
Age, mean (SD), years	41.76 (15)
Previously treated, n (%)	27 (20.3)
Side affected, n (%)	
Right nostril	37 (27.8)
Left nostril	46 (34.6)
Bilateral	50 (37.6)
Type of rhinomanometric impairment n (%)	
Structural	41 (30.82)
Mixed	92 (69.7)

Table II. Comparative pre-operative and post-operative baseline investigation results.

Variable	Pre-operative	Post-operative	p
AAR Pa/cm^3/s median (range)			
Baseline right nostril			
Inspiration	0.74 (0.00-61.00)	0.27 (0.00-1.77)	0.000
Expiration	0.61 (0.00-28.40)	0.00 (0.00-1.29)	0.000
Baseline left nostril			
Inspiration	1.14 (0.00-240.00)	0.26 (0.00-2.44)	0.000
Expiration	1.00 (0.00-553.0)	0.00 (0.00-1.75)	0.000
AR cm^2 median (range)			
Baseline right nostril			
MCA 1	0.32 (0.08-0.96)	0.44 (0.16-2.08)	0.000
MCA 2	0.31 (0.03-1.11)	0.50 (0.14-1.13)	0.000
Baseline left nostril			
MCA 1	0.32 (0.05-0.87)	0.38 (0.09-1.94)	0.002
MCA 2	0.28 (0.04-0.96)	0.41 (0.13-0.93)	0.000

tive techniques for caudal septal deviation. In case of suboptimal results, conservative remodelling of the quadrangular cartilage and respect of the majority of cartilaginous structures can be useful for grafting during secondary or revision rhinoseptoplasty. An immediate intra-operative check-up of the straightening of the caudal septum is possible with low risk of deviation recurrence. In fact, this technique completely avoids the cartilage memory from bending, which is a major issue because cartilage has a strong tendency to return to its original shape.

The operative time that we measured in the study is also a strong point. Hardy et al. [12] in a cohort of 1753 patients who underwent a broad range of complex plastic surgical procedures concluded that surgical duration is an independent predictor of complications, with a significantly increased risk after 3 hours. Septoplasty is usually associated with other surgical procedures such as functional endoscopic sinus surgery and rhinoplasty. For this reason we consider it important to measure surgical time, to organise the surgical schedule and operative time when the intervention is associated with other procedures.

The drawbacks of this procedure are swelling of the mucosa, restenosis of the nasal valve area, septal haematoma and saddle nose development. They can be avoided by correct suture technique to straight the mucosa, especially in the valve area and give support to the repositioned cartilage and prevent saddle nose development. This purpose can be achieved when a sufficient amount of septal cartilage is available for reconstruction such as in primary septoplasty. In case of revision surgery, we are used to positioning the silicone splints into nasal cavities, leaving them for at least 15 days to maintain the septum in a straight position. When there is a shortage of cartilage, we reinforce the neo-septum using calvarian cartilage grafts. In these particular situations, we also consider the use of internal nasal valve reconstruction techniques such as butterfly graft, flaring suture techniques, spreader grafts and Gassner type graft [13-16]. These techniques include the interruption of the T-bar or septal- triangular unit and the use of grafts. Grafts are somewhat intended for partial reabsorption, are at risk for potential destabilization and accompany a donor site morbidity. For all these reasons, we prefer not to use these techniques in primary surgery cases.

Conclusions

The extracorporeal septoplasty technique is a successful surgical technique for anterior deviations of the septum. We emphasise that the replacement of removed cartilage with the described suture technique is an important step in the surgery that must be taken into consideration to get good functional and aesthetic results. Extracorporeal septal reconstruction is the advocated procedure for correction of a markedly deviated nasal septum. The technique has been demonstrated to be safe and effective in restoring nasal patency and the results remain stable during long-term follow-up. Some variants of the classic procedure may be useful to stabilise the reconstructed septum, above all in the valve angle, and preserve structured support of the nasal archway to avoid nasal dorsal irregularity or collapse of the mid-nasal vault.

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OSAHS

Olfactory evaluation in obstructive sleep apnoea patients

Valutazione olfattiva di pazienti affetti da sindrome delle apnee ostruttive del sonno

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SUMMARY

The sense of smell has a high impact on the quality of life. The aim of the present study was to investigate olfactory dysfunction in patients with obstructive sleep apnoea syndrome (OSAS) and correlate the severity of disease with olfactory dysfunction. The relationships between nasal obstruction, nasal mucociliary cleareance and olfactory tests were also evaluated. Sixty patients with a diagnosis of OSAS were enrolled and underwent olfactory function evaluation. In all patients olfactory performance was tested with the Sniffin' Sticks method. Mucociliary transport times and anterior rhinomanometry were performed to identify eventual nasal obstruction and deficits in nasal mucociliary clearance. Olfactory dysfunction was present in 22 (36.6%) patients of the study group: of these, hyposmia was present in 19 (86.4%) and anosmia in 3 (13.6%). The mean TDI score in the study group was 30. A strong correlation between the olfactory dysfunction and severity of sleep apnoea measured using the AHI was found. Patients with OSA would seem to have a high incidence of olfactory dysfunction. The degree of olfactory dysfunction appears to be related to the severity of disease. However, other co-factors such as nasal obstruction and reduced mucociliary clearance might also play a role in of the aetiology of this condition.

KEY WORDS: Obstructive sleep apnoea syndrome • Olfactory dysfunction • Sniffin' Sticks • Nasal mucociliary clearance • Anterior rhinomanometry

RIASSUNTO

Il senso dell'olfatto ha un alto impatto sulla qualità della vita. Lo scopo di questo studio è stato quello di investigare la disfunzione olfattiva in pazienti con sindrome delle apnee ostruttive del sonno (OSA) e correlare la gravità della malattia con la perdita di olfatto. Inoltre, è stata valutata la relazione esistente tra ostruzione nasale, cleareance mucociliare nasale e i risultati dei test olfattivi. Sessanta pazienti con diagnosi di OSA sono stati arruolati in questo studio e hanno eseguito una valutazione olfattiva. In tutti i pazienti la valutazione olfattiva è stata eseguita attraverso lo Sniffin' Sticks test. Il tempo di trasporto mucociliare e una rinomanometria anteriore sono stati eseguiti per identificare una possibile ostruzione nasale e un deficit nella cleareance mucociliare. Una disfunzione olfattiva era presente in 22 (36,6%) pazienti dello studio. In questi pazienti con disfunzione olfattiva l'iposmia era presente in 19 (86,4%) casi e l'anosmia in 3 (13,6%) casi. Il TDI medio del gruppo di studio presentava uno score di 30. Si è riscontrata una correlazione statistica tra la disfunzione olfattiva e la severità delle apnee notture misurata con AHI. I pazienti con OSA sembrerebbero avere un'alta incidenza di disfunzione olfattiva. Il grado di disfunzione olfattiva sembrerebbe essere correlato alla gravità della malattia. Tuttavia, altri fattori come una ostruzione nasale e una ridotta cleareance mucociliare sembrerebbero avere un ruolo nell'eziologia di questa condizione.

PAROLE CHIAVE: *Sindrome delle apnee ostruttive del sonno • Disfunzione olfattiva • Sniffin' Sticks • Cleareance mucociliare nasale • Rinomanometria anteriore*

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Introduction

Obstructive sleep apnoea syndrome (OSAS) is a condition characterised by pauses in breathing and episodes of reduction (hypopnea) or absence (apnea) of airflow in the upper airways during sleep [1-4]. OSAS is becoming more commonly recognised because of its significant negative effects on daily life and its association with neurocognitive and psychological problems, such as memory and new learning, attention, executive function and depres-

sion [1 5 6]. According to the study published by Heinzer et al. [7] the incidence of moderate-to-severe sleep-disordered breathing (≥ 15 events per hour) is 23.4% in women and 49.7% in men.

The sense of smell is a sensorineural system with a high impact on the quality of life [8-10]. Data regarding the incidence of olfactory dysfunctions in the healthy population vary between 10% and 46% of the healthy population [10-15]. A recent study of a large population showed that the overall incidence of olfactory dysfunction was 19.1%, consisting of 13.3% and 5.8% of patients with hyposmia and anosmia, respectively [16]. Similar data were reported by Hummel et al. [11] with a 20% incidence of olfactory dysfunction in subjects between 36 and 55 years of age.

Sinonasal diseases, nasal obstruction, head trauma and central nervous system disorders that affect neurocognitive functions, drugs, and toxins are the most important causes of olfactory dysfunction (OD) [10 15 18 19]. The conditions listed above may be variously present in OSAS patients, making these subjects more inclined to developing an olfactory dysfunction [1-4 20-22].

To our knowledge, only a few authors have evaluated olfactory dysfunction in OSAS patients [23-25] and there is a scarcity of data concerning the relationship between clinical findings and OD in the medical literature.

The aim of the present study was to investigate: 1) incidence of olfactory dysfunction in OSAS patients; 2) correlation between the severity of OSAS and OD; 3) relationships between nasal obstruction, nasal mucociliary clearance and olfactory test results in patients with OSAS.

Materials and methods

This prospective study was performed at the of Organi di Senso Department (Sleep Disorders Unit) of Sapienza University in Rome between January 2016 and February 2017.

The participants were selected from patients who were referred to us with a clinical suspicion of sleep apnoea syndrome. Initially, clinical data, including height and weight, in order to calculate body mass index (BMI), medical history, tobacco use and a list of current medications were collected for each patient.

Subsequently, each patient was submitted to an ENT physical examination with nasal endoscopy (2.7 mm 0° rigid endoscope) to evaluate the features of nasal structures and detect any rhino-sinusal pathologies.

The exclusion criteria for this study included patients with a history of upper respiratory infections within the previous 3 weeks, sinonasal disorders (nasal polyps, chronic rhinosinusitis, allergic rhinitis), asthma, malignancy, head trauma, neurological and psychiatric disorders, metabolic and endocrine disorders, or a recent history of smoking more than 3 cigarettes per day.

Patients who underwent sleep surgery or currently using continuous positive airway pressure were not included in the study.

All patients selected for the study had previously undergone a polysomnographic (PSG) study to confirm diagnosis of OSAS and assess its severity.

All parameters, sleep stage scoring and event scoring were evaluated in accordance with the AASM Manual for the Scoring of Sleep and Associated Events [26].

Diagnosis of OSAS was confirmed when the number of obstructive events (apnoeas, hypopneas + respiratory event related arousals) on PSG were > 15 events/hour or > 5/hour in patients reporting typical symptomatology (unintentional sleep episodes during wakefulness; daytime sleepiness; unrefreshing sleep; fatigue; insomnia; waking up holding breath, gasping or choking; or loud snoring, breathing interruptions or both during the patient's sleep as described by the bed partner) [26 27].

In accordance with American Academy of Sleep Medicine (AASM) guidelines, OSAS severity was classified on the basis of the apnoea + hypopnoea index (AHI). The grade of OSAS was classified as normal (AHI < 5/h), mild (AHI ≥ 5 and < 15 plus typical symptomatology), moderate (AHI ≥ 15 and < 30) or severe (AHI ≥ 30) [26 27].

Once the exclusion criteria was applied, 60 patients with a PSG diagnosis of OSAS were enrolled in the study. During enrollment of these patients, 20 subjects were selected from each OSA category (mild, moderate, severe) to form three homogeneous groups for better comparison of olfactory results.

The same exclusion criteria adopted for the study group were applied for selection of a control group of patients without OSAS. In all the patients of the control group the typical symptomatology of OSAS patients was excluded and the results of PSG examination showed AHI < 5. Forty volunteer subjects were enrolled in this control group.

All OSAS patients enrolled in the study group underwent evaluation of olfactory function. Anterior rhinomanometry and mucociliary transport time were performed to evaluate nasal obstruction and mucociliary clearance. The same study protocol was adopted for the control group.

The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the local Ethics Committee of the University Sapienza of Rome.

All patients gave written informed consent for the PSG, olfactory test, rhinomanometry and saccharin test.

Evaluation of olfactory function

Olfactory performance was tested with the Sniffin' Sticks (Burghart, Wedel, Germany) method [28-30]. This test includes 3 sub-tests that evaluate the olfactory threshold (OD), olfactory discrimination (ODs) and identification (OI). The sum of the results from each of three different sub-tests results in a total score defined as TDI. In the analysis of Sniffin' Sticks tests, the results of the TDI score indicated hyposmia when the total TDI score was < 30.5, anosmia when < 16.5 and no OD when the TDI score was > 30.5 [28-30].

Rhinomanometry

Nasal resistance was evaluated in both OSAS patients and in the control group using anterior rhinomanometry. It has been reported in the literature that the mean total resistance in normal subjects ranges between 0.15 and 0.3 Pa/cm^3/s [31]. For this reason, total nasal airway resistance > 0.3 Pa/cm^3/s was considered pathological.

Mucociliary transport time

Mucociliary transport times were evaluated to identify possible deficits of nasal mucociliary clearance. Mucociliary transport time was evaluated in each patient using the saccharin test, which is a very simple, quick, non-invasive and reproducible method [32 33]. About 2.5 mg of granulated saccharin was placed 1 cm posterior and inferior to the head of the inferior turbinate with patients placed in the sitting position with the head bent forward. Patients were instructed to swallow every 30 seconds and not to sniff, sneeze or wipe their noses until they tasted the saccharin. The time from placement to perception of sweet taste was recorded as the nasal mucociliary clearance time (NMCT) [32 33]. The same test was performed in OSAS patients and the study group.

Statistical analysis

For comparison of data between groups, the chi-square, Student's T test and regression analysis were performed using XLSTAT software (Addinsoft, 2015). A p value < 0.05 was considered as statistically significant.

Results

The characteristics of the 60 patients of the study group are summarised in Table I.

Regarding the 60 patients of the study group, 38 were male and 22 were female, with an average age of 53.1 years (range 32-77). The average age of the three subgroups was 51.8, 54.5 and 53.4 in the mild, moderate and severe OSA subgroups respectively. The average age of the control group was 48.4 years. No difference emerged regarding mean patient age between the study and control groups (p = 0.1). Moreover, no differences were seen in mean age between the OSA subgroups (p > 0.05 for all).

Olfactory evaluation

Olfactory dysfunction was present in 22 (36.6%) patients of the study group. Of these, hyposmia was present in 19 (86.4%) cases and anosmia in 3 (13.6%) cases. In the control group, 7 (17.5%) patients showed olfactory dysfunction. There was a statistically difference between the study and control groups (p = 0.04).

The mean TDI score of the study group was 30 (S.D. = 8.09; Hi = 39.0; Low = 12.0),whereas the mean TDI score in the control group was 33.3 (S.D. = 5.31; Hi = 39.0; Low = 14.0) with a significant difference between groups (p = 0.03).

Regarding olfactory data, it was interesting to note that 50% of patients with a diagnosis of olfactory dysfunction has severe OSAS. In addition, all 3 patients with a diagnosis of anosmia belonged to this subgroup.

Table I. Clinical characteristics of the study group.

	OSAS group N = 60	Control group N = 40
Middle age	Total group: 53.1 years; range: 32-76 years Mild OSAS: 51.8 Moderate OSAS:54.5 Severe OSAS: 53.4	48.4 years
Sex	38 Male 22 Female	26 Male 14 Female
BMI (mean)	31.1	
OSAS severity	Mild OSAS: 20 pts (mean AHI = 10.6) Moderate OSAS: 20 pts (mean AHI = 20.7) Severe OSAS: 20 pts (mean AHI = 38.9.)	No OSAS Mean AHI = 3.9

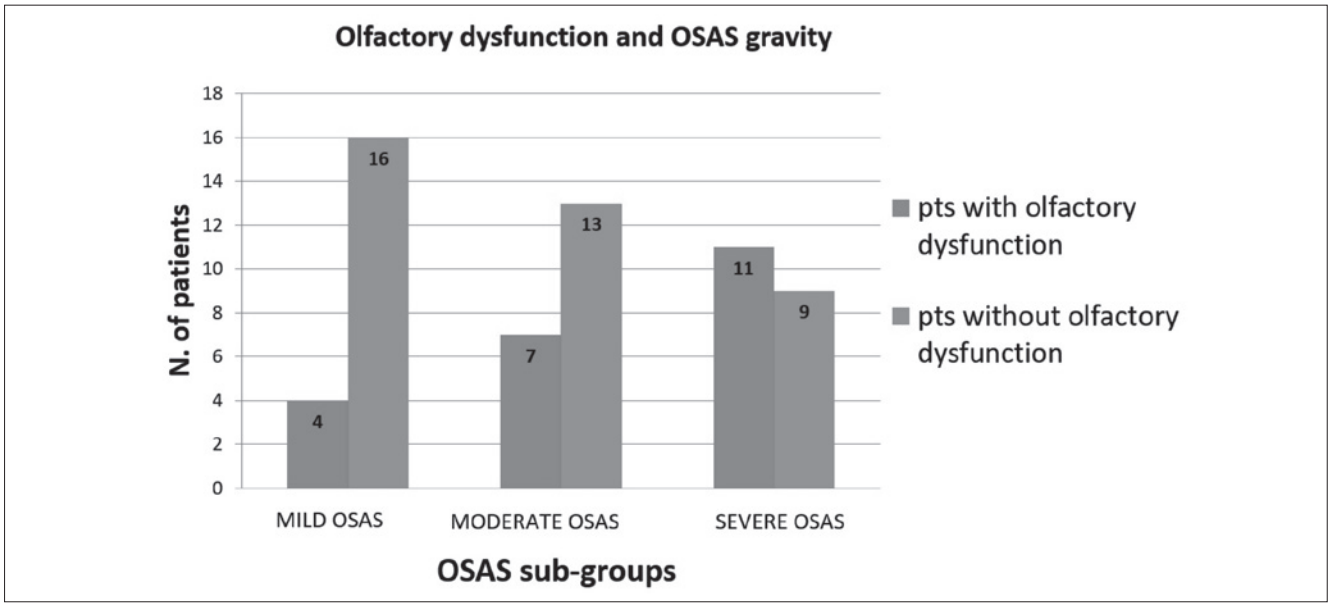

Fig. 1. Distribution of patients with olfactory dysfunction according to the OSAS subgroups.

The data regarding the distribution of olfactory dysfunction according to OSAS severity is summarised in Figure 1. A significant difference (p = 0.04) in olfactory dysfunction emerged only between mild and severe OSAS patients.

A strong correlation (regression analysis; p = 0.04; R2 = 0.05) between the olfactory dysfunction (TDI) and the severity of sleep apnoea using the AHI was found (Fig. 2).

Regression analysis between BMI and TDI did not show any correlation between high BMI and lower TMC scores (p = 0.07; R2 = 0.06). Moreover, no correlation was present between TDI score and middle age (p = 0.7; R2 = 0.002).

Rhinomanometry

Thirty-seven patients (61.6%) presented pathological bilateral values of nasal airway resistance at rhinomanometric examination. No differences regarding rhinomanometry results emerged for the different subclasses of OSAS patients. Nine patients (22.5%) showed pathological values in the control group.

The difference in the incidence of pathological rhinomanometry between the study and control group was significant (p = 0.0002).

Mucociliary transport time

The mean value of NMCT in OSAS patients was 13.2 min (S.D. = 3.13; Hi = 20.0; Low = 7.00; Median = 14.0). On the other hand, in the control group a lower NMCT mean value (Hi = 15 min; Low = 5 min) of 9.8 min was found. The difference between the two groups was statistically significant (p = 0.0001).

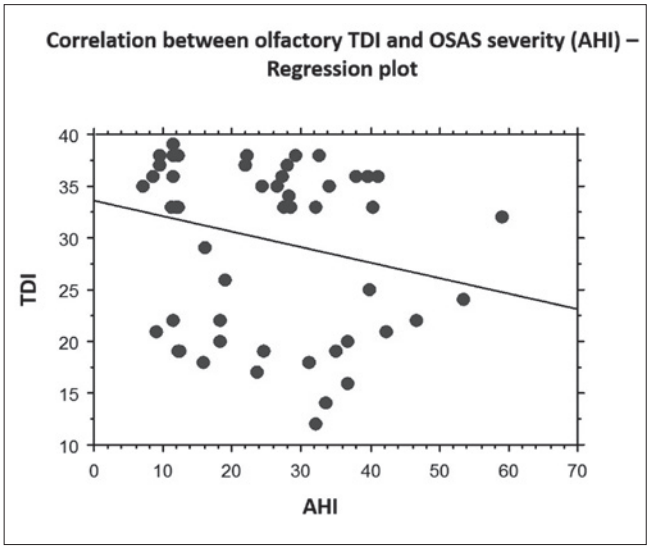

Fig. 2. Linear regression between olfactory TDI and OSAS severity (AHI): TDI had a significant and strong correlation with AHI (p = 0.001).

TDI score, rhinomanometry and mucociliary transport time

Comparing the results regarding nasal obstruction and olfactory evaluation (Table II): 86.3% of OSAS patients with olfactory dysfunction presented a pathological rhi-

Table II. TDI score, rhinomanometry and mucociliary transport time of the study group divided according to the presence or absence of an olfactory dysfunction.

	Middle age	P-value	Pathological rhinomanometry	Normal rhinomanometry	P-value	Mucociliary transport time (min)	P-value
OSAS with olfactory dysfunction (N = 22)	52.8 years	p = 0.9	19 (86.3%)	3 (13.7%)	p = 0.003	14.7	p = 0.005
OSAS without olfactory dysfunction (N = 38)	53.2 years		18 (47.3%)	20 (52.7%)		12.4	

nomanometry outcome. There was a significant difference between patients with and without olfactory dysfunction in terms of rhinomanometry results (p = 0.003) This data might indicate that nasal obstruction is correlated to OSAS in patients with OD.

The average mucociliary transport time in patients with OD was greater than the mean value of OSAS patients without OD (14.7 min vs 12.4 min). A significant difference between these two groups was seen (p = 0.005). Moreover, regression analysis between the TDI score and NMTC showed a significant correlation (p = 0.008) between increase of the NMTC and lower values of the TDI score (Fig. 3).

Discussion

The available data regarding the incidence and characteristics of olfactory dysfunctions in OSA patients are scarce [23-25]. Salihoglu et al. [23] in a clinical study regarding the effects of obstructive sleep apnoea on olfactory functions demonstrated that OSAS has significant negative effects on olfactory capacity, especially on threshold values and discrimination. Recently, Günbey et al. [24] in another clinical study regarding olfactory function in OSA patients, confirmed that patients with severe OSAS had scarce total odour scores with respect to non-snorers.

In accordance with these previous studies, we found that olfactory function declined in patients with OSAS. Olfactory dysfunction was present in 22 (36.6%) patients in our clinical study. Hyposmia and anosmia were present in 86.4% and 13.6% of OSAS patients with OD respectively, indicating that hyposmia is the most common olfactory disorder affecting these patients.

The mean TDI score of the study group was 30 with a statistically significant difference between OSAS patients and the control group. In addition, our data regarding total TDI scores and their values in OSAS subclasses (Table II) appeared similar to those reported by Salihoglu et al. [23] with mean values of 33.4 ± 1.4 in mild and moderate OSAS and 31.6 ± 0.9 in severe OSAS.

In our study, 50% of patients with a diagnosis of olfactory dysfunction had severe OSAS. A strong negative correlation emerged between the olfactory TDI score and the severity of the OSAS measured according to the apnoea–hypopnoea index (AHI). A negative correlation between the olfactory parameters and the severity of sleep apnoea was also found in the study by Salihoglu et al. [23].

Regarding BMI values and olfactory function in the study by Günbey et al. [24], no significant differences emerged in terms of odour parameters, when the subjects were evaluated as obese (BMI ≥ 28 kg/m^2) and non-obese (BMI < 28 kg/m^2). Furthermore, Salihoglu et al. [23] did not observe any significant effect of BMI on the sum score of the Sniffin' Sticks subtests. The data observed in our study would seem to confirm this aspect.

Olfactory functions are generally negatively associated with age and tend to deteriorate further in the elderly [33-35].

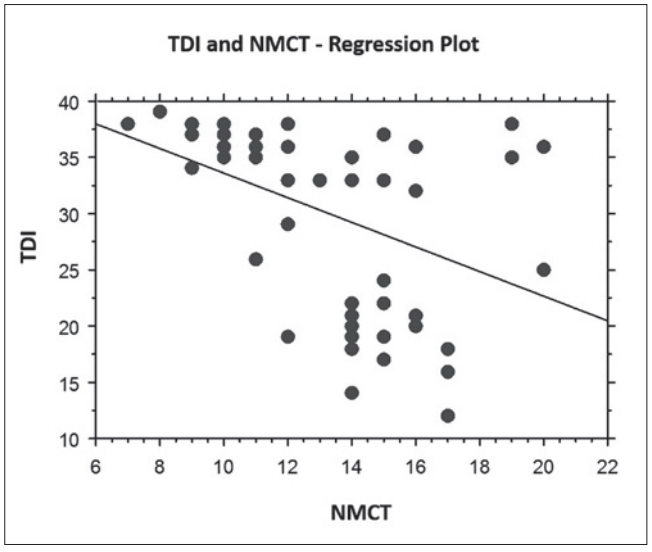

Fig. 3. Linear regression between olfactory TDI and nasal mucociliary clearance time: TDI had a significant and strong correlation with nasal mucociliary clearance time (p = 0.008).

Table III. OSAS group vs control group: olfactory evaluation, rhinomanometry and mucociliary transport time.

	OSAS group N = 60	Control group N = 40	P-value
Olfactory dysfunction: Hyposmia Anosmia	22 (36.6%) 19 (86.4%) 3 (13.6%)	7 (17.5%) 6 (85.7%) 1 (14.3%)	0.04
TDI score (mean value) Mild OSAS Moderate OSAS Severe OSAS	30 33 30.7 26.2	33.3	0.03
Rhinomanometric examination	37 61.6%)	9 (22.5%)	0.0002
NMCT (mean value)	13.2 min	9.8 min	0.0001

In our study, OSAS patients with OD did not have a higher mean age than those without OD (Table III). Regression analysis between TDI score and middle age did not identify any significant correlation (p = 0.7). This aspect would seem exclude age as a factor influencing olfactory results in OSAS patients. Moreover, no significant difference in mean age between the study group and control group was observed.

