Acta Otorhinolaryngologica Italica, XXXVII/6, 447-524, 2017

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

Volume 37

6

December 2017

PACINI EDITORE MEDICINA

www.actaitalica.it

S.I.O.e.C.H.C-F.

Volume 37 – Number 6 – December 2017

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

Publisher
Pacini Editore Srl
Via Gherardesca, 1
56121 Pisa, Italy
Tel. +39 050 313011
Fax +39 050 3130300
info@pacinieditore.it
www.pacinimedicina.it

Acta Otorhinolaryngologica Italica is cited in Index Medicus, MEDLINE, PubMed Central, Science Citation Index Expanded, Scopus, DOAJ, Open-J Gate, Free Medical Journals, Index Copernicus, Socolar

Journal Citation Reports:
Impact Factor 1.530
Acta Otorhinolaryngologica Italica is available on Google Scholar

PACINI EDITORE MEDICINA

www.actaitalica.it

Contents

Information for authors including editorial standards for the preparation of manuscripts available on-line: www.actaitalica.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:447-453; doi: 10.14639/0392-100X-1520

Review

Cost-effectiveness strategies in OSAS management: a short review

Strategie e costi/benefici nella gestione dell'OSAS

D.M. TORALDO[1], D. PASSALI[2], A. SANNA[3], F. DE NUCCIO[4], L. CONTE[5], M. DE BENEDETTO[6]

[1] Hospital Rehabilitation, Dept Cardio-Respiratory Care Unit, "V Fazzi", ASL Lecce, Italy; [2] ENT Clinic, University of Siena, Italy; [3] Pneumology Unit, San Jacopo Hospital, Azienda USL Toscana Centro, Pistoia, Italy; [4] Laboratory of Human Anatomy and Neuroscience, Dept. of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy; [5] Laboratory of Interdisciplinary Research Applied to Medicine (DReAM), University of Salento at the Hospital "V Fazzi", ASL Lecce, Italy; [6] ENT Unit, "V. Fazzi" Hospital, ASL Lecce, Italy

SUMMARY

Obstructive sleep apnoea (OSAS) is an underdiagnosed chronic disease with a high prevalence in adults. It is becoming a significant social problem, since it is associated with a worsening in quality of life and increase in mortality. The cost-effectiveness ratio of diagnostic and therapeutic management of OSAS is a strategic issue to counteract the expected increasing demand of objective testing. OSAS patients with any clinical evidence of comorbidities must be studied using simplified and less expensive systems such as Home Sleep Testing (HST). On the other hand, Sleep Laboratory Polysomnography (PSG) is the gold standard to manage OSAS patients with comorbidities. It should be pointed out that the use of HST can lead to incorrect diagnosis in poorly selected OSAS subjects. This short review discusses various topics for the proper diagnosis and treatment of OSAS in view of epidemiological factors and results in terms of costs and social benefit of the disease. Whatever the strategy chosen and/or the organisational model adopted for managing OSAS, it cannot and should not take into account only cost-effectiveness. Long-term prospective studies evaluating cost-effectiveness ratios and outcomes of OSAS treatment of hospital management models versus home care models are needed.

KEY WORDS: Obstructive sleep apnoea syndrome • Cost-effectiveness • Teleservice • Home sleep testing • Polysomnography • Quality of Life

RIASSUNTO

L'apnea ostruttiva del sonno (OSAS) è una malattia cronica eccessivamente sotto-diagnosticata con un'alta prevalenza negli adulti. L'OSAS sta diventando un problema sociale significativo perché associata ad un peggioramento della qualità della vita ed un aumento della mortalità. Il rapporto costo-efficacia nella gestione diagnostica e terapeutica dell'OSAS è un problema strategico per contrastare la crescente domanda di test oggettivi. I pazienti OSAS che non presentano comorbilità clinicamente evidenti devono essere studiati utilizzando un sistema semplificato e poco costoso, come l'Home Sleep Testing (HST). Al contrario, la Sleep Laboratory Polisomnography (PSG) rimane il gold standard per la gestione dei pazienti con OSAS in presenza di comorbidità. Occorre sottolineare che l'uso di HST potrebbe portare ad una diagnosi errata in soggetti OSAS non ben selezionati. Questa breve rassegna si propone di offrire argomenti di riflessione sulla corretta diagnosi e trattamento dell'OSAS, in rapporto ai dati di prevalenza e alle ricadute sui costi/benefici sociali della malattia. Attualmente non può essere solo il rapporto costo/efficacia a definire il modello organizzativo adottato per la gestione dell'OSAS, in quanto si rendono necessari ulteriori studi prospettici a lungo termine, volti a validare in maniera definitiva tale rapporto nonché il confronto tra il trattamento con modelli di gestione ospedaliera versus l'assistenza domiciliare.

PAROLE CHIAVE: *Sindrome delle apnee ostruttive nel sonno • Costi/benefici • Controllo medico a distanza • Test Ambulatoriale sul sonno • Polisonnografia • Qualità della vita*

Acta Otorhinolaryngol Ital 2017;37:447-453

Introduction

OSAS is an underdiagnosed chronic disease characterised by recurrent episodes of apnoeas and hypopnoeas due to complete or partial occlusion of the upper airway during sleep [1]. A recent epidemiological study in adults showed that 49.7% of men and 23.4% of women have moderate to severe OSAS defined as an apnoea-hypopnoea index (AHI) ≥ 15/hour [2]. Untreated patients are at significantly increased risk of developing cardiovascular [3 4], metabolic [5-7] and neurocognitive diseases [8], as well as motor vehicle (MVAs) [9 10] and/or work accidents [10 11]. Concern that OSAS is a health issue of great relevance with having an adverse impact on the quality [12] and life expectancy [13] and on economic systems is growing [14 15]. Furthermore,

in Europe, it is expected that there will be an exponential increase in the number of diagnosed OSAS subjects. Two main reasons account for this: 1) the increase in OSAS prevalence in the last decades is associated with increasing prevalence and severity of obesity, the first risk factor for OSAS [16]; 2) in EU countries, according to the Commission directive 2014/85/EU, testing for OSAS is mandatory before granting or renewing a driver's license. Applicants or drivers with moderate or severe OSAS under treatment shall be subjected to a periodic medical review, with intervals not exceeding three years for drivers of group 1 and one year for drivers of group 2 [17]. The huge expected increase in number of diagnosis of OSAS is a challenge for health systems, leading to the need to manage OSAS and related problems by simplified tests and developing new models based on cost-effectiveness. Although a simplified and cost-effectiveness approach may help to meet the increase in number of OSAS diagnoses, it must be pointed out that the directive requires a mandatory cut-off (AHI ≥ 15 with excessive daytime sleepiness) for OSAS testing [18]. It is therefore imperative, for clinical and regulatory reasons, to make a proper diagnosis and offer appropriate treatment. Objective sleep studies for OSAS may be of two types [19-21]: Sleep Laboratory Polysomnography (PSG) and Home Sleep Testing (HST), the former isconsidered the diagnostic "gold standard" although highly time consuming and expensive. As a result, suspected OSAS patients may be left waiting for months before being diagnosed and able to initiate treatment. American Academy Sleep Medicine (AASM) and the American Thoracic Society (ATS) recommend the management of OSAS by HST in pre-test subjects with high OSAS suspicion without notorious morbidity or suspicion of neurological disorders, as stated in their guidelines for the use of portable monitors [18]. In addition, HST is considered a cost-effective alternative for OSAS diagnosis in selected patients [22]. Our aim is to review models based on cost-effectiveness to meet the increasing request for OSAS diagnosis and treatment. In 2016, the Italian Ministry of Health produced a document aimed to prevent and assess the clinical pathways for OSAS patients by proposing the creation of a dedicated interdisciplinary network of care [23].

The care of OSAS

International statements on care of OSAS are: a) diagnosis should be confirmed by objective testing [19 20]; b) therapeutic choice comes from a multidisciplinary assessment [19 24]; c) all patients should undergo long term follow-up to monitor treatment effectiveness and adherence to therapy [19 20 25]. Developed in the early 1980's, CPAP has become established as the treatment of choice for OSAS [26]. It is effective as a treatment of OSAS symptoms and among all available treatments, it has the strongest evidence for a beneficial cardiovascular effect [3]. It has also been proven to be effective in reducing MVAs [27] and to improve quality and expectancy of life [3]. The full-night attended sleep laboratory PSG is the gold standard for the OSAS diagnosis and for CPAP titration aimed to determine the optimal positive airway pressure [19 20 24]. More recent studies have shown that alternatives OSAS therapies, like upper airway surgery and even oral appliances [19 28], are as effective as CPAP in mild and moderate OSAS. Indeed, although CPAP is established as a highly efficacious treatment for OSAS, its effectiveness has been limited by poor adherence [29]. Users may experience nasal discomfort, congestion, mask leak and claustrophobia which lead to variable levels of long term compliance ranging from 46% to 85% depending of the criteria used to define it [30]. It has been described a fairly linear dose response relationship such that the greater the CPAP usage, the greater the improvement in sleepiness, quality of life (QoL) and blood pressure outcomes [31]. As a result, there has been much research on methods to optimise CPAP adherence. Interventions that have been conducted include the verbal-visual instruction by health professionals, the application of the nasal and oral-nasal masks as well as the importance of the disease and its health effects [32] with standardised audiovisual presentations and practical demonstrations on performing standards treatments at home [33]. Up to now, gold standard training programs in literature have steadily improved the adherence to CPAP treatment. Many of these clinical trials [34-36] with double arms (control and study) have given controversial results. These latter studies were also criticised due to the higher level of education of the control arm compared to the study arm vs. normal routine care. Consequently, results on the adherence in the study arm appeared worse. However, the majority of the experts still recommend to all patients that starting CPAP requires a high level of intensive instruction. Any educational approach, however, necessary to achieve the best possible adherence to long-term treatment, is time and money consuming.

Sleep laboratory polysomnography *vs* home sleep testing

HST is validated for diagnosis of OSAS as well as for titration of positive airway pressure (PAP) and oral appliances (OA) devices [19 20], and auto titrating PAP (APAP) devices may be used in an unattended way to determine the therapeutic continuous PAP value [37]. Some studies have compared the cost-utility of different diagnostic/therapeutic strategies for the diagnosis of OSAS [38 39]. Pietzsch et al. [40] have assessed the cost-benefit ratio by comparing the three most used diagnostic/therapeutic strategies: a) full-night attended sleep laboratory PSG with manual CPAP titration; b) split-night PSG along with manual CPAP titration; c) HST with subsequent treatment with auto-ti-

trating PAP (APAP). In this study, for a patient with moderate-to-severe OSAS, CPAP therapy has an incremental cost-effectiveness ratio (ICER) of $15,915 per QALY (Quality Adjusted Life Years) gained for the lifetime horizon. Over the lifetime horizon in a population with 50% prevalence of OSAS, full-night polysomnography in conjunction with CPAP therapy is the most economically efficient strategy at any willingness-to-pay greater than $17,131 per QALY gained, because it dominates all other strategies in comparative analysis.

In a more recent study [41], 191 suspected OSAS patients were studied in advance using a pre-clinical test. More than half (56.5%) were suspected of having OSAS. Without involvement of a sleep medicine specialist, obstructive sleep apnoea was not identified in only 5.8% of the sample. The probability to obtain an accurate diagnosis using pre-clinical tests seems not to be influenced by the presence/absence of a specialist sleep physician in accordance with the severity of the disease. The authors concluded that severe OSAS can be reliably identified with HST in a non-referred sample, irrespective of the pretest probability of the disease. Although these studies [42] support, even from an economic point of view, the widespread use of the HST for OSAS diagnosis, it should be pointed out that, as reported by the American Academy of Sleep Medicine, HST may underestimate the seriousness of the hypopnoeic events compared to a full night attended sleep laboratory PSG [43]. This remark is not only important from a clinical and therapeutic point of view for the individual patient, but also for regulatory reasons. Indeed, as indicated by the Directive, an underestimation of the seriousness and number of apnoeas and hypopnoeas with an AHI < 15, can result in a lack of diagnosis for an OSAS subject at risk for MVAs if the driver is not in treatment for OSAS. The AASM also remarks that HST is not indicated in case of suspected sleep related breathing disorders other than OSAS, major comorbid conditions including moderate to severe pulmonary disease, neuromuscular disease, congestive heart failure and sleep disorder. These are almost all high-prevalence diseases. Furthermore, it must pointed out some relevant limitations about HST when used in a long-term management strategy. These include: a) the need to review/reevaluate the raw data that come automatically without performing a manual analysis of the nocturnal polygraphic tracings; b) uncertainties about the long-term use of this outpatient strategy regarding the overall cost-effectiveness compared to a hospital diagnostic plan that is based on supervised polysomnography at 1st level.

Of note, these trials for OSAS diagnosis in primary care excluded patients with comorbidities, including chronic obstructive pulmonary disease or congestive heart failure. For these latter, the concordance between HST and PSG is inadequate [44], due to either poor oximetry and flow recordings in a significant number of patients [45].

A randomised, controlled, non-inferiority study involved patients with OSAS who were treated with HST and compared with a specialist model (Sleep Laboratory Polysomnography) [46]. Among patients with OSAS, treatment under HST did not result in worse sleepiness scores and general quality of life measures, suggesting that the two treatment models may be comparable. Andreu et al. [47] evaluated the efficacy of a home-based programme on clinical response, (CPAP) compliance and cost in a population of high pre-test probability of suffering (OSAS). Patients were randomised into the following three groups with no between-group differences. Group A: home respiratory polygraphy (RP) and home follow-up; Group B: hospital PSG and hospital follow-up; and Group C: home RP and hospital follow-up. Evaluation during 6 months included Epworth Sleepiness Scale (ESS), Functional Outcomes Sleep Questionnaire (FOSQ) and daily activity and symptom questionnaires. Compliance was assessed by memory cards (group A) and using an hourly counter (groups B and C). The randomised prospective study in 65 patients demonstrated that patients with a high initial probability of having OSAS can be diagnosed and treated in a home setting, with a high level of CPAP compliance and lower cost than either a hospital-based approach or home RP/hospital follow-up.

Health-care providers and models of care to manage OSAS

Different approaches and strategies have been proposed to counteract the increasing demand for access to diagnosis and therapy for OSAS [48]. The US created a home care model, based on HST and refundable by the insurance agencies, that deals with both diagnosis and therapeutic care of OSAS patients. This services company, called the Affordable Care Act (ACA) aims to provide high quality healthcare to OSAS patients [49 50]. The ACA is gearing up towards a diagnostic model that focuses on the doctor-patient relationship. In this home care model, the company puts a network of healthcare services at the centre of this relationship, where primary care is subsequently and rapidly integrated after diagnosis. Basically, once diagnosis and the treatment are determined, the Agency rapidly provides home care technical support. Home care diagnosis and treatment is performed by health care professionals along with a consultation with a sleep specialist. This approach reduces the costs of medical staff and simplifies the delivery steps in providing therapeutic equipment at home [51].

Telemedicine is a remote communication system of Information Technology IT/medical data that is used to save time and reduce costs for managing a home care service for chronic diseases [52]. A number of clinical studies have been carried out to evaluate the effectiveness of telemedicine interventions on adherence to CPAP treatment [53 54].

The IT reports were transmitted and received from patient CPAP treatment home units to the reference provider centre wirelessly, and data from the study were collected and processed (Home ↔ Provider). The data collected were: a) loss of pressure in the mask during sleep to CPAP treatment; b) residual AHI during CPAP treatment; c) number of hours of CPAP use. Errors in performing the treatment were easily detected from the technician, who was able to call the patients the next morning through the central Provider and resolve problems about the low efficiency of treatment. In a multicentre randomised controlled trial [55], telemedicine was used to study the economic and clinical impact as well the improvement of the QoL with CPAP treatment compared to traditional follow-up with face-to-face doctor-patient controls. The 139 enrolled patients were sufficiently confident with the IT world. The quality of sleep, side effects of treatment with CPAP and QoL were evaluated at 1, 3 and 6 months. It was observed that a strategy based on telemedicine for follow-up of CPAP in patients with severe OSAS was as effective as therapy performed in hospital in accordance with the gold standard, in terms of the compliance with CPAP and improvement of symptoms, with comparable side effects and satisfaction rates. It was also found that a strategy based on telemedicine resulted in reduction of transport service and productivity costs.

PREDICT, a multicentre randomised controlled study [56], used the telemedicine to assess the clinical and economic aspects of CPAP treatment in OSAS patients older than 65 years. It was found that CPAP treatment reduced subjective and objective sleepiness to that observed in younger patients. Secondary goals were to determine CPAP clinical efficacy, cost-effectiveness ratio and real usefulness of treatment (model-based cost-effectiveness analysis) compared to alternative treatments with APAP/Bilevel/C FLEX (BSC). The QoL at 12 months of treatment was measured by the European Quality of Life-5 Dimensions (EQ-5D). In elderly patients with OSAS, CPAP treatment reduced somnolence more significantly compared to treatment with APAP/Bi-Level/C FLEX (BSC) over a period of 12 months, improving the EQ-5D. Although IT telemonitoring systems saved operating costs and managed several patients simultaneously (at least 100), by using a single provider they hinted at possible medico-legal disputes [57]. First of all, there is no international standard of care for telemedicine [58]. Standards of care exist only for services for the individual, but there are still not many e-Health practices. Medico-legal issues are: a) respect for personal privacy, b) inaccuracies of self-reporting of patients in data recording, c) the resolution limits of data to be recorded and consequent delays due to failure/delayed treatment after recording of data, d) failure of systems that do not work correctly.

In the US, there is a national society in telemedicine called TelaDoc [59] that features a American National Committee to guarantee certification of electronic systems used in tel-

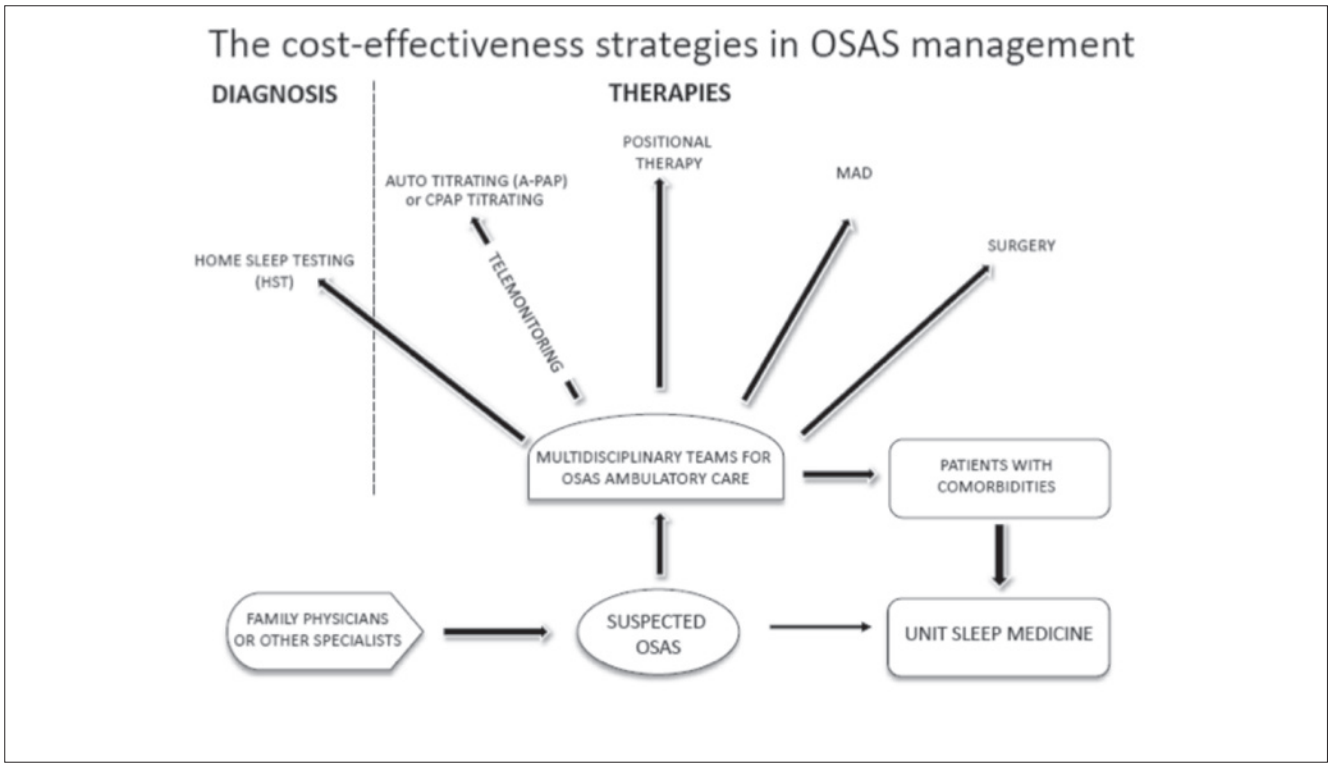


Fig. 1. Model of OSAS management resulting from cost-effectiveness strategies.

emedicine, along with the production of evidence-based clinical practice guidelines for registering data quality [60]. There are emerging results in the literature that support the role of non-medical health professionals who are expert in sleep study, such as nurses, caregivers and IT/health care, who are able to manage home care OSAS in a cost-effective way. We still need more long-term prospective studies that can evaluate the cost-effectiveness ratio, including direct and indirect costs of hospital management models versus models that take into account new, qualified non-medical personnel care.

Another randomised, prospective, controlled study included OSAS patients who underwent CPAP treatment and HST during follow-up. The primary outcome was CPAP compliance at 6 months. Secondary outcomes were ESS score, EuroQoL, patient satisfaction, body mass index (BMI), blood pressure and cost-effectiveness. For patients with OSAS, the treatment provided did not result in worse CPAP compliance compared with a specialist model (Sleep Center Polysomnography) and was shown to be a cost-effective alternative [61].

Conclusions

The huge expected increase in the prevalence and incidence of OSAS is a challenge for healthcare systems. The model of OSAS management resulting from cost-effectiveness strategies is shown in Figure 1. Healthcare systems must ensure rapid access to diagnosis and treatment for each individual with suspected OSAS and avoid exposure to the risk of MVAs and work accidents for both OSAS subjects and others involved in accidents caused by OSAS subjects. HST, health-care providers and the proposed model aimed to manage OSAS are a possible effective response to counteract the increasing demand for access to diagnosis and therapy for OSAS. It takes priority to involve non-medical healthcare professionals and create training courses for all health workers on the management of OSAS and OSAS-related problems. Whatever the strategy chosen and/or organisational model adopted for managing OSAS, it cannot and should not take into account only cost-effectiveness. Long-term prospective studies aimed at evaluating the cost-effectiveness ratio, accuracy of diagnosis and outcomes of OSAS treatment of hospital management models versus home care models are needed.

References

1 White DP. *Pathophysiology of obstructive sleep apnoea*. Thorax 1995;50:797-804.

2 Heinzer R, Vat S, Marques-Vidal P, et al. *Prevalence of sleep-disordered breathing in the general population: the HypnoLaus study*. Lancet Respir Med 2015;3:310-8.

3 Marin JM, Carrizo SJ, Vicente E, et al. *Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study*. Lancet 2005;365:1046-53.

4 Yaggi HK, Concato J, Kernan WN, et al. *Obstructive sleep apnea as a risk factor for stroke and death*. N Engl J Med 2005;353:2034-41.

5 Toraldo DM, DE Nuccio F, DE Benedetto M, et al. *Obstructive sleep apnoea syndrome: a new paradigm by chronic nocturnal intermittent hypoxia and sleep disruption*. Acta Otorhinolaryngol Ital 2015;35:69-74.

6 Passàli D, Tatti P, Toraldo M, et al. *OSAS and metabolic diseases*. Round Table, 99th SIO National Congress, Bari 2012. Acta Otorhinolaryngol Ital 2014;34:158-66.

7 Kendzerska T, Gershon AS, Hawker G, et al. *Obstructive sleep apnea and incident diabetes. A historical cohort study.* Am J Respir Crit Care Med 2014;190:218-25.

8 Peppard PE, Szklo-Coxe M, Hla KM, et al. *Longitudinal association of sleep-related breathing disorder and depression*. Arch Intern Med 2006;166:1709-15.

9 Sanna A. *Obstructive sleep apnoea, motor vehicle accidents, and work performance*. Chron Respir Dis 2013;10:29-33.

10 Garbarino S, Pitidis A, Giustini M, et al. *Motor vehicle accidents and obstructive sleep apnea syndrome: a methodology to calculate the related burden of injuries*. Chron Respir Dis 2015;12:320-8.

11 Garbarino S, Guglielmi O, Sanna A, et al. *Risk of occupational accidents in workers with obstructive sleep apnea: systematic review and meta-analysis*. Sleep 2016;39:1211-8.

12 Garbarino S, Lanteri P, Durando P, et al. *Co-morbidity, mortality, quality of life and the healthcare/welfare/social costs of disordered sleep: a rapid review*. Int J Environ Res Public Health 2016;13(8). pii: E831. doi: 10.3390/ijerph13080831.

13 Punjabi NM, Caffo BS, Goodwin JL, et al. *Sleep-disordered breathing and mortality: a prospective cohort study*. PLoS Med 2009;6:e1000132.

14 Jennum P, Kjellberg J. *Health, social and economical consequences of sleep-disordered breathing: a controlled national study*. Thorax 2011;66:560-6.

15 Leger D, Bayon V, Laaban JP, et al. *Impact of sleep apnea on economics*. Sleep Med Rev 2012;16:455-62.

16 Garvey WT, Mechanick JI, Brett EM, et al. *American association of clinical endocrinologists and american college of endocrinology comprehensive clinical practice guidelines for medical care of patients with obesity*. Endocr Pract 2016;22:1-203.

17 Bonsignore MR, Randerath W, Riha R, et al. *New rules on driver licensing for patients with obstructive sleep apnoea: EU Directive 2014/85/EU*. Eur Respir J 2016;47:39-41.

18 Kuna ST, Badr MS, Kimoff RJ, et al. *An official ATS/AASM/ACCP/ERS workshop report: Research priorities in ambulatory management of adults with obstructive sleep apnea*. Proc Am Thorac Soc 2011;8:1-16.

19 Epstein LJ, Kristo D, Strollo PJ, et al. *Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults*. J Clin Sleep Med 2009;5:263-76.

20 Sanna A, Bellofiore S, Di Michele L, et al. *Indicazioni per la diagnosi e cura della sindrome delle apnee ostruttive del sonno nell'adulto*. Documento dell'Associazione Scientifica

Interdisciplinare per lo studio delle Malattie Respiratorie (AIMAR). www.aimarnet.it. Last access 14 November 2016.

[21] Passali D, Caruso G, Arigliano LC, et al. *Database application for patients with obstructive sleep apnoea syndrome*. Acta Otorhinolaryngol Ital 2012;32:252-5.

[22] Mansfield DR, Antic NA, McEvoy RD. *How to assess, diagnose, refer and treat adult obstructive sleep apnoea: a commentary on the choices*. Med J Aust 2013;199.

[23] Italian Ministery of Health. *Documento di Indirizzo: Sindrome Apnee Ostruttive nel Sonno (OSAS): quale prevenzione possibile*. In press. Rome, Febr 2016.

[24] Durán-Cantolla J, Zamora Almeida G, Vegas Diaz de Guereñu O, et al. *Validation of a new domiciliary diagnosis device for automatic diagnosis of patients with clinical suspicion of OSA*. Respirology 2017;22:378-85.

[25] Aurora RN, Collop NA, Jacobowitz O, et al. *Quality measures for the care of adult patients with obstructive sleep apnea*. J Clin Sleep Med 2015;11:357-83.

[26] Malhotra A, Orr JE, Owens RL. *On the cutting edge of obstructive sleep apnoea: where next?* Lancet Respir Med 2015;3:397-403.

[27] George CF. *Reduction in motor vehicle collisions following treatment of sleep apnoea with nasal CPAP*. Thorax 2001;56:508-12.

[28] Ramar K, Dort LC, Katz SG, et al. *Clinical practice guideline for the treatment of obstructive sleep apnea and snoring with oral appliance therapy: an update for 2015*. J Clin Sleep Med 2015;11:773-827.

[29] Jean Wiese H, Boethel C, Phillips B, et al. *CPAP compliance: video education may help!* Sleep Med 200;6:171-4.

[30] Wozniak DR, Lasserson TJ, Smith I. *Educational, supportive and behavioural interventions to improve usage of continuous positive airway pressure machines in adults with obstructive sleep apnoea*. Cochrane Dtabase Syst Rev 2014:CD007736.

[31] Salvaggio A, Lo Bue A, Isidoro SI, et al. *Gel pillow designed specifically for obstructive sleep apnea treatment with continuous positive airway pressure*. J Bras Pneumol 2016;42:362-36.

[32] Lettieri CJ, Walter RJ. *Impact of group education on continuous positive airway pressure adherence*. J Clin Sleep Med 2013;9:537-41.

[33] Lai AYK, Fong DYT, Lam JCM, et al. *The efficacy of a brief motivational enhancement education program on CPAP adherence in OSA: a randomized controlled trial*. Chest 2014;146:600-10.

[34] Smith I, Nadig V, Lasserson TJ. *Educational, supportive and behavioural interventions to improve usage of continuous positive airway pressure machines for adults with obstructive sleep apnoea*. Cochrane Database Syst Rev 2009:CD007736.

[35] Haniffa M, Lasserson TJ, Smith I. *Interventions to improve compliance with continuous positive airway pressure for obstructive sleep apnoea*. Cochrane Database Syst Rev 2004:CD003531.

[36] Sedkaoui K, Leseux L, Pontier S, et al. *Efficiency of a phone coaching program on adherence to continuous positive airway pressure in sleep apnea hypopnea syndrome: a randomized trial*. BMC Pulm Med 2015;15:102.

[37] Kapoor M, Greenough G. *Home sleep tests for obstructive sleep apnea (OSA)*. J Am Board Fam Med 2015;28:504-9.

[38] Billings ME, Kapur VK. *Medicare long-term CPAP coverage policy: a cost-utility analysis*. J Clin Sleep Med 2013;9:1023-9.

[39] Weatherly HLA, Griffin SC, Mc Daid C, et al. *An economic analysis of continuous positive airway pressure for the treatment of obstructive sleep apnea-hypopnea syndrome*. Int J Technol Assess Health Care 2009;25:26-34.

[40] Pietzsch JB, Garner A, Cipriano LE, et al. *An integrated health-economic analysis of diagnostic and therapeutic strategies in the treatment of moderate-to-severe obstructive sleep apnea*. Sleep 2011;34:695-709.

[41] Aurora RN, Putcha N, Swartz R, et al. *Agreement between results of home sleep testing for obstructive sleep apnea with and without a sleep specialist*. Am J Med 2016;129:725-30.

[42] Collop NA, Anderson WM, Boehlecke B, et al; Portable Monitoring Task Force of the American Academy of Sleep Medicine. *Clinical guidelines for the use of unattended portable monitors in the diagnosis of obstructive sleep apnea in adult patients*. J Clin Sleep Med 2007;3:737-47.

[43] Cooksey JA, Balachandran JS. *Portable monitoring for the diagnosis of obstructive sleep apnea*. Chest 2015;149:1074-81.

[44] Oliveira MG, Nery LE, Santos-Silva R, et al. *Is portable monitoring accurate in the diagnosis of obstructive sleep apnea syndrome in chronic pulmonary obstructive disease?* Sleep Med 2012;13:1033-8.

[45] Suárez M, Osorio J, Torres M, et al. *Should the diagnosis and management of OSA move into general practice?* Breathe 2016;12:243-7.

[46] Chai-Coetzer CL, Antic NA, Rowland LS, et al. *Primary care vs specialist sleep center management of obstructive sleep apnea and daytime sleepiness and quality of life: a randomized trial*. JAMA 2013;309:997-1004.

[47] Andreu AL, Chiner E, Sancho-Chust JN, et al. *Effect of an ambulatory diagnostic and treatment programme in patients with sleep apnoea*. Eur Respir J 2012;39:305-12.

[48] Chai-Coetzer CL, Antic NA, McEvoy RD. *Ambulatory models of care for obstructive sleep apnoea: diagnosis and management*. Respirology 2013;18:605-15.

[49] Glied S, Solís-Román C, Parikh S. *How the ACA's health insurance expansions have affected out-of-pocket cost-sharing and spending on premiums*. Issue Brief (Commonw Fund) 2016;28:1-16.

[50] Riggs KR, Buttorff C, Alexander GC. *Impact of out-of-pocket spending caps on financial burden of those with group health insurance*. J Gen Intern Med 2015;30:683-8.

[51] Moro M, Westover MB, Kelly J, et al. *Decision modeling in sleep apnea: the critical roles of pretest probability, cost of untreated obstructive sleep apnea, and time horizon*. J Clin Sleep Med 2016;12:409-18.

[52] Hwang D. *Monitoring progress and adherence with positive airway pressure therapy for obstructive sleep apnea: the roles of telemedicine and mobile health applications*. Sleep Med Clin 2016;11:161-71.

[53] Isetta V, León C, Torres M, et al. *Telemedicine-based approach for obstructive sleep apnea management: building evidence*. Interact J Med Res 2014;3:e6.

[54] Isetta V, Negrín MA, Monasterio C, et al. *A Bayesian cost-effectiveness analysis of a telemedicine-based strategy for the management of sleep apnoea: a multicentre randomised controlled trial*. Thorax 2015;70:1054-61.

[55] Fox N, Hirsch-Allen AJ, Goodfellow E, et al. *The impact of a telemedicine monitoring system on positive airway pressure adherence in patients with obstructive sleep apnea: a randomized controlled trial*. Sleep 2012;35:477-81.

[56] McMillan A, Bratton DJ, Faria R, et al. *A multicentre randomised controlled trial and economic evaluation of continuous positive airway pressure for the treatment of obstructive sleep apnoea syndrome in older people: PREDICT*. Health Technol Assess 2015;19:1-188.

[57] McDaid C, Griffin S, Weatherly H, et al. *Continuous positive airway pressure devices for the treatment of obstructive sleep apnoea-hypopnoea syndrome: a systematic review and economic analysis*. Health Technol Assess 2009;13:143-274.

[58] Gallegos A. *Frontline Medical News*. *Telemedicine poses novel legal risks for doctors.* Featur Top Chest online Oct 6 2015.

[59] Uscher-Pines L, Mehrotra A. *Analysis of Teladoc use seems to indicate expanded access to care for patients without prior connection to a provider*. Health Aff (Millwood) 2014;33:258-64.

[60] Uscher-Pines L, Mulcahy A, Cowling D, et al. *Access and quality of care in direct-to-consumer telemedicine*. Telemed J E Health 2016;22:282-7.

[61] Sanchez-de-la-Torre M, Nadal N, Cortijo A, et al. *Role of primary care in the follow-up of patients with obstructive sleep apnoea undergoing CPAP treatment: a randomised controlled trial*. Thorax 2015;70:346-52.

Received: November 30, 2016 - Accepted: March 11, 2017

Address for correspondence: Domenico Maurizio Toraldo, via A.C. Casetti 2, 73100 Lecce, Italy. E-mail: d.torald@tin.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:454-457; doi: 10.14639/0392-100X-1502

HEAD AND NECK

Transoral robotic surgery in Eagle's syndrome: our experience on four patients

La chirurgia robotica transorale nella sindrome di Eagle: nostra esperienza su quattro pazienti

D.H. KIM, Y.H. LEE, D. CHA, S.H. KIM

[1]Department of Otorhinolaryngology, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

SUMMARY

Eagle's syndrome is characterised by focal pain in the tonsillar fossa on wide mouth opening or head rotation and various accompanying symptoms. While the syndrome is difficult to diagnose, shortening the styloid process via a transoral or transcervical surgical approach has been shown to be the most effective treatment. The aim of this article was to document our experience with a transoral robotic approach to treat Eagle's syndrome and to present the outcomes of four patients. We reviewed the cases of four patients with Eagle's syndrome who underwent transoral robotic surgery (TORS). The average age of patients was 53.75 years, and there were equal numbers of males and females. The styloid processes were reconstructed in 3D from the preoperative CT scans and were measured as an average of 4.18 cm (range 3.3-5.1). The mean set-up time and operation times were less than 10 minutes and 30 minutes, respectively. All patients were completely relieved of symptoms, and were able to restart an oral diet on post-operative day 1. No patient suffered intraoperative or postoperative complication, including cranial nerve injury, haemorrhage, or deep neck infection. In our experience, transoral excision of the styloid process via a robotic approach can be considered as a feasible treatment option for Eagle's syndrome.

KEY WORDS: Robotics • Eagle's syndrome • Transoral robotic surgery

RIASSUNTO

La sindrome di Eagle è caratterizzata da dolore a livello della loggia tonsillare all'apertura della bocca o alla rotazione della testa, e da vari altri sintomi associati. Nonostante la sindrome sia difficile da diagnosticare, è stato osservato che l'accorciamento del processo stiloideo per via transorale o trancervicale è il trattamento più efficace. L'obiettivo di questo studio è stato quello di portare la nostra esperienza con la chirurgia robotica transorale nel trattamento della sindrome di Eagle, presentando i risultati di quattro pazienti. Abbiamo dunque revisionato i casi di quattro pazienti con Sindrome di Eagle sottoposti a chirurgia robotica transorale (TORS). L'età media dei pazienti era 53.75, e i due sessi erano rappresentati in ugual misura. I processi stiloidei sono stati ricostruiti in 3D a partire dalle scansioni TC preoperatorie e sono stati misurati: la media è risultata pari a 4.18 cm (range 3.3-5.1 cm). I tempi medi di preparazione e di intervento sono risultati pari a 10 e 30 minuti rispettivamente. Tutti i pazienti hanno avuto una completa risoluzione dei sintomi e tutti hanno ripreso la dieta orale in prima giornata postoperatoria. Non c'è stata nessuna complicanza intraoperaatoria o postoperatoria, come emorragie, infezioni o lesioni di nervi cranici. Dalla nostra esperienza si evince che l'accorciamento del processo stiloideo con chirurgia robotica transorale può essere una valida opzione per il trattamento della sindrome di Eagle.

PAROLE CHIAVE: *Chirurgia robotica transorale (TORS) • Sindrome di Eagle*

Acta Otorhinolaryngol Ital 2017;37:454-457

Introduction

Eagle's syndrome was first described by Watt W. Eagle in 1937 as the symptomatic elongation of the styloid process or mineralisation (ossification or calcification) of the stylohyoid ligament complex [1 2]. The syndrome is characterised by focal pain in the tonsillar fossa on wide mouth opening or head rotation and various accompanying symptoms such as a foreign body sensation in the throat, dysphagia and odynophagia.

The aetiology of this disease is unknown, although it is presumed to be caused by impingement of the styloid process on the internal/external carotid arteries, involving the nerve plexus [2 3].

Due to variable and nonspecific symptoms, the syndrome is difficult to diagnose; thus, its prevalence is underestimated in the population. However, once a correct diagnosis is made, shortening the styloid process via a transoral or transcervical surgical approach has been shown to be the most satisfactory and effective treatment [4].

The aim of this article was to document our experience with a transoral robotic approach to treat Eagle's syndrome and to present the outcomes of four patients.

Materials and methods

Patient population

We reviewed the cases of four patients with Eagle's syndrome who underwent transoral robotic surgery (TORS) at Yonsei Head and Neck Cancer Center, Severance Hospital, a tertiary care medical centre, from March 2011 to December 2013. All patients complained of throat pain as the major symptom. One of the patients had undergone previous tonsillectomy. The diagnostic work-up was completed with 3-dimensional computed tomography (CT) reconstruction. Intraoperative time, estimated blood loss, days of hospital stay and cosmetic satisfaction survey were collected and analysed for all four patients.

Surgical procedures

The configuration of the operating room and of the Si da Vinci robotic system (Intuitive Surgical Inc., Sunnyvale, CA) used to conduct TORS have been previously established by robotic surgeons [5-7]. The operation was performed under general anesthesia with the patient in the Rose position. A Crowe-Davis mouth gag retractor (Storz, Munich, Germany) was applied for better transoral exposure. A 0° endoscope was used to visualise the surgical field, and two robotic instrument arms, equipped at both sides of the endoscopic arm with 5-mm Maryland forceps and 5-mm spatula monopolar cautery, were utilised throughout the operation (Fig. 1A) [7]. The assistant handled the suction equipment and rongeur forceps.