The olfactory epithelium is the first odour identification site. Odourants carried by the air enter into the nasal cavity and usually reach the olfactory mucosa through airflow. Subsequently, odourants dissolve within the surface of the olfactory mucosa and then bind to olfactory receptors in the surface of olfactory mucosa, producing nerve impulses [10 19].

In our opinion, especially in OSA patients, two important aspects related to the transport of odourants at the olfactory epithelium, should be analysed and discussed. These are possible nasal obstruction and nasal mucociliary clearance.

Patients with OSA most often present concurrent nasal obstruction [21 22]. The latter has been implicated as an independent, aetiological factor in the pathogenesis of OSA, although no definite conclusions on the role of nasal obstruction in OSA pathogenesis have been reported [21 22]. It would seem that nasal obstruction results in an increase in upstream airflow resistance, which makes the pharynx vulnerable to collapse. During sleep, breathing is primarily nasal, but patients with nasal airway obstructions favour oral breathing, which decreases the hypopharyngeal space, leading to increased upper airway resistance and more frequent apnoeic and hypopnoeic episodes [36-39].

Clearly, nasal obstruction is frequently associated with OD because it results in a reduction of the number of odourants that enter the nose during breathing [17 38]. Only Fu et al. [25] reported a relationship between nasal structure and olfactory function in patients with obstructive sleep apnoea. They investigated OSAS patients using acoustic rhinometry and found a correlation between nasal obstruction and lower TDI score. Based on these data they suggested that nasal structure affects parameters of olfactory function, probably via alterations in nasal airflow.

In our study, we preferred to measure nasal air-flow directly via an anterior rhinomanometry. The OSAS patients in our study showed pathological bilateral values of nasal airway resistance in 61.6% of cases. Moreover, 86.3% of OSAS patients with olfactory dysfunction presented pathological rhinomanometry values. In our study, there was a significant difference between patients with and without olfactory dysfunction regarding rhinomanometry results, indicating that nasal obstruction could be a cofactor that is responsible for OD in these patients (Table III).

Inflammation of the nasal mucosa with alterations in nasal mucociliary clearance are a frequent cause of olfactory neuroepithelium dysfunction. Usually, poor mucociliary clearance hinders the interaction between odourous molecules contained in inspired air and the olfactory epithelium [16 37-40].

Most OSAS patients present a reduced mucosal clearance even in the absence of evident sinonasal inflammatory diseases [39-43]. In a recent clinical study regarding nasal mucociliary clearance in obstructive sleep apneoa syndrome patients, Deniz et al. [44] demonstrated that the nasal mucociliary system presents significant deterioration in severe OSAS patients.

The mean value of NMCT in the OSAS patients of our study was 13.2 min with a significant difference vs to the control group.

It is interesting to note that the mean mucociliary transport time in OSAS patients with OD was greater than the mean NMCT value of the OSAS patients without OD (14.7 min vs 12.4 min). A statistically significant difference between these two groups emerged. Furthermore, regression analysis between TDI score and NMTC showed a significant correlation between the increase of the NMTC and lower values of the TDI score (Fig. 3). These findings could indicate that reduced mucociliary clearance represents another cofactor in the OD aetiology of OSAS patients.

Gastro-oesophageal reflux disease (GERD) or laryngopharyngeal reflux disease (LPRD) may be comorbidities in patients with OSAS and an alteration of taste, due to acid aggression, might be possible in these patients [45-48]. However, such conditions do not seem to alter the results of mucociliary transport time measured using the saccharin test, because as reported by Altundag et al., no impairment in sweet taste is present in OSAS patients with LPRD [47 48]. To avoid reflux and other possible subjective interferences, a composition of vegetable charcoal powder and saccharin powder at 3% could be used in alternative to the traditional saccharin test [49].

Finally, regarding the aetiopathogenetic factors of OD in OSAS patients, it should be remembered that intermittent nocturnal hypoxia/reoxygenation episodes represent a trigger for upper airway inflammation and the increase of pro-inflammatory markers such as interleukin-8, tumour necrosis factor-α, or C-reactive protein [40 50 51]. It is possible that these inflammatory mediators might contribute to the harmful effects on olfactory neuroepithelium that occur in these patients. An extensive study through evaluation of inflammatory mediators (e.g. cytokines, interleukins, chemokines, TNF etc.) and nasal cytological aspects is under way in order to broaden current knowledge about olfactory damage in patients with sleep apnoea syndrome.

Conclusions

Patients with OSAS appear to suffer from olfactory dysfunction more than adults who are not affected by sleep apnoea syndrome. The degree of olfactory dysfunction appears to be related to severity of disease. However, other factors such as nasal obstruction and reduced mucociliary clearance play a role in of the aetiology of this condition.

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Audiology

Frontal brain asymmetries as effective parameters to assess the quality of audiovisual stimuli perception in adult and young cochlear implant users

Asimmetria nell'attività cerebrale frontale come parametro efficace della qualità percettiva degli stimoli audiovisivi in portatori di impianto cocleare giovani e adulti

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SUMMARY

How is music perceived by cochlear implant (CI) users? This question arises as "the next step" given the impressive performance obtained by these patients in language perception. Furthermore, how can music perception be evaluated beyond self-report rating, in order to obtain measurable data? To address this question, estimation of the frontal electroencephalographic (EEG) alpha activity imbalance, acquired through a 19-channel EEG cap, appears to be a suitable instrument to measure the approach/withdrawal (AW index) reaction to external stimuli. Specifically, a greater value of AW indicates an increased propensity to stimulus approach, and vice versa a lower one a tendency to withdraw from the stimulus. Additionally, due to prelingually and postlingually deafened pathology acquisition, children and adults, respectively, would probably differ in music perception. The aim of the present study was to investigate children and adult CI users, in unilateral (UCI) and bilateral (BCI) implantation conditions, during three experimental situations of music exposure (normal, distorted and mute). Additionally, a study of functional connectivity patterns within cerebral networks was performed to investigate functioning patterns in different experimental populations. As a general result, congruency among patterns between BCI patients and control (CTRL) subjects was seen, characterised by lowest values for the distorted condition (vs. normal and mute conditions) in the AW index and in the connectivity analysis. Additionally, the normal and distorted conditions were significantly different in CI and CTRL adults, and in CTRL children, but not in CI children. These results suggest a higher capacity of discrimination and approach motivation towards normal music in CTRL and BCI subjects, but not for UCI patients. Therefore, for perception of music CTRL and BCI participants appear more similar than UCI subjects, as estimated by measurable and not self-reported parameters.

KEY WORDS: Approach/Withdrawal • Alpha rhythm • Electroencephalography • Music • Functional connectivity • Graph Theory

RIASSUNTO

Come è percepita la musica dai portatori di impianto cocleare (CI)? Questa domanda sorge come la "prossima sfida", date le impressionanti prestazioni ottenute da questi pazienti nella percezione del linguaggio. Inoltre, come valutare la percezione della musica oltre il dichiarato verbale, così da ottenere dati misurabili? Per rispondere a tale domanda la stima dell'asimmetria dell'attività elettroencefalografica (EEG) in banda alfa, acquisita tramite una cuffia a 19 canali, risulta un mezzo adatto a misurare la tendenza all'approccio o al rifiuto (indice AW) verso uno stimolo. Specificamente, un maggior valore di AW indica una maggiore propensione all'approccio, viceversa un minor valore di AW una tendenza ad evitare un determinato stimolo. Inoltre, a causa dell'acquisizione prelinguale e postlinguale della sordità, bambini ed adulti rispettivamente potrebbero differire nella percezione della musica. Scopo del presente studio è stato quello di indagare due popolazioni di portatori di impianto cocleare di diversa età, bambini e adulti, nelle condizioni di impianto cocleare unilaterale (UCI) e impianto cocleare bilaterale (BCI), durante l'esposizione a tre condizioni sperimentali di stimolo musicale (Normale, Distorto e Muto). Inoltre, è stato effettuato uno studio di modelli di connettività funzionale tra reti cerebrali, così da investigare eventuali pattern funzionali peculiari delle diverse popolazioni. Come risultato generale, sia negli adulti che nei bambini, è stato dimostrata una congruenza tra i pattern elettroencefalografici riportati in pazienti BCI e soggetti di controllo normoudenti (CTRL), caratterizzata da valori più bassi per la condizione Distorto (rispetto alle condizioni Normale e Muto) nell'indice AW e nell'analisi di connettività. Inoltre, la condizione Normale e Distorta risultavano differenti in modo statisticamente significativo per il gruppo degli adulti con impianto cocleare e nei CTRL, così come nel gruppo bambini CTRL, ma non nei bambini con impianto cocleare. Queste evidenze suggeriscono una maggiore capacità di discriminazione e di motivazione all'approccio verso la musica Normale per i soggetti CTRL e BCI, a causa della somiglianza nella percezione della musica per questi due gruppi, in particolare per gli adulti, ma non per i pazienti UCI. Di conseguenza soggetti CTRL e BCI appaiono più simili che gli UCI nella percezione della musica Normale, come stimato da parametri misurabili, non derivanti da dati auto-dichiarati dai pazienti.

PAROLE CHIAVE: *Approccio/Evitamento • Ritmo Alfa • Elettroencefalografia • Musica • Connettività funzionale • Teoria dei Grafi*

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Introduction

Musical sounds, since prehistory, have always exerted rewarding and emotional effects on humans [1], and research on music perception and its effects have seen a large rise in recent years [2]. In this field, cochlear implant recipients are receiving growing interest, particularly in relation to the study of patients' quality of life [3], evaluation of bilateral implantation in adults as a good clinical practice [4] and possible development of specific training for patients [5 6]. Concerning this latter point, an intriguing example has been shown in a recent electroencephalographic (EEG) event related potentials study, where singing was associated with increased development of neural networks for attention and more accurate neural discrimination in cochlear implanted (CI) children [7]. Additionally, musically trained deaf children (hearing aid and/or CI users) showed better performance in auditory scene analysis, auditory working memory and phonetic discrimination tasks in comparison to untrained peers, and the better performances on these tasks might at least be partly driven by music lessons [8].

It is well-known from literature that CI users experience difficulties in perceiving music. This is probably due to the constraint of the CI in transmission of the spectral information of music, and to the complexity of pitch relationships between notes, both at the basis of the perception of the melody [9-12]. Several neuroimaging studies investigating the cortical activation of CI users during speech-language perception showed an increased activation of already present auditory networks, i.e. brain areas traditionally employed for auditory processing; in addition, CI users demonstrate plastic reorganisation of normally occurring networks, including recruitment of brain areas not traditionally utilised for auditory processing [9]. Furthermore, studies on experienced CI users suggest that the degree of activation (in terms of both extent and intensity) of auditory cortex in response to speech stimuli corresponds to the degree of success in speech perception [10]. In fact, even if language perception has reached impressive levels in this population, also characterised by high auditory cortical activation [14], music perception still remains challenging. In fact, intriguing evidence of higher and lower cortical activity for rhythm and melody perception has been found [14]. Therefore, the study of cortical activation in response to music exposure in CI users appears worthy, since it has been repeatedly shown altered cortical activity in CI subjects in comparison to normal hearing ones. Additionally, due to prelingually and postlingually deafened pathology acquisition, which frequently depicts the condition in children and adults, respectively, they would probably result in a different music perception. This statement is suggested by studies showing peculiar frontal cortex activity in prelingual and postlingual deafness. For instance, it has been shown a larger mismatch negativity, generated by the frontal cortex, in CI prelingually deafened patients, related to better speech performances [15]. In addition, CI postlingually deafened patients showed increased cortical activity in comparison to normal hearing subjects, probably due to the use of already existing speech sound networks [16].

This hypothesis is also indirectly suggested by the evidence that later cochlear implantation is correlated with an improved hearing cues in music ability as tested by the Montreal Battery of Evaluation of Amusia (MBEA) in unilateral CI (UCI) children, probably due to the low frequency access in older implanted patients [17]. Evidence also suggests poorer music perception in CI children in comparison to adults [18]. Instead, with respect to normal hearing (NH) peers, UCI children were less accurate but best able to discern rhythm changes and to remember musical pieces [17]. Concerning music perception and the relative induced pleasantness, the available data have shown that both children and young adults CI recipients succeed in identifying the original and instrumental versions of familiar recorded songs. Although favourably evaluated, they were not able to identify different melody versions [19].

In addition, the study of cerebral activations in CI recipients may provide additional perspectives to investigate the possible benefits of surgery and musical therapies. In particular, motivation and affective processes could play an important role for a better comprehension of musical messages. In this regard, the prefrontal cortex (PFC) appears to play a pivotal role in a larger overall circuit involved in emotional and motivational processes [20]. In fact, frontal EEG alpha activity is frequently used to detect intra- and between-subject asymmetries in cortical activation. According to the ''withdrawal/approach'' model, analyses of the EEG power spectrum suggest a different lateralisation for the anterior cerebral hemispheres in approach and withdrawal motivational tendencies and emotions [21]. Specifically, a relative power suppression of the alpha rhythm across the left PFC is associated with a propensity to engage toward a stimulus. The application of EEG withdrawal/approach analysis to the reaction to music exposure has been investigated, highlighting a correlation between the reported valence (pleasantness/unpleasantness) and arousal (intensity/energy) of musical stimuli with frontal alpha (8-13 Hz) asymmetry [22]. In particular, these authors revealed a greater relative left frontal EEG activity to joy and happy musical pieces and greater relative right frontal EEG activity to fear and sad ones.

Experimental evidence in adult CI users in line with this theory have been already provided. The approach towards musical video clips, indexed by the frontal EEG alpha activity imbalance, in a NH population and in bilateral CI (BCI) users presented similar range of variation across different stimuli conditions (normal, distorted and mute), in particular with a total excursion of 0.5 and 0.55 z-score units of the alpha frontal asymmetry index among the conditions; UCI patients reported a higher range of variation in comparison to the other groups, presenting a total excursion of 3.5 z-score units [23].

EEG alpha asymmetries have also been investigated through estimation of the cortical sources of prelingually deaf implanted children during music exposure. In particular, the inconsistency with the approach/withdrawal model in comparison to NH subjects was seen in the UCI condition, suggesting an impairment in discriminating normal from dissonant music and appreciating the pleasantness of normal music [24]. A preliminary study suggested less approach and interest in music for one UCI user with respect to one BCI user and one NH child [25]. Additionally, the pattern of the EEG power spectrum in the alpha band in UCI children indicated more approach (as reflected by the higher alpha synchronization) for mute and distorted music listening than for the Normal audio condition, while NH children and BCI users showed cortical activation that was more similar among the two groups, characterised by higher approach for normal audio in comparison to both mute and distorted audio conditions. Such findings suggested a closer to normal music perception for the BCI users [26].

Moreover, it appears extremely interesting and informative to extend such investigations to different technical approaches. A relation between cerebral rhythms and connectivity has been suggested in several articles, and synchronisation in different frequency bands may correspond to different networks and different cognitive functions [27], also enabling the discrimination between healthy and pathological brain functioning [28].

The brain is a complex system where spatially segregated areas are interconnected. Starting from this perspective, brain network properties can be represented by functional connectivity patterns, and graph theory provides useful quantitative indices to measure these patterns [29]. Previous findings in NH subjects provided evidence for an increase of the number of functional connections and a more random network structure in a portion of the alpha band during music perception, in comparison to both noise and silence conditions. This result supports the hypothesis of a positive effect (higher efficiency) of music on human brain functional networks [30]. Furthermore, a principal component analysis study showed asymmetry in the pre-frontal cortex relating to a number of emotions induced by different kinds of music (pleasantness, energy, tension, anger, fear, happiness, sadness and tenderness) [31]. Despite its importance in understanding physiological and pathophysiological conditions, functional connectivity estimation has not been applied to study music perception in CI users.

In the light of the aforementioned background, the objective of the present study was twofold. Firstly, because of the differences due to the unilateral and bilateral amplification and to the prelingually and postlingually deafened acquisition, we investigated two age populations in unilateral and bilateral cochlear implantation conditions, children and adult implant users, during three experimental situations of music exposure. Secondly, given the absence of published data concerning the functional connectivity during music exposure in these populations, we also characterised connectivity patterns in the same musical experimental conditions (normal, distorted and mute).

Towards these aims, two age populations, children and adults, were recruited according to three experimental groups (BCI, UCI and NH participants), who underwent EEG recording during exposition to three conditions (normal, distorted and mute) of a movie with explicit musical content. The frontal brain asymmetry in the alpha band activity was calculated to quantify the approach expressed by subjects in the three experimental conditions. Furthermore, brain asymmetry calculation was also performed via graph theory on the functional brain connectivity patterns investigated in the same testing conditions.

To our knowledge, this is the first study on music perception in cochlear implanted patients that also investigates functional brain connectivity.

Materials and methods

Experimental design with paediatric subjects

The paediatric population was composed of 6 NH control subjects, 7 UCI and 4 BCI patients (Tables I and II). All children in the study were affected by bilateral, profound, sensorineural hearing loss. None had usable residual hearing (i.e. auditory threshold better than 100 dB HL) in either ear before cochlear implantation. In the unilateral CI condition, patients had no usable residual hearing in the non-implanted ear, and were poor contralateral hearing aid users. All patients included in the study had been receiving auditory-verbal therapy for a minimum of three years. With unilateral CI, they achieved a 20 dB HL PTA for all tested pure tone frequencies. By the time they were included in the study, they had good speech comprehension skills, i.e. 100% score obtained in quiet (assessed

Table I. Paediatric groups age and time of cochlear implant experience.

Group	Age at EEG recording (mean years±SD)	Amplification experience 1st CI (mean years±SD)	Amplification experience 2nd CI (mean years±SD)
CTRL	7.67 ± 4.5		
UCI	4.9 ± 2.15	1.73 ± 0.85	
BCI	5.42 ± 1.92	2.73 ± 1.12	0.31 ± 0.16

Table II. Paediatric clinical data.

Participant	Gender	Atiology	Age at 1st CI	Side 1st CI	Age at 2nd CI	Side 2nd CI
1	M	Unknown	1.92	R		
2	F	Unknown	7.58	R		
3	F	Unknown	4.17	L	7.25	R
4	F	Unknown	1.42	R	4.33	L
5	F	Unknown	1.75	R		
6	F	Unknown	2.17	R	2.92	L
7	M	Prematurity	2.67	R	5.58	L

by speech audiometry, simplified bisyllabic word lists by Turrini, [32]) for children > 5 years and an "appropriate for age" score, assessed by the Sentence Comprehension Test in Italian [33]. All patients wore Nucleus® cochlear implants produced by Cochlear Ltd. (Cochlear Ltd, Sydney, Australia) and used ACE strategy for the encoding of the sound. All CI electrodes were active with normal impedance levels, 900 pps stimulation rate, and ADRO preprocessing algorithm. On the day that the EEG registration was performed, all the patients previously underwent warble-tone free-field audiometry and speech audiometry to ensure their hearing and speech recognition abilities were adequate. The EEG recordings were performed at IRCCS Fondazione Santa Lucia in according to the Declaration of Helsinki after receiving the approval by the local ethical committee. Informed consent was obtained from the parents of all experimental subjects.

Study participants underwent EEG recordings during an audio-video stimulation lasting 3 minutes, extracted from the cartoon Fantasia (Walt Disney, 1940) with the original music of D. Paradisi. This specific cartoon was chosen because it is characterised by a close association between music and images. Additionally, the cartoon nature of the stimulation is particularly suitable to engage the attention of young children, as it is the type of video usually watched by this population. In the selected video extracts, there were ostriches, dressed and dancing like classical dancers, dancing on the Paradisi's music. All the experimental groups performed the test sitting on a comfortable chair, placed at a distance of one meter from the screen used for the stimulation. The audio of the video was set so that it did not have intensity peaks greater than 65 dB. In particular, three versions of the video clip were proposed during the EEG recordings: the first was composed of original audio and video and it will be called hereafter as "normal" movie (normal condition). The second version of the movie was obtained in the following way from the original normal movie: 1) the video was maintained unchanged; 2) the audio signal was played in reverse mode, from the last note to the first one, in order to generate an undecipherable sound but maintaining the same global acoustic pressure generated in the normal condition. The software Audacity was used to realise this stimulus, which will be called hereafter as "distorted". The third version of the movie was generated by presenting the same video than in the normal and distorted stimuli but without sound. In this case, the movie was called "mute". The three stimulations (normal, distorted, mute) were counterbalanced among subjects to avoid a sequence effect.

Experimental design with adult subjects

The adult population was composed of 7NH control subjects, 7 UCI and 6 BCI patients (Tables III and IV). Several of the adult patients had residual hearing in both ears before any implantation, as indicated in Table IV by the PTA calculated as the average threshold among 250 Hz, 500 Hz, 1 kHz, 2 kHz frequencies for each ear. As for the paediatric group, the UCI adult sample was also composed of poor contralateral hearing aid users. Five of 7 adult patients enrolled in the study received speech therapy for at least 1 yeast before the testing, two of whom did not undergo speech therapy. All patients wore coch-

Table III. Adult groups age and time of cochlear implant experience.

Group	Age at EEG recording (mean years±SD)	Amplification experience 1st CI (mean years±SD)	Amplification experience 2nd CI (mean years±SD)
CTRL	37.57 ± 14.55		
UCI	48.87 ± 14.74	2.89 ± 3.51	
BCI	49.71 ± 15.82	3.87 ± 3.55	0.65 ± 0.35

Table IV. Adult clinical data (PTA calculated as the average threshold among 250 Hz, 500 Hz, 1 KHz, 2 KHz frequencies for each ear).

Participant	Gender	Deafness	Age at 1st CI (mo)	PTA Pre-1st CI Right side	PTA Pre-1st CI Left side	Side of 1st CI	Age at 2nd CI (mo)	PTA Pre-2nd CI Right side	PTA Pre-2nd CI Left side	Side of 2nd CI
1	M	Postverbal	61.08	62.5	95	R	61.33	88.75	95	L
2	F	Periverbal	52.08	95	95	L	54.33	95	108.33	R
3	M	Postverbal	32.67	81.67	120	R	34.58	100	120	L
4	M	Postverbal	47.08	57.5	91.25	L		57.5	103.75	
5	F	Periverbal	20.58	95	95	R	25.42	120	102.5	L
6	M	Periverbal	41.00	101.25	87.5	R	50.50	115	97.5	L
7	M	Postverbal	67.33	80	120	L	68.08	120	120	R

lear implants produced by Cochlear Ltd. (Cochlear Ltd, Sydney, Australia), used ACE strategy for the encoding of the sound, and all CI electrodes were active with normal impedance levels, 900 pps stimulation rate and ADRO preprocessing algorithm. On the day that the EEG registration was performed, all patients previously underwent warble-tone free-field audiometry and speech audiometry to ensure their hearing and speech recognition abilities were adequate. The EEG recordings were performed at IRCCS Fondazione Santa Lucia in according to the Declaration of Helsinki after receiving the approval by the local ethics committee. Informed consent was obtained from all participants.

For the adult population, the visual stimuli consisted of a 3-minute-length piece from the musical West Side Story. The extract of the musical was about a very animated scene of a sort of dance challenge, with many dancers and a strongly rhythmical music. In particular, in the video clip there is no speech, but only music and dance strictly related. As for the paediatric population, three versions of the video clip were watched by each participant and the three movies were named according to the labeling already described in the previous section (normal, distorted and mute).

Behavioural rating

Adult subjects were asked to rate how much they liked or disliked each condition (normal, distorted and mute) on a scale ranging from 0 (lowest) to 10 (highest) immediately after watching the video. This self-report investigation was conducted only on adult subjects because children were not able to express on a number scale their like/dislike rating.

EEG data recording and signal processing

The EEG data were acquired using the BeMicro device (EBNeuro spa, Italy), equipped with a 16 electrode cap according to the 10-20 International System. In particular, the signal was gathered from the following locations: F7, F8, F3, Fz, F4, T7, C3, Cz, C4, T8, P3, Pz, P4, O1, O2. The signals were acquired maintaining the value of the impedance below the 10 kΩ and at a sampling frequency of 256 Hz. This EEG activity at rest (in the present experiment corresponding to the open eyes condition looking at a black screen without any stimuli played) was used to calculate the Individual Alpha Frequency (IAF) to accordingly define individual alpha and band ranges of the EEG spectrum [34].

The pre-processing of the EEG data was performed using the EEGLAB software [35] according to the following steps. The first was to perform a band-pass filtering of the EEG data in the range 2-30 Hz, in order to exclude most muscular artifacts (beyond 30 Hz) and the drift due to the changing of the contact impedance (below 2 Hz). Successively, the independent component analysis (ICA) was performed to remove signal artifacts mainly due to eye movements. Therefore, components related to eye-blinks were first detected and then removed. The EEG trace was segmented into trials lasting 1 sec, thus obtaining 180 tri-

als for each subject and for each movie condition analysed. EEG spectral analysis was performed by calculating the power spectral density [36] (PSD) of the acquired signals during the different conditions with a frequency resolution of 0.3 Hz. The EEG PSD values obtained were averaged within the alpha frequency band whose range was calculated in accordance with the definition of the IAF [34], i.e. alpha range is between [IAF-4, IAF+2].
In order to analyse the EEG activity when the novelty effect of the stimulation was ended, the following analyses were conducted considering the time interval corresponding to the second minute of stimulation (from trial 61 to 120).

PSD frontal imbalance index
The use of the approach/withdrawal theory has been validated over the last 20 years by a large number of studies since its formulation in the early 1990s [37-41]. In the present study, the imbalance of the EEG spectral activity in the alpha frequency band over the prefrontal areas has been chosen as the main index for the evaluation of approach/withdrawal towards the stimuli. This index was then estimated for each subject and for each condition analysed. In particular, the approach/withdrawal (AW) index is defined as follows:
AW = PSDR – PSDL
being the PSDR the mean value of PSD calculated on the frontal right electrodes F8, F4, and the PSDL the average value of PSD related to frontal electrodes F7, F3. The AW index was calculated in each population for each movie condition. Positive values would indicate relative greater right alpha frontal activity, so suggesting an approach tendency; vice versa negative values would underline relative higher left alpha frontal activity, suggesting a withdrawal tendency.