After palpating the elongated styloid process, the location of the lesion is marked on the oral mucosa, and a peritonsillar mucosa incision was placed 1 cm lateral to the anterior pillar [8]. The styloid process was carefully dissected with the 5 mm spatula monopolar from the surrounding connective tissues and the internal carotid artery (Fig. 1D). Prudent blunt dissection was done with cottonoid gauze placed posteriorly to the styloid process

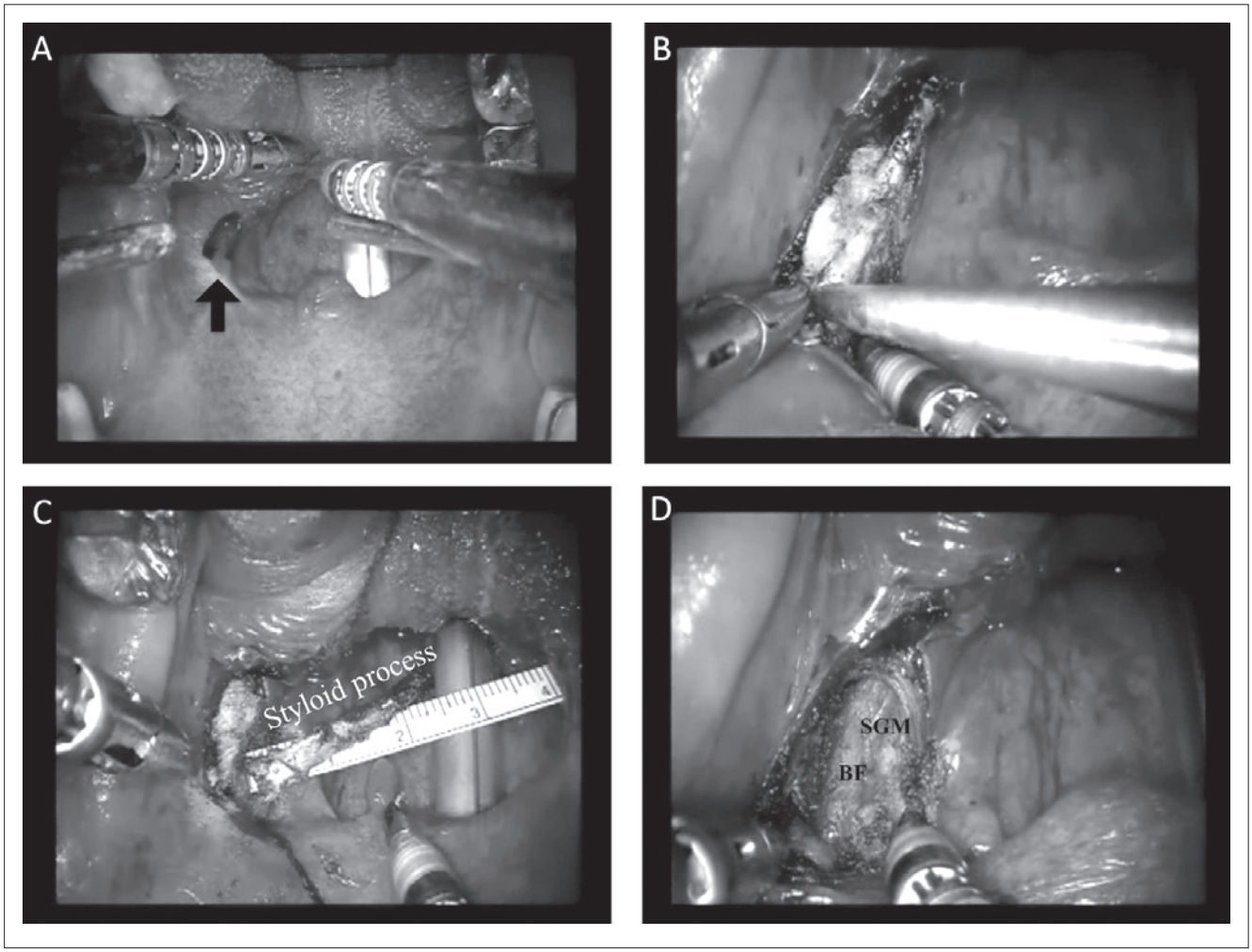


Fig. 1. Operative field of view of the robot. A) Robotic setting. Maryland forceps, spatula monopolar cautery, and two suction equipment. Palpable styloid process (black arrow) is marked. B) Styloid process being cut with the rongeur forcep. C) The styloid process measured 3.1 cm. D) Postoperative view after excision of the styloid process. Styloglossus muscle (SGM) and buccal fat (BF) is noted.

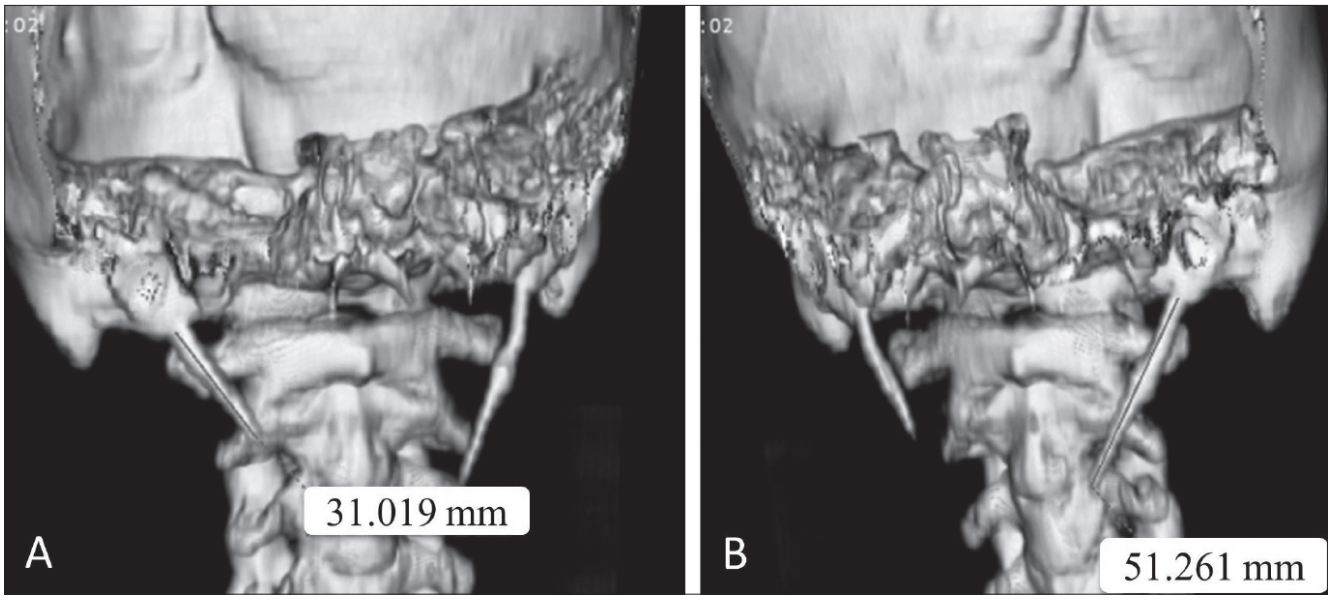


Fig. 2. Preoperative 3D CT of Eagle's syndrome. A, B) Preoperative 3D CT. The left styloid process measured 3.1 cm, while the right was 5.1 cm.

and retracted toward the cephalic direction. When the superior portion was reached, the styloid process was cut by the assistant with the rongeur forceps (Fig. 1B). Bleeding control was done, and the mucosa was sutured with absorbable vicryl. A tonsillectomy procedure was unnecessary for visualisation in three cases; the remaining case had previously undergone tonsillectomy 10 years prior.

Results

Four patients diagnosed with Eagle's syndrome were treated with transoral robotic surgery. The average age of patients was 53.75 years, and there were equal numbers of males and females. The styloid processes were reconstructed in 3D from the preoperative CT scans (Fig. 2) and were measured as an average of 4.18 cm (range 3.3-5.1). The set-up for the robotic approach required less than 10 minutes. The operation required approximately 30 minutes with minimal blood loss (5 mL) in three cases; however, one case required 50 minutes of operation time due to a mucosal bleeding tendency caused by underlying disease.

All patients were completely relieved of symptoms, and were able to restart an oral diet on post-operative day 1 (Table I). No patient suffered intraoperative or postoperative complications such as cranial nerve injury, postoperative bleeding, or deep neck infection.

Discussion

Due to variable and nonspecific symptoms, Eagle's syndrome is difficult to diagnosis; thus, the prevalence of the syndrome is underestimated in the general population. However, once a diagnosis is made, the treatment of Eagle's syndrome can be either non-surgical or surgical [1 9 10]. Various non-surgical treatments such as steroid injection or long-acting analgesics have been used, but long-term symptom relief has been difficult to achieve with these approaches. For patients who do not respond to medical treatments, a transoral or transcervical surgical approach has been shown to be the most satisfactory and effective treatment [1 4].

Transoral approaches have been thought to be 'blind' in that they can damage the neurovascular structures and have been heavily criticised because of the increased risk of deep space neck infection and poor visualisation of the surgical field [11].

Table I. Clinical characteristics of cases.

Case	Age (years)	Sex	Diagnosis	Styloid process length (cm)	Set-up time (minutes)	Operation time (minutes)	EBL (mL)	Follow-up (month)	Oral diet (days)	Hospital stay (days)	Preop VAS	Postop VAS
1	57	F	Elongated styloid process	4.8	4	35	5	55	1	4	9	1
2	55	F	Elongated styloid process	3.1	9	33	5	36	1	4	9	2
3	38	M	Elongated styloid process	5.1	9	29	< 10	13	1	3	8	1
4	65	M	Elongated styloid process	3.5	5	50	60	15	2	10	10	1

VAS scores: 0 = none, 5 = moderate, 10 = severe EBL, estimated blood loss; F, female; M, male.

External approaches might give a lower possibility of deep neck infections and improved exposure than a classic naïve transoral approach. However, these invasive methods require more time than the transoral approach and can leave the patients with a visible scar on the face or neck [11 12]. In a transoral approach with the robotic system, the robot's endoscopic view offers a 15- to 20-fold magnified view, enabling the surgeon to easily distinguish even tiny neurovascular structures from soft tissue. This magnified view and 360° rotating instruments provides surgical safety with regards to preservation of neurovascular structures without iatrogenic injury [8]. It should be noted that none of the cases experienced neurovascular injury, deep cervical infection, or failure to complete the procedure from an intraoral approach.

Transoral robotic surgery has advantages over conventional transoral surgery in that the assistant can have the same view as the operator, and the operator can be in control of four arms: two robotic arms and the two assistant's arms. The endoscope can also be closely approached stably and rotated during bone resection, allowing maximal resection of the styloid process. This allows sequelae caused by the remnant styloid process to be minimised.

Nonetheless, there is controversy regarding the robotic approach due to the cost burden on the patient. However, its advantages and the surgical convenience of this approach make it a favourable treatment option for Eagle's syndrome with safe and satisfying results.

Conclusions

In conclusion, given the superb visualisation and effective preservation of the ICA and neurovascular structures, transoral excision of the styloid process via a robotic approach is a safe surgical alternative treatment option for Eagle's syndrome.

References

1. More CB, Asrani MK. *Eagle's syndrome: report of three cases.* Indian J Otolaryngol Head Neck Surg 2011;63:396-9.
2. Eagle WW. *Symptomatic elongated styloid process; report of two cases of styloid process-carotid artery syndrome with operation.* Arch Otolaryngol 1949;49:490-503.
3. Costantinides F, Vidoni G, Bodin C, et al. *Eagle's syndrome: signs and symptoms*. Cranio 2013;31:56-60.
4. Torres AC, Guerrero JS, Silva HC. *A modified transoral approach for carotid artery type Eagle syndrome: technique and outcomes.* Ann Otol Rhinol Laryngol 2014;123:831-4.
5. O'Malley BW Jr, Weinstein GS, Snyder W, et al. *Transoral robotic surgery (TORS) for base of tongue neoplasms.* Laryngoscope 2006;116:1465-72.
6. Weinstein GS, O'Malley BW Jr, Snyder W, et al. *Transoral robotic surgery: supraglottic partial laryngectomy.* Ann Otol Rhinol Laryngol 2007;116:19-23.
7. Byeon HK, Duvvuri U, Kim WS, et al. *Transoral robotic retropharyngeal lymph node dissection with or without lateral oropharyngectomy.* J Craniofac Surg 2013;24:1156-61.
8. Park YM, De Virgilio A, Kim WS, et al. *Parapharyngeal space surgery via a transoral approach using a robotic surgical system: transoral robotic surgery.* J Laparoendosc Adv Surg Tech A 2013;23:231-6
9. Strauss M, Zohar Y, Laurian N. *Elongated styloid process syndrome: intraoral versus external approach for styloid surgery.* Laryngoscope 1985;95:976-9.
10. Chase DC, Zarmen A, Bigelow WC, et al. *Eagle's syndrome: a comparison of intraoral versus extraoral surgical approaches.* Oral Surg Oral Med Oral Pathol 1986;62:625-9.
11. Al Weteid AS, Miloro M. *Transoral endoscopic-assisted styloidectomy: how should Eagle syndrome be managed surgically?* Int J Oral Maxillofac Surg 2015;44:1181-7.
12. Muderris T, Bercin S, Sevil E, et al. *Surgical management of elongated styloid process: intraoral or transcervical?* Eur Arch Otorhinolaryngol 2014;271:1709-13.

Received: November 16, 2016 - Accepted: March 11, 2017

Address for correspondence: Se Heon Kim, Department of Otorhinolaryngology, Yonsei University College of Medicine, 03722 Seoul, Korea. Tel. +82 2 2228 3600. Fax +82 2 393 0580. E-mail: SHKIMMD@yuhs.ac

HEAD AND NECK

Prognostic factors in head and neck cancer: a 10-year retrospective analysis in a single-institution in Italy

Fattori prognostici del tumore testa-collo: un'analisi retrospettiva monocentrica di 10 anni

G. CADONI[1], L. GIRALDI[2], L. PETRELLI[1], M. PANDOLFINI[1], M. GIULIANI[1], G. PALUDETTI[1], R. PASTORINO[2], E. LEONCINI[2], D. ARZANI[2], G. ALMADORI[1], S. BOCCIA[3]

[1] Institute of Otorhinolaryngology, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "Agostino Gemelli", Rome, Italy; [2] Section of Hygiene, Institute of Public Health, Università Cattolica del Sacro Cuore, Rome, Italy; [3] Section of Hygiene, Institute of Public Health, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "Agostino Gemelli", Rome, Italy

SUMMARY

This study was undertaken to evaluate the association between demographics, lifestyle habits, and clinical data and overall survival (OS), recurrence and second primary cancer (SPC) in patients with first primary head and neck cancer (HNC). We retrospectively reviewed data from 482 patients treated at the "Agostino Gemelli" Teaching Hospital, Rome, between 2002-2012 for primary HNC. Individual parameters were evaluated for association with specific outcomes such as OS, cancer recurrence and second primary cancer (SPC) appearance using hazard ratios (HR) and 95% confidence intervals (CIs). Five-year OS was 60.6% for all HNC cases, 49.0% for oral cavity, 54.8% for oropharynx, 50.0% for hypopharynx and 63.4% for larynx. Predictors of OS were older age (HR = 1.04; 95% CI: 1.02-1.05) and advanced tumour stage (HR = 2.00; 95% CI: 1.41-2.84). The risk of recurrence was associated with drinking 8-14 drinks per week (HR = 1.73; 95% CI: 1.00-2.97). The risk of developing SPC increased with advanced tumour stage (HR = 2.75; 95% CI: 1.39-5.44) and with smoking for more than 40 years (HR = 3.68; 95% CI: 1.10-12.30). OS differed among HNC sites. Increasing age was an unfavourable predictor of HNC OS. Tumour stage was a prognostic factor both for OS and for risk of developing SPC. Alcohol and tobacco consumption were prognostic factors for recurrence and SPC, respectively.

KEY WORDS: Head and neck cancer • Prognostic factors • Epidemiology

RIASSUNTO

È stata condotta un'analisi retrospettiva su 482 pazienti con diagnosi di tumore testa-collo arruolati presso l' ospedale "Agostino Gemelli" di Roma. L'associazione tra fattori demografici, clinici e comportamentali con la overall survival (OS), il rischio di ricorrenza ed il rischio di un secondo tumore primitivo è stata stimata usando gli Hazard Ratio (HR) e gli intervalli di confidenza al 95% (CIs). La OS considerando tutte le sedi tumorali è stata del 60%, mentre considerando le singole sedi tumorali è risultata del 49.0% per il cavo orale, 54.8% per l'orofaringe, 50.0% per l'ipofaringe e 63.4% per la laringe. Un'età avanzata alla diagnosi (HR = 1.04; 95% CI: 1.02-1.05) ed un avanzato stadio del tumore (HR = 2.00; 95% CI: 1.41-2.84) sono risultati fattori significativamente associati con la OS. Il rischio di ricorrenza è risultato associato con il consumo di alcolici (HR = 1.73; 95% CI: 1.00-2.97). Il rischio di sviluppare un secondo tumore primitivo è risutlato associato con uno stadio avanzato del tumore primario (HR = 2.75; 95% CI: 1.39-5.44) e con l'aver fumato per più di 40 anni (HR = 3.68; 95% CI: 1.10-12.30). In conclusione abbiamo notato che la OS differisce tra le sedi tumorali del tumore testa-collo. Lo stadio tumorale è risultato essere associato sia con la OS che con il rischo di sviluppare un secondo tumore primitivo. Il consumo di alcol e di tabacco sono risultati essere fattori prognostici, rispettivamente, per la ricorrenza e per l'insorgenza di un secondo tumore primitivo.

PAROLE CHIAVE: *Cancro della testa e del collo • Fattori prognostici • Epidemiologia*

Acta Otorhinolaryngol Ital 2017;37:458-466

Introduction

Squamous cell carcinoma of the head and neck (HNC) is the sixth common cancer worldwide affecting 600,000 new cases diagnosed each year [1]. In 2009 in Italy there were an estimated 1008 new HNC cases (790 males and 218 females) with a 5-year survival rate of 55% (2000-2004) [2].

Various genetic and environmental factors are related to HNC [3]. The incidence of HNC has a large geographical variability related to different prevalence of lifestyle risk factors, such as alcohol drinking, tobacco smoking and dietary factors [4-12]. Other known risk factors are human papillomavirus (HPV) [13] and Epstein-Barr virus (EBV)

infections [14]. Men have a higher risk having a HNC than women [1].
The most frequent tumour sites of HNC are the larynx, oral cavity and pharynx. Head and neck cancers also include salivary gland tumours as well as nasopharyngeal cancer and paranasal and nasal sinus cancer. These tumours are less frequent and will not be discussed in this report.
The management of HNC is often a clinical challenge, since in more than 60% of patients the disease is locally advanced at diagnosis: a combined modality therapy with surgery, radiotherapy and chemotherapy is generally recommended [15]. When the disease is deemed unresectable or an organ-preservation goal is pursued, the current standard treatment is represented by the combination of radiation and chemotherapy [16].
In head and neck oncology, the lack of well-defined prognostic and predictive factors limits the possibility to tailor the best therapeutic approach on an individual basis.
In this study, we retrospectively evaluated the association of individual parameters with specific outcomes in terms of disease control in a cohort of 482 patients treated at our Italian centre to identify predictive and prognostic factors that could help clinicians in deciding the most appropriate treatment.

Materials and methods

Subjects with histologically confirmed primary squamous cell carcinoma of the head and neck cancer were consecutively recruited at the Gemelli Hospital, from 2002 to 2012. The study was approved by the Ethics Committee of the University. Details of the tumour classification used have been described previously [17].

Data collection

Patients were interviewed face-to-face by trained interviewers or physicians on demographic, alcohol and tobacco consumption, and other relevant lifestyle factors. Interviews were conducted from 15 to 5 days prior to treatment. Questions assessed information at one year before diagnosis. Participant were also followed from the date of diagnosis to the date of death, or loss to follow up, whichever occurred first. Death certificate data were also used for mortality, and the cause of death was coded according to the International Classification of Diseases, Ninth Revision [18]. Cancer recurrence and SPC were collected from medical records and cancer registries. Data on tumour pathology and treatment were obtained from pathology records.

Diagnostic and therapeutic guidelines

Pre-treatment work up included: complete head and neck exam, biopsy, chest imaging, CT with contrast and/or MRI with contrast of primary and neck; FDG-PET /CT for stage III-IV disease, nutrition, speech and swallowing evaluation, multidisciplinary consultation. Tumour HPV testing was performed in oropharynx primary in the last years.
The therapeutic guidelines used were as follows: oral cavity, early stage T1-T2 N0 surgery; advanced stage (T3N, T1-T3 -N1-N3, T4a any N) surgery or multidisciplinary approach.
Oropharynx: early stage (T1-T2, N0-1) definitive radiotherapy (RT) or surgery; advanced stage (T3, T4a, N0-N1) concurrent systemic therapy/RT or surgery or induction chemotherapy followed by radiotherapy or systemic therapy/RT.
Hypopharynx: early stage (T1-selected T2 N0) definitive RT or surgery; advanced stage (T2-3, any N and t1 N+; T4a any N) induction chemotherapy or surgery or concurrent systemic therapy/RT.
Larynx: early stage (T1-T2 or selected T3) surgery or RT; T3 requiring total laryngectomy any N concurrent systemic therapy/RT or RT if patient not candidate for systemic therapy/RT or surgery or induction chemotherapy; T4 any N surgery.

Outcome definitions

The primary endpoint was overall survival (OS) measured as the time from the date of initial diagnosis of index primary tumours to the date of death from any cause. All observations were censored at loss to follow-up and at the end of the study period. Recurrence was defined as the local, regional or distant return of cancer after that the patient was defined as disease free. By definition, a second primary tumour of the same histologic type as the first had to be separated from it by more than 2 cm of normal epithelium or had to occur at least 3 years after diagnosis of the first primary tumour. Any new tumour of a different histologic type was characterised as a second primary tumour without the requirement of separation of more than 2 cm [19].

Statistical analysis

We used the Kaplan–Meier method to calculate the cumulative proportion surviving and to plot survival curves. We used multivariable Cox's proportional hazards model to determine independent predictors of OS, recurrence and SPC. We formally tested the Cox proportional hazards assumption for each covariate using Schoenfeld residuals [20]. Hazard ratios (HR) for all-cause mortality were adjusted for age, tumour stage and lymph nodes.
Models to predict SPC were adjusted for age, tumour stage, years of smoking and presence of lymph nodes. Models to predict recurrence were adjusted for age, treatment and lymph nodes. With respect to smoking, patients were classified as never, former or current smokers. Cumulative tobacco consumption was calculated as intensity of smoking (never smokers, ≤ 20 cigarettes/day, > 20 ciga-

rettes/day), and smoking duration in years (never smokers, ≤ 20, 21-40, > 40). With respect to alcohol, subjects were classified as never drinkers, former and current, and according to alcohol consumption (none or < 1 drink equivalent/week, 1-7 drinks/week, 8-14 drinks/week, 15-21 drinks/week, 22-28 > 29 drinks/week). The standard definition for one drink equivalent was 14 g ethanol, which approximately corresponds to 150 mL wine, 330 mL beer, and 36 mL spirits [21]. Analysis were performed for overall HNC. Statistical analyses were performed using Stata software, version 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

Results

A total of 482 subjects were included in the study. Characteristics of the patients are reported in Table I. Disease location was the oral cavity in 83 (17.2%) patients, oropharynx in 84 (17.4%), hypopharynx in 20 (4.2%), larynx in 290 (60.2%). For 5 patients (1.0%) the disease location was oral cavity or pharynx not otherwise specified. There was a high predominance of male cases (79.1%) and median age at diagnosis was 64 (interquartile range (IQR): 57-70) years old overall. Most patients presented an advanced tumour at the time of diagnosis (Stage III-IV = 54.2%). Lymph nodes are not involved frequently

Table I. Characteristics of head and neck cancer cases by tumour site.

Tumor site	Subjects n (%)	Gender (% male)	Age (median, IQR*)	Stage n (%)		Lymph node n (%)	Tumour size n (%)	
Oral cavity	83 (17.2)	59.0%	64 (51-71)	1-2	29 (34.9)	0 (0)	1	11 (13.9)
							2	29 (36.7)
				3-4	54 (65.1)	26 (51.0)	3	10 (12.7)
							4	29 (36.7)
				Missing	0 (0)	4 (4.8)		4 (4.8)
Oropharynx	84 (17.4)	75.3%	61 (52-68.5)	1-2	31 (36.9)	0 (0)	1	13 (15.5)
							2	32 (38.1)
				3-4	53 (63.1)	41 (77.4)	3	7 (8.3)
							4	32 (38.1)
				Missing	0 (0)	0 (0)		0 (0)
Hypopharynx	20 (4.2)	65.0%	61 (58-66)	1-2	1 (5)	0 (0)	1	1 (5.0)
							2	3 (15.0)
				3-4	19 (95)	14 (73.7)	3	3 (15.0)
							4	13 (65.0)
				Missing	0 (0)	0 (0)		0 (0)
Larynx	290 (60.2)	86.9%	65 (58-71)	1-2	157 (54.1)	0 (0)	1	94 (32.4)
							2	68 (23.5)
				3-4	133 (45.9)	47 (35.3)	3	53 (18.3)
							4	75 (25.9)
				Missing	0 (0)	0 (0)		0 (0)
OC, OP, HP NOS	5 (1.0)	80.0%	64 (51-69)	1-2	3 (60.0)	1 (33.3)	1	1 (20.0)
							2	3 (60.0)
				3-4	2 (40.0)	2 (100.0)	3	-
							4	1 (20.0)
				Missing	0 (0)	0 (0)		0 (0)
Total	482	79.1%	64 (57-70)	1-2	221 (45.9)	0 (0)	1	120 (25.1)
							2	135 (28.2)
				3-4	261 (54.2)	130 (50.4)	3	73 (15.3)
							4	150 (31.4)
				Missing	0 (0)	4 (0.8)		4 (0.8)

** IQR: interquartile range (25°-75° percentile). OC, oral cavity; OP, oropharynx; HP, hypopharynx; NOS, not otherwise specified*

Table II. Treatment of head and neck cancer cases according to tumour stage.

Treatment	Stage n (%)	
	1-2	3-4
Radiation	50 (22.7)	4 (1.5)
Surgery	134 (60.9)	52 (20.1)
Chemotherapy and Radiation	16 (7.3)	69 (26.6)
Surgery and Radiation	19 (8.6)	73 (28.2)
Surgery and Chemotherapy	1 (0.5)	7 (2.7)
Surgery and Chemotherapy and Radiation	-	54 (20.9)
Missing	1	2
Total	221 (45.9)	261 (54.1)

in laryngeal cancer (16.2%), unlike oral cavity (31.3%), oropharyngeal (48.9%) and hypopharyngeal (70.0%) cancers. Treatment of HNC cases according to tumour stage is reported in Table II: for patients with an early stage tumour exclusive surgery was the treatment of choice (60.9%), while in the advanced stages (SIII-SIV) the most widely performed treatments were combined surgery with post-operative radiotherapy (Surg/RT, 28.2%) and chemotherapy with radiotherapy (CHT/RT, 26.6%).

Overall survival

Median follow-up time since cancer diagnosis was 49 months (IQR: 19-86) (Table III). During follow-up, 190 of 482 patients (39.4%) died, of which 97 (51.1%) died for HNC cancer, 26 (13.7%) for other cancer and the remaining 67 for other causes (data not shown). Percentages of death for other causes were as follows: oral cavity 10.1%, oropharynx 11.8%, hypopharynx 8.3% and larynx 15.0%. Five-years OS for all HNC sites combined was 60.6%: oral cavity 49.0%, oropharynx 54.8%, hypopharynx 50.0% and larynx 63.4%. Median survival time in months was higher for laryngeal cancer than for the other tumour sites [(59 vs 23 months, oral cavity $p = 0.002$), 43 (oropharynx $p = 0.023$), 35 (hypopharynx $p = 0.03$)] (Fig. 1). When stratifying the survival time by tumour stages, we did not find significant differences for any cancer sites for early stage tumour sites, while for the advanced tumour stages we observed higher survival for laryngeal cancer than for oral cavity cancer (p=0.008) (Figs. 2, 3).
Table IV reports the distribution for selected covariates and the HR for all-cause mortality adjusted for age, stage and lymph node status. Reduced survival was associated with increasing age of diagnosis (HR = 1.04; 95% CI: 1.02-1.05), and advanced tumour stage (HR = 2.00; 95% CI: 1.41-2.84).

Cancer recurrence and second primary cancer

Demographic, clinical and lifestyle characteristics and the association with recurrence and SPC are reported in Table V. Among the 482 HNC patients included, disease recurrence was unknown for 26 (5.4%) patients. and 12 (2.5%) patients did not report the date of recurrence. A total of 181 (39.7%) patients developed clinical recurrence. The risk of recurrence was associated with drinking 8-14 drinks per week (HR = 1.73; 95% CI: 1.00-2.97).
Among the 482 HNC patients included, information on SPC were unknown for 17 (3.5%) patients and 17 (3.5%) patients did not report the date of second primary, therefore there were 448 patients with complete information on SPC. A total of 169 (40.0%) patients developed SPC. The risk of developing SPC increased with advanced tumour stage (HR = 2.75; 95% CI: 1.39-5.44) and with smoking for more than 40 years (HR = 3.68; 95% CI: 1.10-12.30).

Discussion

Many therapeutic options are available for patients with HNC, but the appropriate regimen for the individual patient is still a difficult and often controversial choice. Worldwide, HNC is the sixth most common neoplasm, and despite advances in therapy, long-term survival in

Table III. Median survival time and deaths by tumour site.

Follow-up time (months)					Deaths	
	N	Median	1Q	3Q	n	%
Oral cavity (OC)	83	23	10	60	34	41.0%
Oropharynx (OP)	84	43	16	82	38	45.2%
Hypopharynx (HP)	20	35	16	72	10	50.0%
Larynx	290	59	26	94	106	36.6%
OC, OP, HP NOS	5	71	63	82	2	40.0%
Total	482	49	19	86	190	39.4%

1Q: first quartile, 3Q: third quartile. NOS, not otherwise specified

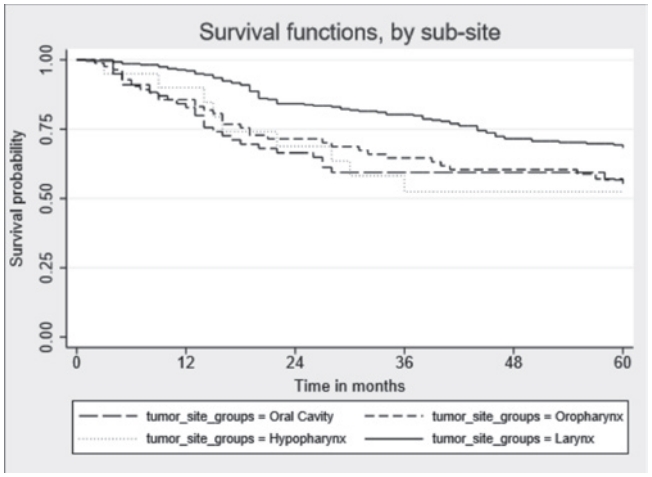


Fig. 1. Kaplan-Meier unadjusted overall 5-year survival by head and neck-cancer site.

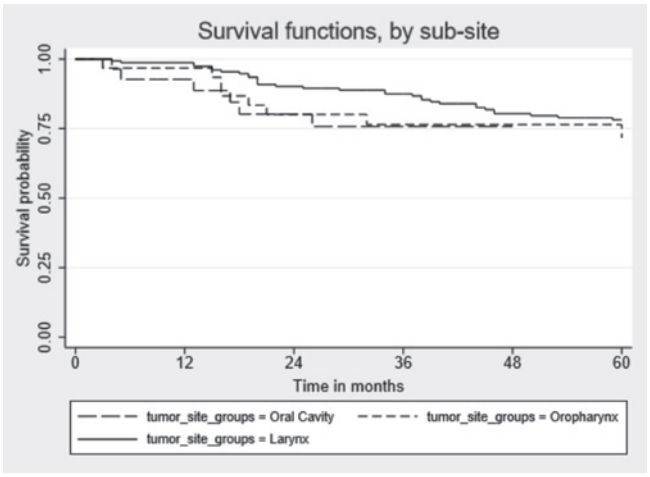


Fig. 2 Kaplan-Meier unadjusted overall 5-year survival by head and neck cancer site for early stage tumours.

HNSCC patients is poor. The prognosis for HNC overall has improved slightly since the 1990s, and is influenced by site, stage, molecular markers and HPV status. Prognostic factors can guide the physician in selecting the best possible treatment for each patient, possibly increasing the therapeutic index [22 23] .

In our analysis, many factors showed an association with one or more of the outcomes. Many are well known in the literature.

For instance, age has a negative impact on the prognosis of patients included in our analysis. Other authors observed that older patients have similar survival outcomes compared with their younger peers; however, they may experience worse toxicity, especially with treatment intensification [24]. As life expectancy increases, surgeons can expect an increasing number of geriatric patients. Management of this subpopulation has become a source of debate because there is a paucity of randomised data regarding the effect of age on treatment response and morbidity associated with the treatment of HNC [25 26].

The heterogeneity of HNC creates various difficulties, first of all for tumour site classification. The site of origin is an important prognostic factor, both because of the different stage at diagnosis and because of the different possibilities of surgical treatment. One must pay attention to laryngeal and oropharyngeal classification for suprahyod larynx or oral and oropahryngeal classification for the base of the tongue.

Tumour staging is a well identified prognostic factor for HNC. A large proportion of patients with HNC are often not diagnosed until their disease has reached an advanced stage, requiring aggressive and costly treatment that may not be curative, with relapse occurring in around 50% of cases with locally advanced HNC [27 28].

We report a significant association between advanced tumour stage (S III-IV) and poor overall and disease-specific survival. The choice of treatment for advanced HNC did not influence time-survival as for other authors [29]. We were unable to find

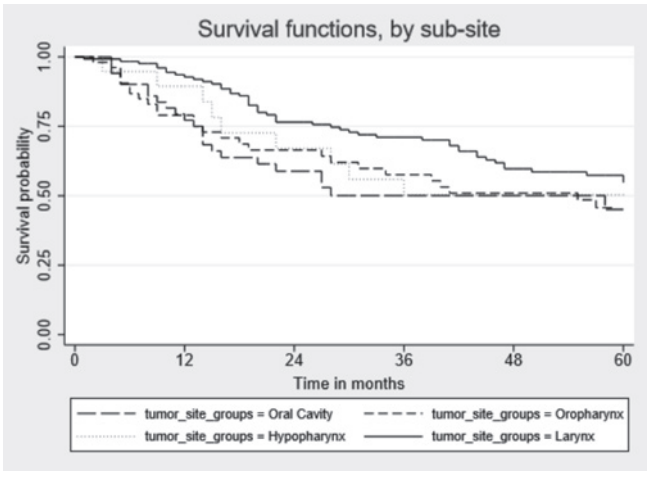


Fig. 3. Kaplan-Meier unadjusted overall 5-year survival by head and neck cancer site for advanced stage tumours.

any significant factor that may constitute a contraindication for a combined approach with RT and CHT or surgery/CHT-RT, and identify advanced HNC patients for whom a palliative approach or best supportive care could be preferred.

Smoking and high alcohol consumption habits are not only well-known causative but also prognostic factors in HNC [30]. In our experience, a long history of smoking impacted SPC development. HNC patients have a higher risk of second primary tumours than the general population. The most frequent locations are the head and neck, lung and oesophagus, decreasing long-term survival. The incidence of SPC has increased in the last decade, with a negative effect on survival. Since no specific early diagnostic tool is available, alcohol and tobacco avoidance along with scheduled follow-up are suggested to reduce its incidence [31-33].

In recent years, HPV status was found to play a major role as a prognostic and predictive parameter, especially

Table IV. Predictors of overall survival among head and neck cancer cases by multivariate analysis.

	Subjects		Death from all causes (total/deaths 482/190)
	n	%	HR* (95% CI)
Demographics			
Age at diagnosis (ordinal)	482	100	**1.04 (1.02-1.05)**
Gender			
Men	381	81.2	1.00
Women	88	18.8	0.92 (0.63-1.35)
Tumour characteristics			
Stage			
I/II	221	45.9	1.00
III/IV	261	54.1	**2.00 (1.41-2.84)**
Cigarette smoking and alcohol consumption			
Smoking status			
Never	68	14.6	1.00
Former	259	55.6	1.15 (0.73-1.81)
Current	139	29.8	1.20 (0.73-1.98)
Years of smoking			
Never smokers	68	14.1	1.00
≤ 20	56	11.6	0.93 (0.49-1.75)
21-40	199	41.3	1.16 (0.72-1.86)
> 40	159	33.0	1.22 (0.76-1.97)
Cigarettes per day			
Never smokers	68	14.1	1.00
≤ 20	234	48.5	1.16 (0.74-1.84)
> 20	180	37.3	1.14 (0.71-1.85)
Drinking status			
Never	100	21.4	1.00
Former	19	4.1	0.83 (0.29-2.35)
Current	348	74.5	1.27 (0.86-1.87)
Drinks per week			
Never drinkers or < 1	81	16.8	1.00
1-7	48	10.0	1.33 (0.74-2.39)
8-14	113	23.4	1.36 (0.86-2.16)
15-21	32	6.6	0.98 (0.49-1.98)
22-28	58	12.0	1.15 (0.66-1.99)
> 28	150	31.1	1.52 (0.94-2.45)

*Text in bold indicates statistically significant risk factors. HR: hazard ratio; CI: confidence interval. * HR adjusted by age, stage, lymph nodes.*

in oro-pharyngeal cancer. HPV-positivity is a consistent determinant of superior survival irrespective of the treatment approach used [34-36]. Unfortunately, evaluation of HPV status in HNC entered our clinical practice only a few years ago. For this reason, these data are not available for most patients in our series, making it impossible to obtain a significant analysis.

Apart from HPV, many other biological parameters have been studied as prognostic factors in HNC patients. For istance, a recent meta-analysis on HNC patients confirmed the negative impact of epidermal-growth factor (EGFR) overexpression [37]. In the near future, analysis of these parameters could help physicians in selection of the best treatment approach for each individual patient [38]. Unfortunately, as for HPV, our analysis did not investigate such parameters, because data were missing in a signifi-

Table V. Predictors of recurrence and second primary among head and neck cancer cases by multivariate analysis.

	Recurrence n = 169	Second primary n = 60
	HR (95% CI)[a]	HR (95% CI)[b]
Demographics		
Age at diagnosis (ordinal)	1.00 (0.98-1.02)	1.02 (0.99-1.05)
Gender		
Men	1.00	1.00
Women	0.69 (0.42-1.13)	1.57 (0.76-3.23)
Tumour characteristics		
Stage		
I/II	1.00	1.00
III/IV	0.99 (0.61-1.60)	**2.75 (1.39-5.44)**
Cigarette smoking and alcohol consumption		
Smoking status		
Never	1.00	1.00
Former	0.93 (0.54-1.58)	2.64 (0.80-8.69)
Current	0.61 (0.33-1.14)	3.28 (0.94-11.47)
Years of smoking		
Never smokers	1.00	1.00
≤ 20	0.77 (0.38-1.58)	1.91 (0.42-8.75)
21-40	0.90 (0.51-1.57)	2.34 (0.69-7.93)
> 40	0.71 (0.39-1.30)	**3.68 (1.10-12.30)**
Cigarettes per day		
Never smokers	1.00	1.00
≤ 20	0.81 (0.47-1.39)	2.72 (0.82-8.99)
> 20	0.81 (0.45-1.44)	2.97 (0.87-10.12)
Drinking status		
Never	1.00	1.00
Former	1.21 (0.42-3.48)	0.61 (0.08-4.78)
Current	1.11 (0.71-1.74)	0.73 (0.35-1.51)
Drinks per week		
Never drinkers or < 1	1.00	1.00
1-7	1.33 (0.66-2.67)	0.85 (0.27-2.67)
8-14	**1.73 (1.00-2.97)**	0.99 (0.41-2.39)
15-21	1.07 (0.46-2.51)	0.44 (0.09-2.12)
22-28	0.84 (0.41-1.73)	0.78 (0.27-2.24)
> 28	0.91 (0.51-1.63)	0.83 (0.33-2.11)

Text in bold indicates statistically significant risk factors. HR: Hazard Ratio; CI: Confidence Interval. a HR adjusted by age, treatment, lymph nodes. b HR adjusted by age, stage, years of smoking, lymph nodes.

cant proportion of patients included in this series. Another limitation of the study is represented by not having performed the analysis by HNC subsite and not having available data on comorbidities: due to this latter limitation we could not justify the reason for the elevated number of deaths due to causes other than tumour, although is likely that the advanced age of these patients could be the main reason.

Despite these limitations, we feel that our study contributes in identifying prognostic factors for HNC patients, because the data we considered in this series are easy to collect in any department. The number of patients included in this analysis is small, considering the long-time period studied. However, we preferred a more limited number of patients, but with all the required information available and with sufficient follow-up time.