Partial directed coherence
The estimation of the functional connectivity by means of partial directed coherence (PDC) [42] allows to analyse the causal relationships between the EEG signals acquired from the different electrodes during the execution of a task. PDC is a frequency domain representation that allows the inference of functional relationships between electrodes, and so between localised cortical areas. Once estimated the connectivity between the EEG signal gathered from the different electrodes, it was necessary to apply a statistical validation method to distinguish the real connections from those arising due to random fluctuations and measurement errors. The value of effective connectivity for a given pair of electrodes, obtained by computing PDC [43 44], must be statistically compared with a threshold level which is related to the lack of transmission between considered regions of interest (ROIs) (null hypothesis). Threshold values were estimated using asymptotic statistic [45 46]. Details of the applied methodology have been provided elsewhere [47 48]. After the validation process, the PDC estimation is averaged within the alpha band defined according to the IAF [34] to take into account the variability among subjects of the alpha peak in the spectrum. Specifically, we defined the alpha band ranging between [IAF-4, IAF+2].

Graph theory
A graph consists of a set of vertices (or nodes) and a set of edges (or connections) indicating the presence of some sort of interaction between the vertices. The PDC previously obtained in the present study was selected as the current adjacency matrix. The adjacency matrix contained the information about the connectivity structure of the graph. In graph theory, a path or a walk is a sequence of vertices, in which from each of its vertices, there is a connection to the next vertex in the sequence. Such adjacency matrix can be used for the extraction of salient information about the characteristic of the investigated network, by defining several indexes based on the elements of such matrix.
In order to obtain a characterisation of the global cerebral networks via graph theory, we used the following indices already present in literature [49]: degree and local efficiency. Degree: a measure of "centrality", it is calculated as the number of links connected to a node, somehow a measure of the "importance" of a node in a network Local efficiency: a measure of the "segregation" of the network, related to the shortest path between two nodes both neighbours of the node object of measurement of its local efficiency and it is calculated as the average of the local efficiency of all nodes belonging to the network [50].
In addition, we defined the following two new ones to obtain a specific characterization of the frontal asymmetries, analogously to the AW index. In particular, we defined the imbalance of out degree as the ratio between the differences of the out degrees (i.e. outgoing connections) of the two hemispheres of frontal areas and the total out degree computed across frontal nodes. Specifically:

$$[IMB]\_(Out\ Degree)=([Out\ Degree]\_R - [Out\ Degree]\_L)/[Out\ Degree]\_Tot$$

where the out degreeR is the average degree among nodes in frontal right hemisphere, out degreeL is the average degree among nodes in frontal left hemisphere and out degreeTot is the total degree of the nodes across all frontal nodes.

According to the formula, the minimum value that this index can assume is -1. In this case, all the outgoing connections comes from the left hemisphere. Analogously, its maximum value is equal to 1, and is representative of the opposite situation with all the outgoing connections coming from the right hemisphere. If both hemispheres have the same number of outgoing connections, this index is zero. Since the out degree index represents the number of links outgoing from a node, it would reflect the flow of information from a certain brain region, and finally a measure of cerebral activity in that area. In the just introduced index, the imbalance of out degree would shape the difference in outgoing flow information level between the right and left frontal areas.

The second index is the imbalance of local efficiency, which is defined as the ratio between the difference of the mean local efficiency computed among right and left frontal nodes and the local efficiency areas across all frontal nodes.

[IMB]_(Loc Eff)=([loc eff]_R - [loc eff]_L)/[loc eff]_Tot

where:

loc eff_R is the mean local efficiency across nodes of the frontal right hemisphere, loc eff_L is the mean local efficiency across nodes of the frontal left hemisphere, whereas the loc eff_Tot represents the mean local efficiency across all frontal nodes. The imbalance index of local efficiency ranges between [-1; 1] and, depending on the prevailing hemisphere, it can reach -1 (only the left hemisphere contributes) or +1 (only the right hemisphere contributes). When the contribution of the two hemispheres is balanced this index is zero. The local efficiency reflects the tendency of a network to create subgroups of elements (clusters) strongly related among them, probably suggesting a sort of specialisation within the network. This index constitutes a measure of efficiency, so the introduced imbalance of local efficiency index would show the difference in efficiency between the right and left frontal areas. The indices were calculated for all subjects and conditions. Further results also showed an interesting pattern in the evolution of functional network complexity: networks were relatively closer to random in the youngest and the oldest groups, and relatively more 'small-world' like in the intermediate age group [51]. This suggests that network evolution during development may be characterised by the gradual acquisition of order in random networks, converting them to optimal 'small-world' networks. A study in children, who had EEG recorded at age five and age seven, supports this hypothesis on brain network development [52].

Statistical analysis

For each experimental group (CTRL, UCI, BCI) and cerebral index (AW, IMB out degree, IMB loc eff) a repeated measures ANOVA was carried out, in which the dependent variables were represented by the indices employed to summarise the estimated brain network, and the independent factor by the movie conditions (normal, distorted and mute). The statistical analysis was completed by performing the post-hoc Duncan test at the 0.05 level of significance.

Results

Behavioural rating

Concerning the rating of the different conditions within each experimental group, only cochlear implant patients showed a significant difference between conditions (UCI: $p = 0.03$, $F = 4.8$; BCI: $p < 0.001$, $F = 15.07$; Control: $p = 0.09$, $F = 2.96$) (Fig.1).

The comparison between normal, distorted and mute conditions in UCI and BCI subjects revealed a significant difference between normal and distorted conditions ($p = 0.023$ and $p = 0.002$ respectively) and between normal and mute conditions ($p = 0.021$ and $p < 0.001$ respectively). The Control group, although without reaching statistical significance, showed absolute values with a trend similar to the one reported in UCI and BCI. In both UCI and BCI groups, the comparison between distorted and mute conditions did not reach statistical significance. However, the mute condition for both the UCI and BCI groups showed the lowest ratings, while the Control group showed the lowest value for the distorted condition.

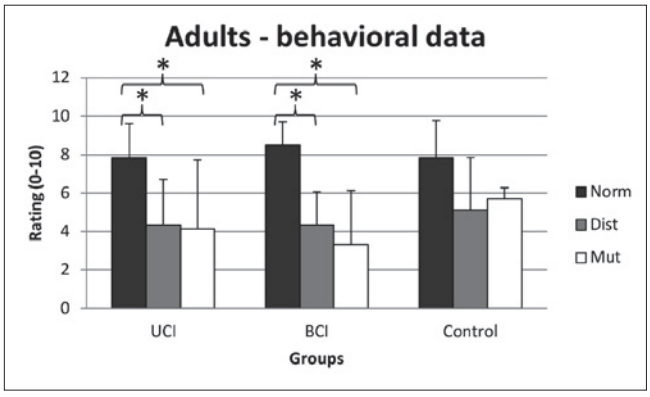

Fig. 1. Adult subjects rating of the different video versions (normal, distorted and mute) on a scale ranging from 0 (lowest pleasantness) to 10 (highest pleasantness). The comparison between normal, distorted and mute conditions in unilateral cochlear implant (UCI) and bilateral cochlear implant (BCI) subjects revealed a significant difference between normal and distorted conditions ($p = 0.023$ and $p = 0.002$, respectively) and between normal and mute conditions ($p = 0.021$ and $p < 0.001$, respectively). "Control" stands for normal hearing control subjects.

PSD alpha asymmetries

In general, in both adults and children, a congruency among patterns between BCI patients and CTRL subjects was observed. In particular, both the BCI and CTRL groups showed lowest approach values for the distorted condition, in comparison to normal and mute ones. All paediatric groups, UCI, BCI and CTRL, reported the same pattern, characterised by lowest values for the distorted condition and highest values for the mute one. All adult groups showed a statistically significant difference between the normal and distorted condition and between distorted and mute, and UCI and BCI groups reported highest values for the normal condition. Concerning paediatric groups, only the CTRL group showed a significant difference in the above-cited comparisons, and the BCI group showed a significant difference only in the comparison between mute and the other conditions, while the UCI group did not show any significant difference between conditions.

Unilateral group (UCI)

Paediatric population. There was no significant difference between the experimental conditions ($p = 0.84$, $F = 0.17$) (Fig. 2a). It was possible to see a trend of the pattern shown by the Control group (Fig. 2e) (see below): lowest values for the distorted condition, then the normal and mute conditions progressively higher.

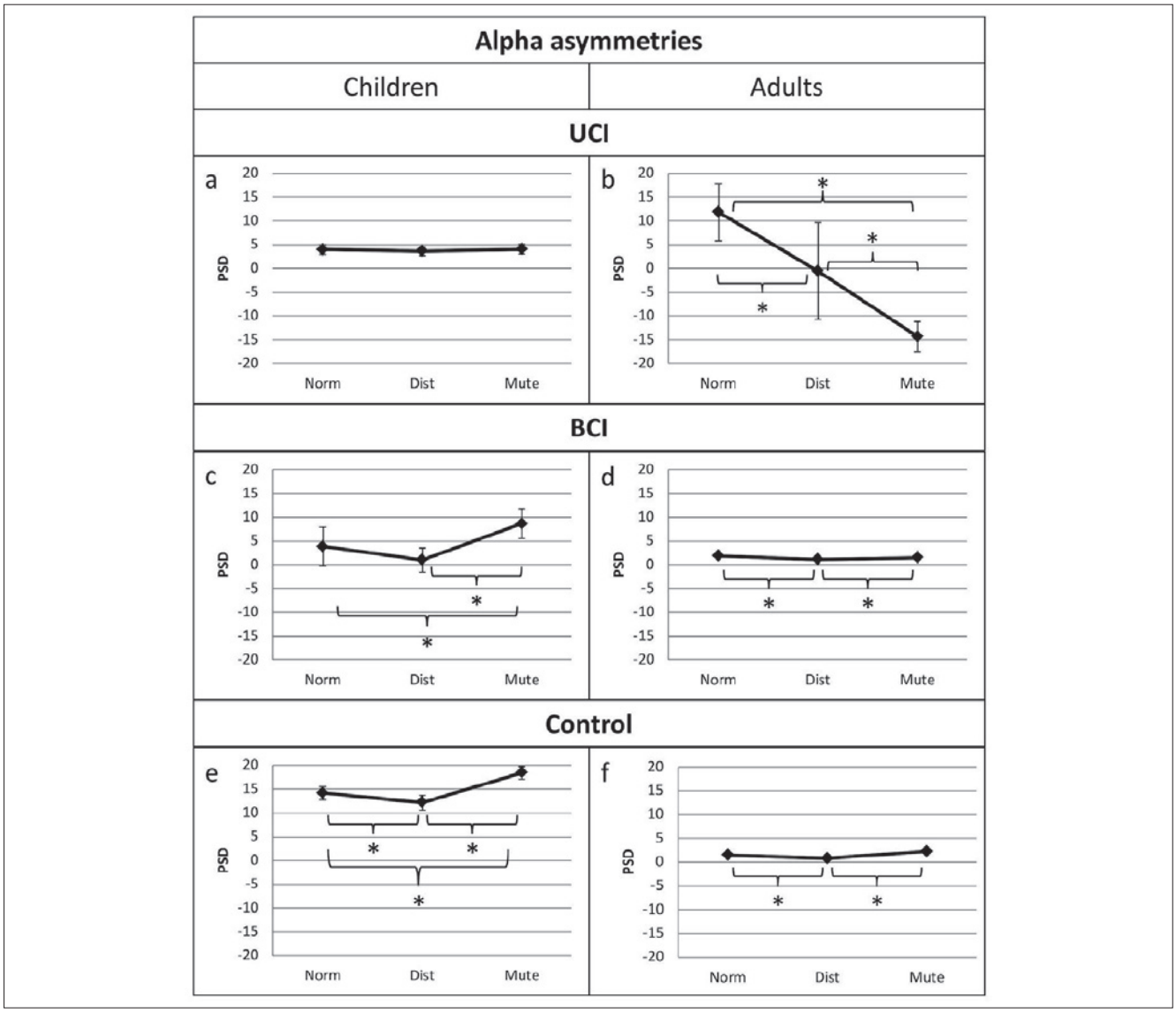

Fig. 2. EEG alpha asymmetries reported in the paediatric (left panels) and adult groups (right panels). Asterisks indicate statistically significant differences. Control = normal hearing control subjects; UCI = unilateral cochlear implant subjects; BCI = bilateral cochlear implant subjects.

Adult population. There was a significant difference between the three movie conditions ($F = 12.94$, $p < 0.001$) (Fig. 2b). The mute condition presented the lowest value, followed by the distorted and normal movies, and the pairwise comparisons were all characterised by significant differences (Duncan's test, normal and mute $p = 0.004$; normal and distorted $p = 0.0449$; distorted and mute $p < 0.001$).

Bilateral group (BCI)

Paediatric population. There was a significant difference between the experimental movies ($p < 0.002$, $F = 6.6$). Furthermore, a significant difference was present in the comparison between normal and mute (Duncan's test $p < 0.05$) and distorted and mute (Duncan's test $p < 0.05$). The above cited pattern concerning frontal imbalance (distorted→normal→mute) was also seen in this group.

Adult population. There was a significant difference between all movies ($p < 0.001$, $F = 28.31$) (Fig. 2d). The pairwise comparisons also showed statistically significant differences between normal and distorted $p = 0.025$) and between mute and distorted ($p = 0.009$).

Control group

Paediatric population. There was a significant difference between all the experimental conditions ($p = 0.001$, $F = 19.3$) (Fig. 2e). In particular, concerning the distorted condition, the front imbalance index value was significantly lower than the normal and mute condition (Duncan's test, $p = 0.049$ and $p < 0.001$, respectively), and there was also a significant difference between the normal and mute conditions ($p < 0.001$).

Adult population. A significant difference was seen between all types of movies ($p = 0.0311$, $F = 4.16$) (Fig. 2f). Additionally, the distorted movie presented a frontal imbalance index that was statistically significantly lower than normal (Duncan's test, $p = 0.0179$) and mute (Duncan's test, $p = 0.0424$) conditions.

Graphs indices asymmetries

The aforementioned connectivity indices were computed and compared for each population (paediatric, adult) and experimental condition. Below, we report the results related to the indices that were significantly modulated across populations and conditions: the imbalance of out degree and the imbalance of local efficiency.

Concerning the imbalance of out degree, the BCI and UCI groups showed the lowest values for the distorted condition, which was significantly lower for the distorted condition in comparison to normal except for the paediatric UCI group, where there was only a trend. Both UCI groups showed the highest values for the normal condition and lowest values for the mute video.

Concerning the imbalance of local efficiency, the BCI and CTRL groups showed the lowest values for the distorted condition. Furthermore, UCI children BCI adults and both CTRL groups had the highest values for the normal condition.

Unilateral group (UCI)

Paediatric population. The indices that showed a significant difference in the CTRL group were analysed in the UCI group. The imbalance of out degree index showed a significant difference ($p=0.02$, $F=5.78$) (Fig. 2a), but Duncan's post-hoc comparisons did not show significant differences, even if the comparison between the mute and the distorted conditions was just below the significance threshold ($p = 0.057$). The analysis of the imbalance of local efficiency index reached statistical significance ($p = 0.025$, $F = 5.875$) (Fig. 4a), and the comparison between normal and mute ($p = 0.045$) and between distorted and mute ($p = 0.002$) conditions were also significantly different.

Adult population. The indices that showed a significant difference in the CTRL group were analysed in the UCI group. Neither the imbalance of out degree index (Fig. 3b) ($p = 0.07$, $F = 3.29$) nor the imbalance of local efficiency index (Fig. 4b) reached statistically significant differences ($p = 0.84$, $F = 0.177$).

Bilateral group (BCI)

Paediatric population. The indices that showed a significant difference in the CTRL group were analysed in the BCI group. The imbalance of out degree index analysis showed a trend similar to the that present in CTRL subjects ($p = 0.89$, $F = 0.46$) (Fig. 3c), characterised by distorted condition values lower than mute and normal conditions; similarly the imbalance of local efficiency index analysis did not reveal any significant difference ($p = 0.478$, $F = 0.857$) (Fig. 4c).

Adult population. The indices that showed a significant difference in the CTRL group were analysed in the BCI group. Both the imbalance of output degree index ($p = 0.024$, $F = 5.58$) (Fig. 3) and the imbalance of local efficiency index ($p = 0.046$, $F = 4.64$) (Fig. 4d) showed a significant difference. In the imbalance of local efficiency index, the distorted condition value was significantly lower than the normal and mute conditions (Duncan's test, $p = 0.038$ and $p = 0.022$, respectively). Concerning the imbalance of out degree index, similar results were obtained. The distorted condition value was significantly lower than that in the normal and mute conditions ($p = 0.025$ and

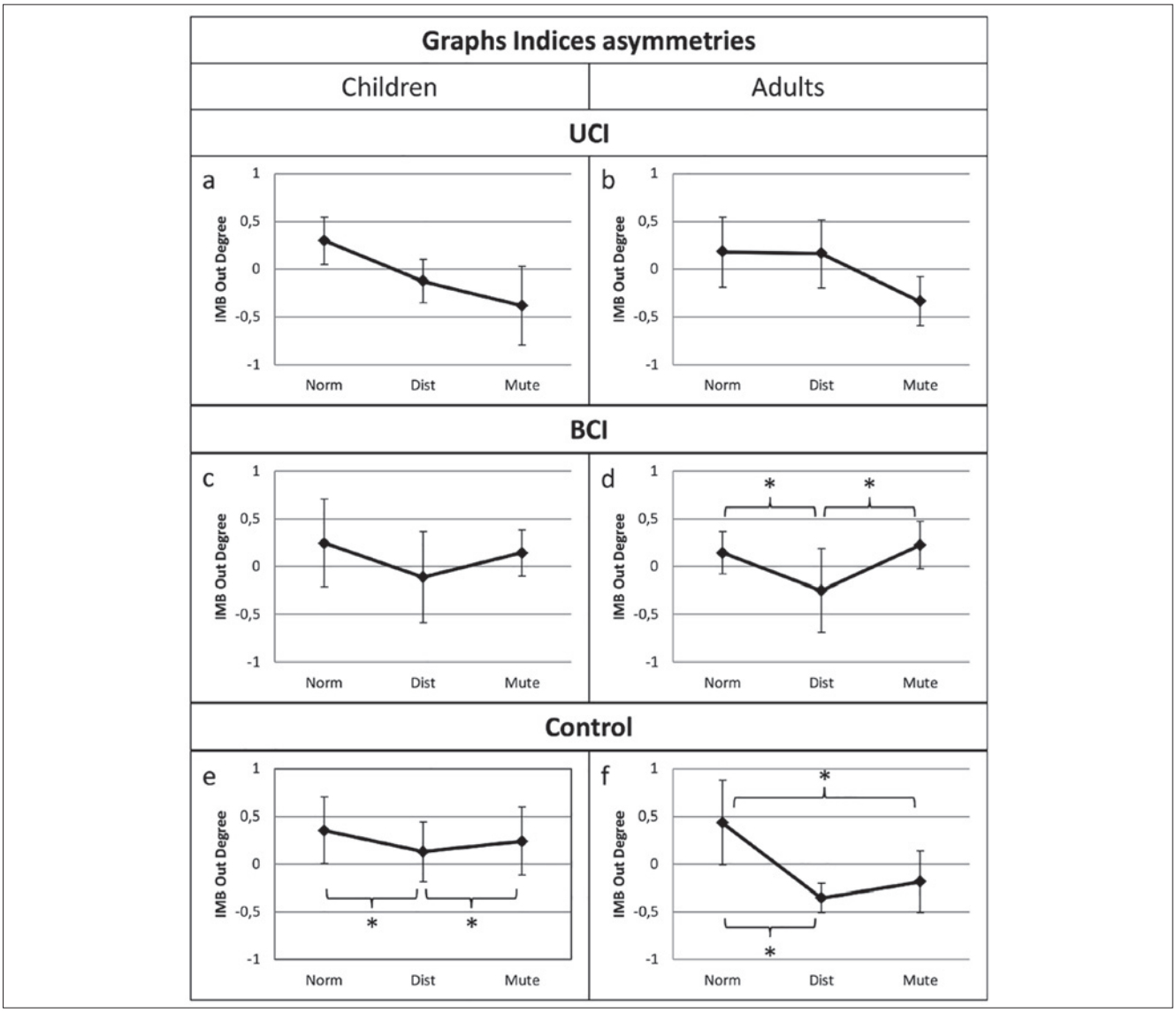

Fig. 3. EEG imbalance of out degree graph index reported in the paediatric (left panels) and adult groups (right panels). Asterisks indicate statistically significant differences. Control = normal hearing control subjects; UCI = unilateral cochlear implant subjects; BCI = bilateral cochlear implant subjects.

p = 0.017). Finally, the trend resulting in the imbalance of out degree index was similar to that observed in CTRL subjects.

Control group

Paediatric population. The analysis of the imbalance of the out degree showed a significant difference (p = 0.02, F = 6.64), showing higher activity in the normal and mute conditions with respect to the distorted condition (p = 0.005 and p = 0.016, respectively) (Fig. 3e).

A significant difference was seen in the imbalance of local efficiency index analysis (p = 0.03, F = 5.6) (Fig. 4e). Duncan's post-hoc test highlighted a significant reduction in the distorted condition, which was characterised by a negative value vs. the positive values of the normal and mute conditions (p = 0.016 and p = 0.025).

No significant differences were seen in the analysis of: the global efficiency index (p = 0.89, F = 0.11), local efficiency index (p = 0.87, F = 0.14), asymmetry between hemispheres index (p = 0.58, F = 0.59), degree index (p = 0.63, F = 0.48), input degree index (p = 0.61, F = 0.48), out degree index (p = 0.61, F = 0.49), imbalance degree index (p = 0.43, F = 0.91) or imbalance of input grade index (p = 0.65, F = 0.45).

Adult population. The imbalance of the out degree index analysis showed a significant difference (p = 0.0144,

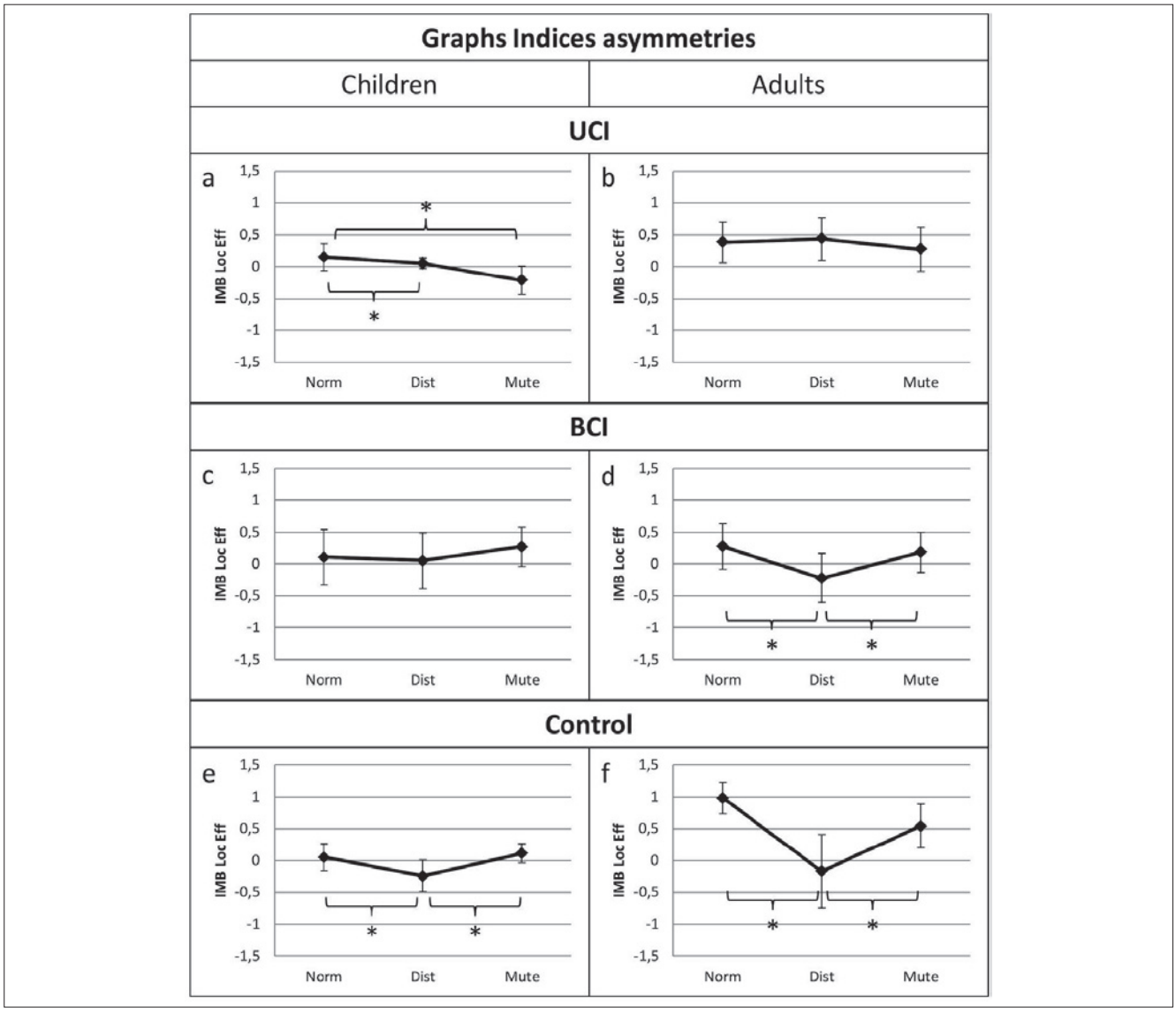

Fig. 4. EEG imbalance of local efficiency graph index reported in the paediatric (left panels) and adult groups (right panels). Asterisks indicate statistically significant differences. Control = normal hearing control subjects; UCI = unilateral cochlear implant subjects; BCI = bilateral cochlear implant subjects.

F = 4.25) with the Normal condition expressing a higher value in comparison to the Distorted and Mute conditions (p = 0.001 and p = 0.0289, respectively) (Fig. 3f).

The analysis of the imbalance of local efficiency index showed a significant difference (p = 0.0284, F = 7.49), and the post-hoc comparisons revealed significantly lower values for the distorted condition in comparison to the normal and mute conditions (p = 0.0019 and p = 0.0449, respectively) (Fig. 4f).