Conclusions

HNC is the sixth most common neoplasm, and despite advances in therapy, long-term survival in HNC patients is poor. Age and tumour staging are considered the most important prognostic factors for overall survival. Recurrence and second primary tumours are influenced negatively by high alcohol consumption and cigarette smoking. Clinical characteristics remain the best known prognostic factors in HNC. A major effort in HNC prevention and modification of patients' behaviour could lead to early diagnosis and reduction of recurrence and second primary tumours.

Funding

This work was supported by the Associazione Italiana per la Ricerca sul Cancro - AIRC IG 2011 (contract no. 10491) and IG2013 (contract no. 14220), and Fondazione Veronesi. The funders had no role in study design or data analysis.

Acknoledgements

We acknowledge financial support by the Associazione Italiana per la Ricerca sul Cancro and Fondazione Veronesi.

References

1 Ferlay J, Soerjomataram I, Ervik M, et al. *International Agency for Research on Cancer*. World Health Organisation. Globocan: 2012 Estimated cancer incidence, mortality and prevalence worldwide in 2012. Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr.

2 AIRTUM. *ITACAN: Tumori in Italia, Versione 2*.0. Associazione Italiana dei Registri Tumori (http://www.registritumori.it).

3 Cadoni G, Boccia S, Petrelli L, et al. *A review of genetic epidemiology of head and neck cancer related to polymorphisms in metabolic genes, cell cycle control and alcohol metabolism*. Acta Otorhinolaryngol Ital 2012;32:1-11.

4 Black RJ, Bray F, Ferlay J, et al. *Cancer incidence and mortality in the European Union: Cancer registry data and estimates of national incidence for 1990*. Eur J Cancer 1997;33:1075-107.

5 Ferlay J, Shin HR, Bray F, et al. *GLOBOCAN 2008 v1*.2, *Cancer Incidence and Mortality Worldwide.* IARC CancerBase No. 10 [Internet] Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr, accessed on 30/05/2013.

6 Tuyns AJ, Estève J, Raymond L, et al. *Cancer of the larynx/hypopharynx, tobacco and alcohol: IARC international case-control study in Turin and Varese (Italy), Zaragoza and Navarra (Spain), Geneva (Switzerland) and Calvados (France)*. Int J Cancer 1988;41:483-491.

7 Toporcov TN, Znaor A, Zhang ZF, et al. *Risk factors for head and neck cancer in young adults: a pooled analysis in the INHANCE consortium*. Int J Epidemiol 2015;44:169-85.

8 Galeone C, Edefonti V, Parpinel M, et al. *Folate intake and the risk of oral cavity and pharyngeal cancer: a pooled analysis within the International Head and Neck Cancer Epidemiology Consortium*. Int J Cancer 2015;136:904-14.

9 Leoncini E, Edefonti V, Hashibe M, et al. *Carotenoid intake and head and neck cancer: a pooled analysis in the International Head and Neck Cancer Epidemiology Consortium*. Eur J Epidemiol 2016;31:369-83.

10 Leoncini E, Ricciardi W, Cadoni G, et al. *Adult height and head and neck cancer: a pooled analysis within the INHANCE Consortium*. Eur J Epidemiol 2014;29:35-48.

11 Wyss A, Hashibe M, Chuang SC, et al. *Cigarette, cigar, and pipe smoking and the risk of head and neck cancers: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium*. Am J Epidemiol 2013;178:679-90.

12 Leoncini E, Vukovic V, Cadoni G, et al. *Clinical features and prognostic factors in patients with head and neck cancer: Results from a multicentric study*. Cancer Epidemiol 2015;39:367-74.

13 Mork J, Lie AK, Glattre E, et al. *Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck* . N Engl J Med 2001;344: 1125-31.

14 Chang ET, Adami HO. *The enigmatic epidemiology of nasopharyngeal carcinoma*. Cancer Epidemiol Biomarkers Prev 2006;15:1765-77.

15 Berneir J, Domenge C, Ozsahin M et al. *European organization for research and treatment of cancer trial 22931 postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer*. N Engl J Med 2004;350:1945-52.

16 Franceschini D, Paiar F, Saieva C et al. *Prognostic factors in patients with locally advanced head and neck cancer treated with concurrent radiochemotherapy*. Radiol Med 2016;121:229-37.

17 Leoncini E, Vukovic V, Cadoni G, et al. *Clinical features and prognostic factors in patients with head and neck cancer: Results from a multicentric study*. Cancer Epidemiol 2015;39:37-74.

18 http://www.cdc.gov/nchs/icd/icd9cm.htm

19 Warren S, Gates O. *Multiple primary malignant tumours: a survey of literature and statistical study*. Am J Cancer 1932;16:1358-414.

20 Schoenfeld D. *Partial residuals for the proportional hazards regression model*. Biometrika 1982;69:239-41.

21 IARC. *Alcoholic beverage consumption and ethyl carbamate (urethane)*. IARC Monogr Eval Carcinog Risks Hum 2007;96:72-84.

22 Bussu F, Miccichè F, Rigante M, et al. *Oncologic outcomes in advanced laryngeal squamous cell carcinomas treated with different modalities in a single institution: a retrospective analysis of 65 cases*. Head Neck 2012;34:573-9.

23 Castaldi P, Rufini V, Bussu F, et al. *Can "early" and "late"18F-FDG PET-CT be used as prognostic factors for the clinical outcome of patients with locally advanced head and neck cancer treated with radio-chemotherapy?* Radiother Oncol 2012;103:63-8.

24 VanderWalde NA, Mary Fleming M, Jared Weiss, et al. *Treatment of older patients with head and neck cancer: a review*. Oncologist 2013;18:568-578.

[25] Genden EM, Rinaldo A, Shaha AR, et al. *Treatment considerations for head and neck cancer in the elderly*. J Laryngol Otol 2005;119:169-74.

[26] Syrigos KN, Karachalios D, Karapanagiotou EM, et al. *Head and neck cancer in the elderly: an overview on the treatment modalities*. Cancer Treat Rev 2009;35:237-45.

[27] Argiris A, Eng C. *Epidemiology, staging, and screening of head and neck cancer*. Cancer Treat Res 2003;114:15-60.

[28] Bussu F, Miccichè F, Rigante M, et al. *Oncologic outcomes in advanced laryngeal squamous cell carcinomas treated with different modalities in a single institution: a retrospective analysis of 65 cases*. Head Neck 2012;34:573-9.

[29] Adelstein DJ, Lavertu P, Saxton JP, et al. *Mature results of a phase III randomized trial comparing concurrent chemoradiotherapy with radiation therapy alone in patients with stage III and IV squamous cell carcinoma of the head and neck*. Cancer 2000;88:876-83.

[30] Leoncini E, Vukovic V, Cadoni G, et al. *Clinical features and prognostic factors in patients with head and neck cancer: Results from a multicentric study*. Cancer Epidemiol 2015;39:367-74.

[31] Lee DH, Roh JL, Baek S, et al. *Second cancer incidence, risk factor, and specific mortality in head and neck squamous cell carcinoma*. Otolaryngol Head Neck Surg 2013;149:579-86.

[32] Schwartz LH1, Ozsahin M, Zhang GN, et al. *Synchronous and metachronous head and neck carcinomas*. Cancer 1994;74:1933-8.

[33] Herranz González-Botas J, Varela Vázquez P, Vázquez Barro C. *Second primary tumours in head and neck cancer*. Acta Otorrinolaringol Esp 2016;67:123-9.

[34] Boscolo-Rizzo P, Del Mistro A, Bussu F, et al. *New insights into human papillomavirus-associated head and neck squamous cell carcinoma*. Acta Otorhinolaryngol Ital 2013;33:77-87.

[35] Sedghizadeh PP, Billington WD, Paxton D, et al. *Is p16-positive oropharyngeal squamous cell carcinoma associated with favorable prognosis? A systematic review and meta-analysis*. Oral Oncol 2016;54:15-27.

[36] Lassen P, Eriksen JG, Krogdahl A, et al. *The influence of HPV-associated p16-expression on accelerated fractionated radiotherapy in head and neck cancer: evauatio of the randomized DAHANCA 6&7 trial*. Radiother Oncol 2011;100:49-55.

[37] Keren S, Shoude Z, Lu Z, et al. *Role of EGFR as a prognostic factor for survival in head and neck cancer: a meta-analysis*. Tumour Biol 2014;35:2285-95.

[38] Gollin SM. *Cytogenetic alterations and their molecular genetic correlates in head and neck squamous cell carcinoma: a next generation window to the biology of disease*. Genes Chromosomes Cancer 2014;53:972-90.

Received: May 25, 2016 - Accepted: January 15, 2017

Address for correspondence: Gabriella Cadoni, Institute of Otorhinolaryngology, Università Cattolica del Sacro Cuore, Fondazione Policlinico Universitario "Agostino Gemelli", Rome, Italy. Tel. +39 06 30154439. E-mail: gabriella.cadoni@rm.unicatt.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:467-474; doi: 10.14639/0392-100X-851

HEAD AND NECK

A miRNA signature suggestive of nodal metastases from laryngeal carcinoma

miRNA signature predittivo di metastasi linfonodali da carcinoma della laringe

F. RICCIARDIELLO[1], R. CAPASSO[2], H. KAWASAKI[2,3], T. ABATE[4], F. OLIVA[1], A. LOMBARDI[2], G. MISSO[2], D. INGROSSO[2], C.A. LEONE[5], M. IENGO[1], M. CARAGLIA[2]

[1] Ear Nose and Throat Unit, Cardarelli Hospital, Naples, Italy; [2] Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Naples, Italy; [3] Drug Discovery Laboratory, Wakunaga Pharmaceutical Co., Ltd., Akitakata, Hiroshima, Japan; [4] Ear Nose and Throat Unit, University of Naples Federico II, Naples, Italy; [5] Ear Nose and Throat Unit and Neck Surgery, Monaldi Hospital, Naples, Italy

SUMMARY

The discovery that miRNAs are frequently deregulated in tumours offers the opportunity to identify them as prognostic and diagnostic markers. The aim of this multicentric study is to identify a miRNA expression profile specific for laryngeal cancer. The secondary endpoint was to identify specific deregulated miRNAs with potential as prognostic biomarkers for tumour spread and nodal involvement, and specifically to search for a miRNA pattern pathognomonic for N+ laryngeal cancer and for N- tissues. We identified 20 miRNAs specific for laryngeal cancer and a tissue-specific miRNA signature that is predictive of lymph node metastases in laryngeal carcinoma characterised by 11 miRNAs, seven of which are overexpressed (upregulated) and four downregulated. These results allow the identification of a group of potential specific tumour biomarkers for laryngeal carcinoma that can be used to improve its diagnosis, particularly in early stages, as well as its prognosis.

KEY WORDS: Laryngeal cancer • miRNA • Nodal metastasis • Expression profile of miRNA • Prognostic factor

RIASSUNTO

La scoperta che i microRNA sono frequentemente deregolati nei tumori consente di utilizzarli come marker prognostici e diagnostici. Lo scopo di questo studio multicentrico è stato stilare un profilo di espressione di miRNA specifico per il carcinoma della laringe. L'obiettivo secondario è stato identificare particolari miRNA deregolati da usare come potenziali biomarker predittivi di diffusione tumorale e di coinvolgimento linfonodale, nello specifico è stato ricercare un pattern di miRNA patognomonico per tessuto di carcinoma della laringe N+ e per N- rispettivamente. Gli Autori hanno identificato venti miRNA specifici per carcinoma della laringe ed inoltre una miRNA signature tessuto-specifica predittiva di metastasi linfonodali da carcinoma della laringe caratterizzata da 11 miRNA, sette dei quali over-espressi (up-regolati) e quattro down-regolati. Questi risultati permettono l'identificazione di un gruppo di potenziali biomarker tumore-specifici per il carcinoma della laringe che potrebbe essere usata per migliorare la sua diagnosi, in particolare negli stadi iniziali, e soprattutto per la sua prognosi.

PAROLE CHIAVE: *Carcinoma della laringe • miRNA • Metastasi linfonodali • Profilo di espressione dei miRNA • Fattori prognostici*

Acta Otorhinolaryngol Ital 2017;37:467-474

Introduction

Laryngeal squamocellular carcinoma (LSCC) accounts for approximately 2% of all tumours [1], with an incidence of 39,900 new cases per 100,000 people in 2012 and a male-female ratio of 8.8:0.8 (9:0.7 in Italy) [2]. It is considered the second most frequent neoplasia of the respiratory system after lung cancer.

Mortality estimated for LSCC was 19,800 cases per 100,000 in 2012 in Europe, with a male-female ratio of 4.3:0.3 (3.3:0.3 in Italy) [2].

Specific survival for larynx tumour is conditioned by many prognostic factors; the presence of cervical nodal metastasis represents the single most important prognostic factor [3 4]. The 2-year overall survival of pN+s patients is reduced by 40-50% (88.01% in the pN0 vs. 41.54% in pN+) [4].

At present, there are no valid prognostic factors that can systematically drive the choice of nodal treatment in laryngeal carcinoma [5].

It is a consensus opinion in the literature that biomolecular markers can fill this deficiency [6 9], especially considering the high potential of the studies on miRNAs.

miRNAs are small non-coding RNAs that regulate post-transcriptional gene expression through mechanisms of degradation of the messenger (only in vegetables and

bugs) or simple sequestration with inhibition of translation (the mechanism present in humans) [10 11].
An important feature of miRNAs is their ability to take part simultaneously in different pathways through the contemporary interaction with multiple messenger targets. Currently there are many studies that show the key role of miRNAs in the genesis, progression and metastatic ability of tumours [12 14].
Different miRNAs are implicated in tumorigenesis by acting through oncogenes or through tumor-suppressor genes, therefore their expression in tumoural tissues, in comparison to healthy tissue, can reveal under- or over-expression [15].
The discovery that miRNAs are frequently deregulated in tumours offers the opportunity to identify them as prognostic and diagnostic markers.
The aim of this multicentric study wasn to identify a miRNA expression profile specific for laryngeal cancer. The secondary endpoint was to identify specific deregulated miRNAs with potential as prognostic biomarkers of tumour spread and nodal involvement, and specifically a miRNA pattern pathognomonic for N+ laryngeal cancer and for N- tissues.

Materials and methods

Patient enrollment
This study included 24 patients suffering from laryngeal carcinoma, 22 males and 2 females, with an average age of 60 years (39-77). All the patients came from Campania and were treated for a laryngeal tumour at the Complex Operative Unit (COU) of Otorhinolaryngology of the University Hospital Policlinico "Federico II", at the COU of Otorhinolaryngology and Cervico-facial Surgery of the Hills Specialist Hospital Monaldi-Cotugno-CTO and at the COU of Otorhinolaryngology of the "A. Cardarelli" National Relief Hospital from January to June 2014. All patients underwent the following diagnostic procedures:

1. Laryngeal endoscopy.
2. Computerised tomography (CT) of the neck and chest with and without contrast.
3. Multiple laryngeal biopsies with histological examination.

The TNM classification was applied in all cases according to the 2010 AJCC criteria.All patients were submitted to "open" laryngeal surgery and bilateral nodal cervical emptying. In all cases a sample of approximately 1 cm x 0.5 cm was withdrawn both from healthy tissue and macroscopically tumoral tissue from the removed larynx, which was immediately introduced into RNA later® tubes. The same patients were submitted to blood draw, and serum was subsequently cryopreserved at -80°C.
The study was approved by the respective Ethics Committees.

Extraction of miRNAs
The RNA was drawn out using the mirVana PARIS kit (Ambion) according to the protocol described by the supplier. A 0.5 mg sample of tumour tissue and the same quantity of healthy tissue were used. The concentration of the RNA was determined using a Nano Drop spectrophotometer by nanodrop reading.

Expression profile of miRNAs
The miRNA expression profile was determined using the TaqMan Array Card Type A (Life Technologies) according to the protocol Megaplex pool A. Experiments were performed on a thermocycler Viia7 (Life Technologies, Inc.), and the relative expression was computed by using the $2^{-\Delta\Delta CT}$ formula and normalised using the endogenous U6. For the determination of miRNAs, we used standard cards that allow assessment of 382 different miRNAs of known function. The cards were provided by the manufacturer and used following the manufacturer's instructions (Life Technologies, Inc.).

Analysis of miRNA expression profile in laryngeal tumoural tissues
The RNA extracted from patients' samples was assembled into two pools, the first including patients with stage T3 and T4 tumours and nodal involvement (N+) and the second comprising patients with stage T3 and T4 tumours without nodal involvement (N-). The control pool consisted of RNA extracted from healthy biopsy tissue taken from the same patients enrolled for pools N+ and N-.

Results

All patients were submitted to "open" surgery of the larynx (2 OSL, 3 CHEPs, 19 total laryngectomies).
In all patients, histological examination led to a diagnosis of squamous cell carcinoma. After histological examination, patients were classified according to both the TNM and histological grading as detailed in Table I.
The pTNM scores of the 24 treated patients and grading were as follow:

- 17 pT3, 7 pT4;
- 12 pN0, 3 pN1, 7 pN2, 2 pN3;
- 11 G2, 12 G3, 1 G4.

All patients included in the study of miRNA expression were selected with homogeneous characteristics regarding both T (pT3 and pT4) and grading. These 24 patients were then divided into two homogenous groups with respect to age, T stage and histological grade on the basis of lymph node involvement found in histologi-

cal examination: 12 patients were pN+ and 12 patients pN-, respectively.

The characteristics of patients based upon the degree of T and presence of lymph node involvement in selected patients were:

- 9 patients pT3N0;
- 8 patients pT3N+;
- 3 patients pT4N0;
- 4 patients pT4N+.

The miRNAs extracted from the 24 selected patients were analysed, and the results of differential expression of miRNAs are described below and shown in Tables II to VI.

Expression analysis showed that normal tissues expressed 180/382 miRNAs, the N- pool expressed 207/382 miRNAs and the N+ pool expressed 200/382 miRNAs.

Comparative analysis between the N+ and N- pools and the control pool showed that in both groups of patients, 89 miRNAs were overexpressed compared to normal tissue counterparts, and are collected in three groups in Table II on the basis of their relative expression; 17 miRNA were downregulated, and are shown in Table III.

Analyzing the N+ and N- pools separately and comparing them to healthy control tissues from the same patient, it is

Table I. p TNM, Grading and laryngectomy type of the 24 patients enrolled in the study.

Number	pTNM	Grading	Laryngectomy type
1	T3N2	G3	Total
2	T4N2	G2	Total
3	T3N3	G2	Total
4	T4N2	G3	Total
5	T3N0	G2	Total
6	T4N0	G2	Total
7	T3N0	G2	Total
8	T3N2	G3	Osl
9	T3N0	G3	Chep
10	T3N1	G3	Total
11	T4N0	G3	Total
12	T4N1	G2	Total
13	T3N0	G3	Total
14	T4N3	G3	Total
15	T3N1	G4	Total
16	T3N0	G3	Total
17	T3N2	G2	Chep
18	T3N2	G3	Osl
19	T3N0	G2	Total
20	T3N2	G2	Total
21	T3N0	G3	Total
22	T4N0	G3	Total
23	T3N0	G2	Total
24	T3N0	G2	Chep

Table II. miRNAs overexpressed in tumour tissues in comparison with healthy tissues in patients with LSCC.

miRNAs with fold change >10	miRNAs with 5 < fold change < 10	miRNAs with 2 < fold change < 5
hsa-miR-106b	hsa-let-7d	hsa-let-7a-
hsa-miR-10b	hsa-miR-101	hsa-let-7e-
hsa-miR-130b	hsa-miR-103	hsa-let-7 g-
hsa-miR-15b	hsa-miR-106a	hsa-miR-10a-
hsa-miR-185	hsa-miR-135b	hsa-miR-125b-
hsa-miR-19a	hsa-miR-141	hsa-miR-127-
hsa-miR-205	hsa-miR-142-5p	hsa-miR-130a-
hsa-miR-20a	hsa-miR-15a	hsa-miR-132-
hsa-miR-21	hsa-miR-17	hsa-miR-138
hsa-miR-221	hsa-miR-181a	hsa-miR-148a
hsa-miR-25	hsa-miR-182	hsa-miR-149
hsa-miR-299-5p	hsa-miR-193a-5p	hsa-miR-152
hsa-miR-455	hsa-miR-19b	hsa-miR-155
hsa-miR-494	hsa-miR-210	hsa-miR-192
hsa-miR-511	hsa-miR-223	hsa-miR-194
hsa-miR-598	hsa-miR-23b	hsa-miR-199a-3p
hsa-miR-708	hsa-miR-27a	hsa-miR-200a
hsa-miR-9	hsa-miR-27b	hsa-miR-200b
	hsa-miR-340	hsa-miR-203
	hsa-miR-34a	hsa-miR-24
	hsa-miR-429	hsa-miR-26b
	hsa-miR-532	hsa-miR-28-3p
	hsa-miR-655	hsa-miR-29a
	hsa-miR-660	hsa-miR-29b
	hsa-miR-886-3p	hsa-miR-29c
	hsa-miR-92a	hsa-miR-301b
	hsa-miR-99b	hsa-miR-30b
		hsa-miR-30c
		hsa-miR-32
		hsa-miR-324-5p
		hsa-miR-331
		hsa-miR-335
		hsa-miR-337-5p
		hsa-miR-374
		hsa-miR-422a
		hsa-miR-425-5p
		hsa-miR-454
		hsa-miR-483-5p
		hsa-miR-508
		hsa-miR-532-3p
		hsa-miR-590-5p
		hsa-miR-744
		hsa-miR-758
		hsa-miR-99a

Table III. miRNAs downregulated in tumor tissues in comparison with healthy tissues of patients with LSCC.

miRNA	N- fold change	N+ fold change
hsa-miR-1	0.072	0.040
hsa-miR-126	0.528	0.592
hsa-miR-133a	0.016	0.009
hsa-miR-133b	0.118	0.046
hsa-miR-139-5p	0.210	0.354
hsa-miR-140-3p	0.378	0.333
hsa-miR-186	0.857	0.497
hsa-miR-204	0.514	0.507
hsa-miR-375	0.175	0.742
hsa-miR-449	0.125	0.013
hsa-miR-449b	0.445	0.139
hsa-miR-486	0.403	0.450
hsa-miR-489	0.588	0.605
hsa-miR-539	0.195	0.154
hsa-miR-574-3p	0.705	0.385
hsa-miR-628-5p	0.549	0.440
hsa-miR-885-5p	0.222	0.130

Table IV. Twelve miRNAs with different expression between the two groups of patients (N-, N+). miRNAs overexpressed are in red, miRNAs downregulated compared to healthy control tissue of patients with LSCC are in blue.

miRNA	N- fold change	N+ fold change
hsa-let-7b	1.391	2.437
hsa-miR-135a	0.631	3.538
hsa-miR-20b	1.467	3.981
hsa-miR-212	0.147	0.756
hsa-miR-324-3p	1.375	2.476
hsa-miR-328	1.482	0.519
hsa-miR-365	3.353	1.352
hsa-miR-376a	1.338	0.586
hsa-miR-493	1.986	0.539
hsa-miR-500	2.771	1.297
hsa-miR-642	0.452	1.375
hsa-miR-886-5p	1.221	3.049

Table V. miRNAs expressed only in pathological tissue and not in control healthy tissue from the same patients.

hsa-miR-181c	hsa-miR-509 5p
hsa-miR-183	hsa-miR-512 3p
hsa-miR-18a	hsa-miR-517a
hsa-miR-22	hsa-miR-517c
hsa-miR-331 5p	hsa-miR-523
hsa-miR-362 3p	hsa-miR-548c 5p
hsa-miR-363	hsa-miR-570
hsa-miR-424	hsa-miR-576 3p
hsa-miR-455 3p	hsa-miR-579
hsa-miR-502 3p	hsa-miR-583 3p

evident that 12 miRNAs were differentially expressed in the two groups of patients and compared to healthy control tissue (Table IV). In particular, 4 miRNAs were overexpressed in N+ patients with respect to both the N- and healthy tissues, 3 miRNAs were downregulated in N+ patients compared to both the N- and healthy tissues, 2 were overexpressed in N- patients with respect to both the N+ and healthy tissues, 2 miRNAs were downregulated in N- patients compared to both N+ and healthy tissues, 1 was overexpressed in N+ patients compared to healthy tissues and downregulated in N- patients compared to the healthy tissue of 24 selected patients with LSCC.

Twenty miRNAs were expressed only in the two groups of patients (N+ and N-) and not in healthy control tissues from the same patients (Table V). Therefore, these miRNAs are expressed only in tumour tissues.

Fifteen miRNAs were expressed only in the N+ group, and 23 miRNAs were expressed only in the N- group (Table VI).

Table VI. Twenty-three miRNAs expressed only in the N- group, 15 miRNAs expressed only in the N+ group. Red: overexpression with respect to healthy control tissue from the patients with LSCC; blue: downregulation with respect to healthy control tissues from the patients with LSCC; n.e.c.: no expression change.

N-	Fold change	N+	Fold change
hsa-miR-146b-3p	1879	hsa-miR-190	0787
hsa-miR-148b	2455	hsa-miR-486-3p	0047
hsa-miR-338-3p	1043	hsa-miR-542-5p	2795
hsa-miR-339-5p	0359	hsa-miR-618	13980
hsa-miR-485-3p	2172	hsa-miR-198	n.e.c.
hsa-miR-518b	0829	hsa-miR-342 5p	n.e.c.
hsa-miR-518f	0509	hsa-miR-369 3p	n.e.c.
hsa-miR-627	0827	hsa-miR-373	n.e.c.
hsa-miR-216b	n.e.c.	hsa-miR-433	n.e.c.
hsa-miR-296	n.e.c.	hsa-miR-450b 5p	n.e.c.
hsa-miR-323 3p	n.e.c.	hsa-miR-487b	n.e.c.
hsa-miR-372	n.e.c.	hsa-miR-545	n.e.c.
hsa-miR-382	n.e.c.	hsa-miR-597	n.e.c.
hsa-miR-503	n.e.c.	hsa-miR-876 3p	n.e.c.
hsa-miR-518c	n.e.c.	hsa-miR-876 5p	n.e.c.
hsa-miR-529a	n.e.c.		
hsa-miR-522	n.e.c.		
hsa-miR-548d	n.e.c.		
hsa-miR-582 5p	n.e.c.		
hsa-miR-636	n.e.c.		
hsa-miR-651	n.e.c.		
hsa-miR-873	n.e.c.		
hsa-miR-137	n.e.c.		

Discussion

Laryngeal tumours identical in site, subsite and clinical stage, and subjected to the same treatment may have different clinical outcomes and prognosis, especially when considering nodal spreading.

In this study, we analysed the expression of miRNAs in tissues resulting from carcinomas of the larynx to identify a tissue-specific miRNA signature predictive of unfavourable development toward lymph node metastases.

Even if the population under study is very limited in number, the results are of considerable interest. The comparative data show that the miRNA expression profiles in pathological tissues compared to healthy tissues exhibit a clear majority of overexpressed miRNAs with only a few hypoexpressed miRNAs.

Some of the miRNAs overexpressed in the diseased tissues have already been described in the literature, also in relation to cancer of the larynx:

- **miR19a:** Marioni et al., recently, have demonstrated its higher expression in malignant glottis lesions than in benign conditions [16]. It was previously correlated with neck nodal metastasis, poor differentiation and advanced stage when overexpressed [17].
- **miR 27a**: it has been shown that miR27a promotes proliferation and suppresses apoptosis [18 19].
- **miR 155:** the expression of tissue and plasma miR155 is significantly upregulated in patients with LSCC [20]; furthermore, it seems to play a role in development of LSCC in terms of promotion of proliferation and invasion [21].
- **miR 21:** some authors have described its overexpression in laryngeal cancer tissues [22 23], and its ratio with miR375 (miR21/miR375) has been related with worse prognosis if high [24 25]; and its high expression in serum is associated with nodal metastasis in LSCC [26]. Recently, miR21 was shown to be deregulated by acidic bile and implicated in precancerous lesiosn of laryngeal mucosa [27].
- **miR 106b:** it was found to be upregulated in LSCC tissues, together with miR21, and their level were found to be increased in poorly/moderately differentiated (G2-G3) cancer tissues and associated with lymph node metastasis [28].
- **miR 375:** according to Wu et al., increased expression of miR375 is associated with a more aggressive phenotype of LSCC; moreover, a high-level expression of miR375 and miR148a in patients with laryngeal dysplasia may predict malignant transformation [29]. In our study, it was downregulated in tumour tissues in agreement with Hu [25].
- **miR 708:** it is upregulated in tumour tissues as is miR21 and miR205 [30] according to our data.
- **miR 205:** it is upregulated in tumour tissues (as in our study), and in addition it significantly induces cell proliferation and invasion by suppressing CDK2AP1 [31].
- **miR 221:** Yilmaz demonstrated that it is upregulated in LSCC plasma samples, but was at normal levels in postoperative plasma; he proposed it as a diagnostic marker of LSCC [32].

Among the overexpressed miRNAs (fold change between 2 and 5) in tumour tissues, some have described to be down-regulated in patients with LSCC.

- **miR 203**: according to Tian et al., its lower expression is related to poor differentiation, advanced clinical stage, lymph node involvement and decreased 5-year overall survival [33]. Recently, it has been shown to correlate with local disease recurrence after radiotherapy in a series of patients with laryngeal cancer [34].
- **miR 152:** it was described as significantly downregulated in supraglottic laryngeal carcinoma tissues and its expression was correlated with p T and p N stages in patients with supraglottic LSCC [35].
- **miR 24**: its upregulation, similar to miR27a, leads to promotion of proliferation and early apoptosis inhibition in LSCC [18]; according to Xu et al., miR24 expression is significantly lower in LSCC cell lines and it inhibits growth-related apoptosis and enhances radiosensitivity in LSCC [36].

Of considerable interest are miRNAs detected in our study and not yet associated with cancer of the larynx, even though they have been previously associated with other tumours. This is the case of six miRNAs: **mir9** [37], **mir511** [38], **mir494** [39], **mir25** [40], **mir20** [41], and **mir10b** [42], which are greatly overexpressed in several tumour tissues compared to healthy control tissues from the same patients.

Interestingly, we identified some miRNAs with specific expression in either N+ or N- cases.

The analysis of the N+ group detected the following miRNAs:

- **miR618:** strongly overexpressed in our study in N+ patients; it is considered by Hui to be a prognostic factor for HNSCC (head and neck squamous cell carcinoma) [43]. It has been also correlated with thyroid cancer [44].
- **miR 542-5p:** overexpressed in tumour tissues in our series of N+ patients, it was previously reported in rhabdomyosarcoma [45] and osteosarcoma [46].
- **miR 486-3p:** downregulated in tumour tissues of patients with nodal metastases compared to healthy control tissues from the same patients, its decreased level has been associated with metastasis in cervical cancer patients [47].
- **miR 135 a:** on the basis of our data, this miRNA is overexpressed in tumour tissues of N+ patients compared to healthy control tissues and downregulated in tumour tissues of patients without lymph node involvement compared to healthy tissue from the same patients. Its high expression in gastric cancer tissues is more likely to have aggressive characteristics, among which lymphatic metastasis [48].
- **miR 20b:** overexpressed in tumour tissues of N+

patients, it was associated with laryngeal cancer in 2010 [49]; its upregulation promotes proliferation, migration and invasiveness in oesophageal tumours [50].

- **miR 324-3p:** overexpressed in tumour tissues of N+ patients, it is upregulated in plasma of stage I of lung squamous cell carcinoma compared to healthy controls [51]; furthermore, its low expression might be an important marker for prediction of low response to RT/CRT and poor overall survival and recurrence-free survival [52].

Analyzing the 12 N- patients, the most interesting miRNAs for their biological functions are the following:

- **miR 148b:** overexpressed in the diseased tissue of pN- patients, it has been linked with melanoma [53].
- **miR 339-5p:** downregulated in tumour tissues of pN- patients, it has been described as a regulator of breast cancer progression [54].
- **miR 485-3p:** overexpressed in the diseased tissue of patients without lymph node metastasis, it is described as a suppressor of breast cancer metastasis [55].
- **miR 518f:** downregulated in tumour tissues of pN- patients compared to control tissue, it is related to endometrial cancer in which it is downregulated [56].

Through analysis of these results, we may define a tissue-specific miRNA signature that is predictive of lymph node metastases in laryngeal carcinoma characterised by 11 miRNAs, seven of which are overexpressed (upregulated) and four downregulated, in particular: miR618, miR542-5p, let 7b, miR135a, miR20b, miR324-3p, and miR886-5p are overexpressed; and miR486-3p, miR328, miR376a and miR493 are downreguated. This signature is suggestive to be predictive of lymph node involvement even if the validation of these results on a wider series of patients is strongly warranted.

Conclusions

We have identified a group of miRNAs with characteristic expression profiles in diseased tissues compared to matched healthy tissue from the same patients; in addition, we have highlighted a miRNA pattern specific of N+ laryngeal cancer cases compared to N- cases and healthy tissues. Furthermore, the authors have detected a miRNA pattern expressed specifically in laryngeal cancer tissues (and not in healthy tissues), one expressed exclusively in laryngeal cancer with N+ and another one present in N-. These results are largely innovative, at least in our opinion, and allow the identification of a group of potentially specific tumour biomarkers for laryngeal carcinoma that can be used to improve its diagnosis, particularly at early stages, and to detect patients with minimal residual disease or recurrence if the miRNA pattern specific of laryngeal cancer is present; but, overall, they can be useful to predict prognosis atient in early stages on the basis of the identification of the miRNAs signature suggestive for nodal involvement. In this case, the miRNAs could lead to tailored treatment.

The technologies of molecular biology are not yet available in all centres, so that the use of miRNA profiling with microarray techniques on large scale in diagnosis of laryngeal carcinoma is not readily possible. However, the methods of real-time PCR are presently relatively cheap and easy to perform. The bottleneck in this type of study is, in fact, the identification of differentially expressed miRNAs through the use of low-density arrays (as in our case) and their subsequent validation in a large population of patients. Once validated, the miRNA biomarkers are easy to detect in the tissue of patients with cancer and other neoplasms. Another advantage of miRNAs is their presence in all body fluids, and in particular in plasma and serum of patients, in which they can be easily detected and quantified [57]. A further phase of the present study is, in fact, the determination of an array of circulating miRNA in serum from the same patients, which will be determined and cross-referenced with those obtained in tissues of the same patients. In this way, we can outline a limited group of very reliable miRNAs that can be validated (or not) together with a "portfolio of prognostic factors" (clinical and pathological) for routine use in clinical evaluation.

Acknowledgments

M. C. received funding from MIUR for a project (FIRB-PROGRAM AGREEMENTS 2011) entitled: "Application of high-throughput technology platforms for the characterization of new biomarkers and molecular targets in nanovectors for the diagnosis and treatment of human cancer." M. C. has also received funding from the Campania Region with a project entitled "Public Laboratories Project Hauteville".

References

1 Succo G, Crosetti E, Bertolin A, et al. *Benefits and drawbacks of open partial horizontal laryngectomies, Part A: early-intermediate stage glottic carcinoma*. Head Neck 2016;38 Suppl 1:E333-40.

2 Ferlay J, Steliarova-Foucher E, Lortet-Tieulenta J, et al. *Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012*. Eur J Cancer 2013;49:1374-403.

3 Spriano G, Manciocco V, Marchesi P, et al. *Il trattamento dell'N nel carcinoma della laringe*. Attualità in Oncologia Laringea 2010;553-74.

4 Barroso Ribeiro R, Ribeiro Breda E, Fernandes Monteiro E. *Prognostic significance of nodal metastasis in advanced tumours of the larynx and hypopharynx*. Acta Otorrinolaringol Esp 2012;63:292-8.

5 Spriano G, Piantanida R, Pellini R, et al. *Elective treatment of the neck in squamous cell carcinoma of the larynx: clinical experience*. Head Neck 2003;25:97-102.

6 Paludetti G, Almadori G, Bussu F, et al. *Prognosi del cancro della laringe*. Identificazione dei Marcatori prognostici nel Tumore della Laringe; 2009. p. 63-68.

[7] Albera R, Martone T, Cortesina G. *Fattori prognostici clinici e molecolari dei carcinomi squamosi del distretto testa- collo (HNSCC)*. Identificazione dei Marcatori prognostici nel Tumore della Laringe; 2009. p. 81-96.

[8] Almadori G, Bussu F, Galli J, et al. *Diminished expression of S100A2, a putative tumour suppressor, is an independent predictive factor of neck node relapse in laryngeal squamous cell carcinoma*. J Otolaryngol Head Neck Surg 2009;38:16-22.

[9] Bolzoni Villaret A, Barbieri D, Peretti G, et al. *Angiogenesis and lymphangiogenesis in early-stage laryngeal carcinoma: prognostic implications*. Head Neck 2013;35:1132-7.

[10] Bartel DP. *miRNAs: target recognition and regulatory functions*. Cell 2009;136:215-33.

[11] Carthew RW, Sontheimer EJ. *Origins and mechanisms of miRNAs and siRNAs*. Cell 2009;136:642-55.

[12] Pencheva N, Tavazoie SF. *Control of metastatic progression by miRNA regulatory networks*. Nat Cell Biol 2013;15:546-54.

[13] Li Y, Ahmad A, Kong D, et al. *Targeting miRNAs for personalized cancer therapy*. Med Princ Pract 2013;22:415-7.

[14] Liu C, Kelnar K, Liu B, et al. *The miRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44*. Nat Med 2011;17:211-5.

[15] Calin GA, Croce CM. *MiRNA signatures in human cancers*. Nat Rev Cancer 2006;6:857-66.

[16] Marioni G, Agostini M, Cappellesso R, et al. *miR-19° and SOCS-1 expression in the differential diagnosis of laryngeal (glottic) verrucous squamous cell carcinoma*. J Clin Pathol 2016;69:415-21.

[17] Wu T, Zhang T, Qu L, et al. *MiR-19a is correlated with prognosis and apoptosis of laryngeal squamous cell carcinoma by regulating TIMP-2 expression*. Int J Clin Exp Pathol 2013;7:56-63.

[18] Wang Y, Zhang ZX, Chen S, et al. *Methylation status of SP1 sites within miR-23a-27a-24-2 promoter region influences laryngeal cancer cell proliferation and apoptosis*. Biomed Res Int 2016;2016:2061248.

[19] Tian Y, Fu S, Qui GB, et al. *miRNA-27a promotes proliferation and suppresses apoptosis by targeting PLK2 in laryngeal carcinoma*. BMC Cancer 2014;14:678.

[20] Wang JL, Wang X, Yang D, et al. *The expression of MicroRNA-155 in plasma and tissue is matched in human laryngeal squamous cell carcinoma*. Yonsei Med J 2016;57:298-305.

[21] Zhao XD, Zhang W, Liang H, et al. *Overexpression of miR -155 promotes proliferation and invasion of human laryngeal squamous cell carcinoma via targeting SOCS1 and STAT3*. PLoS One 2013;8:e56395.

[22] Cybula M, Wieteska L, Jòzefowicz-Korczynska M, et al. *New miRNA expression abnormalities in laryngeal squamous cell carcinoma*. Cancer Biomark 2016;16:559-68.

[23] Zhou P, Zeng F, Liu J,et al. *Correlation between mir-21 expression and laryngeal carcinoma risks*. J Evid Based Med 2015 Dec 12. doi: 10.1111/jebm.12184 [Epub ahead of print].

[24] Hu A, Huang JJ,Xu WH, et al. *MiR-21/miR-375 ratio is an independent prognostic factor in patients with laryngeal squamous cell carcinoma*. Am J Cancer Res 2015;5:1775-85.

[25] Hu A, Huang JJ,Xu WH, et al. *miR-21 and miR-375 microRNAs as candidate diagnostic biomarkers in squamous cell carcinoma of the larynx: association with patient survival*. Am J Transl Res 2014;6:604-13.

[26] Wang J, Zhou Y, Lu J,et al. *Combined detection of serum exosomal miR-21 and HOTAIR as diagnostic and prognostic biomarkers for laryngeal squamous cell carcinoma*. Med Oncol 2014;31:148.

[27] Sasaki CT, Vageli DP. *miR-21, miR-155, miR-192, and miR-375 deregulations related to NF-kappa B activation in gastroduodenal fluid-induced early preneoplastic lesions of laryngeal mucosa in vivo*. Neoplasia 2016;18:329-38.

[28] Yu X, Wu Y, Liu Y, et al. *miR-21, miR-106b and miR-375 as novel potential biomarkers for laryngeal squamous cell carcinoma*. Curr Pharm Biotechnol 2014;15:503-8.

[29] Wu Y, Yu J, Ma Y, et al. *MiR-148a and miR-375 may serve as predictive biomarkers for early diagnosis of laryngeal carcinoma*. Oncol Lett 2016;12:871-8.