No statistically significant differences were found in the analysis of: the Global Efficiency index (p = 0.763, F = 0.275); the Local Efficiency index (p = 0.55, F = 0.622); Degree (p = 0.553, F = 0.586); the Input Degree index (p = 0.57, F = 0.56); the Out Degree index (p = 0.55, F = 0.58); the Imbalance Degree index (p = 0.925, F = 0.078); the Imbalance of Input Degree index (p = 0.977, F = 0.023).

Discussion

PSD alpha asymmetries

The most evident result was represented by the pattern of activation characterising the CTRL and BCI groups in both the adult and paediatric populations. This pattern showed the lowest values of the approach-withdrawal

index, sign of less approach, for the distorted condition, in accordance with previous studies [24 26]. This suggests a higher capacity of discrimination and approach motivation towards normal music in the CTRL and BCI groups, in particular in the adult population, due to the similarity in music perception for these two groups, but not for the UCI group. Previous studies support this hypothesis, providing evidence of a higher capability of enjoyment of music by BCI vs. UCI subjects. In particular, subjects who underwent sequentially bilateral cochlear implantation reported that music generally sounded better: 90% reported it was more natural and 85% reported it was more pleasant after the second CI [53]. Additionally, it is interesting to note that, regarding CTRL and BCI patients, the adult population reported the highest alpha PSD (or approach) values for the normal condition. This could be explained by the fact that adult subjects usually have a past of some type of hearing, especially in the low frequency ranges. It has been hypothesised that access to early acoustical hearing in the lower frequency ranges appears to establish a basis for music perception, which can be accessed with later electrical CI hearing [17]. In addition, it has been shown that low-frequency acoustic hearing improves pitch discrimination performances in comparison to electric-only stimulation in CI adult users [11]. This cueing strategy relying on low frequencies would of course be denied to children selected for cochlear implantation because of the frequent absence of residual hearing in these patients. Moreover, also EEG show the presence of residual neural skills for music processing in adult CI users. These enable patients to automatically process changes in sound features in a musical context, except for automatic rhythm discrimination [54].

In the literature, there is some evidence of the major interest and relying on visual stimuli than on auditory stimuli expressed by CI children [55-57]. In the present study, in contrast to expectations, NH and not only CI children groups also presented the highest values for the mute condition. This result could be explained by the observation that, in general, values obtained in CTRL subjects were lower than BCI or UCI subjects, probably suggesting a higher approach of all sensory modalities involved in CI groups. Furthermore, BCI children reported a value very close to zero for the distorted condition suggesting a not well-defined approach or withdrawal motivation toward the distorted movie, but higher and positive values for the normal and mute conditions. These results could suggest a deficit in the discrimination of "alternative versions" of music, as previously reported [17], thus explaining a lacking response of interest or disinterest. Concerning UCI children, the patterns of the approach-withdrawal index were in general flatter than NH and BCI children, and did not show significant differences between conditions, supporting the hypothesis of the insufficient amplification for music perception provided by only one implant in these types of patients [26]. Furthermore, this evidence could be linked to the sensitive period that is necessary for correct establishment of auditory-visual integration in CI children [58].

Finally, it is interesting to note the unique pattern displayed by the adult UCI group, not presenting a "V shape" but a "flat shape", characterised by a zero value for the distorted condition and negative values for the mute condition. The first observation could be explained by a deficit in discriminating the distorted condition due to insufficient amplification in the UCI group. The second observation could be mirrored by the behavioural rating data, reporting lowest values for the mute condition in cochlear implanted patients; finally, this scenario could reflect the sensation of "fear" that is incidentally and behaviourally self-reported by UCI subjects. This feeling shapes the neural correlates of withdrawal suggested by negative values.

Graph indices asymmetries

The results regarding the CTRL and BCI groups presented lowest values in the distorted condition, similarly to the frontal imbalance analysis for the same groups. Concerning the BCI adult group, there was a consistency in the pattern expressed in the two graph indices, and in the frontal asymmetry index, suggesting accordance and reliability between the first ones and among all indices. Concerning the UCI groups, in both the indices, they reported the same pattern characterised by lowest values for the mute condition (Fig. 4), both in children and the adult population. As discussed above, that could be linked to the "fearful" reaction of subjects to the absence of sound self-reported by patients. This aversive reaction could be expressed by UCI subjects and not by BCI subjects because of a more demanding and distressful approach to sound as a baseline condition in UCI patients, in whom the amplification provided may not be sufficient. An indirect proof of this hypothesis is that normal-like audiovisual segregation is possible in highly skilled cochlear implant users [59], so that UCI individuals cannot rely on the same set of audio and visual cues, finally producing withdrawal in UCI in the mute condition.

Finally, considering the graph indices in the normal condition, it is interesting to note that positive values were ubiquitous in all the experimental groups and reaching significance in BCI and CTRL groups, showing a relative greater activity in the right frontal hemisphere. This con-

sideration shows that the normal condition elicited positive graph index values and therefore approach behaviour, as suggested by brain functionality in the frontal areas. This brain functionality would reflect the normal hearing condition and the analogous condition restored by neuroplasticity in the BCI groups, but not in the UCI groups, even if a tendency toward the pattern is suggested even in unilateral implantation. As suggested by the graph indices (both the out degree and the local efficiency), similar considerations but in an opposite direction can be made for the neural activity underlying the reaction to the distorted condition, characterised by a relatively greater activity in the left frontal hemisphere and therefore a propensity to withdraw from the stimulus by CTRL and (mainly) BCI groups. Evidence of an alteration in connectivity has been already identified in neurological disorders, such as Alzheimer's disease, multiple sclerosis, traumatic brain injury and epilepsy, challenging the classical concept of neurological disorders producing either "local" or "global" efficiency alterations, and pointing to the overload and failure of hubs as a possible final common pathway in neurological disorders [28].

Abstracting the EEG functional activity from the direction of the connection among nodes, and generalising to the level of synchronisation activity among them, the reaction to the listening of music pieces has been investigated. In particular, listening to pleasant music (by Bach and Mahler) characterised by melodic features following expected rules produced an increase in the left cognitive area activity; on the other hand, exposure to unpleasant music lacking predictable melodic features produced an higher right frontopolar activity [60]. These data could support the hypothesis that, as indexed by the imbalance of out degree and the imbalance of local efficiency in our study, the almost ubiquitous higher right alpha relative functional connectivity in response to normal music would underlie the catching of its predictive melodic features, while the higher left relative functional connectivity in response to the distorted condition mainly expressed by BCI and CTRL groups may underlie the catching of the novel/unpredictable nature of that musical version. The last statement, again, would imply an insufficient melodic cue detection by UCI subjects, so to enable them of the unpredictable nature of the distorted condition.

The sum of these evidences supports the hypothesis that the connectivity study is an alternative suitable approach for assessing approach tendency towards music in adults and children, although the clinical implications of altered connectivity pattern must be further explored.

Conclusions

Responses in the BCI groups were more similar to those shown by NH groups, thus supporting the evidence for lack of information provided by one cochlear implant. All indices investigated, frontal alpha band asymmetry and graphs (connectivity) indices reported accordance among results, suggesting the worthiness of also applying this type of analysis to approach/withdrawal motivation studies.

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AUDIOLOGY

Hearing threshold estimation by auditory steady state responses (ASSR) in children

Stima di soglia mediante potenziali evocati uditivi di stato stazionario in età pediatrica

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SUMMARY

Hearing threshold identification in very young children is always problematic and challenging. Electrophysiological testing such as auditory brainstem responses (ABR) is still considered the most reliable technique for defining the hearing threshold. However, over recent years there has been increasing evidence to support the role of auditory steady-state response (ASSR). Retrospective study. Forty-two children, age range 3-189 months, were evaluated for a total of 83 ears. All patients were affected by sensorineural hearing loss (thresholds ≥ 40 dB HL according to a click-ABR assessment). All patients underwent ABRs, ASSR and pure tone audiometry (PTA), with the latter performed according to the child's mental and physical development. Subjects were divided into two groups: A and B. The latter performed all hearing investigations at the same time as they were older than subjects in group A, and it was then possible to achieve electrophysiological and PTA tests in close temporal sequence. There was no significant difference between the threshold levels identified at the frequencies tested (0.25, 0.5, 1, 2 and 4 kHz), by PTA, ABR and ASSR between the two groups (Mann Whitney U test, $p < 0.05$). Moreover, for group A, there was no significant difference between the ASSR and ABR thresholds when the children were very young and the PTA thresholds subsequently identified at a later stage. Our results show that ASSR can be considered an effective procedure and a reliable test, particularly when predicting hearing threshold in very young children at lower frequencies (including 0.5 kHz).

KEY WORDS: Sensorineural hearing loss • ASSR • ABR• Audiometry • Children

RIASSUNTO

Effettuare una stima di soglia in età pediatrica è compito spesso difficile e complesso. A tal proposito, i potenziali evocati uditivi del tronco (ABR) rappresentano ancora la tecnica più affidabile per la definizione di soglia, sebbene il ruolo dei potenziali evocati uditivi di stato stazionario (ASSR) sia stato rivalutato negli ultimi anni. In questo studio retrospettivo sono stati valutati 42 bambini, di età compresa tra 3 e 189 mesi, per un totale di 83 orecchie. Tutti i pazienti inclusi sono risultati affetti da ipoacusia neurosensoriale (≥ 40 dB HL in base alla valutazione click-ABR). Tutti i pazienti sono stati sottoposti ad ABR, ASSR ed audiometria tonale, quest'ultima eseguita appena lo sviluppo psico-fisico del bambino lo ha consentito. I soggetti sono stati suddivisi in due gruppi: gruppo A e B, quest'ultimo ha effettuato tutte le indagini audiologiche quasi nello stesso momento, in quanto i bambini erano più grandi rispetto ai soggetti del gruppo A (in particolare sia le valutazioni elettrofisiologiche che l'audiometria tonale sono state eseguite quasi contemporaneamente). Non sono risultate differenze significative tra i livelli di soglia individuati, alle frequenze testate (0,25, 0,5, 1, 2 e 4 kHz), mediante audiometria tonale, ABR e ASSR, tra i due gruppi (test di U Mann Whitney, $p < 0,05$). Inoltre, considerando i bambini del gruppo A, non si è rilevata alcuna differenza significativa tra le soglie identificate mediante ASSR e ABR rilevate quando i bambini erano molto piccoli, e le soglie di audiometria tonale identificate successivamente. Nella nostra esperienza quindi, i potenziali evocati uditivi di stato stazionario possono essere considerati una metodica efficace ed affidabile soprattutto nella stima di soglia dei bambini molto piccoli e per le basse frequenze (compreso 0.5 kHz).

PAROLE CHIAVE: *Ipoacusia neurosensoriale • ASSR • ABR • Audiometria • Bambini*

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Introduction

Since normal hearing is a necessary condition for the spontaneous acquisition of oral ability and cognitive development [1], hearing loss detection should be performed at the earliest possible age in order to provide timely interventions such as hearing aids or cochlear implants. In 2007, the Joint Committee on Infant Hearing (JCIH) recommended action as of 6 months, when treating congenital hearing loss in infants, in order to promote cognitive

and linguistic development (2007 position statement). However, obtaining precise and objective hearing information in very young children, especially at mid-to-low frequencies, is still a challenging task. Conventional pure tone audiometry (PTA) remains a crucial test to describe the degree of hearing loss in subjects who are able to respond and cooperate; behavioural audiometry and/or visual reinforcement audiometry can be used when evaluating younger children [2], while electrophysiological tests such as auditory brainstem responses (ABRs) are still the gold standard in very young and non-cooperating children. However, in recent years, an increasing number of clinical studies have examined the role of the auditory steady-state response (ASSR) for estimating hearing thresholds. ASSR are scalp-recorded potentials elicited by continuous amplitude and/or frequency-modulated tones [3]. These can be recorded by using two stimulation techniques: single frequency and multifrequency stimulation [4]. The application of ASSR has been studied in normal and hearing-impaired adults, children and infants [5-7], and several advantages of this objective procedure have already been reported. In particular, the stimulus seems to be more frequency range-specific, compared to clicks-evoked ABR [8], and the measurement can provide hearing information even for individuals with profound hearing loss (> 90 dB) [9]. Some authors have investigated the correlation between ASSR threshold prediction and hearing level determined by standard audiometry [10-14]. However experimental evidence in this area remains limited, especially in very young children affected by sensorineural hearing loss.

The aim of the present study is to verify, in a population of young children, the reliability of ASSR in: (i) identifying hearing threshold levels and (ii) predicting hearing threshold levels in very young children particularly at mid-to-low frequencies.

Materials and methods

Retrospective study. Forty-two children (22 males and 20 females), for a total of 83 ears tested, were included in this study: 41 children were affected by bilateral sensorineural hearing loss and 1 child by monolateral sensorineural hearing loss. Depending on age and time of audiological assessment, subjects were divided into two groups: we included 22 children, 43 ears (*group A*) born between November 2007 and May 2012, and 20 subjects, 40 ears (*group B*) born between October 1995 and August 2006. In group A, ASSR was carried out when subjects were between 3 and 121 months (mean age 27.22 months). In group B, ASSR was performed in older subjects, between 86 and 189 months (mean age 133.75 months). Pure tone audiometry was performed between 22 months and 131 months (median age 47.86) within group A, and between 86 and 189 months (median age 138.25 months) within group B. Therefore, the average time-interval between investigation by ASSR and pure tone audiometry was 22.41 months for group A and 4.37 months for group B.

All clinical charts were reviewed and data were collected retrospectively. Informed consent was obtained from all participants' parents, at the time of the first audiological investigation, according to current Italian law, and the search was carried out in compliance with the Helsinki Declaration.

Both groups were affected by moderate to severe sensorineural hearing loss (thresholds ≥ 40 dB HL according to a click-ABR assessment). Inclusion criteria were: normal ENT clinical findings, transient otoacoustic emissions (TEOAEs) failure and ABR threshold ≥ 40 dB HL in at least one ear. All patients underwent TEOAEs, ABR, ASSR, behavioural audiometry and pure tone audiometry (PTA), the latter performed later according to the child's mental and physical development. Subjects were included in group B based on the same criteria, but were older than those of group A at the time of audiological assessment: it was then possible to perform ASSR and PTA on the same session or in close temporal sequence. All investigations were performed at the ENT & Audiology Department of the University Hospital of Ferrara.

TEOAEs were recorded using an AccuScreen device (Otometrics, Madsen). The clicks in the stimulus train followed a non- linear protocol and the click frequency was 60 Hz. The stimulus level was set between 70-90 dB SPL. The automatic evaluation was based on the determination of the weighted average of the noise-level and the number of significant signal peaks. In all examined ears TEOAEs recorded failure.

ABR and ASSR responses were recorded by ICS Chartr (GN Otometrics, Mercury, Italy). ASSR responses were registered at single carrier frequencies (500, 1000, 2000, 4000 Hz) using pure tone frequency with 25% modulation and by 100% amplitude modulation. ASSR values were detected To a 5% error-margin. The amplification used for better detection of the signal was 200 k.

Carrier frequencies were 500, 1000, 2000 and 4000 Hz, and were modulated respectively at 97, 81, 95 and 88 Hz for the right ear and 95, 79, 94 and 85 Hz for the left ear. Signals were filtered using a high pass filter of 65 Hz, and a low pass filter of 105 Hz. Signal/noise average ratio was + 2 dB across all measurements. At each frequency the time limit for signal detection was set at 3 minutes. ABR were obtained by using transient clicks (0.1 msec with alternating polarity). ABR and ASSR were recorded

Table I. Risk factors for congenital hearing loss across the group of children studied.

Risk factors (JCIH 2007)	Group A (22 subjects)	Group B (20 subjects)
Family history of hearing loss	1	5
In-utero infections	1	0
Craniofacial anomalies	1	3
Syndromes associated with hearing loss	4	1
Neonatal intensive care >5 days	2	2
Prematurity < 37 weeks	2	2
Hyperbilirubinaemia	1	0
Total	*12*	*13*

in spontaneously sleeping subjects using silver chloride cup electrodes, with the active and reference electrodes applied to the vertex and the mastoid, respectively. ABR and ASSR threshold identification were performed by decreasing steps of 10 dB SPL starting from the maximum intensity of stimulation (90 dB HL). For both techniques, threshold detection was identified as the minimum intensity level that could allow the identification of a clear electrophysiological response by the operator; each run was replicated at or near threshold.

PTA was performed by placing the child in a soundproof room, as soon as he/she was able to provide reliable responses. It was performed using headphones to assess air conduction and a bone vibrator for bone conduction; the better ear was evaluated first (sound-proof cabin model E2X2, roll 01008 220V 10A; Mercury, Milan, Italy; Amplaid audiometer, Amplaid, Milan, Italy, calibrated to ISO 9001 standards). The examination was conducted by an experienced audiometric technician or an experienced technician and an audiologist. The intensity of the acoustic signal was progressively reduced, using 5dB steps, to assess the threshold level, frequency by frequency [14].

Statistical analysis

The data collected were examined using the program SPSS, version Windows Base System (SPSS Inc., Chicago, IL, USA); The strength of association between the variables was quantified by Pearson's correlation test. To evaluate the differences between subpopulations we used the non-parametric Wilkoxon's test for pairwise comparisons and Mann Whitney's test for independent samples. The results were considered statistically significant for p-values < 0.05 (*).

Results

Twenty-two children (43 ears) were included in group A and 20 children (40 ears) in group B; risk factors for con-

Table II. Hearing threshold levels identified by ASSR, ABR and pure tone audiometry within groups A and B, across all the tested frequencies (0.25, 0.5, 1, 2 and 4 kHz).

		assr500	assr1k	assr2k	assr4k	aud500	aud1k	aud2k	aud4k	abr
GROUP B	N	32	14	14	28	40	37	37	39	33
	Min-Max	20-90	35-105	40-100	40-95	10-110	15-105	10-105	15-110	20-90
	Mean	48.1	65.7	75.7	70.9	44.9	53.9	61.7	62.2	61,2
	St dev	22.2	21.1	18.1	15.4	26.1	25.8	23.0	25.0	17.6
U test sig		0.49	0.65	0.04*	0,03*	0,05	0,19	0,52	0,60	0,36
GROUP A	N	38	17	12	30	43	43	43	43	40
	Min-Max	25-85	35-100	40-95	20-100	15-80	15-100	15-110	15-115	20-85
	Mean	49.3	63.0	61.7	63.5	49.8	60.7	66.3	65.9	59.1
	St. dev	16.8	21.6	13.5	13.1	14.7	19.1	19.2	23.2	13.1
Total	N	70	31	26	58	82	80	80	82	73
	Mean	48.8	64.2	69.2	67.1	46.6	57.6	64.2	64.1	60,1
	St. Dev	19.3	21.1	17.3	14.6	20.0	22.5	21.0	24.0	15,2

genital hearing loss were identified in 25 cases (59.5%), 12 in group A (54.5%) and 13 in group B (65%), according to Joint Committee on Infant Hearing (JCIH 2007) criteria (Table I).

In Table II, we report the average hearing threshold levels identified by ASSR, ABR and PTA within both groups with the respective standard deviations (frequency range = 0.5, 1, 2 and 4 kHz). Groups A and B showed overlapping threshold profiles for PTA and ASSR (Fig. 1). Comparison of the threshold levels identified at the different frequencies tested by pure tone audiometry and ABR revealed a significant difference among the two groups only at 2 and 4 kHz (independent samples Mann Whitney U test, $p < 0.05$).

Nonetheless, in order to evaluate the reliability of ASSR, we calculated the differences, in decibels, between the thresholds obtained by PTA and ASSR (see also Table III). These data were obtained by subtracting threshold levels obtained by ASSR and PTA at each frequency (0.5, 1, 2 and 4 kHz). The average differences between the values obtained are shown as mean ± standard deviation in Table III. This method has been applied for both groups. In particular, negative values indicate that the ASSR overestimates the threshold level, while positive values indicate that the ASSR underestimates the threshold level; where the values were close to 0, the two methods did not show differences in threshold estimation. For each group and for each frequency we checked for significant differenceS ($p < 0.05$) among threshold levels, identified by PTA and ASSR, using a nonparametric test (Wilcoxon Matched-Pair Signed-Rank test). There were no statistically-significant differences between groups of patients (group A vs. group B) using a Mann Whitney test except at 4 kHz. Moreover, in group A no statistically significant differences were found between the threshold levels obtained by the two methods. Additionally, there was no statistical significance at 0.5 kHz or 1 kHz among group B subjects, while a significant difference was found at 2 and 4 kHz (Table III).

Our analysis revealed that the two methods are substantially equivalent and reliable in determining the hearing threshold and that the correlation between the two methods is greater for low and mid frequencies.

The degree of correlation between threshold levels, obtained by PTA and ASSR, was also studied applying Pearson's test for both groups. As reported in Figure 2, there is a strong and positive correlation at all frequencies investigated, in particular at 0.5 and 4 kHz (those with more data available). In particular, this relationship was significant ($p < 0.001$) in both groups at 0.5 kHz (Pearson = 0.9 ($p < 0.001$) group B; 0.61 ($p < 0.001$) group A) and at 4 kHz (Pearson = 0.86 ($p < 0.001$) group B; 0.81 ($p < 0.001$) group A). Also, the threshold levels obtained by ABR and by ASSR were compared using Pearson's test for both groups: the correlation coefficient was 0.63 and 0.74 ($p < 0.001$) at 2 and 4 kHz among group A, and was 0.60 and 0.56 ($p < 0.001$) in group B.

Discussion

Congenital hearing loss has been reported to be the most prevalent human communication disorder worldwide [15]. The early identification of hearing loss, through universal neonatal hearing screening programs, and therefore the objective hearing threshold assessment, are crucial to reduce the development of linguistic and cognitive impairment [16 17].

Currently, ABR is still considered the gold standard for prediction of hearing threshold in very young and non-cooperative children [18-20]. Nonetheless, it is difficult to ascertain precise and objective information about hearing thresholds in very young children and at certain frequency ranges [21]. Other objective methods tested for the evaluation of mid-low frequencies include the use of tone pip ABR, CE-Chirps and Low-Chirp BERA (LCBERA); very recently, Frank et al. reported that LC-BERA appears to possess a high degree of accuracy in detecting threshold values at low frequencies in their experience with 25 adults with normal hearing. In addition, CE-Chirps have been reported to offer good signal-to-noise ratio when testing mid-low frequencies. However, most of the studies available in the literature so far report experiments on young adults or newborns with normal hearing [22-27].

In recent years, an increasing number of clinical studies have examined the role of ASSR [3 20 27-30]. ASSR offers evoked potentials that can explore both ears simultaneously across several frequencies (range 250 to 8000 Hz), including middle-to-low frequencies [7 9 31]; in the present study, 4 cases with uncertain ABR responses showed a better hearing threshold definition by ASSR testing.

Correlation between threshold levels identified by ABR, ASSR and PTA have already been investigated in children with normal hearing [31-33], while the experiences available in those affected by hearing loss are still very limited [20 34]. Only a few studies have already demonstrated a good correlation between threshold levels identified by ASSR and ABR [18 19]; Lin [35] showed a high correlation between ASSR and ABR estimates at 4 kHz; Swanepoel et al. found a correlation between ABR and ASSR for frequencies between 2 and 4 kHz in normal hearing patients or with conductive hearing loss, and between 1 and 4 kHz in those with sensorineural hearing loss [2]. Also, the relation between ASSR and subject protocols (i.e. play audiometry and/or visual

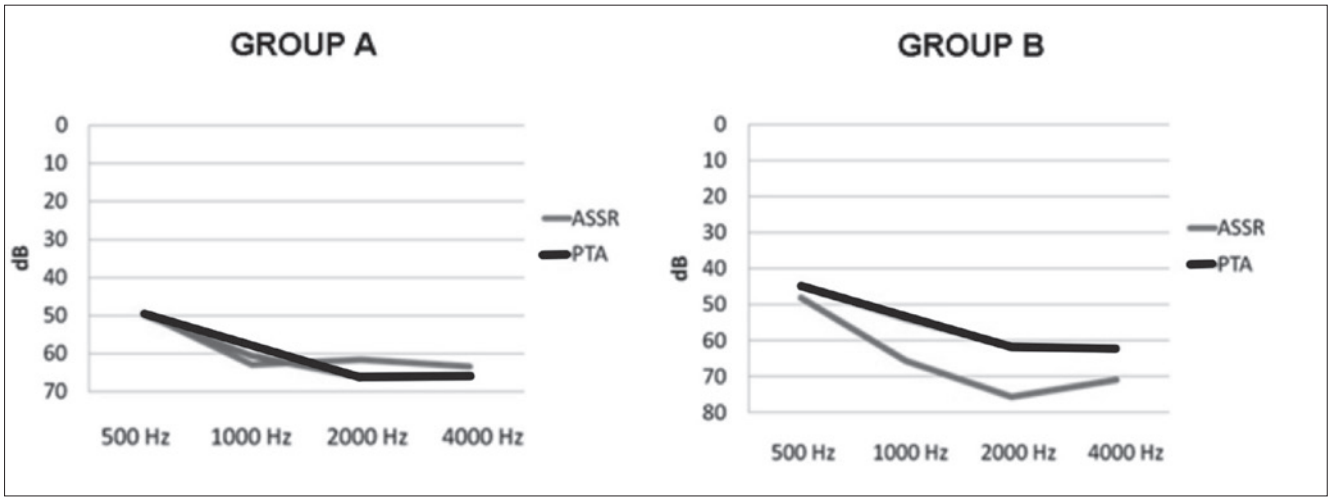

Fig. 1. Groups A and B, showed overall overlapping threshold profiles using pure tone audiometry and ASSR; the average time-interval between audiological tests was 22.41 months for subjects in group A and 4.37 months for group B.

Table III. Differences, in decibels, between the threshold levels obtained by the pure tone audiometry and by ASSR, in groups A and B, at the tested frequencies (0.25, 0.5, 1, 2 and 4 kHz).