[30] Cao P, Zhou L, Zhang J, et al. *Comprehensive expression profiling of microRNAs in laryngeal squamous cell carcinoma*. Head Neck 3013;35:720-8.

[31] Zhong G, Xiong X. *miR-205 promotes proliferation and invasion of laryngeal squamous cell carcinoma by suppressing CDK2AP1 expression*. Biol Res 2015;48:60.

[32] Yilmaz SS, Guzel E, Karatas OF, et al. *MiR-221 as a pre- and postoperative plasma biomarker for larynx cancer patients*. Laryngoscope 2015;125:E377-81.

[33] Tian L, Li M, Ge J, et al. *MiR-203 is downregulated in laryngeal squamous cell carcinoma and can suppress proliferation and induce apoptosis of tumours*. Tumor Biol 2014;35:5953-63.

[34] De Jong MC, Ten Hoeve JJ, Grenman R, et al. *Pretreatment microRNA expression impacting on epithelial-to-mesenchimal transition predicts intrinsic radiosensitivity in head and neck cancer cell lines and patients*. Clin Cancer Res 2015;21:5630-8.

[35] Song Y, Tian Y, Bai W, et al. *Expression and clinical significance of miRNA-152 in supragalottic laryngeal carcinoma*. Tumor Biol 2014;35:11075-9.

[36] Xu L, Chen Z, Xue F, et al. *MicroRNA-24 inhibits growth, induces apoptosis, and reverses radioresistance in laryngeal squamous cell carcinoma by targeting X-linked inhibitor of apoptosis protein*. Cancer Cell Int 2015;15:61.

[37] Minor J, Wang X, Zhang F, et al. *Methylation of miRNA-9 is a specific and sensitive biomarker for oral and oropharyngeal squamous cell carcinomas*. Oral Oncol 2012;48:73-8.

[38] Cao G, Dong W, Meng X, et al. *MiR-511 inhibits growth and metastasis of human hepatocellular carcinoma cells by targeting PIK3R3*. Tumour Biol 2015;36:4453-9.

[39] Yang YK, Xi WY, Xi RX, et al. *MiRNA494 promotes cervical cancer proliferation through the regulation of PTEN*. Oncol Rep 2015;33:2393-401.

[40] Zhao Z, Liu J, Wang C, et al. *MiRNA-25 regulates small cell lung cancer cell development and cell cycle through cyclin E2*. Int J Clin Exp Pathol 2014;7:7726-34.

[41] Zhang GJ, Li Y, Zhou H, et al. *miR20a is an independent prognostic factor in colorectal cancer and is involved in cell metastasis*. Mol Med Rep 2014;10:283-91.

[42] Wang YF, Li Z, Zhao XH, et al. *MiRNA-10b is upregulated and has an invasive role in colorectal cancer through enhanced Rhoc expression*. Oncol Rep 2015;33:1275-83.

[43] Hui L, Wu H, Yang N, et al. *Identification of prognostic microRNA candidates for head and neck squamous cell carcinoma*. Oncol Rep 2016;35:3321-30.

[44] Yi L, Yuan Y, et al. *MicroRNA-618 modulates cell growth via targeting PI3K/Akt pathway in human thyroid carcinomas*. Indian J Cancer 2015;52 Suppl 3:E186-9.

[45] Yang Z, Tien P. *MiR373 and miR542-5p regulate the replication of enterovirus 71 in rhabdomyosarcoma cells*. Sheng Wu Gong Cheng Xue Bao 2014;30:943-53.

[46] Cheng DD, Yu T, Hu T, et al. *MiR-542-5p is a negative prognostic factor and promotes osteosarcoma tumorigenesis by targeting HUWE1*. Oncotarget 2015;6:42761-72.

[47] Ye H, Yu X, Xia J, et al. *MiR-486-3p targeting ECM1 represses cell proliferation and metastasis in cervical cancer*. Biomed Pharmacother 2016;80:109-14.

[48] Yan LH, Chen ZN, Li-Li, et al. *MiR-135a promotes gastric cancer progression and resistance to oxaliplatin*. Oncotarget 2016;7:70699-714.

[49] Wang P, Fu T, Wang X, et al. *Primary, study of miRNA expression patterns in laryngeal carcinoma by microarray*. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2010;24:535-8.

[50] Wang B, Yang J, Xiao B. *MicroRNA-20b (miR-20b) promotes yhe proliferation, migration,invasion, and tumorigenicity in esophageal cancer cells via the regulation of phosphatase and tensin homologue expression*. PLoS One 2016;11:E0164105.

[51] Gao X, Wang Y, Zhao H, et al. *Plasma miR-342-3p and miR-1285 as diagnostic and prognostic biomarkers for early stage lung squamous cell carcinoma*. Oncotarget 2016;7:59664-75.

[52] Xu J, Ai Q, Cao H, et al. *MiR-185-3p and miR-324-3p predict radiosensitivity of nasopharyngeal carcinoma and modulate cancer cell growth and apoptosis by taergeting SMAD7*. Med Sci Monit 2015;2:2828-36.

[53] Mirzaei H, Gholamin S, Shahidsales S, et al. *MicroRNAs as potential diagnostic and prognostic biomarkers in melanoma*. Eur J Cancer 2016;53:25-32.

[54] Yan H, Zhao M, Huang S, et al. *Prolactin inhibits BCL6 expression in breast cancer cells through a microRNA-339-5p-dependent pathway*. J Breast Cancer 2016;19:26-33.

[55] Lou C, Xiao M, Cheng S, et al. *MiR-485-3p and miR-485-5p suppress breast cancer cell metastasis by inhibiting PGC-1α expression*. Cell Death Dis 2016;7:e2159.

[56] Dong P, Ihira K, Xiong Y, et al. *Reactivation of epigenetically silenced miR-124 reverses the epithelial-to-mesenchymal transition and inhibits invasion in endometrial cancer cells via the direct repression of IQGAP1 expression*. Oncotarget 2016;7:20260-70.

[57] Mitchell PS, Parkin RK, Kroh EM, et al. *Circulating miRNAs as stable blood-based markers for cancer detection*. Proc Natl Acad Sci U S A 2008;105:10513-8.

Received: September 24, 2015 - Accepted: March 11, 2017

Address for correspondence: Filippo Ricciardiello, ENT U.O.C. - Cardarelli Hospital, Cardarelli Square, 80131 Naples, Italy. Tel. +39 081 7116287. Fax +39 081 7114644. E-mail: filipporicciardiello@virgilio.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:475-478; doi: 10.14639/0392-100X-1091

LARYNGOLOGY

Enhanced recovery program (ERP) in major laryngeal surgery: building a protocol and testing its feasibility

Elaborazione e applicazione di un protocollo di enhanced recovery program (ERP) in chirurgia oncologica laringea

M. GEMMA[1], S. TOMA[2], F. LIRA LUCE[2], L. BERETTA[1], M. BRAGA[3], M. BUSSI[2]

[1] Head and Neck Department, Anesthesia and Neurosurgical Intensive Care Unit, San Raffaele Hospital, Vita-Salute University, Milan, Italy; [2] Head and Neck Department, Otorhinolaryngology Unit, San Raffaele Hospital, Vita-Salute University, Milan, Italy; [3] Department of Surgery, San Raffaele Hospital, Vita-Salute University, Milan, Italy

SUMMARY

Enhanced recovery programs (ERP) represent a multimodal approach to perioperative patient care. The benefits of ERP are well demonstrated in colorectal surgery and Enhanced Recovery After Surgery (ERAS®) programs, that epitomise the ERP concept, have being introduced in different specialties, including vascular, gastric, pancreatic, urogynecologic and orthopaedic surgery. However, no ERP has been proposed for head and neck surgery. We developed an expert-opinion-based ERP for laryngeal surgery based on the key principles of colorectal surgery ERAS®. Twenty-four patients undergoing major laryngeal surgery (total and partial laryngectomies or surgical removal of oropharyngeal tumour with muscle flap reconstruction) were treated according to such an ERP protocol, which differed under several respects from our previous standard practice (described in 70 consecutive patients who underwent major laryngeal surgery before ERP implementation. The adherence rate to the different ERP items is reported. Adherence to ERP items was high. Nutritional assessment, antibiotic prophylaxis, postoperative nausea and vomit (PONV) prophylaxis and postoperative speech therapy targets were applied as required in 100% of cases. Some ERP items (antibiotic prophylaxis, intraoperative infusion rate, and postoperative speech therapy) were already frequently implemented before ERP adoption. Postoperative medical complications occurred in 8.3% of patients. Our expert opinion-based ERP protocol for major laryngeal surgery proved feasible. The degree of benefit deriving from its implementation has yet to be assessed.

KEY WORDS: Enhanced Recovery After Surgery • Enhanced Recovery Program • Head and neck surgery • Larynx cancer

RIASSUNTO

Con il termine Enhanced Recovery Program (ERP) si fa riferimento a protocolli, sempre più utilizzati in ambito chirurgico, che introducono un approccio multimodale evidence-based alla gestione perioperatoria del paziente. In particolare, i benefici derivanti dall'applicazione dei protocolli di Enhanced Recovery After Surgery (ERAS®) sono stati ampiamente dimostrati nella chirurgia colon-rettale, dove hanno determinato una riduzione della durata della degenza e delle complicanze postoperatorie. Ulteriori protocolli ERP sono stati introdotti in vari campi chirurgici, tra cui la chirurgia vascolare, gastroenterologica, pancreatica, ginecologica, urologica e ortopedica. Nel campo della chirurgia otorinolaringoiatrica, non è ancora stato intrapreso un tentativo di implementazione di un protocollo basato sui principi ERAS®. Lo scopo del nostro lavoro è stato sviluppare un programma ERP per la chirurgia laringea maggiore (laringectomie parziali e totali, rimozione di tumori orofaringei con ricostruzione con lembo nuscolare a cielo aperto), basato sui principi fondamentali del protocollo ERAS® validato nella chirurgia colon-rettale. Ventiquattro pazienti sottoposti a chirurgia oncologica laringea maggiore sono stati trattati con tale protocollo ERP, che differiva sotto molti aspetti dalla nostra precedente pratica standard (descritta sulla scorta di settanta pazienti sottoposti a chirurgia laringea oncologica a cielo aperto prima dell'introduzione del nuovo protocollo). La percentuale di aderenza dei pazienti al protocollo ERP è stata elevata. In particolare gli "items" valutazione nutrizionale preoperatoria, profilassi antibiotica, profilassi PONV (nausea e vomito postoperatori), riabilitazione logopedica post-operatoria, sono stati applicati nel 100% dei casi. Alcune voci del protocollo ERP (profilassi antibiotica, tassi di infusione intraoperatoria e logopedia postoperatoria) erano state già spesso implementate prima dell'adozione ERP. Si sono presentate poche complicanze postoperatorie di tipo medico (8,3% dei casi). Il nostro protocollo ERP per la chirurgia laringea maggiore si è rivelato possibile. Il grado di beneficio derivante dalla sua applicazione potrà essere valutato mediante un ulteriore implementazione del campione di studio.

PAROLE CHIAVE: *Enhanced Recovery After Surgery • Enhanced Recovery Program • Chirurgia testa collo • Tumore laringeo*

Acta Otorhinolaryngol Ital 2017;37:475-478

Introduction

Different perioperative strategies have been recently developed in an effort to reduce the impact of surgery on hospitalisation. In particular, multimodal approaches to perioperative care, such as Enhanced Recovery After Surgery (ERAS®) programs, are now frequently implemented in the management of patients undergoing elective surgery. ERAS® protocols include different items, mostly evidence-based, designed to reduce the intra- and perioperative stress response and to support recovery of organ functions, ultimately aiming at help-

ing patients to recover sooner and with less discomfort after surgery [1–4].

In the last two decades, the benefits from enhanced recovery programs (ERP) have been well demonstrated in colorectal surgery, where ERAS® is associated with lower morbidity and shorter length of hospital stay [5-14]. New emerging evidence supports the possible advantage that could derive from the implementation of ERPs in other surgical areas. Examples of ERP application can be found in a great number of specialties, such as vascular, gastric, pancreatic, orthopaedic and uro-gynaecological surgery [15-23]. It has recently been suggested that the introduction of ERP could benefit patients undergoing major head and neck surgery [24].

In the present work, we generated an ERP for major laryngeal surgery and prospectively tested its feasibility in a series of patients.

Materials and methods

Protocol design

In a preliminary phase of the study, we developed an ERP for laryngeal surgery. An expert panel (MB, LB, MG) reviewed the items and the general principles of the ERAS® colorectal surgery protocols [25] with the purpose of adapting them to laryngeal surgery. Some items could be directly applied to laryngeal surgery, and were kept unchanged with respect to colon surgery protocols. Other items needed some adaptation due to the relevant differences between colorectal and laryngeal surgery. At the end of this phase we obtained an expert opinion based ERP that could be implemented in the perioperative management of patients undergoing laryngeal surgery.

The 11 items constituting the protocol were:

1. Psychological counseling

Preoperative and postoperative meetings with professional psychologists.

2. Nutritional assessment

Evaluation of the nutritional status using the MUST (Malnutrition Universal Screening Tool) score system.

3. Preoperative high carbohydrate drink

Carbohydrate enteral loading administration on the evening before and 2-3 hours before surgery.

4. Temperature control

Intraoperative measurement of patients' temperature and maintenance of normothermia by air blanket and warm intravenous fluid infusions.

5. Antibiotic prophylaxis

Administration of iv cefoxitin (2 g if body weight > 50 kg; 1 g if body weight < 50 kg) and clindamycin (600 mg) 0-30 minutes before surgery, to be repeated every 3 hours for cefoxitin and every 6 hours for clindamycin.

6. Postoperative nausea and vomiting (PONV) prophylaxis

Intraoperative administration of iv ondansetron (4 mg) and dexamethasone (4 mg) 2 hours before the end of surgery.

7. Intraoperative iv infusions

Targeting 6 ml/kg/h mean intraoperative fluids infusion by the end of surgery.

8. Postoperative pain control

Administration of iv paracetamol (1 g every 6 hours) and morphine by Patient Controlled Infusion (PCA - 1 mg/10 min, max 4 mg/h).

9. Early enteral nutrition

Start of enteral nutrition on the first postoperative day.

10. Early mobilisation

Start of patient mobilisation (sitting position and ambulation) on the first postoperative day.

11. Postoperative speech therapy

Postoperative meetings with speech therapists, including speech and breathing exercises.

Protocol evaluation

In a second phase of the study consecutive patients undergoing elective major laryngeal surgery (total and partial laryngectomies or surgical removal of oropharyngeal tumour with muscle flap reconstruction) between October 2011 and May 2014 in our hospital were considered. Exclusion criteria were: refusal to sign the informed consent form, pregnancy and age less than 18 years. Moreover, patients living outside the area of Milan, where our hospital is located, were not considered, since in this phase preoperative and postoperative protocol items could be difficult to implement.

Patient adherence to each protocol item was recorded in a dedicated database as a no/yes variable, except for the intraoperative iv infusions that were recorded as ml/kg/h. We also recorded the postoperative day (POD) of first liquid oral assumption, first solid food oral assumption, nasogastric tube removal, hospital discharge, daily hours of mobilization during postoperative day 1-4, postoperative need for vasopressor and transfusion and occurrence of medical complications. Medical complications were meant to include respiratory complications, cardiovascular events and urinary tract complications.

In order to sketch the differences between our ERP and our routine pre-ERP practice, we retrieved data from the 75 consecutive patients who underwent major laryngeal surgery before the ERP protocol implementation, from October 2008 to September 2011. For these cases, we could retrieve data about all of the 11 ERP items, nasogastric tube removal, hospital discharge, postoperative vasopressor and transfusion need and medical complications. Pre-ERP data are reported exclusively for documentary purposes in order to show that the ERP protocol represented a change from our previous practice. No formal comparison is attempted between pre-ERP and ERP data. Continuous data are reported as mean ± SD. Discrete variables were reported as number-percentage (95% CI). The statistical software Stata 11.1 (StataCorp, College Station, Texas, USA) was used to analyse data.

Results

During the study period 76 patients underwent elective major laryngeal surgery in our Hospital. Thirty-nine (51%) lived outside the area of Milan, 10 (13%) refused to sign the informed consent form and 3 (4%) were less than 18 years old, so that 24 (32%) patients were enrolled.

Table I reports on the implementation of our 11 ERP items. Adherence to ERP items was high. Nutritional assessment, antibiotic prophylaxis, PONV prophylaxis and postoperative speech therapy targets were applied as required in 100% of cases. Early mobilisation was the item with the lowest adherence to protocol target (70.8% (51.2-90.4) of cases).

In ERP patients, oral intake of fluids started on POD 11 ± 5.7 and oral intake of solid food on POD 12 ± 5.3. These patients were mobilised 1.8 ± 2.1 hours on POD 1, 3.8 ± 2.9 hours on POD 2, 5.8 ± 3.2 hours on POD 3 and 6.2 ± 3.1 hours on POD 4.

Postoperative nasogastric tube removal occurred on the 16 ± 5 POD. Vasopressors were needed in 8.3% of ERP patients and postoperative transfusions were necessary in 12.5% of cases. Hospital discharge occurred on the 21 ± 8 postoperative day.

Postoperative medical complications occurred in 8.3% of cases. The majority of our ERP items were infrequently or never implemented before the adoption of the ERP protocol, except antibiotic prophylaxis, intraoperative infusion rate and postoperative speech therapy, which were already implemented in a high percentage of cases before the adoption of the ERP protocol.

Table I. ERP items implementation.

ERP protocol		ERP	Pre-ERP
Item	**Target**	**(n = 24)**	**(n = 75)**
1. Psychological counseling *	100%	23 95.8% (87.2-1.0)	31 41.3% (29.9-52.7)
2. Nutritional assessment *	100%	24 100%	0 0% (0-0)
3. Preoperative glucose drink *	100%	20 83.3% (67.3-99.4)	0 0% (0-0)
4. Temperature control *	100%	23 95.8% (87.2-100)	47 62.7% (51.5-73.9)
5. Antibiotic prophylaxis *	100%	24 100%	73 97.3% (93.6-100)
6. PONV prophylaxis *	100%	24 100%	41 54.7% (43.1-66.2)
7. Intraoperative iv infusions (ml/kg/h) §	6	7.2 ± 3.0	7.8 ± 3.1
8. PO Morphine PCA *	100%	23 95.8% (87.2-100)	48 65.3% (52.9-75.2)
9. Early enteral nutrition *	100%	19 79.2% (61.7-96.7)	68 90.7% (82.1-98.7)
10. Early mobilisation *	100%	17 70.8% (51.2-90.4)	26 34.7% (23.6-45.7)
11. Postoperative logopaedia *	100%	24 100%	68 90.7% (82.1-98.7)

*ERP = Enhanced Recovery Program; PO = postoperative; PONV = postoperative nausea and vomiting.; PCA = patient controlled analgesia * Number and percentage (95% CI) of patients satisfying the ERP target. For 100% values no CI is reported. § Mean ± SD.*

Discussion

ERP principles, epitomised in ERAS® protocols, are increasingly adopted in many surgical settings, but no ERP has been yet proposed in otolaryngology, although its usefulness has been strongly suggested [24].

It is conceivable that the favourable results of ERP implementation in several surgical settings [5-23] fostered a positive attitude towards ERP principles in physicians and nurses involved in the perioperative care of major surgery patients. This possibly accounts for the high adherence to protocol items that we easily obtained in our series. Some items were even satisfied in 100% of cases (namely nutritional assessment, antibiotic prophylaxis, PONV prophylaxis and postoperative speech therapy), exhibiting the highest degree of feasibility. As an example, it is noteworthy that some items were already implemented in our pre- ERP patients, plainly reflecting good common clinical practice. This holds true for both antibiotic prophylaxis and postoperative speech therapy.

The mean intraoperative infusion rate in ERP patients was only slightly lower than in pre-ERP patients and approached the 6 ml/kg/h target without meeting it. We believe that this reflects a tendency to administer less intraoperative fluids independently from ERP protocols, but this certainly also points at some difficulty in coping with intraoperative fluid restriction by anaesthesiologists.

Our study did not address the issue of ERP outcomes and was not adequately powered for this.

Moreover, the best choice of consistent ERP outcome variables in laryngeal surgery may be challenging. We reported the timing of postoperative nasogastric tube removal and hospital discharge in our series. With respect to these issues, although the reduction of both postoperative fasting and hospital length of stay is a cornerstone of ERAS® programs, the optimal timing for nasogastric tube removal after major laryngeal surgery is clearly dictated by anatomical reasons and its evaluation as a possible ERP outcome is questionable. Similar considerations may be appropriate for hospital discharge, as surgical postoperative evaluation may require specific timing.