	Difference	250 Hz	500 Hz	1 kHz	2 kHz	4 kHz
GROUP B	N	2	32	13	14	28
	Range	-25:-10	-20:+25	-20:+15	-30:+5	-35:+15
	Mean	-17.5	-2.2	-5.4	-8.6	-11.2
	St dev	10.6	10.7	11.4	10.6	11.2
	W (sig) assr vs aud	--	(0.18ns)	(0.10ns)	(0.01*)	(0.00**)
U Mann Whitney sig Cases vs controls		0.80 ns	0.40 ns	0.43 ns	0.21 ns	0.002*
GROUP A	N	3	38	17	12	30
	Min-Max	-30:-15	-20:+30	-20:+25	-25:+20	-35:+20
	Mean	-21.7	0.9	-1.8	-0.8	-1.5
	St. dev	7.6	13.6	12.4	14.6	11.7
	W (sig) assr vs aud	--	(0.76 ns)	(0.51ns)	(0.79 ns)	(0.56 ns)
		250	500	1	2	4
TOTAL	N	5	70	30	26	58
	Mean	-20	-0.5	-3.3	-5.0	-6.2
	St. Dev	7.9	12.4	11.9	13.0	12.4
	W (sig)	0.03*	(0.53 ns)	(0.11 ns)	(0.04 *)	(0.00 **)

reinforcement audiometry) seems to be reliable [36 37]; however, some authors showed differences in threshold levels at 0.5 kHz and 2 kHz [18 19 32 38], while estimates for higher frequencies (2-4 kHz) seem to be more reliable [39 40]. When comparing ASSR threshold levels with tonal audiometry, the correlation was found to be reliable at 0.5 kHz and 2 kHz, but was only confirmed in adult subjects [3 11 41 42-47].

In the present study, we investigated the relationship between ABR and ASSR threshold level estimates and PTA hearing thresholds in children. Our findings reveal slight differences in threshold levels at 2 and 4 kHz when comparing PTA to ABR, and when comparing PTA to ASSR, among the groups studied. Furthermore, when considering subjects in group A, there was no significant differ-

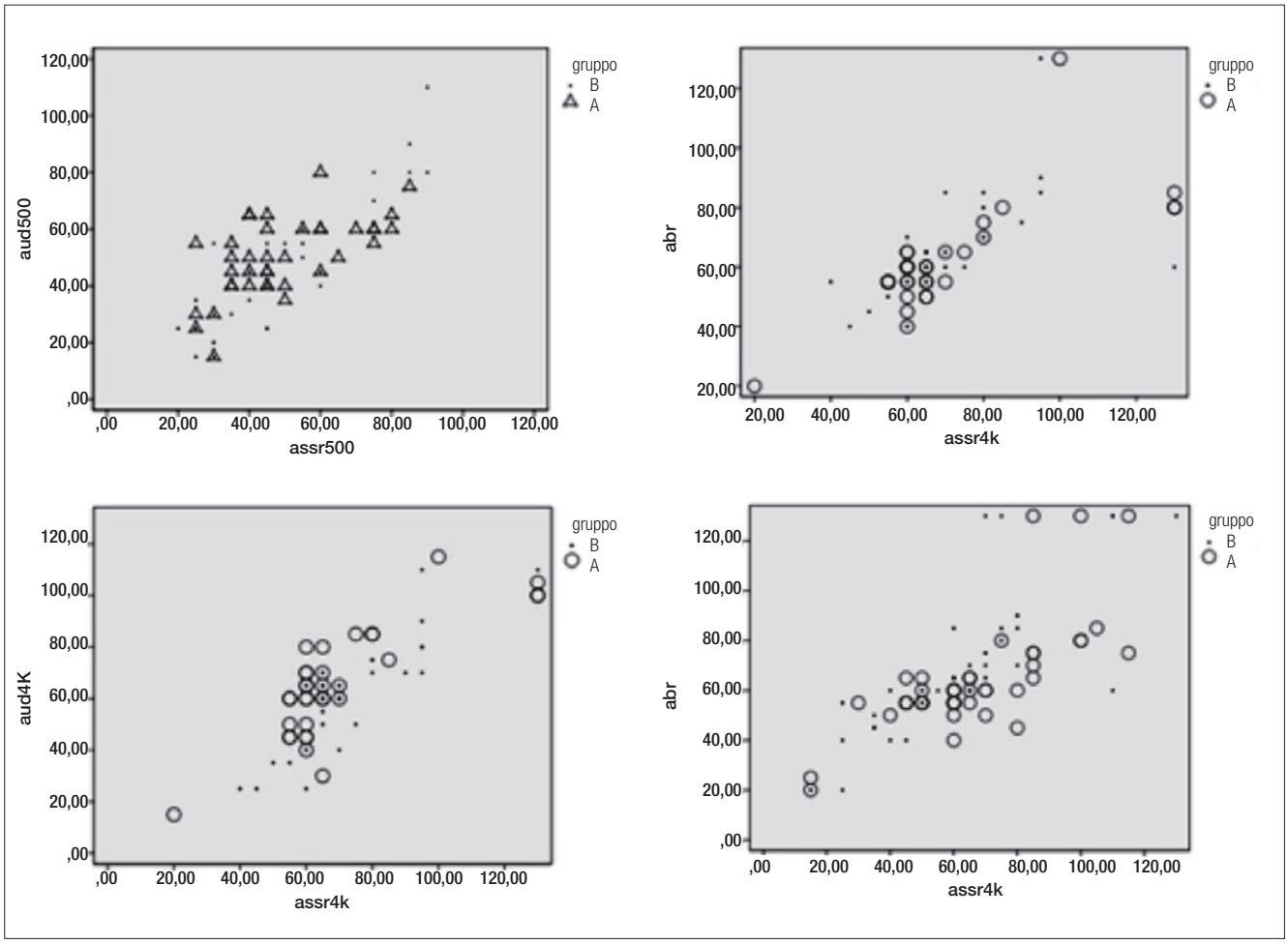

Fig. 2. Degree of correlation between threshold levels, obtained at 0.5 and 4 kHz, by PTA and ASSR, as well as those obtained by ABR and by ASSR, applying Pearson's test for both groups (ABR threshold was obtained using a broadband click).

ence between the ASSR and ABR thresholds detected when the children were very young, and the PTA thresholds subsequently identified, later in time, within the same group of children. We also were able to report, for the first time, a reliable correlation between threshold estimations at low frequencies (0.5 kHz) in young children. In previously reported experiments, this correlation was not clear; several authors (i.e. Savio, 1997 and Lins, 1996) argued that this evaluation was difficult probably due to poor neural synchronisation at this frequency, or to a masking effect produced by background noise.

As for small (10 dB) threshold differences between ASSR and PTA at 2 and 4 kHz among group B subjects, we believe this may represent a genuine difference in sensitivity between the two methods in detecting the hearing threshold, since: (i) other authors have already indicated this difference at 2 and 4 kHz [2] and (ii) in our experiments, both procedures were performed within this group within a limited time-lag and under the same test conditions. Therefore, ASSR can be used to predict hearing thresholds even in younger children and particularly at mid-to-low frequencies.

A drawback of this study is the absence of tympanometric data: since it is a retrospective study, tympanometry was not available for all cases. Indeed, it is possible that part of the threshold difference between ASSR and PTA at 2 and 4 kHz among group B subjects could also be due to the presence of middle ear effusion (certainly frequent during infancy) at the time of ASSR testing. The presence of middle ear effusion might have some influence on the fact that differences were noted across different frequencies, as also described by other authors [48]. The same consideration could apply for the differences in threshold levels at 2 and 4 kHz in-between PTA and ABR. Finally, the tests used herein (ASSR vs. PTA vs. ABR) were performed in different sessions, and therefore in possibly different middle ear conditions.

Conclusions

In conclusion, in light of the present study, ASSR can be considered to be an effective and reliable procedure particularly to predict hearing threshold in children at different ages, and even at lower frequencies (including 0.5 kHz). Furthermore, in our opinion, ASSR can be particularly useful in order to: (i) confirm hearing threshold when performed together with other hearing tests (i.e. ABR and/or otoacoustic emissions) ; (ii) to predict hearing threshold also at lower frequencies (including 0.5 kHz) especially in younger and non-collaborative children; (iii) to facilitate procedures for the fitting of hearing-aids, particularly focusing on mid-to-low frequencies in very young children.

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VESTIBOLOGY

MR imaging of endolymphatic hydrops in Ménière's disease: not all that glitters is gold

MR imaging dell'idrope endolinfatica nella malattia di Ménière: non è oro tutto quel che luccica

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SUMMARY

Ménière's disease (MD) is a chronic condition characterised by fluctuating hearing loss, intermittent vertigo, tinnitus and aural fullness. Its anatomical and pathological counterpart is represented by endolymphatic hydrops (EH). Recent development and progress in magnetic resonance (MR) imaging techniques has enabled visualisation of EH in living human subjects using a 3 Tesla (T) scanner and gadolinium-based contrast-agent (GBCA) via intravenous (IV) or intra-tympanic (IT) administration. Data emerging from the literature about MR imaging of EH in MD patients are limited, and we therefore reviewed the most common MR imaging findings in the study of the endolymphatic space in both MD and non-MD patients.

KEY WORDS: Magnetic resonance imaging • Endolymphatic hydrops • Ménière's disease • Cochlea • Vestibule

RIASSUNTO

La malattia di Ménière è una condizione cronica caratterizzata da sordità, vertigini, acufeni e sensazione di aumento della pressione intra auricolare. La sua controparte anatomo-patologica è l'idrope endolinfatica. I recenti progressi in campo di imaging di risonanza magnetica (RM) hanno permesso di visualizzare la presenza di idrope endolinfatica in vivo mediante l'acquisizione di immagini su scanner 3 Tesla dopo la somministrazione di mezzo di contrasto per via endovenosa o intratimpanica. I recenti dati di letteratura sull'imaging RM della sindrome di Ménière e la caratterizzazione dell'idrope endolinfatica sono tuttavia contradditori. Obiettivo di questo lavoro è la revisione dei reperti radiologici RM più comuni nello studio dell'idrope endolinfatica in pazienti affetti e non affetti da sindrome di Ménière.

PAROLE CHIAVE: *Imaging di Risonanza Magnetica • Idrope endolinfatica • Malattia di Ménière • Coclea • Vestibolo*

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Introduction

Ménière's disease (MD) is a chronic condition characterised by fluctuating hearing loss, intermittent vertigo, tinnitus and aural fullness. It is a relatively common disorder, with a prevalence of 200-500 per 100,000 [1]. Its anatomical and pathological counterpart is represented by endolymphatic hydrops (EH), a distension of the endolymphatic space of the inner ear into areas that are normally occupied by the perilymphatic space. The most common affected areas are the cochlear duct and the saccule, but EH may also involve the utriculum and semicircular canals [2]. Guidelines for diagnosis of this syndrome were established in 1995 by the American Academy of Otolaryngology - Head and Neck Surgery (AAO-HNS). The most recent and revised classification considers two major categories: definite and probable Ménière's disease [3]. Diagnosis of definite MD is made by the presence of two or more episodes of vertigo, audiometrically documented low-to-medium frequency sensorineural hearing loss in one hear, and fluctuating aural fullness. Probable MD is defined by two or more episodes of vertigo, and fluctuating aural fullness in the affected ear [4]. To evalu-

ate the presence of EH, both electronystagmography and electrocochleography tests can be used, which reflect the reduced vestibular response and the elevation of inner ear pressure through distension of the basilar membrane, respectively. An emerging technique in assessment of EH is vestibular evoked myogenic potential (VEMP), a neuroelectrophysiological test that evaluates the otolithic organs of the utricle and saccule. VEMP may be abolished in patients with EH in the vestibule. Imaging studies in the past were mainly used to exclude retro cochlear disorder, such as schwannoma. However, the recent development and progress of magnetic resonance (MR) imaging techniques has enabled visualizsation of EH in living human subjects using a 3 Tesla (T) scanner and gadolinium-based contrast-agent (GBCA) via intravenous (IV) or intratympanic (IT) administration [5 6]. Data from the literature about MR imaging of EH in MD patients are limited due to discrepancies in patient selection and MR assessment criteria. For this reason, our purpose is to provide insight of the current MD imaging scenario. We reviewed the main techniques in assessment of EH and most common MR imaging findings in study of the endolymphatic space in both MD and non-MD patients.

MR findings in MD patients

In June 2017, a structured search was performed in PubMed using the following key words: "MRI" AND "endolymphatic hydrops" (n = 153), "MRI" AND "Ménière's disease" (n = 213). The search was filtered for studies on human subjects and published in English language. In order to have a clearer view of the potential diagnostic role of MR imaging in the assessment of patients with MD, we analysed only MR findings of studies on patients with definite MD according to the AAO-HNS criteria, who underwent MR imaging at 3 Tesla scanner with IT or IV administration of GBCA and volumetric acquisition. We excluded from the review: 1) case reports, reviews and metanalysis; 2) studies on patients enrolled according to diagnostic criteria other than those of the AAO-HNS, or in which clinical evidence of diagnosis ("definite", "probable" or "possible") according to those criteria was not specified; 3) studies in which the hydrops was not assessed by MR standardised qualitative, quantitative or semi-quantitative methods; 4) studies in which a per-ear analysis (symptomatic ear versus asymptomatic ear) of MR findings was not performed for each subject.

After the titles and abstracts of preliminary articles were read, 18 articles were deemed eligible. Two readers (F.L. and S.C., radiology residents with 1-3 years of experience in otoradiology, respectively) then read the articles in their entirety, confirming the eligibility for 17 of them. They subsequently extracted the following information from eligible studies: first author, total number of subjects (MD patients and controls), MR acquisition and analysis methods and MR findings of particular interest for the evaluation of the hydrops in the symptomatic/asymptomatic ears of the enrolled subjects.

Results are reported separately for studies adopting IT-GBCA administration and those adopting IV-GBCA administration. The results of the search are summarised in Tables I and II.

Results and discussion

MR technique

MR assessment of the endolymphatic space can be performed using both IT and IV administration of GBCA [7]. The IT-GBCA technique consists in IT administration of 0.3-0.6 ml of GBCA, diluted 8-fold, into the tympanic cavity by puncture of the tympanic membrane. The contrast medium diffuses into the perilymph, but not in the endolymph, depending on the permeability of the round window, giving a perilymph positive image (PPI) [8]. MR imaging is usually performed at 24 hours after administration, and a heavily T2-weighted 3D-FLAIR sequence with variable flip angle is usually preferred. The endolabyrinth appears to have a lower signal compared to the surrounding perilymph. The inversion time of the 3D-FLAIR can also be shortened to suppress the signal of the perilymph and increase that of the endolymph, thus giving a positive endolymphatic image (PEI) [9]. 3D inversion-recovery turbo spin-echo with real reconstruction (3D-real IR) allows to separate the signals from the perilymph (positive), endolymph (negative) and surrounding bone (zero) using an inversion time between the null point of the perilymph containing the contrast medium and the endolymph. However, this sequence is less sensitive to low GBCA concentrations than 3D-FLAIR [9].

The IV-GBCA technique consists in IV administration of a recommended dose of GBCA (usually 0.1 or 0.2 ml/Kg) that slowly accumulates in the perilymph, but not in the endolymph, depending on the permeability of the blood-labyrinthine barrier, thus giving a PPI [10]. Two types of sequences are recommended: 3D-real IR or heavily T2-weighted 3D-FLAIR [7]. The optimal time for MR acquisition is about 4 hours from the contrast-medium administration as demonstrated in another study concerning various fluid-containing spaces [10]. It is important to note that the visibility of the endolymphatic space depends on the inversion time. For this reason, it is recommended to acquire MR images in control subjects, in order to establish the normal standard for these sequences. MR images can be directly evaluated after acqui-

Table I. MR findings for IV-GBCA technique.

Articles	MR sequence (TR/TI/TE)	Reference MR criteria for the assessment of EH	Percentage (%) of EH in symptomatic MD ear	Percentage (%) of EH in asymptomatic MD ear	Percentage (%) of EH in ears with other audiological disorders	Percentage (%) of EH in healthy ears
Pakdamn et al [19]	3D-FLAIR (9000/2350/534)	vEH if VES/vestibule > 50% cEH: not investigated	vEH: 22/32 (68%)	vEH: 0/32 (0%)	vEH: 0/11 (0%)	vEH: 0/11 (0%)
Sano et al. [15]	3D-FLAIR (9000/2400/540)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	vEH 1/1 (100%) cEH: 1/1 (100%)	vEH: 1/1 (100%) cEH: 1/1 (100%)	vEH: 4/6 (66%) cEH: 4/6 (66%)	vEH: 2/4 (50%) cEH: 0/4 (0%)
Barath et al. [21]	3D-real IR (6000/2000/177)	vEH if VES/vestibule > 50% cEH if cochlear duct is dilatated	EH: 41/43 (95%)	EH: 10/45 (22%)	NA	NA
Sepahdari et al. [13]	3D-FLAIR (9000/2350/534)	vEH if VES/vestibule > 45% cEH: not reported	vEH: 6/12 (50%)	NA	NA	NA
Yoshida et al. [16]	3D-FLAIR (not specified)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	vEH: 49/52 (94%) cEH: 45/52 (86%)	vEH; 17/32 (53%) cEH: 15/32 (46%)	NA	vEH: 3/42 (7%) cEH: 16/42 (33%)
Tagaya et al. [17]	3D-FLAIR (not specified)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	vEH: 7/7 (100%) cEH: 5/7 (71%)	vEH: 3/5 (60%) cEH: 1/5 (20%)	NA	
Attyè et al. [18]	3D-FLAIR (7600/2300/345)	vEH if VES/vestibule > 50% cEH if cochlear duct is dilatated	vEH: 14/30 (46%) cEH: 11/30 (36%)	NA	NA	vEH: 9/30 (30%) cEH: 4/30 (13%)
Sepahdari et al. [22]	3D-FLAIR (9000/2350/534)	vEH if VES/vestibule > 50% cEH if cochlear duct is dilatated	EH: 7/7 (100%)	EH: 0/7 (0%)	NA	NA
Attyè et al. [20]	3D-FLAIR (8000/2400/316)	vEH if VES/vestibule > 50% cEH if cochlear duct is dilatated	EH: 73/95 (77%)	EH: 9/41 (21%)	vEH: 15/128 (11%) cEH: 31/128 (24%)	NA

EH = endolymphatic hydrops; cEH = cochlear endolymphatic hydrops; vEH = vestibular endolymphatic hydrops; NA = not available; TE = echo time; TI = inversion time; TR = time of repetition.

sition or read post-processed. For example, in HYDROPS images (Hybrid of the reversed image of the positive endolymph signal and native image of positive perilymph signal) the PEI is subtracted from the PPI [10]. HYDROPS2 is reconstructed by subtracting the MR cisternography sequence (usually 3D heavily T2-weighted Turbo spin echo sequence with variable flip-angle) from the PEI [11]. HYDROPS and HYDROPS2 can be multiplied for the MR cisternography image to further increase the contrast-to-noise ratio (CNR) obtaining the HYDROPS-Mi2 and HYDROPS2-Mi2 images, respectively [12]. Some authors suggest that the use of maximum intensity projection (MIP) reconstruction of heavily T2-weighted 3D-FLAIR represents a robust and accurate method for assessment of EH [13].

Although IT-GBCA technique has a big advantage of creating a stronger perilymph signal, the IV-GBCA technique is preferred as it is less invasive, requires only four hours to complete the MR examination and allows study of both ears in the same session [7].

Findings for IV-GBCA administration

The most common method for assessment of the vestibular and cochlear endolymphatic spaces was firstly described by Nakashima et al. [14] (Figs. 1-3). This method assesses the vestibular endolymphatic space (VES) by calculating the ratio of the area of the VES to the entire vestibule (VES/vestibule ratio) in the axial plane and defining the vestibular EH (vEH) absent if this ratio is < 33%, mild if between 33% and 50% and significant if > 50%. In addition, Nakashima et al. [14] evaluated the cochlear endolymphatic space looking at displacement of Reissner's membrane and defining the cochlear EH (cEH) as mild if there is a Reissner's membrane displacement with the area of the endolymphatic compartment not exceeding the area of the scala vestibule, and as significant when the endolymphatic compartment exceeds the area of the scala vestibule [8]. While the aforementioned diagnostic criteria for cEH are used by almost all authors, different cut-offs of the VES/vestibule ratio for the identification vEH were further proposed.

Mild vEH, according to Nakashima's criteria, was reported from 94% to 100% of symptomatic ears of MD patients, but also from 53% to 100% of asymptomatic ears [15-17]. Sano et al. detected mild vEH in 4/6 (66%) symptomatic ears of patients with other otological diseases [15]. Attyè et al. identified at least mild vEH in 27/30 (90%) ears of healthy volunteers [18]. These data suggest that a VES/vestibule ratio > 33% has a low specificity in identifying the

Table II. MR findings for IT-GBCA technique.

Articles	MR sequence (TR/TI/TE)	Reference MR criteria for the assessment of EH	Percentage (%) of EH in symptomatic MD ear	Percentage (%) of EH in asymptomatic MD ear	Percentage (%) of EH in ears with other audiological disorders	Percentage (%) of EH in healthy ears
Hornibrook et al. [29]	3D-FLAIR (TI 2500)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	EH: 14/30 (47%)	NA	EH: 3/45 (7%)	NA
Wu et al. [25]	3D-FLAIR (6000/2100/387)	vEH if VES/vestibule > 50% cEH: if cochlear duct is dilatated	vEH: 75/108 (69%)	vEH: 1/108 cEH: 9/108	NA	NA
Claes et al. [24]	3D-FLAIR (9000/1700/134)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	vEH 2/12 (16%) cEH: 3/12 (25%)	NA	NA	NA
Bykowski et al. [30]	2D-FLAIR (9454/2500/122)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	vEH 6/6 (100% cEH: 6/6 (100%)	NA	NA	NA
Naganawa et al. [27]	3D-FLAIR (9000/2250/544)	vEH if VES/vestibule > 33% cEH: note reported	vEH: 8/9 (89%) cEH: 6/9 (67%)	vEH: 5/9 (55%) cEH: 4/9 (44%)	NA	NA
Lida et al. [26]	3D-FLAIR (9000/2500/130)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	vEH: 9/11 (81%) cEH:9/11 (81%)	vEH; 6/9 (67%) cEH: 6/9 (67%)	NA	NA
Shi et al. [23]	3D-FLAIR (9000/2500/128)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	EH: ¾ (75%)	NA	NA	NA
Suga et al. [28]	3D-FLAIR (NA)	vEH if VES/vestibule > 33% cEH if cochlear duct is dilatated	vEH 4/6 (67%) cEH 4/6 (67%)	vEH 1/1 (100%) cEH 1/1 (100%)		

EH = endolymphatic hydrops; cEH = cochlear endolymphatic hydrops; vEH = vestibular endolymphatic hydrops; NA = not available; TE = echo time; TI = inversion time; TR = time of repetition.

affected ear of MD patients with unilateral symptoms and in differentiating MD from other otological diseases in which a secondary vEH can occur. However, this cut-off could represent a rule out factor in MD patients in which a define diagnosis has not been clinically reached, due to its high sensitivity.

Severe vEH, according to Nakashima's criteria, was found in 46% and 68% of the symptomatic MD ears in two stud-

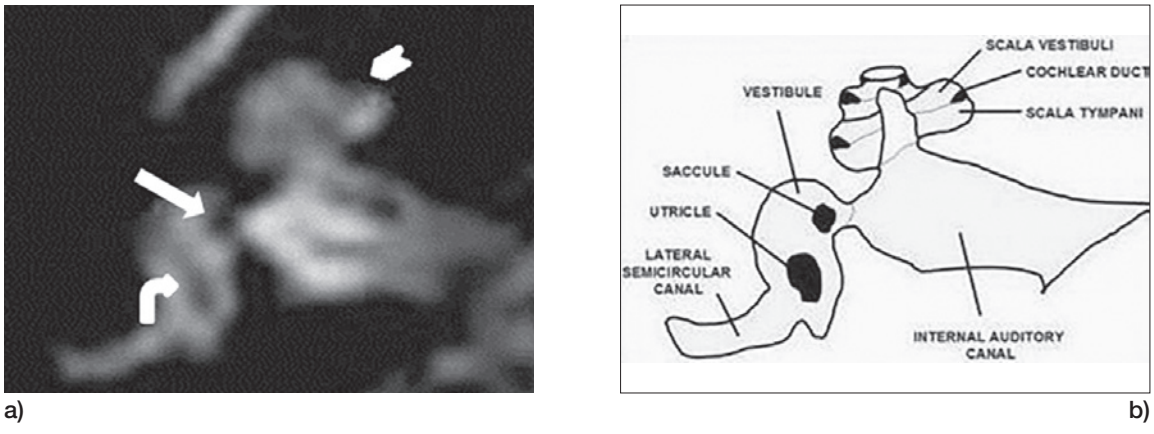

Fig. 1. a) and **b)** T2-weighted FLAIR and schematic illustration image depicting the normal appearance of the vestibular endolymphatic space: the saccule (straight arrow) and the utricule (curved arrow) occupy less than 33% of the vestibular space (VES/vestibule ratio); there is no enlargement of endolymphatic space in the cochlea.

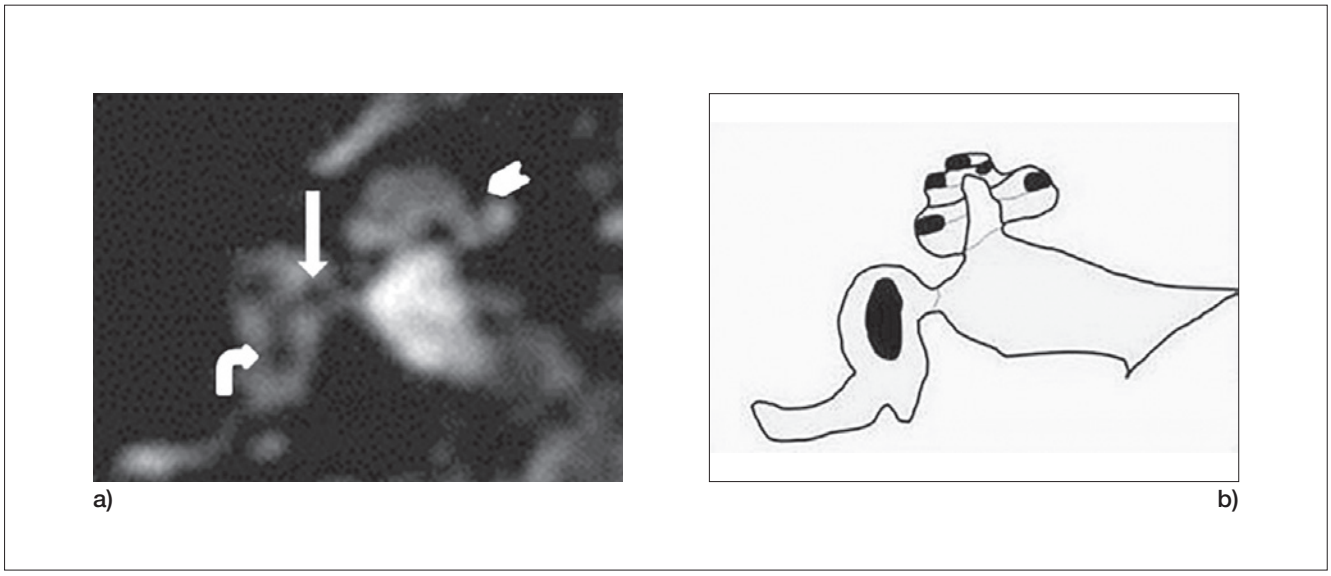

Fig. 2. a) and **b)** T2-weighted FLAIR and schematic illustration of the endolymphatic space image demonstrate the presence of mild vEH, with a VES/vestibule ratio > 33% (straight arrow points to saccule; curved arrow points to utricle); there is also mild cEH, represented by displacement of the Reissner's membrane with the area of the endolymphatic compartment not exceeding the area of the scala vestibule (arrow-head).

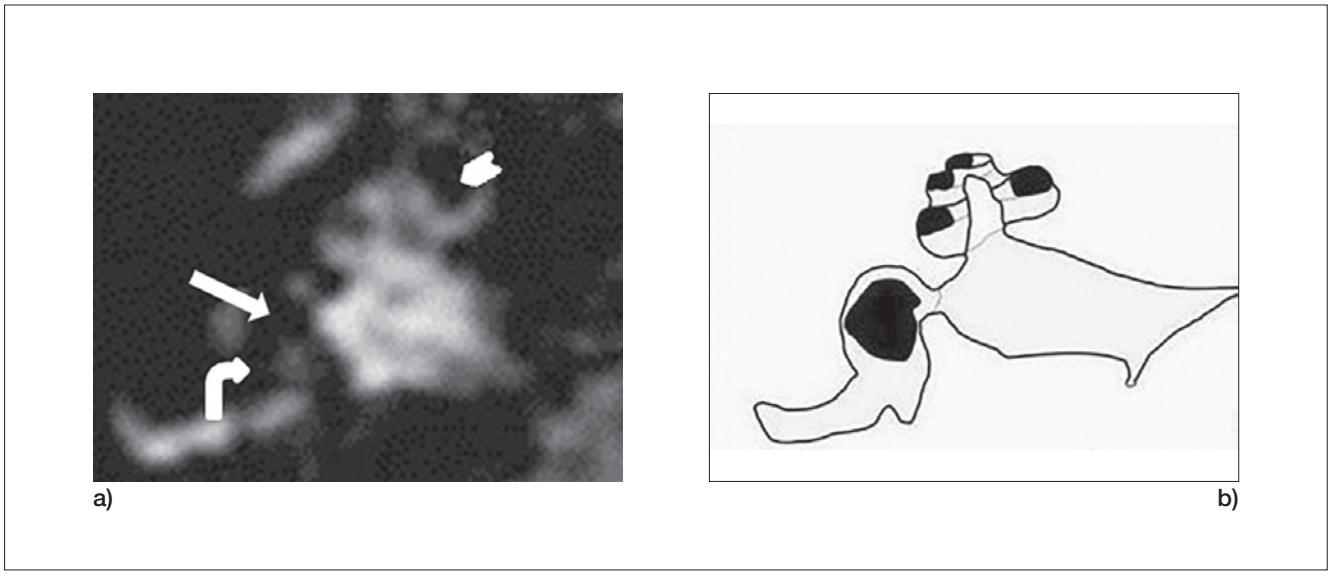

Fig. 3. a) and **b)** T2-weighted FLAIR and schematic illustrations of the endolymphatic space image show both significant vEH and cEH. VES/vestibule ratio is > 50% (straight arrow points to saccule; curved arrow points to utricle) and the endolymphatic compartment exceeds the area of the scala vestibuli (arrow-head).

ies [18 19]. However, Pakdaman et al. did not find it in 32 asymptomatic ears of MD patients [19]. Furthermore, vEH was reported in a range from 0% to 30% of asymptomatic ears of healthy volunteers [18 19]. Severe vEH was identified in 15/128 (11%) and 0/11 (0%) symptomatic ears of patients with other otological diseases, respectively [19 20].

Although the VES/vestibule ratio > 50% may have a lower sensitivity to detect MD ears, these data suggest that it may represent a rule in criteria for MD, since it has a good specificity in both differentiating MD ears from healthy ears and those affected by other otological disorders.

Other potential cut-off values were investigated. Sepahdari et al. calculated a VES/vestibule ratio of 45% as two standard deviations above the mean of a group of patients

with sensorineural hearing loss [13]. Using this cut-off value, they found vEH in 6/12 of the symptomatic ears of MD patients (50%) suggesting that this diagnostic criterion cannot be used to exclude a form of MD presenting with sudden hearing loss [13]. Yoshida et al. reported that a VES/vestibule cut-off value of 41.9%, calculated by the ROC curve, has a sensitivity of 88.5% and a specificity of 100% in differentiating MD from healthy ears [16].

According to Nakashima's criteria, cEH was found in 36%-86% of symptomatic ears in three large cohorts of MD patients [16-18]. Yoshida et al. also assessed asymptomatic ears of both MD patients and healthy volunteers, detecting cEH in 46% and 33% of them, respectively [16]. Attyè et al. found cEH in 13% of healthy volunteers [18]. The latter research group found cEH in 24% of ears of patients with recurrent peripheral vestibulopathy [20]. These data suggest that cEH, as defined by Nakashima's criteria, cannot be used to rule out MD, and can be found in approximately up to one-third of patients with other otological disease of otherwise healthy subjects, resulting less specific and preventing radiologists from diagnosing MD without the support of clinical data.

EH according to Nakashima's, either vEH (VES/vestibule ratio > 33%) or cEH, was reported in all 60 ears (30 MD patients and 30 healthy subjects) studied by Attyè et al., giving a sensitivity of 100% but a specificity of 0% [18]. In contrast EH, defined by the presence of either vEH (VES/vestibule ratio > 50%) or cEH, was reported in 77%-100% of symptomatic ears of MD patients 18,24,25, in 0%-22% of asymptomatic ears (14,20-22), and in 24% of ears of patients with recurrent peripheral vestibulopathy [20]. This means that the presence of EH, independently from the definition of vEH, could be a good criterion/parameter for detecting the affected side of MD patients with unilateral symptoms, but it does not allow radiologists to differentiate MD from other otological disorders. In addition, EH often is not a pathologic finding, having been reported in a large number of healthy subjects.

More recently, another research group assessed hydrops using a saccular morphology-based method. The authors defined saccular hydrops as a saccule to utricle ratio (SURI) ≥ 1, reaching a sensitivity of 50% (15/30 patients) and a specificity of 100% in differentiating the symptomatic ears of patients with Ménière's disease from the asymptomatic ears of 30 healthy volunteers [18]. However, it still remains unclear what the diagnostic role of this method is in differentiating MD from other otologic disorders.

Findings for IT-GBCA administration

When studying MD patients by IT-GBCA administration, Nakashima's method to investigate and assess the vestibular and cochlear endolymphatic spaces should be used, as it represents a suitable and reliable method [14].

Based on the common knowledge of drugs entry through an oval window pathway in rats, Shi et al. were among the first able to demonstrate a compromised passage through the oval window, showing vEH in 3 of 4 patients with definite MD [23]. Claes et al., in contrast, did not find any added value from the use of the IT method in evaluating the presence of EH after the injection of GBCA after a surgical procedure: the presence of cEH and vEH was demonstrated in a small percentage of patients (25% and 16% respectively) [24]. A possible explanation for this low rate of positive findings can be found in the dilution factor of the administered GBCA. IT administration is still off-label and many patients are reluctant to receive a puncture to the tympanic membrane, so that unilateral IT injection is usually performed even in cases when bilateral EH is suspected, underestimating the possible involvement of the contralateral ear. The study from Wu et al. is one of the few in which MD patients underwent to a bilateral IT administration: presence of vEH was found in 75 of 108 symptomatic ears (69%) and found cEH in 9 of 108 contralateral ears (8%) [25]. The presence of EH in the contralateral asymptomatic ears was demonstrated by Lida et al. by using both IT and IV administration: 67% of asymptomatic ears were shown to have both vEH and cEH [26]. A comparison between the results obtained after simultaneous IV-IT administration was made by Naganawa et al., by using 3D-real IR images for IT-IV side and HYDROPS2 for IV [27]. Only HYDROPS images were able to demonstrate EH in all ears VEH and cEH were demonstrated in 89% and 67% of ears, respectively. A significant rate of vEH (55%) and cEH (44%) was also found in the contralateral asymptomatic ears [27].

Differences may also exist between the times of delayed postcontrast imaging, most likely being performed after 24 hours, but in some cases after 4 hours [28]. The impact of this discrepancy on the imaging evaluation of MD patients is unclear: Suga et al. were able to assess the presence of vEH in 4 of 6 patients with definite MD independently from the time of acquisition [28]. The presence of EH in other audiological disorders can be shown by IT administration: Hornibrook et al. assessed the presence of EH not only in patients with definite MD (14/30 ears), but also in patients with other audiological disorders (3/45 ears), even if at a very low rate (6%) [29]. To address potential pitfalls in the acquisition or in the interpretation of the images, Bykowski et al. used an 8 channel surface coils and acquired MR images 26 hours after monolateral IT administration in 6 definite MD patients [30]. Variable FLAIR inversion time images were used to determine the

impact on fluid-suppression interpretation. 100% of definite MD patients (6/6) showed both vEH and cEH in the symptomatic ears.

Conclusions

MR imaging of the endolabyrithine space can be easily performed using a 4 hour-delayed volumetric acquisition after IV administration of GBCA. Thus, IT administration of GBCA, which is more invasive, is not recommended. Recent achievements in this technique have allowed radiologists to detect cochlear or vestibular EH on MR imaging. However, neuroimaging evidence from the literature and pathological findings described in cadavers suggest that EH does not represent exclusive findings of MD patients and is probably not always pathological, since it has been often described in normal ears. For this reason, quantitative MR assessment is most likely not sufficient in diagnosis of MD, and morphology-based criteria should be investigated. In line with this view, the more recent SURI method, described by Attyè et al. [18] represents a promising tool in differentiating MD ears from ears affected by other pathologies, but further studies should investigate this method and confirm its accuracy. Along with this, it is mandatory to use only a 3 Tesla MR in the diagnostic workup of MD. We are still far from being able to use MR imaging as a new diagnostic tool for MD and its role remains mainly to exclude other diseases when clinical manifestations are not clear, and a definitive diagnosis of MD has not been reached.

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Otology

Anatomical and functional results of ossiculoplasty using titanium prosthesis

Risultati anatomici e funzionali dell'ossiculoplastica con protesi in titanio

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SUMMARY

Titanium ossicular chain replacement prosthesis is often used for rehabilitation of the columellar effect in otologic surgeries. This retrospective study aims to analyse the anatomical and functional results of surgeries in which a titanium prosthesis was used. Two hundred and eighty procedures in 256 patients operated on in a tertiary referral center were analysed. Aetiologies, preoperative audiograms, peroperative data and postoperative outcomes at 2 and 12 months postoperatively were reviewed. Chronic suppurative otitis media with or without cholesteatoma was the main aetiology (89%). There was no difference in anatomical results between partial and total ossicular replacement prosthesis, with an overall dislocation rate of 6%, and an overall extrusion rate of 3%. Regarding functional results, a postoperative air-bone gap ≤ 20 dB was achieved in 65% of cases, with a better result for partial compared to total ossiculoplasty (p = 0.02). A significant difference in air bone gap closure was found when comparing aetiologies, with a higher air-bone gap closure in malformation cases compared to chronic suppurative otitis media with cholesteatoma or retraction cases (p = 0.03). Ossiculoplasty using titanium prosthesis is a safe and effective procedure for rehabilitation of hearing loss, which allows reaching an air-bone gap ≤ 20 dB in the majority of patients.

KEY WORDS: Ossiculoplasty • Chronic suppurative otitis media with cholesteatoma • Malformation • Ossicular chain • Otology

RIASSUNTO

Le protesi ossiculari in titanio sono sempre più frequentemente utilizzate per ristabilire la continuità della catena ossiculare nella chirurgia otologica. Il presente studio retrospettivo ha come scopo quello di analizzare i risultati anatomici e funzionali di questa tecnica. Sono state studiate 280 procedure di ossiculoplastica con protesi in titanio, realizzate su 256 pazienti in un centro ospedaliero universitario. Sono stati raccolti e analizzati i dati riguardanti l'eziopatogenesi, l'audiometria preoperatoria e i risultati postoperatori anatomici e audiologici a 2 e 12 mesi. La prima patologia riscontrata in ordine di frequenza è stata l'otite cronica con o senza colesteatoma (80% dei casi), ma non è stata riscontrata nessuna differenza significativa nei dati audiologici preoperatori in funzione della patologia. Nel 65% dei casi è stato raggiunto nel postoperatorio un gap aereo ≤ 20 dB, con risultati significativamente migliori nelle ossiculoplastiche parziali rispetto alle totali (p = 0,02). Al contrario, per quanto riguarda i risultati anatomici non è stata riscontrata alcuna differenza utilizzando le protesi parziali o totali, con un tasso globale di dislocazione del 6% e di estrusione della protesi del 3%. È stata trovata una differenza significativa nei risultati audiologici postoperatori in funzione della patologia otologica di base, con una variazione maggiore del gap aereo nei casi di malformazione rispetto ai casi di otite cronica colesteatomatosa o patologia retrattiva (p = 0,03). Ne consegue che il tipo di chirurgia non influenza il risultato postoperatorio. L'ossiculoplastica con protesi in titanio si è rivelata una procedura valida ed efficace che permette un miglioramento dell'udito postoperatorio nella maggior parte dei pazienti.

PAROLE CHIAVE: *Ossiculoplastica • Colesteatoma • Malformazione • Catena ossiculare • Otologia*

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Introduction

Restoring a columellar effect with ossiculoplasty for rehabilitation of hearing loss in case of ossicular chain discontinuity is one of the main objectives of tympanoplasty procedures. Different materials are used for ossiculoplasty, including homologous materials (ossicules, cartilage) and synthetic materials (plastipore, ceravital, hydroxyapatite, metal, titanium) [1 2]. Titanium has many advantages in terms of biocompatibility, weight and acoustic performance [3-5]. It is also compatible with MRI at 1.5 and 3 te-

sla [6 7], which is of primary importance, especially in cholesteatoma surgery. Many studies have shown that titanium prosthesis gave better anatomical and functional results than autologous materials [8 9], and the results seem to be comparable to those with hydroxyapatite prosthesis [10 11].
Despite many reports describing encouraging results with the use of titanium prosthesis, we aimed to analyse the anatomical and functional results of a large series of patients operated on for ossiculoplasty; moreover, we aimed to look for prognostic factors influencing these results.

Materials and methods

Study design

All patients operated on for an ossiculoplasty from January 2004 to December 2008 in a tertiary referral centre were retrospectively reviewed. This study was authorised by the local institutional review board (CPP Île-de-France VI) and all patients gave their informed consent to the use of clinical data. Two hundred and eighty procedures in 256 patients were included. Titanium prosthesis were used in all procedures. All patients had a preoperative audiometric test and at least a postoperative test 8 weeks after surgery. Preoperative assessment included otoscopy, pure-tone audiometry with headphone and HRCT-scan study. Post-operative assessment included anatomical results with otoscopy (presence of a well-healed tympanic membrane), functional results (audiometry) and complications (residual perforation of the tympanic membrane, retraction, cholesteatoma recurrence, prosthesis extrusion, or displacement).

Audiometric assessment

Preoperative and postoperative data were recorded according to the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) standards [12]. Audiometric outcomes were recorded at 2 months and 12 months after surgery.
Air conduction (AC) and bone conduction (BC) thresholds were recorded. Thresholds at 500; 1,000; 2,000 and 3,000 Hz were used to calculate the pure-tone average (PTA). If the 3,000 Hz threshold was not available, it was calculated as the mean between 2,000 and 4,000 Hz. The preoperative and postoperative Air-Bone Gap (ABG) were calculated as BC PTA minus AC PTA. The differences between the preoperative and the postoperative ABG (ABG closure) were recorded and noted as ΔABG. The differences between the preoperative and the postoperative BC threshold at 4,000 Hz were calculated; postoperative labyrinthisation was defined by a difference of more than 30 dB.

Surgery

A retroauricular approach was used in 248 cases (88%), and a trans-canal approach through an ear speculum in 32 cases (12%). The procedure started with systematic exploration of the middle ear, with attico-mastoidectomy in case of cholesteatoma, using a canal-wall-up (CWU) technique whenever possible. Ossiculoplasty was performed in a one-stage procedure, even in the case of cholesteatoma [13]. Kurz® (Heinz Kurz GmbH Medzintechnik, Dusslingen, Germany) titanium Bell partial ossicular replacement prosthesis (PORP) was used when the stapes superstructure was mobile and intact (Fig. 1A). Kurz® titanium Aerial total ossicular replacement (TORP) was used when the stapes was absent (Fig. 1B). In case of fixed stapes footplate, a stapedotomy with perichondrium or fascia temporalis interposition was achieved and the TORP was then positioned. The AC-sizer system was used in all cases to choose the length of the prosthesis.
A thinned cartilage graft was placed in all cases between the tympanic membrane and the head plate of the prosthesis (Fig. 2). Furthermore, in 187 cases (67%), the tympanic membrane was reinforced by a cartilage graft. At the end of the procedure, the tympanomeatal flap was positioned, and pieces of Merogel® (Medtronic Xomed, Jecksonville, FL) and an ear wick were placed above it. The ear wick was removed one week later. All patients had post-operative eardrops of ofloxacine for 2 weeks.

Statistical analysis

Results are presented as mean ± standard deviation (SD). Statistical tests were performed using Statview (SAS Inc. Carey). Fisher test, ANOVA, Bonferroni, and t-test were used depending on data compared. Differences were considered statistically significant when $p < 0.05$.

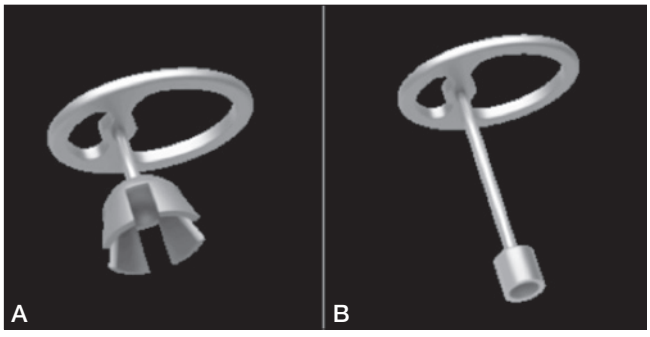

Fig. 1. Partial **(A)** and total **(B)** ossicular replacement prosthesis used in this study.

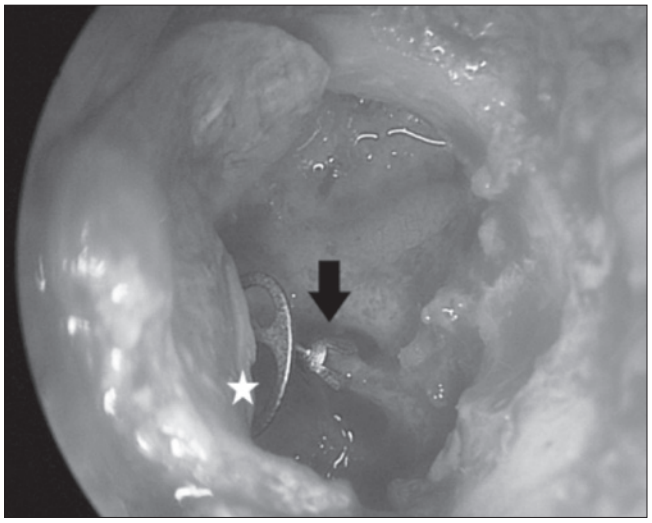

Fig. 2. An endoscopic view of a right ear undergoing a canal-wall-down tympanoplasty with cartilage graft (white star) and partial ossicular replacement prosthesis (black arrow).

Results

Two hundred and eighty procedures in 256 patients were included. The mean age was 44 ± 14 years (17-74). Demographic data, aetiologies and surgical procedures are detailed in Table I. Chronic suppurative otitis media with or without cholesteatoma and retraction pocket were the main aetiologies, with 89% of cases (Table I). Other aetiologies were revision surgery for otosclerosis with a lysis of the long process of the uncus, traumatism, ossicular malformation and middle ear tumours (one paraganglioma and two facial nerve schwannomas).

Preoperatively, the mean PTA was 50 ± 18.1 dB, and the mean ABG was 27 ± 11.9 dB. There was no difference in preoperative audiologic data between aetiology (ANOVA and Bonferroni tests) (Table II).

PORP were used in 163 cases (58%), and TORP were used in 117 cases (42%). Stapedotomy with perichondrium or fascia interposition was necessary in 10 cases (9%) in the TORP group in case of a fixed stapes footplate. The mean preoperative ABG was 26 ± 10.5 dB in PORP group and 30 ± 13.2 dB in TORP group (p = 0.001, t-test).

Postoperative outcomes were available for 280 procedures at 2 months postoperatively (100%), and for 180 procedures at 12 months postoperatively (64%).

Anatomical results

Residual perforations of the tympanic membrane were reported in 5 cases at 2 months (2%) and in no case at 12 months. Three cases of postoperative retraction were noted at 12 months (1%). At 12 months, extrusion of the prosthesis was present in 6 cases (3%) and dislocation in 11 cases (6%), of which 3 cases occurred before 2 months (Table III). All cases of extrusion or dislocation were described for patients with chronic suppurative otitis media with or without cholesteatoma. There was no significant

Table I. Demographic and pathological characteristics.

		N	%
Sex	Men	123	48
	Women	133	52
Side	Right	158	56.4
	Left	122	43.6
Causing pathology	Chronic suppurative otitis media with cholesteatoma	125	45
	Chronic suppurative otitis media	85	30
	Retraction pocket	40	14
	Traumatism	12	4
	Otosclerosis (revision)	11	4
	Malformation	4	1
	Tumor	3	1
Surgery	Revision surgery	161	57
	Primary surgery	119	43
	No mastoidectomy	160	57
	CWU mastoidectomy	74	26
	CWD mastoidectomy	46	17
	Trans-canal atticotomy	187	67

CWU: canal-wall-up; CWD: canal-wall-down.

Table II. Audiologic results depending on the pathology preoperatively, at 2 months, and at 12 months after surgery.

Pathology	PTA			ABG		
	Pre	2 months	12 months	Pre	2 months	12 months
All Chronic suppurative otitis media with cholesteatoma (n = 125)	49 ± 18.4	40 ± 13.3	40 ± 14.9	27 ± 11.9	19 ± 10.5	18.7 ± 10.5
Recurent chronic suppurative otitis media with cholesteatoma (n = 54)	52 ± 19.3	43 ± 19.3	43 ± 20.7	30 ± 12.8	21 ± 11.8	19 ± 10.9
Chronic suppurative otitis media (n = 85)	54 ± 17.8	43 ± 19.5	45 ± 19.5	30 ± 10.9	18 ± 11.2	18 ± 10.9
Retraction pocket (n = 40)	44 ± 14.9	37 ± 14.6	38 ± 19.8	22 ± 10.1	17 ± 8.9	15 ± 10.3
Traumatism (n = 12)	51 ± 20.5	33 ± 17.1	29 ± 17.1	25 ± 14.8	13 ± 9.3	10 ± 9.1
Otosclerosis (n = 11)	55 ± 4.4	44 ± 16.4	39 ± 14.3	26 ± 10.1	15 ± 10.2	15 ± 10.1
Malformation (n = 4)	55 ± 15.4	28 ± 21.1	19 ± 6.9	37 ± 12.2	10 ± 5.3	7 ± 1.9
Tumour (n = 3)	32 ± 21.9	35 ± 7.3	26 ± 8.9	12 ± 9.5	10 ± 5.1	12 ± 7.1

PTA = Pure-Tone Average; ABG = Air-Bone Gap.

Table III. Anatomic and audiological outcomes depending on the surgical technique at 12 months after surgery.

	Ossicular prosthesis			Primary or revision			Mastoidectomy			
	PORP (n = 106)	TORP (n = 74)	p	Primary (n = 77)	Revision (n = 103)	p	No (n = 109)	CWU (n = 45)	CWD (n = 26)	p
Preoperative ABG (dB)	26 ± 10.5	30 ± 13.2	0.001	25 ± 11.6	29 ± 11.9	0.03	26 ± 11.9	24 ± 9.7	33 ± 12.8	0.03
Dislocation N (%)	3 (3)	8 (11)	0.05	6 (7.8)	5 (4.9)	0.5	5 (5)	5 (11)	1 (4)	0.2
Extrusion N (%)	2 (2)	4 (5)	0.2	4 (5)	2 (2)	0.4	3 (3)	2 (4)	1 (4)	0.8
Surgical success N (%)	76 (72)	41 (55)	0,02	58 (75)	59 (57)	0.02	74 (68)	34 (76)	9 (35)	0.002
ABG closure (dB)	11 ± 1.1	10 ± 1.5	> 0.5	11 ± 1.5	10 ± 1	> 0.5	12 ± 1.1	11 ± 1.8	7 ± 1.9	0.07

ABG = Air-Bone Gap; CWU = Canal-Wall-Up; CWD = Canal-Wall-Down.

difference in extrusion rate or dislocation rate for TORP and PORP group at 12 months (Fisher tests), although the rate of dislocation was higher for TORP (n = 8, 11%) compared to PORP (n = 3, 3%) (p = 0.05, Fisher test). There was no difference in extrusion rate or dislocation rate comparing primary or a revision surgery, even for recurrent cholesteatoma (Fisher test) (Table III).

Audiometric results

The mean PTA was 40 ± 18.3 dB and 40 ± 19.5 dB at 2 months and 12 months after surgery, respectively. The mean ABG was 18 ± 10.5 dB at 2 months after surgery and 17 ± 10.6 dB at 12 months after surgery. Table II shows the audiometric results depending on the pathology.

Surgical success, defined as a postoperative ABG ≤ 20 dB, was observed in 59% (n = 166) of cases at 2 months postoperatively, and in 65% (n = 117) of cases at 12 months after surgery. There was a significant difference between the PORP group and the TORP group at 2 and 12 months after surgery: at 2 months, surgical success was achieved for 108 cases (66%) in the PORP group and in 57 cases (49%) in the TORP group (p = 0.004, Fisher test); at 12 months, it was achieved for 76 procedures (72%) in the PORP group and in 41 cases (55%) in the TORP group (p = 0.02, Fisher test). There was no difference in ABG closure in the PORP and TORP groups. At 2 months, mean ABG closure was 9 ± 0.8 dB in the PORP group and 9 ± 1.2 dB in the TORP group (p > 0.5, t-test). At 12 months, the mean ABG closure was 11 ± 1.1 dB in the PORP group, and 10 ± 1.5 dB in the TORP group (p > 0.5, t-test). A difference in ABG closure at 2 months after surgery was found depending on the pathology (Fig. 3): ABG closure in malformation cases was significantly greater than in cholesteatoma cases (respectively 27 ± 10.7 and 7 ± 11.7; p = 0.03, t-test) and retraction cases (respectively 27 ± 10.7 and 5 ± 9.5; p = 0.02, t-test). This difference was not significant at 12 months (p = 0.06, t-test).

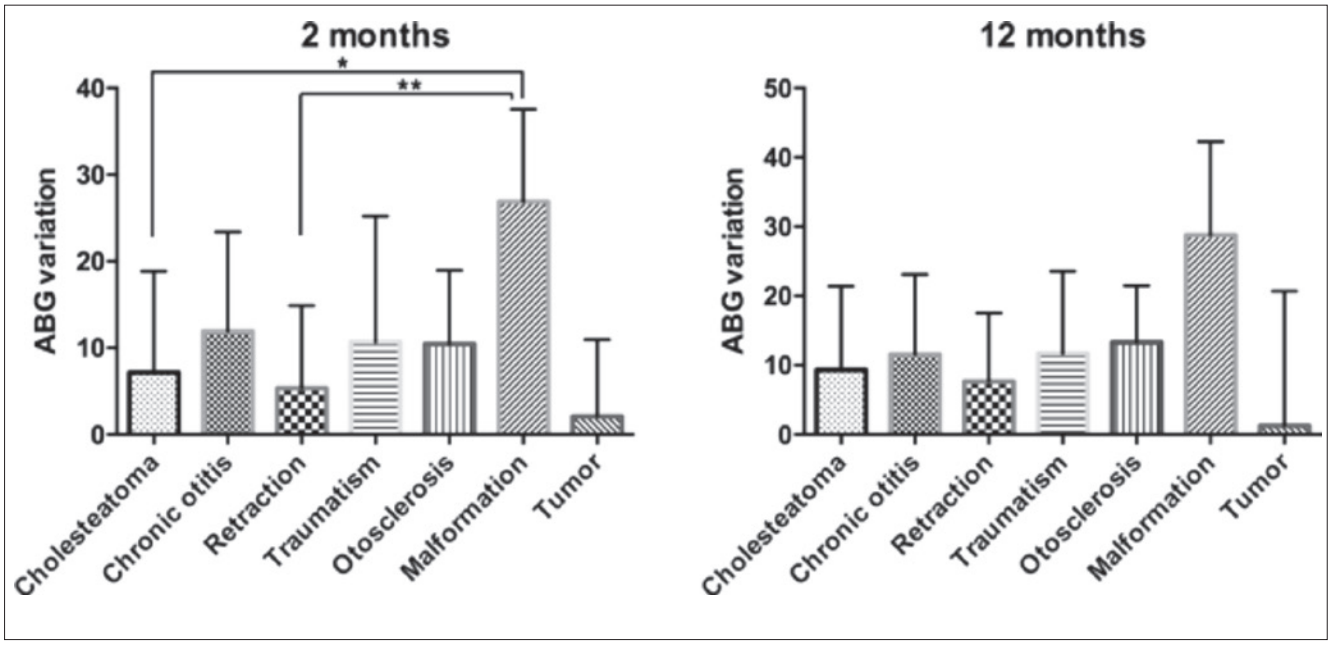

Fig. 3. ABG (Air-Bone Gap) variation depending on the causing pathology at 2 months and 12 months after surgery.

ABG closure was not significantly different in case of primary or revision surgery (t-test), although surgical success was significantly different (Table III): in case of primary surgery, ABG closure was 9 ± 1.2 dB at 2 months after surgery, and 11 ± 1.5 dB at 12 months after surgery. In case of revision surgery, ABG closure was 9 ± 0.5 dB at 2 months after surgery, and 10 ± 1 dB at 12 months after surgery.

Finally, ABG closure was not dependent on the surgical procedure, although surgical success was significantly different (Table III): there was no difference between patients operated on without mastoidectomy, with canal-wall-up, or with canal-wall-down mastoidectomy. At 12 months after surgery, the closure was, respectively, 12 ± 1.1 dB, 11 ± 1.8 dB and 7 ± 1.9 dB (non-significant, t-test).

Concerning postoperative labyrinthisation, there was no variation of the mean postoperative BC threshold at 2 and 12 months compared with the preoperative one (t-test), and no difference between the TORP and the PORP group for the postoperative BC thresholds (t-test). Labyrinthisation occurred in 1 cholesteatoma case.

Discussion

Titanium ossicular prosthesis are routinely used in otologic surgery and seem to give satisfactory postoperative anatomic and audiologic results. Globally, the literature reports surgical success, defined as ABG < 20 dB, between 56% and 81.4% in short-term studies (around 12 months of follow-up) [14-22]. These results are comparable to results presented in this study with 65% success at 12 months postoperatively. Some studies compared results obtained with titanium prosthesis to other materials: titanium appeared to give better audiologic results than gold [23], ceravital [23], or plastipore [24]. Also, ossiculoplasty with titanium prosthesis appears to be more succesfull than autologous materiel (ossicle or cartilage) [8 9]. The results with hydroxyapatite prosthesis are comparable to titanium prosthesis [10 11 25]. Finally, cement ossiculoplasty seems to give similar results compared to partial prosthesis for incudostapedial, maleus to stapes re-bridging [26].

The results presented in this study are also comparable for extrusion and dislocation rates, with 3% of extrusion and 6% of dislocation. In short-term studies, extrusions are estimated between 1 and 5% [17 19 27 28], with dislocation in 2% of cases [28]. Long-term studies show more cases of dislocations (between 3.5 and 10.8% [16 29 30]). This can be caused either by a non-optimal prosthesis size, or by a recurrence of the causing pathology, especially retraction or cholesteatoma [3 29]. In this study, no difference in extrusion or dislocation rate was found for recurrent cholesteatoma compared to other aetiologies, but the follow-up ends at 12 months postoperative. Concerning extrusions, they seem to appear earlier after surgery. All cases in this study were seen early, at 2 months after surgery, and long-term studies also show that extrusions appear in the first year after surgery [3 29].

This study reports results comparable to others in terms of hearing and anatomical results, even if all procedures were one-stage procedures, even in case of cholesteatoma.

This result should be confirmed in a long-term study, but for adult ossiculoplasty, it seems that there is no need to postpone the reconstruction procedure for further surgery. There was no difference between PORP and TORP group concerning extrusion or dislocation rate. Other studies in literature show the same result with no more dislocation or extrusion using TORP or PORP [31 32]. Conversely, a difference in audiologic results was observed for postoperative ABG, with a better result for the PORP group compared to the TORP group, with surgical success significantly higher for former group. This has to take into account that preoperative ABG is also better in the PORP group compared to the TORP group, and that there is no difference in ABG closure between the two groups. This observation suggests that the presence of the stapes superstructure could be important for audiologic results, even if anatomic results are as good for the TORP group as for the PORP group. This could be due to the fact that the absence of the stapes superstructure is usually associated with a more extensive disease, which causes an adverse environment for reconstruction. This result was also observed in the literature in most studies using a titanium [5 16 31 32] or hydroxyapatite prosthesis [2]. In a meta-analysis on a total of 4311 procedures, the superiority of PORP of postoperative ABG was confirmed with a combined RR of 1.28 (95% CI 1.17-1.41) [31]. Thus, this observation is controverted in literature, and other studies showed no difference between results obtained with PORP or TORP [15 29].

In addition to the type of reconstruction, the causing pathology also seems to be a prognostic factor for the postoperative audiologic result. This study showed very good functional results in case of malformation and poorer results in case of cholesteatoma or retraction pocket. In addition to malformation cases, ABG gain seems to be around 10 dB after surgery. ABG gain was greater for malformation cases, but this difference was not significant at 12 months, probably because of the low statistical power due to patients lost during follow-up. Despite this, ABG outcomes seem to be worse in case of chronic suppurative otitis media with or without cholesteatoma (Table II). Gelfand and Chang also found that cholesteatoma or tympanic perforation had a negative impact on the audiologic results [11]. In a longer-term study, Hess-Erga et al. showed that patients without chronic suppurative otitis media media tend to have better audiologic results than patients with chronic suppurative otitis media [3]. Becvarovski described the Middle Ear Risk Index (MERI) that includes poor prognosis factors for middle ear surgery: cholesteatoma, perforation, otorrhoea, granulation, ossicular lysis, revision surgery and smoking [33]. Two studies then showed that cases with a high risk according to the MERI were associated with poorer audiologic results (ΔABG) [34 35]. These studies also showed a deleterious effect of the surgical technique, particularly if mastoidectomy or CWD was used. Unlike these studies, ours shows no effect of the type of surgery on audiologic results, and revision surgery was not related to a poorer result, but this could be because of a lack of statistical power herein.

This study has certain limitations. First, it reports only short-term outcomes of titanium ossiculoplasty, and it would be interesting to look for long term results. In fact, O'Connell et al. showed a significant increase between short-term and long-term results for ABG [29]. Additionally, they showed that surgical revisions were all performed more than 2 years after the first surgery [29]. Second, it is a retrospective study, with a significant proportion of patients lost to follow-up. This could introduce some bias in the results at 12 months.

Conclusions

Ossiculoplasty using titanium prosthesis is a safe and effective procedure both anatomically or functionally. This study suggests that using a PORP gives better results in terms of hearing compared to TORP, and that inflammatory pathologies of the middle ear also have a negative impact on audiologic results. All these results must be confirmed during longer follow-up, which would permit observing stability of outcomes over time.

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Otology

Multi-option therapy *vs* observation for small acoustic neuroma: hearing-focused management

Opzioni di terapia vs osservazione per i piccoli neurinomi dell'acustico: una gestione orientata alla funzione uditiva

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SUMMARY

The current treatment options for acoustic neuromas (AN) – observation, microsurgery and radiotherapy – should assure no additional morbidity on cranial nerves VII and VIII. Outcomes in terms of disease control and facial function are similar, while the main difference lies in hearing. From 2012 to 2016, 91 of 169 patients (54%) met inclusion criteria for the present study, being diagnosed with unilateral, sporadic, intrameatal or extrameatal AN up to 1 cm in the cerebello-pontine angle; the remaining 78 patients (46%) had larger AN and were all addressed to surgery. The treatment protocol for small AN included observation, translabyrinthine surgery, hearing preservation surgery (HPS) and radiotherapy. Hearing function was assessed according to the Tokyo classification and the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) classification. Sixty-one patients (71%) underwent observation, 19 (22%) HPS and 6 (7%) translabyrinthine surgery; 5 patients were lost to follow-up. Median follow-up was 25 months. In the observation group, 24.6% of patients abandoned the wait-and-see policy for an active treatment; the risk of switching from observation to active treatment was significant for tumour growth ($p = 0.0035$) at multivariate analysis. Hearing deteriorated in 28% of cases without correlation with tumour growth; the rate of hearing preservation for classes C-D was higher than for classes A-B ($p = 0.032$). Patients submitted to HPS maintained an overall preoperative hearing class of Tokyo and AAO-HNS in 63% and 68% of cases, respectively. Hearing preservation rate was significantly higher for patients presenting with preoperative favourable conditions (in-protocol) ($p = 0.046$). A multi-option management for small AN appeared to be an effective strategy in terms of hearing outcomes.

KEY WORDS: Acoustic neuroma • Vestibular schwannoma • Wait and see • Hearing preservation • Microsurgery

RIASSUNTO

Le opzioni di trattamento per il neurinoma del nervo acustico (AN), ovvero osservazione, microchirurgia e radioterapia non dovrebbero esporre a una morbidità addizionale i nervi cranici VII e VIII. Esse comportano risultati simili su controllo del tumore e conservazione del nervo facciale, ma differenti e discussi quanto a udito. Dal 2012 al 2016, 169 pazienti con diagnosi di AN sporadico, monolaterale sono stati valutati nel nostro centro. In totale, 91 pazienti hanno soddisfatto i criteri di inclusione, presentando un tumore intracanalare o extrameatale di dimensioni inferiori a 1 cm in angolo pontocerebellare. I restanti 78 pazienti con AN di dimensioni maggiori sono stati tutti trattati con opzione chirurgica primaria. La funzione uditiva è stata determinata sulla base delle classificazioni di Tokyo e dell'American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS). Il controllo post trattamento ha avuto una durata media di 25 mesi. Sessantuno pazienti (71%) sono andati incontro a trattamento conservativo di osservazione, 19 (22%) a chirurgia di preservazione dell'udito (HPS) e 6 (7%) a chirurgia per via translabirintica; 5 pazienti sono stati esclusi per incompletezza di dati. Il 24,6% dei pazienti in osservazione è uscito da tale protocollo durante il follow-up. Il rischio di cambiare da osservazione a trattamento attivo è risultato significativo all'analisi multivariata per i tumori in crescita ($p = 0,0035$). Nello stesso gruppo in osservazione, l'udito è andato incontro a un peggioramento nel 28% dei casi, indipendentemente da una correlazione con la crescita tumorale. La conservazione di un udito in classe A-B si è dimostrata significativamente inferiore rispetto a quella per un udito in classe C-D ($p = 0,032$) nel tempo di osservazione. I pazienti sottoposti a HPS hanno mantenuto la classe uditiva preoperatoria nel 63% e nel 68% dei casi, rispettivamente per le classificazioni di Tokyo e AAO-HNS. Il tasso di preservazione dell'udito inoltre, si è dimostrato significativamente maggiore per i pazienti che si presentavano con caratteristiche preoperatorie più favorevoli (in-protocol) ($p = 0,046$). Un protocollo di trattamento multi-opzionale per i piccoli AN rappresenta una strategia efficace per il risultato chirurgico a breve termine.

PAROLE CHIAVE: *Neurinoma dell'acustico • Schwannoma vestibolare • Wait and scan • Preservazione dell'udito • Microchirurgia*

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Introduction

Thanks to early imaging, acoustic neuromas (AN) are now diagnosed more frequently and are often small, presenting with mild symptoms. The natural history of the tumour supports abstaining from any treatment, shifting to an active policy if it grows or new, invalidating symptoms develop [1 2]. Active therapies such as microsurgery or radiotherapy (RT) may be offered at the time of diagnosis, but only if they provide the patient more benefit than observation alone [3 4]. It is essential to ask why and when a small AN should be actively treated. The goal of treatment should be to assure long-term cure of the disease with no further morbidity on cranial nerves VII and VIII, or post-treatment sequelae.

The three possible treatment options to consider – observation, microsurgery and RT [3] – are not equivalent. The value of each is influenced by the goals of treatment, the patient's expectations and the institution's treating attitude. Previous reports showed similar outcomes in terms of tumour removal and facial function, while the main difference lies in hearing function [3-5]. All three treatment approaches will involve a substantial loss of serviceable hearing over time [3-5]. Surgery has various rates of early hearing loss, while observation and RT reportedly have longer-term serviceable hearing rates [3]. Active treatments may be worthwhile if they can preserve hearing better than long-term observation. Hearing preservation surgery (HPS) is warranted as soon as growth is evident [4].

At our institution, the treatment options currently consist in observation alone, HPS, traditional surgery (possibly associated with hearing rehabilitation with cochlear implants [CI]), and RT. These four options have been adopted in our protocol [6] (Table I) with a view to offering the best chance of long-term cure and preservation of facial function and hearing.

The aim of the present study was to analyse and provide an extended view of the treatment strategies that are available when a small AN is diagnosed, focusing on hearing as the at-risk function in the different therapies. A multioptional treatment was offered to each patient, the choice being based on pre-defined parameters, as well as patient willingness.

Materials and methods

Participants

From January 2012 to June 2016, 169 patients with a first diagnosis of unilateral, sporadic AN were assessed at our institution. Patients diagnosed with neurofibromatosis type 2 or cystic AN were not included. A prospectively-maintained clinical database and the Italian National Health System's electronic medical records were queried for all patients. Only small AN were considered in the present study, i.e. intrameatal or extrameatal tumours up to 1 cm in size in the cerebello-pontine angle (CPA). A total of 91 of 169 patients met this inclusion criteria.

Diagnosis

AN were diagnosed on high-resolution contrast-enhanced magnetic resonance imaging (MRI). Tumour size was measured in the longest diameter (mm) in the CPA on contrast-enhanced T1 sequences [7]. Hearing was assessed with pure tone audiometry, speech audiometry and auditory brainstem responses (ABR). The hearing measures considered were pure tone average (PTA) from 500 to 4000 Hz and speech discrimination score (SDS) obtained at an intensity of 40 dB above detection or at most comfortable threshold. The results were stratified according to the Tokyo classification [7]. Hearing outcome is given with the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) classification [8], where necessary for the purpose of comparisons with data in the literature. Facial nerve function was assessed clinically according to the House-Brackmann (HB) grading system [9] and using electromyography.

Treatment options

The treatment options for small AN (Table I) included observation, RT, HPS and translabyrinthine surgery. Indi-

Table I. Institutional protocol for small sporadic acoustic neuroma management (from Martini et al., 2017 [6], mod.).

Acoustic neuroma size (mm in the CPA angle)	Decision factors	Treatment
< 10 mm	Good hearing (<30 dB, > 70% SDS, normal or slightly modified ABR)	Hearing preservation surgery or observation*
	Good hearing (< 30 dB, > 70% SDS, normal or slightly modified ABR) + surgical risk/unwillingness to undergo surgery	Observation*
	Poor hearing (> 30 dB, < 70% SDS)	Observation* or surgery and hearing rehabilitation with cochlear implant

**Active treatment (surgery or RT) in the event of tumour growth to > 15 mm, or vertigo, or VII cranial nerve impairment. CPA: cerebello-pontine angle*

vidual cases were first discussed, balancing the pros and cons of each option, and the final choice proposed to the patient was based on: (1) tumour size; (2) patient's age and aging (comorbidities); (3) hearing status; (4) tumour growth (defined as stable, growing, or not assessed); (5) contralateral hearing function; (6) facial nerve function; (7) patient's willingness, and other personal aspects such as work, social life and follow-up availability.

Hearing rehabilitation with CI was discussed at the time of planning the therapy, and was considered both in the event of HPS failure and in cases of early translabyrinthine surgery, when the cochlear nerve was preserved. The outcome of patients treated with any type of hearing rehabilitation goes beyond the scope of the present study.

Wait-and-see patients

Observation was indicated in principle for tumours coinciding with hearing impairment (class C or worse on the Tokyo scale), for aged/aging patients, or for patients with good hearing function but unwilling/unable to undergo surgery. Patients under observation underwent clinical examination, pure tone- and speech audiometry and contrast-enhanced MRI planned at 6-month intervals for the first year, then yearly for the next 5 years. The follow-up was then planned every 3 years afterwards. Tumour growth was defined as an increase of more than 2 mm in the tumour's largest extrameatal diameter between the first and latest MRI scans. Hearing deterioration was defined as hearing class change/worsening at the last evaluation. Regarding HPS, it was applied only to cases with good hearing (class AB Tokyo) at diagnosis.

Hearing preservation surgery (HPS)

The indications for HPS included class A-B hearing on the Tokyo scale, normal ABR or slightly increased waves III and V (10) and intrameatal or ≤ 10 mm tumours in the CPA, regardless of their growth and extension to the fundus. Patients who met these inclusion criteria were assigned to an in-protocol group for HPS. Patients strongly motivated to receive HPS, but not satisfying one or more of the above inclusion criteria, were assigned to an off-protocol group. A retro-sigmoid approach with a retrolabyrinthine meatotomy was adopted in all cases of HPS [11 12].

Translabyrinthine surgery

Translabyrinthine surgery was only considered as a primary treatment option in the case of invalidating vestibular symptoms or facial nerve weakness at the time of diagnosis. Otherwise, translabyrinthine surgery was discussed as a secondary option during the observation period in the event of: 1. the onset of invalidating vertigo, or facial nerve weakness; 2. tumour growth with impaired hearing (class C or worse on the Tokyo scale); 3. impaired hearing in cases where functional hearing rehabilitation surgery with CI was planned. The follow-up for surgically-treated patients involved contrast-enhanced MRI scheduled 1 and 3 years after surgery. Further imaging was then planned 6, 10 and 15 years afterwards.

Radiotherapy

No tumour was submitted to primary RT unless there was evidence of growth [6]. Patients with good hearing at diagnosis never received RT as first-choice primary treatment.

Statistical analysis

Categorical variables are presented as frequencies and percentages, continuous data as means and standard deviations or as medians and interquartile range (IQR), according to the Shapiro-Wilk test of normality. Pearson's chi-square test or Fisher's exact test were used to compare categorical variables. The Mann-Whitney U test or the t-test for independent and paired samples were applied to continuous variables, depending on their distribution. The cumulative hazard of hearing impairment was calculated in the wait-and-see group using the Kaplan-Meier method. In this observation group, the association of 5 variables (age, gender, tumour site, tumour growth, and hearing impairment) with the risk of the conservative

Table II. Characteristics of the study population, stratified by tumour site at diagnosis.

	IAC (n = 47) n (%)	< 10 mm CPA (n = 39) n (%)	p value
Male	28 (59.5)	20 (51)	0.441
Age (years), mean ± SD	56.2 ± 12.6	55.2 ± 12.7	0.697
Baseline PTA (dB), mean ± SD	43.2 ± 21.2	41.4 ± 22.7	0.704
Tokyo class A-B hearing	24 (51)	19 (49)	0.829
Observation	34 (72)	27 (69)	0.752
HPS	9 (19)	10 (26)	0.470
Translabyrinthine surgery	4 (8.5)	2 (5)	0.685

IAC: internal auditory canal; CPA: cerebello-pontine angle; PTA: pure tone average; HPS: hearing preservation surgery.

treatment being abandoned was tested with the log-rank test and Cox regression. Statistical significance was assumed when $p < 0.05$ in two-tailed tests, hazard ratios (HR) and confidence intervals at 95% (95% CI) are reported. The statistical analysis was performed using the Statistical Package for the Social Sciences software (IBM SPSS Statistics for Windows, version 20, IBM Corp., Armonk, N.Y., USA).

Results

Of 169 patients presenting with sporadic AN, 91 met our inclusion criteria. Five had incomplete medical records and were consequently excluded, leaving 86 patients for the final analysis.

Patient demographics, signs and symptoms

The sample included 38 female (44.2%) and 48 male (55.8%) with a mean age at diagnosis of 55.7 ± 12.6 years. At diagnosis, more than one symptom was reported by 38.5% of patients. Hearing loss was present in 56% of cases, followed by tinnitus (37%), vertigo (29%) and facial nerve palsy (1%). The most common combinations were hearing loss and tinnitus (35%), or vertigo and tinnitus (27%). Eighty-five patients (99%) presented with a facial nerve function at diagnosis of grade I HB, and one patient with grade II HB.

The treatment strategies adopted at the time of diagnosis are shown in Figure 1. The findings are analysed and grouped by: (1) tumour size at diagnosis; and (2) treatment strategy adopted at diagnosis (observation, surgery, or RT). The median follow-up was 25 months (IQR 12-38 months).

1. Tumour size

Forty-seven patients (54.6%) had a pure intrameatal tumour at diagnosis, while 39 patients (45.4%) presented with extrameatal tumours (Table II). The two groups did not differ significantly in terms of sex, age or hearing status (PTA and Tokyo scores) at presentation. Moreover, tumour size at diagnosis did not influence the initial treatment policy.

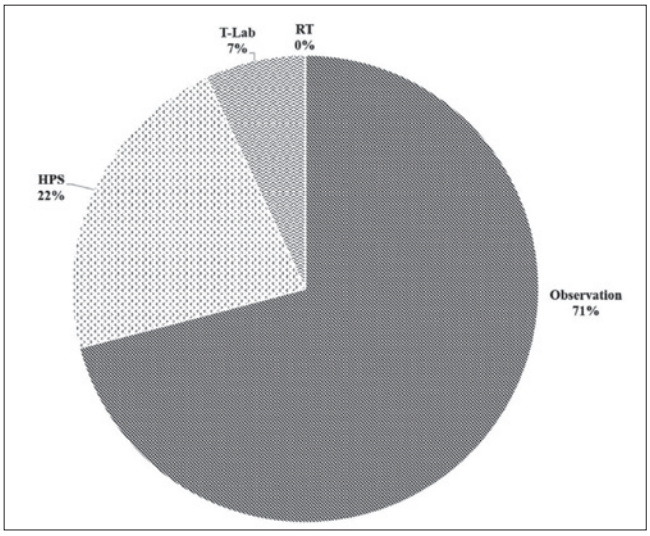

Fig. 1. Distribution of treatment policies adopted for small acoustic neuromas at diagnoses.

2. Treatment strategies

2.1 Wait-and-see

The observation group included 61 patients, 30 female (49%) and 31 male (51%), with a mean age of 58.9 ± 11.4 years. There were 34 cases of intrameatal AN (55.7%), while 27 patients (44.3%) had extrameatal tumours (Table III).

At last observation, tumour growth occurred in 17 patients (28%). Of these, 11 (65%) switched to active treatment, while 6 (35%) remained under observation. As shown in Figure 2, the cumulative hazard of tumour growth after diagnosis was 3.4% for intrameatal tumours and 15.6% for extrameatal tumours in the first year, rising to 12.3% and 26.2%, respectively, in the first two years (HR = 1.93, 95% CI = 0.73-5.04; p = 0.173).

Analysing the overall outcome of conservative management, 46 patients (75.5%) remained under observation, 12 (19.5%) underwent surgical procedures, and 3 patients (5%) were addressed to RT. Of 12 patients submitted to

Table III. Patients' characteristics in the wait-and-see group, by tumour site at diagnosis.

	IAC (n = 34) n (%)	< 10 mm CPA (n = 27) n (%)	p value
Male	18 (53)	13 (48)	0.710
Age (years), mean ± SD	59.1 ± 11.5	59.5 ± 10.8	0.908
Baseline PTA (dB), mean ± SD	45.2 ± 21.1	48.0 ± 19.2	0.597
Tokyo class A-B hearing	15 (44)	9 (33)	0.392
Tumour growth	7 (21)	10 (37)	0.155
Hearing deterioration	11 (32)	6 (22)	0.381
Facial nerve loss	0 (-)	0 (-)	-

IAC: internal auditory canal; CPA: cerebello-pontine angle; PTA: pure tone average

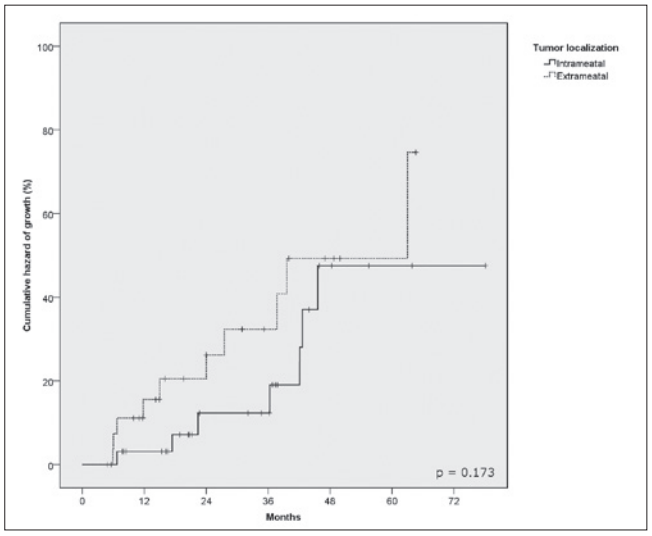

Fig. 2. Cumulative hazard of tumour growth by site at diagnoses.

surgery, all the 11 cases treated with a translabyrinthine approach had extrameatal growth, and the only case with growing intralabyrinthine symptomatic tumour was operated on through a transcanal approach to the vestibule.

The risk of switching from observation to active treatment was significant at univariate analysis (Fig. 3, Table IV) for site at diagnosis (p = 0.0226) and tumour growth (p = 0.0004). Hearing status did not influence the course of the observation treatment policy (p = 0.873). On multivariate analysis (Table IV), only tumour growth was confirmed as having an impact on the probability of remaining under observation, and prompting the switch to active treatment (surgery or RT).

Surgery was indicated for patients initially managed with the wait-and-see policy as a result of: tumour growth (8 cases); patient preference (2 cases); hearing loss (1 case); onset of intractable vertigo (1 case).

Three patients were referred for RT after observation due to tumour growth over a median observation period of 17.4 months (IQR 16-21 months). In two cases, cyberknife treatment was preferred due to the patients' age (mean 70.4 years), comorbidities and impaired hearing status (both class D). The last patient addressed to RT was 56 years old and had a preserved hearing function (class A), but preferred a non-surgical treatment over HPS. After RT, all patients showed disease control in terms of absence of tumour growth at radiological imaging after a mean follow-up of 27.6 months. Hearing function decline was observed in the only patient whose hearing was good at diagnosis.

2.1.1 Hearing outcome in the wait-and-see group

At diagnosis, 24 patients (39.4%) showed preserved hearing, defined as Tokyo class A-B. The other 37 patients were in hearing classes C (27.8%), D (16.4%), E (9.8%), or F (6.6%). There was a significant difference ($p < 0.001$) between median PTA at diagnosis and latest hearing assessment with 43 dB (IQR 31-57 dB) and 53 dB, (IQR 40-62.5 dB), respectively.

Hearing deteriorated in 17 patients (28%). It is noteworthy that hearing deterioration coincided with growing tumours in only 6 cases (35%; p = 0.52). As shown in Figure 4, the cumulative hazard of hearing impairment related to tumour growth was 4.8% for stable tumours and 12.5% for growing tumours in the first year, and 11.9% and 22.2%, respectively, in the first two years (HR = 1.82; 95% CI = 0.59-5.66; p = 0.214).

Preoperative classes A and B were maintained in 58% of cases at last follow-up examination, and preoperative classes C and D in 85% of cases (p = 0.032). Figure 5 shows a statistical trend in hearing deterioration after the first 24 months of observation, which was higher for patients in classes A or B at diagnosis, compared to patients in classes C and D (HR = 2.67; 95% CI = 0.93-7.63; p = 0.081).

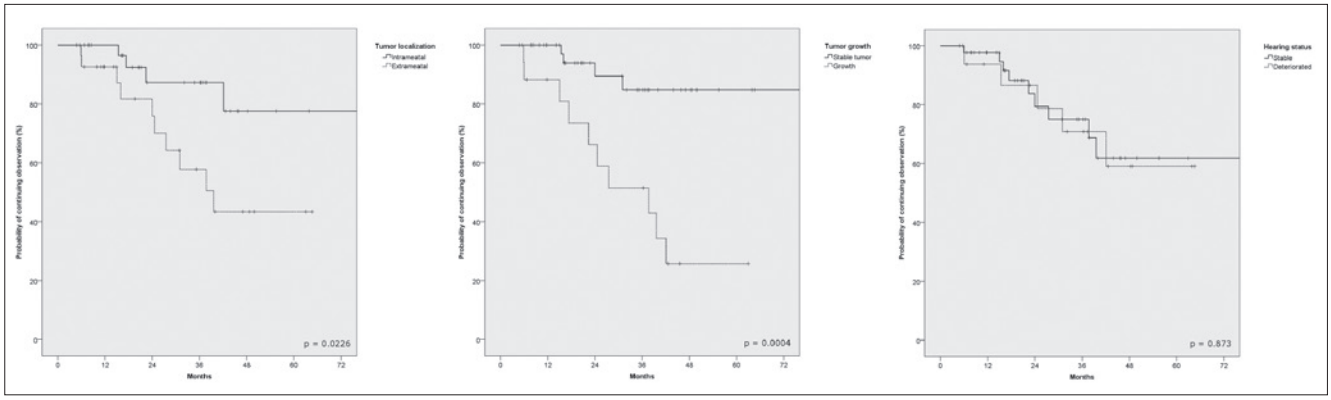

Fig. 3. Probability of remaining under observation by tumour site at diagnoses, tumour growth and hearing deterioration during follow-up.

Table IV. Univariate and multivariate analysis of predictors for switching from the wait-and-see strategy to active treatment.

Variable	HR	95% CI	p value
Univariate analysis			
Age (y)			0.675
< 65	1.00	RG	
≥ 65	0.80	0.28 – 2.26	
Sex			0.912
Male	1.00	RG	
Female	0.94	0.34 – 2.65	
Tumour site			0.0226*
Intrameatal	1.00	RG	
Extrameatal	3.16	1.12 – 8.94	
Tumour growth			0.0004*
No	1.00	RG	
Yes	5.54	1.74-17.07	
Hearing impairment			0.873
No	1.00	RG	
Yes	1.09	0.36-3.24	
Multivariate analysis			
Tumour site			0.058
Intrameatal	1.00	RG	
Extrameatal	3.09	0.96-9.96	
Tumour growth			0.0035*
No	1.00	RG	
Yes	5.66	1.76-18.15	

*HR: hazard ratios; CI: confidence intervals; RG: reference group; *: statistical significance*

2.2 Surgery

The surgical group included 37 patients, 14 female (37.8%) and 23 male (62.2%), with a mean age of 50 ± 12.4 years. There were 17 cases (46%) treated with translabyrinthine surgery, 19 (51.4%) with HPS, and 1 (2.6%) via a trans-canal approach to the vestibular labyrinth. Post-operative major complications occurred in two cases (5.4%), one epidural haematoma, which required revision of the extradural surgical field and one transient cerebellar oedema, which resolved after medical therapy and an early external drain that was removed after 3 days. No neurologic sequalae or other complications were observed. Cerebrospinal fluid leak was observed in two cases (5.4%), which resolved spontaneously in one case and required surgical revision in the other. Postoperative facial nerve function was grade II HB after HPS in one patient with a 10 mm extrameatal tumour, who recovered to grade I HB 4 months after surgery; and grade III HB in the patient with preoperative facial nerve weakness. No recurrences were observed at last follow-up.

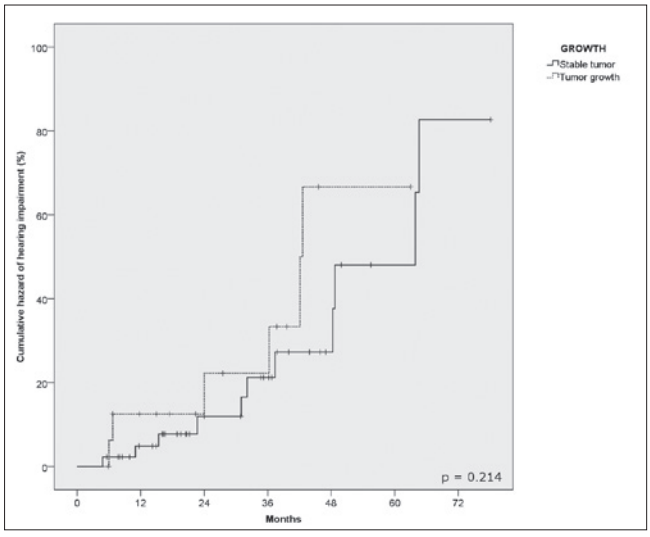

Fig. 4. Cumulative hazard of hearing impairment related to tumour growth.

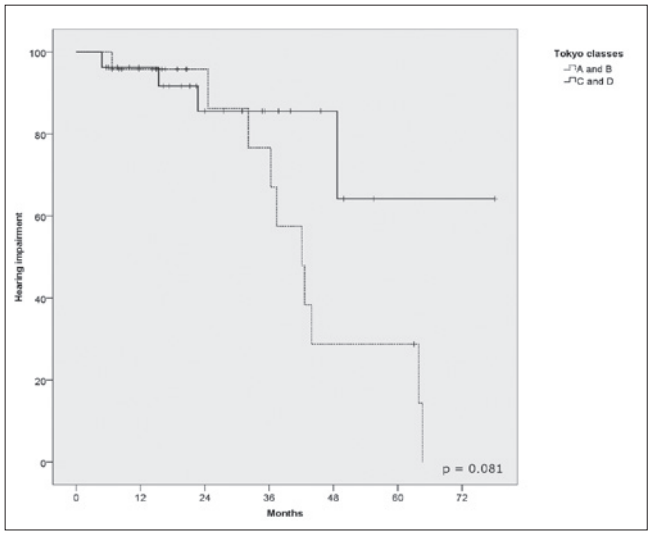

Fig. 5. Hearing impairment in relation to preoperative Tokyo class A-B or C-D hearing for the wait-and-see group of patients.

2.2.1 Translabyrinthine surgery

Seventeen patients – 10 male and 7 female – with a mean age of 52.5 ± 14.0 years underwent translabyrinthine surgery. Eleven (64.7%) patients came from the wait-and-see group, with a median time from diagnosis to treatment of 38.5 months (IQR 21-54 months). The other 6 patients were directly referred for surgery due to intractable vertigo (4 cases), planned CI (1 case), or facial nerve palsy (1 case). Patients arriving from the observation group had a significantly lower median PTA (55 dB, IQR 39-58 dB) than those referred for primary translabyrinthine surgery (75 dB, IQR 62-87.5 dB) (p = 0.005).

2.2.2 Hearing preservation surgery (HPS)

The mean age in the group of 19 patients who underwent HPS was 47.5 ± 10.4 years. All candidates for HPS belonged to Tokyo and AAO-HNS hearing class A or B. The median PTA at diagnosis was 21.5 dB, (IQR 12.5-

Table V. Patients' characteristics in the HPS group, stratified by compliance with protocol.

	In-protocol (n = 13) n (%)	Off-protocol (n = 6) n (%)	p value
Male	9 (69)	4 (67)	1.00
Age at diagnosis (years), mean ± SD	44.7 ± 8.3	52.2 ± 13.0	0.145
Median time from diagnosis to treatment (months)	6.3	5.8	0.357
Extrameatal tumour	6 (46)	6 (100)	0.044*
CPA tumour size (mm), mean ± SD	7.2 ± 2.9	8.7 ± 3.3	0.375
Baseline PTA (dB), median	15.0	33.2	0.244
Postoperative PTA (dB), median	36.2	71.8	0.022*
Maintained Tokyo hearing class A-B	10 (77)	2 (33)	0.129
Maintained AAO-HNS hearing class A-B	11 (85)	2 (33)	0.046*

** Statistical significance*

32.5 dB). Concerning postoperative hearing outcomes, Tokyo class A-B was maintained in 12 of the 19 patients (63%), and AAO-HNS class A-B was maintained in 13 patients (68%); 2 patients became Tokyo class D postoperatively, and were fitted with hearing aids; the remaining 5 cases became deaf.

Our preoperative inclusion criteria for HPS were met for 13 in-protocol cases (68.4%). When hearing outcome was assessed by in- and off-protocol group (Table V), the success rate was 77% for the in-protocol patients, and 33% for the off-protocol group according to the Tokyo classification. When the AAO-HNS hearing classification system was considered, the success rate for in-protocol patients was 85%, differing significantly from the HPS results success rate (33%) for the off-protocol group (p = 0.046). A significant difference in postoperative median PTA between the two groups was also observed (p = 0.022).

Discussion

This report focuses on the indications for the currently-available treatment approaches to small AN, and the results achieved in our series. The outcomes can only be interpreted in the light of the protocol adopted, which gives priority to the hearing function as the only discriminating factor among the various treatment options. Our management of small AN is discussed within this frame.

In the present series, 61 patients were assigned to the wait-and-scan policy, 25 were referred directly for translabyrinthine surgery or HPS. The wait-and-see group showed an overall tumour growth rate of 28% over a median follow-up of 25 months. The patients under observation had small tumours and poor hearing, or were patients who had previously refused or were poor candidates for surgery. When tumour growth warranted active therapy, the indication was for HPS if still feasible, or a translabyrinthine approach or RT otherwise. Hearing deteriorated in 28% of cases in the wait-and-see group, regardless of tumour growth. Tokyo classes A and B were maintained in 58% of cases (14 of 24 patients), while classes C and D were maintained in 85% of cases. These results suggest that adopting a wait and see policy at diagnosis could be more appropriate for patients whose hearing is already impaired, while patients with a good hearing function, likely to worsen over time, could benefit more from active hearing preservation policies.

Our current overall success rate with HPS (postoperative class A-B hearing) was 63% according to the Tokyo classification and 65% to the AAO-HNS hearing grading system. In the literature, the results of hearing preservation surgery differ widely. Overall rates of success are reported after both the retro-sigmoid and middle cranial fossa approaches within a range of 46% and 82%, regardless tumour size [10]. Considering only small AN (≤ 15 mm in the CPA) and according to the AAO-HNS classification, preoperative classes A and B were maintained in 46% to 85% of cases [13-16].

In our series, hearing function was preserved in 77% of cases within the preoperative limits of 30 dB PTA / 70% SDS - normal or slightly altered ABR – size up to 10 mm in the CPA (in-protocol). In the off-protocol group the success rate dropped to 33%. These results are similar to those of previous experiences [10-12 17]. In-protocol patients achieving a class A-B outcome after HPS experienced hearing deterioration in 23% of cases according to the Tokyo classification, and 15% according to the AAO-HNS. This rate appears to be worse than observation and RT in the short term [12 17]. The long-term hearing outcome is not yet available for the present sample, but in a previous series of 200 cases [10] with a 6- to 21-year follow-up (mean 14, median 9 years) it was as follows: postoperative AAO-HNS class A cases maintained class A or B in 92% of cases, and deteriorated to class C or D in 8%; 87%

of class A and B cases remained A or B, while 13% deteriorated to class C or D. Similarly, Wang and colleagues reported an 84% rate of preservation for postoperative AAO-HNS classes A and B at 5-year follow-up [15]. These are the data to compare with the rates of long-term loss of classes A and B reported by Kirchmann (66%), and Stangerup (56%) [2 4].

In principle, it was the natural history of AN that dictated our treatment approach: most small tumours do not grow, but hearing function declines inexorably regardless of tumour growth. The wait-and-see policy is justified by the finding of no tumour growth for a considerable proportion of small tumours [1 2 4 5 18] despite progression of hearing loss. Follow-up seems to be the most predictable variable when assessing hearing outcome – whatever the therapeutic approach – because hearing decline is inescapable when tumours are observed over a long period of time.

Results obtained with RT are likewise promising in the short term [5 19 20], but diminish with time [3], as shown by the 23% of cases with class A and B hearing preservation (serviceable hearing, 50/50%) in the long term. RT remains an option in cases when patients prefer it or if surgery carries a high risk.

The short-term results of microsurgery are worse than with observation or RT, but they merge with the more conservative treatments in the longer term, and it is self-evident that they should be considered more durable [5].

The variability of the results obtained in the surgical series is the main argument supporting the claim that RT is generally superior in terms of preserving hearing function [19 20]. HPS success rates also depend on the surgical team and are strongly influenced by preoperative patient selection [5 14 17]. The comparison of unsuccessful HPS outcomes with that of RT series can be "misleading" [20], since the heterogeneity of results is higher in the surgical group and if only the worse surgical series are considered for comparison, any conclusive statement is biased. Moreover, the effectiveness of RT should be determined in terms of disease control in the long-term, and only confined to tumours with documented growth [21].

Proponents of each therapeutic approach may have their own way of assessing patients and comparing results in the attempt to investigate success and failure rates. It is nonetheless generally agreed that the outcomes in terms of survival, neurological losses and facial nerve preservation are good and much the same whatever the therapy. The difference lies in hearing preservation.

We tried to compare the results obtained with our multi-option strategy as opposed to published data on the observation alone strategy. The most recent paper from a Danish group [4] on the natural history of intrameatal AN reported long-term data on tumour growth and hearing. Despite a high rate of patient loss to follow-up (more than 50%), this study is one of the most relevant long-term reports on the observation strategy for intrameatal AN. Intrameatal AN growth was reported in 37% of cases, extrameatal growth in 23% and the need to switch to active therapy was 15%. AAO-HNS class A hearing (30 dB/70% SDS) was maintained in 47% and 17% of cases at 5- and 10-year follow-up, respectively, and serviceable hearing (50 dB/50% SDS) in 47% and 34%, respectively. Hearing was class C in 66% of cases. The group of cases under observation with 100% SDS and a mean PTA of 46 dB at diagnosis, maintained 70-100% SDS at 10 years in 77% of cases, but no details were provided on the intensity at which the score was obtained. or the worsening PTA [4]. It is generally agreed that combining PTA and SDS enables a better, more complete judgement of the quality of functional hearing.

The main weaknesses of the present study are considered. The article reports on a monocentric series of small AN with a small number of patients and limited follow-up time, too short to draw any definitive conclusion, but enough to define a trend that needs further confirmation over time.

Moreover, the results were at least partially influenced by the specific institutional practices, therefore reducing their generalisability and introducing a bias in the treatment option, even if objective pre-treatment conditioning parameters were defined. A selection bias occurs in the present series as in every planned prospective study where randomisation of treatment is not feasible.

A multicentre collaboration among surgical experienced centres is advocated in order to achieve more robust results.

Conclusions

The various treatment options available for small AN provide good results in terms of disease control and complications, while their benefits on hearing outcome are still debated. A multi-option strategy combining both observation and active treatment (HPS or traditional surgery), according to a pre-treatment selection of patients, as related to our institutional hearing-focused protocol, appeared to provide better results than a single-modality option alone. In patients diagnosed early with small AN, proactive treatment can either preserve the hearing function through HPS or rehabilitate it with a translabyrinthine surgery and hearing aids. Patients with postoperative hearing worse than class A or B might benefit from hearing rehabilitation with hearing aids, so whether a preserved class C aided hearing can be considered success or failure is debatable.

HPS is advisable with an expected good outcome when preoperative hearing and tumour size are within the ranges of

PTA ≤ 30 dB, SDS ≥ 70% and ≤10 mm in the CPA. Otherwise, observation seems to be the choice, as shown by the poor results of pre-treatment unfavourable cases. Under a wait-and-see policy, hearing remains adequately stable in the short term, but seems to become unsatisfactory over time. Long-term hearing results are advisable, as they appear to be the determining outcome measure in selecting treatment options.

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Letter to the Editor

The "Italian way" to counteract obstructive sleep apnoea syndrome in children

Strategie italiane per inquadrare la sindrome delle apnee ostruttive nei bambini

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Dear Editor,

the estimated prevalence of OSAS and habitual snoring in children is 0.1-13% and 6-12%, respectively [1 2]. The lack of treatment of sleep-related breathing disorders puts patients at risk of hypertension, growth lag, hyperactivity, attention deficit, learning disabilities, low levels of education and literacy. Some studies have shown a significant increase in the use of health services (new admissions, accesses to first aid, consumption of drugs) by children with OSAS compared to the control group for all ages. The severity of OSAS correlates directly with total annual costs and is age-independent [3]. Other studies have shown that annual healthcare costs are reduced by one-third for children with OSAS undergoing adenotonsillectomy [4]. The awareness of OSAS and habitual snoring as a highly relevant health issue at the developmental age is rather inadequate. There is also a very significant gap between the estimated number of children with OSAS, as a high percentage of them are undiagnosed, and the ability of the Italian health system to diagnose and treat them. This is why the Italian Minister of Health has approved a new holistic approach that is aimed at improving the health of children with OSAS.

Noisy breathing, habitual snoring with or without breathing pauses during sleep, enuresis, sleeping in the sitting position, cyanosis, headache on awakening, excessive daytime sleepiness, attention deficit, hyperactivity and learning disorder are the symptoms that best make up the clinical picture of OSAS in children [2 5]. Any dentist or paediatrician in any outpatient clinic can easily formulate a clinical suspicion of OSAS by using a structured interview. The next step for a child with suspected OSAS is to refer them as outpatients to a multidisciplinary team that should comprise paediatricians, ear-nose-throat specialists and orthodontists [2]. These specialists, working in a functional unit that is focused on OSAS, should make a collective visit that includes physical examination to investigate adenotonsillar hypertrophy, craniofacial dysmorphisms, oropharyngeal abnormalities (dental malocclusions and jaw contraction) and obesity. In the event of suspicion of comorbidities, further clinical and objective examinations are mandatory [2 6]. Through clinical history and physical examination, patients are subjected to objective testing [2 6]. Although polysomnography is still the gold standard, a less expensive objective testing such as home sleep cardiorespiratory monitoring or night pulse oximetry is validated for the diagnosis of OSAS in children [2 5 7]. By combining the clinical profile and the results of the home sleep testing, and taking into account the predominant risk factor for OSAS, children can be classified into different phenotypes: 1) "classical" phenotype, a child with adenotonsillary hypertrophy, with or without dental and skeletal malocclusions; 2) "adult type" phenotype, characterised by obesity and associated with aspects of the classical phenotype; 3) "congenital" phenotype, with anomalies such as micrognathia or cranio-facial alterations associated with genetic syndromes such as Pierre Robin, Down's, etc. The phenotype should be taken into account, and a patient-tailored therapeutic choice should be offered. OSAS therapeutic hubs are represented by medical therapy (steroids and washing solutions administered by nasal or spray shower), surgical therapy with adenoids and tonsils removal, orthodontic therapy, myofunctional treatment and therapy with positive pressure devices [2]. Surgical therapy with adenotonsillectomy is the first choice for children with severe OSAS and adenoton-

sillar hypertrophy. Short-term improvement can also be seen in terms of school performance and reduction of drug therapies. The surgical indication must be based on clinical and objective testing criteria. In the presence of comorbidities, adenotonsillectomy represents a first stage of the therapeutic program; in these cases, it is necessary to provide post-surgical follow-up to select subjects needing further treatment. Orthopaedic-orthodontic therapy is able to reduce symptoms and alter the natural history of OSAS. This treatment can be integrated with both medical therapy and surgical therapy. Physiotherapists, speech therapists and nutritionists contribute to the implementation of patient-tailored therapy and long-term management of children with OSAS. Children with congenital diseases or severe comorbidities should be studied by overnight polysomnography [8]. Similarly to children requiring upper airway or maxillo-facial surgery, they will be inpatients.

The document approved by the Italian Minister of Health consists of three levels. The first level concerns the formulation of a clinical suspicion of OSAS, actively involving and engaging dentists and paediatricians in outpatient clinics. The second level concerns confirmation of diagnosis and prescription of treatment by ear-nose-throat specialists, orthodontists and paediatricians in outpatient functional units; these specialists are also responsible for multidisciplinary management and long-term care of children. The third level concerns inpatient studies in sleep laboratories and/or surgical or other treatments.

The document highlights the need for the three levels to be functionally connected, such that first-level paediatricians and dentists have at least an adequate knowledge of OSAS and such that second and third-level ear-nose-throat specialists, orthodontists and paediatricians are experts in the diagnosis, treatment and long-term management of these patients. It is expected that this new and holistic approach can meet criteria for effectiveness and efficiency, and will allow easy access to diagnosis and treatment to an increasing number of children with suspected OSAS.

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