A further limitation of our study is that we did not register the POD in which patients were "fit to discharge", but rather the actual discharge POD, which is subject to bias due to administrative and organisational variables.

In building our ERP protocol we adapted a series of ERP items to the laryngeal surgery setting. This process was expert-opinion based and entails some degree of subjectivity. Although other approaches could yield different

ERP protocols, our 11 items seem to adequately epitomise ERP philosophy.

Conclusions

Our expert-opinion-based ERP protocol for major laryngeal surgery proved feasible. The degree of benefit deriving from its implementation has yet to be assessed.

References

1 Calder PC. *Immunonutrition*. BMJ 2003;327:117-8.

2 Stableforth WD, Thomas S, Lewi SJ. *A systematic review of the role of immunonutrition in patients undergoing surgery for head and neck cancer.* Int J Oral Maxillofac Surg 2009;38:103-10.

3 Moskovitz DN, Kim YI. *Does perioperative immunonutrition reduce postoperative complications in patients with gastrointestinal cancer undergoing operations?* Nutr Rev 2004;62:443-7.

4 Bianchini C, Ciorba A, Stomeo F, et al. *Immunonutrition in head and neck cancer: have a look before surgery!* Eur Arch Otorhinolaryngol 2012;269:5-8.

5 Melnyk M, Casey RG, Black P, et al. *Enhanced recovery after surgery (ERAS) protocols: time to change practice?* Can Urol Assoc J 2011;5:342-8.

6 Wind J, Polle SW, Fung Kon Jin, et al. *Systematic review of enhanced recovery programmes in colonic surgery.* Br J Surg 2006;93:800-9.

7 Kehlet H, Mogensen T. *Hospital stay of 2 days after open sigmoidectomy with a multimodal rehabilitation programme.* Br J Surg 1999;86:227-30.

8 Eskicioglu C, Forbes SS, Aarts MA, et al. *Enhanced recovery after surgery (ERAS) programs for patients having colorectal surgery: a meta-analysis of randomized trials.* J Gastrointest Surg 2009;13:2321-9.

9 Lassen K, Soop M, Nygren J, et al. *Consensus review of optimal perioperative care in colorectal surgery: Enhanced Recovery After Surgery (ERAS) Group recommendations.* Arch Surg 2009;144:961-9.

10 Abraham N, Albayati S. *Enhanced recovery after surgery programs hasten recovery after colorectal resections.* World J Gastrointest Surg 2011;3:1-6.

11 Sammour T, Zargar-Shoshtari K, Bhatet A, et al. *A programme of Enhanced Recovery After Surgery (ERAS) is a cost-effective intervention in elective colonic surgery.* N Z Med J 2010;123:61-70.

12 Teeuwen PH, Bleichrodt RP, de Jong PJ, et al. *Enhanced recovery after surgery versus conventional perioperative care in rectal surgery.* Dis Colon Rectum 2011;54:833-9.

13 Varadhan KK, Neal KR, Dejong CHC, et al. *The enhanced recovery after surgery (ERAS) pathway for patients undergoing major elective open colorectal surgery: a meta-analysis of randomized controlled trials.* Clin Nutr 2010;29:434-40.

14 Srinivasa S, Sammour T, Kahokehret A, et al. *Enhanced Recovery After Surgery (ERAS) protocols must be considered when determining optimal perioperative care in colorectal surgery.* Ann Surg 2010;252:409.

15 Sjetne IS, Krogstad U, Ødegård S, et al. *Improving quality by introducing enhanced recovery after surgery in a gynaecological department: consequences for ward nursing practice.* Qual Saf Health Care 2009;18:236-40.

16 Minig L, Biffi R, Zanagnolo V, et al. *Early oral versus "traditional" postoperative feeding in gynecologic oncology patients undergoing intestinal resection: a randomized controlled trial.* Ann Surg Oncol 2009;16:1660-8.

17 Pruthi RS, Chun J, Richman M. *Reducing time to oral diet and hospital discharge in patients undergoing radical cystectomy using a perioperative care plan.* Urology 2003;62:661-5.

18 Lee J, Jeon H. *The Clinical indication and feasibility of the enhanced recovery protocol for curative gastric cancer surgery: analysis of 147 consecutive experiences.* Dig Surg 2014;31:318-323.

19 Braga M, Pecorelli N, Ariotti R, et al. *Enhanced recovery after surgery pathway in patients undergoing pancreaticoduodenectomy*. World J Surg 2014;38:2960-6.

20 Savaridas T, Serrano-Pedraza I, Khan SK, et al. *Reduced medium-term mortality following primary total hip and knee arthroplasty with an enhanced recovery program. A study of 4,500 consecutive procedures.* Acta Orthop 2013;84:40-3.

21 Melnyk M, Casey RG, Black P, et al. *Enhanced recovery after surgery (ERAS) protocols: time to change practice?* Can Urol Assoc J 2011;5:342-8.

22 Podore PC, Throop EB. *Infrarenal aortic surgery with a 3-day hospital stay: a report on success with a clinical pathway.* J Vasc Surg 1999;29:787-92.

23 Feo CV, Romanini B, Sortini D, et al. *Early oral feeding after colorectal resection: a randomized controlled study*. ANZ J Surg 2004;74:298-301.

24 Bianchini C, Pelucchi S, Pastore A, et al. *Enhanced recovery after surgery (ERAS) strategies: possible advantages also for head and neck surgery patients?* Eur Arch Otorhinolaryngol 2014;271:439-43.

25 Gustafsson UO, Scott MJ, Schwenket W, et al. *Guidelines for perioperative care in elective colonic surgery: Enhanced Recovery After Surgery (ERAS®) Society recommendations.* World J Surg 2013;37:259-84.

Received: February 8, 2016 - Accepted: November 18, 2016

Address for correspondence: Salvatore Toma, San Raffaele Hospital, Vita-Salute University, Head and Neck Department, Otorhinolaryngology Unit, via Olgettina 60, 20132 Milan, Italy. E-mail: toma.salvatore@hsr.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:479-485; doi: 10.14639/0392-100X-1420

OSAHS

Orthopaedic treatment effects of functional therapy on the sagittal pharyngeal dimensions in subjects with sleep-disordered breathing and Class II malocclusion

Effetti del trattamento ortopedico-funzionale sulle dimensioni sagittali faringee in soggetti con disturbi respiratori del sonno e malocclusione di Classe II

C. PAVONI[1], E. CRETELLA LOMBARDO[1], R. LIONE[1], P. BOLLERO[2], F. OTTAVIANI[1], P. COZZA[1, 3]

[1] Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata, Italy; [2] Department of Systems Medicine, University of Rome Tor Vergata, Italy; [3] Department of Orthodontics, University Zoja e Këshillit të Mirë, Tirane, Albania

SUMMARY

The purpose of this cephalometric study was to evaluate the craniofacial changes induced by functional treatment of mandibular advancement with special regard to pharyngeal sagittal airway dimensions, tongue and hyoid bone position in subjects with sleep-disordered breathing (SDB) and dentoskeletal Class II malocclusions compared with an untreated Class II control group. 51 subjects (24 female, 27 male; mean age 9.9 ± 1.3 years) with Class II malocclusion and SDB consecutively treated with a functional appliance (Modify Monobloc, MM) were compared with a control group of 31 subjects (15 males, 16 females; mean age 10.1 ± 1.1) with untreated Class II malocclusion. For the study group, mode of breathing was defined by an otorhinolaryngologist according to complete physical examination. The parents of all participants completed a modified version of the paediatric sleep questionnaire, PSQ-SRBD Scale, by Ronald Chervin (the Italian version in 22 items form) before and after the trial. Lateral cephalograms were available at the start and end of treatment with the MM. Descriptive statistics were used for all cephalometric measurements in the two groups for active treatment changes. Significant, favourable skeletal changes in the mandible were observed in the treated group after T2. Significant short-term changes in sagittal airway dimensions, hyoid position and tongue position were induced by functional therapy of mandibular advancement in subjects with Class II malocclusion and SDB compared with untreated controls. After orthodontic treatment, a significant reduction in diurnal symptoms was observed in 45 of the 51 participants who had received an oral appliance. Orthodontic treatment is considered to be a potential therapeutic approach for SDB in children. Orthodontists are playing an increasingly important role in managing snoring and respiratory problems by oral mandibular advancement devices and rapid maxillary expansion.

KEY WORDS: Sleep-disordered breathing • Mandibular advancement device • Functional treatment

RIASSUNTO

Con il termine Sleep disorder breathing (SDB) s'intendono tutte quelle difficoltà respiratorie che si verificano durante il sonno. Si può osservare una grande variabilità nella sintomatologia dei pazienti affetti da SDB, direttamente proporzionale alla resistenza che le vie aeree superiori offrono al passaggio dell'aria quando queste sono ostruite. L'SDB rappresenta un ampio ventaglio di disturbi che vanno dal russamento primario fino ad arrivare alle apnee ostruttive del sonno. I bambini con problemi respiratori tendono a compensare l'ostruzione delle vie aeree assumendo posizioni caratteristiche, tali da garantire il mantenimento della pervietà delle vie aeree durante il sonno. Un'anomalia di posizione nel sonno, durante la fase di crescita e sviluppo, si ripercuote in un'alterazione dello sviluppo occlusale e in una modifica del pattern di crescita. Le principali alterazioni sono a carico del mascellare superiore, dell'altezza facciale, del tono muscolare e della posizione mandibolare; nei bambini con SDB, infatti, è spesso presente un pattern scheletrico di Classe II, con lunghezza mandibolare ridotta ed overbite aumentato. Lo scopo del presente studio è stato quello di valutare i cambiamenti craniofacciali indotti dalla terapia funzionale di avanzamento mandibolare con particolare riferimento alla dimensione sagittale delle vie aeree, superiori ed inferiori, alla posizione dell'osso ioide e alla posizione della lingua in soggetti con SDB e malocclusione di Classe II, messi a confronto con un gruppo controllo in Classe II non trattato. 51 soggetti (24 femmine, 27 maschi; età media 9,9 ± 1,3 anni) con malocclusione dentoscheletrica di Classe II e SDB trattati con il dispositivo funzionale Monoblocco Modificato (MM) sono stati messi a confronto con un gruppo controllo non trattato di 31 soggetti (15 maschi, 16 femmine; età media 10,1 ± 1,1 anni) presentanti la stessa malocclusione senza SDB. Il gruppo di studio è stato valutato da uno specialista in otorinolaringoiatria per la definizione del tipo di respirazione ed è stato sottoposto ad un esame fisico completo. I genitori di tutti i pazienti hanno completato un questionario per valutare la presenza di sintomi notturni e diurni prima e dopo il test clinico (versione italiana in 22 punti del Pediatric sleep questionnaire, ideato da Ronald Chervin). Le teleradiografie in proiezione latero laterale sono state analizzate all'inizio e alla fine del trattamento con MM. Tutte le misurazioni cefalometriche dei due gruppi sono state analizzate attraverso dei test per la valutazione statistica dei cambiamenti avvenuti durante il trattamento. I risultati hanno evidenziato dei cambiamenti scheletrici favorevoli nel gruppo trattato a tempo T2. La terapia funzionale di avanzamento mandibolare ha indotto dei cambiamenti statisticamente significativi nella dimensione sagittale delle vie aeree, nella posizione dell'osso ioide e nella posizione della lingua in soggetti di Classe II affetti da SDB rispetto ai controlli non trattati. Dopo la terapia ortodontica in 45 pazienti del gruppo di studio è stata osservata una riduzione dei sintomi diurni di SDB. Il trattamento

con apparecchiature funzionali, non solo migliora i rapporti tra mascellare superiore e mandibola, ma riduce anche il rischio del collasso delle vie aere superiori. La logica terapeutica si basa sul concetto che tutte le anomalie, legate ad un retroposizionamento mandibolare, beneficiano della terapia funzionale di avanzamento mandibolare, che è in grado di ampliare lo spazio posteriormente alla lingua ed allo stesso tempo promuovere l'avanzamento linguale. Lo spostamento anteriore della mandibola influenza la posizione dell'osso ioide e la posizione della lingua, aumentando lo spazio intermascellare in cui quest'ultima alloggia e migliorando la morfologia delle vie aeree superiori. Ne consegue sia la risoluzione della malocclusione scheletrica di Classe II che il miglioramento dei rapporti retrofaringei, eliminando quei fattori predisponenti per lo sviluppo di disturbi respiratori in età adulta.

PAROLE CHIAVE: *Problematiche respiratorie nel sonno • Dispositivo di avanzamento mandibolare • Terapia funzionale*

Acta Otorhinolaryngol Ital 2017;37:479-485

Introduction

Sleep-disordered breathing (SDB) is a general term for breathing difficulties occurring during sleep. SDB may be defined as a disorder characterised by prolonged increased upper airway resistance, partial upper airway obstruction, or complete obstruction that disrupts pulmonary ventilation, oxygenation, or sleep quality [1].

There is a large variability in the symptoms of SDB directly related to increasing upper airway resistance. It represents a "continuum" of respiratory disorders from primary snoring to obstructive sleep apnoea (OSA) [2].

Some children compensate the airway obstruction by sleeping in a knee-chest position and with their neck hyper-extended to give the best chance of maintaining their airway while asleep [3]. This form of breathing may change the growth pattern of the face and lead to morphological and functional alterations in the organism [4].

Mouth breather children develop abnormalities, such as speech disorders, facial deformities, abnormal body posture and inadequate positioning of the teeth [5 6].

Several studies examined anatomic differences between SDB subjects and nasal breathers using traditional cephalometric tracing, model analysis and three-dimensional (3D) radiography [7-13].

Model analysis revealed that SDB patients had narrower maxilla and mandible compared with the control group; the tongue may compensate for the reduced inter-arch dimensions and such as assume a more upward and backward position [14].

Cozza et al. in 2004 [12] observed that the children with respiratory symptoms demonstrated a skeletal Class II pattern with a reduced mandibular length, deep overbite and the hyoid bone was located superiorly. Guilleminault et al. [15] suggested that children with retroposition of the mandible, steep mandibular plane, high hard palate, long oval-shaped face, or long soft palate were highly likely to have sleep-disordered breathing.

Kim et al. [16] in 2010 showed that the mean total airway volume, extending from the anterior nasal cavity and the nasopharynx to the epiglottis, in retrognathic patients was significantly smaller than that of patients with a normal anteroposterior skeletal relationship.

The most common cause of SDB is adenotonsillar hypertrophy. Children with adenotonsillar hypertrophy are usually treated by adenotonsillectomy (ATE). Children who do not improve after adenotonsillectomy tend to have a narrower epipharyngeal air space, a more poorly developed maxilla and mandibular retrusion [17].

Oral appliances and functional orthopaedic appliances have been used in children to shift the mandible forwards, enlarge the upper airway and improve respiratory function in patients who have OSAS and craniofacial anomalies [17 18].

Mandibular advancement devices, in adult patients with mild or moderate obstructive sleep apnoea, are successful in improving AHI, and comparison with inactive appliances suggests that mandibular advancement is crucial in establishing efficacy [19 20]; whereas only a few studies in the literature have evaluated mandibular advancement for treatment of respiratory difficulties in growing patients [2].

The aim of the present study was to evaluate the craniofacial changes induced by functional treatment of mandibular advancement with special regard to the pharyngeal sagittal airway dimensions, tongue and hyoid bone position in subjects with sleep-disordered breathing and dentoskeletal Class II malocclusions compared with an untreated Class II control group immediately after therapy.

Materials and methods

The study project was approved by the Ethical Committee at the Tor Vergata Hospital in Rome, and informed consent was obtained from the subjects' parents.

A sample of 90 Class II subjects was selected among patients of the Department of Orthodontics at the University of Rome Tor Vergata.

51 consecutive subjects (24 female and 27 male, mean age 9.9 ± 1.3 years), who were seen for sleep-disordered breathing, were selected for the study group and were treated for a mean period of 1.8 years (SD = 1.1) (Table I); 31 Class II subjects without SDB (15 males and 16 females, mean age 10.1 ± 1.1) were selected for comparison and were observed for a mean period of 1.9 years (SD = 0.8) (Table I). The remaining 8 subjects were excluded for the presence of systemic disorders. Demographic data of the treated and control samples are reported in Table I.

Table I. Demographics for treatment and control group.

	Age at T1, y		Age at T2, y		T1-T2 interval, y	
	Mean	SD	Mean	SD	Mean	SD
Treated group	9.9	1.3	11.7	1.9	1.8	1.1
Control group	10.1	1.1	12.0	0.3	1.9	0.8

At T1, all patients had a Class II malocclusion characterised by ANB of 4° or more, overjet greater than 5 mm, full Class II or end-to-end molar relationships, deep overbite, normo-hypo divergence, no adenoidectomy or tonsillectomy, absence of previous orthodontic treatment and the absence of craniofacial syndromes.

All patients were in category 2 or 3 of the cervical vertebrae maturation indices, which indicates that they have not reached the peak pubertal growth spurt [21].

For the study group, mode of breathing was defined by an otorhinolaryngologist according to complete physical examination. The parents of all participants completed a modified version of the paediatric sleep questionnaire, PSQ-SRBD Scale, by Ronald Chervin [22] (the Italian version in 22 items form) before and after the trial. The questions sought information about child's daytime symptoms (including sleepiness, irritability, tiredness, school problems, morning headache, oral breathing, and nasal stuffiness) and night-time symptoms (including habitual snoring, apnoea, restless sleep, and nightmares).

The treatment protocols consisted of a modified monoblock (MM) made by a construction bite that positioned the mandible anteriorly in an edge to edge incisor relationship. It was fabricated from acrylic resin which is physiologically harmless, insoluble in water, odour free and inactive. The central screw was activated only once a month to follow maxillary transversal growth. Appliances were checked at regular recall.

The subjects were instructed to wear their appliances full-time. During treatment, the absence of acrylic on the occlusal surface of posterior mandibular teeth encouraged them to erupt. The MM appliance also incorporated a Tucat's pearl on a sliding wire to determine the reference point for the tip of the tongue. Tucat's pearl allows the placement of the tongue tip against the palatal aspect of the alveolar process, behind the maxillary incisors, to improve muscle function and the habitual position of the tongue [10].

Treatment with the MM appliance ended with the achievement of Class I molar relationship. After this period, subjects used the appliance at night only. To be included in the study, all the subjects had to present with lateral cephalograms available at two time periods: T1, at the start of treatment/observation period and T2, at the end of therapy/observation period.

The success of therapy in correcting the Class II malocclusion in each patient at the end of the observation period was not a determining factor for patient recruitment, an approach that lowered any potential selection bias.

The control group was observed in the same period because therapy with functional appliances was postponed to pubertal growth spurt.

Cephalometric analysis

All lateral cephalograms of each patient were hand traced at a single sitting by one investigator (ECL). Landmark location and the accuracy of the anatomical outlines were verified by a second (CP). A customized digitisation regimen (Viewbox, version 4.0, dHAL Software, Kifissia, Greece) was created and used for cephalometric evaluation.

Lateral cephalograms for each patient at T1 and T2 were digitised, and a custom cephalometric analysis was used.

The cephalometric measurements used were (Fig. 1):

1. Dento-skeletal measurements: SNA°, SNB°, ANB°, Co-Me (mm), SN^Go-Gn°, FMA°, OVJ°, OVB°.
2. Airway dimension: PNS-AD1 (mm): lower airway thickness; distance between the PNS and AD1; AD1-Ba (mm): lower adenoid thickness; PNS-AD2: upper airway thickness; AD2-H: upper adenoid thickness; Upper pharynx dimension: the minimum distance between Phw1 and Psp; Lower pharynx dimension: the minimum distance between Phw2 and Tb.
3. Hyoid bone: AH-C3 horizontal (mm): the horizontal distance from AH to C3; AH-C3 vertical (mm): the vertical distance from AH to C3; AH-FH (mm): the distance from AH to Frankfort horizontal; AH-Rgn (mm): the horizontal position of the hyoid; AH-AH1 (mm): the vertical position of the hyoid on the mandibular plane; AH-SN (mm): the vertical position of the hyoid to the SN line.
4. Tongue: V-T (mm): the distance from the intersection of the epiglottis and the base of the tongue to the tip of the tongue; H perpendicular to V-T: representing tongue height; V-T^FH°: representing the vertical position of the tongue.
5. Soft palate and oropharyngeal dimensions: MPW (mm): middle pharyngeal width measured from the intersection of a perpendicular line from U to the posterior pharyngeal wall; U-PNS (mm): representing the length of the soft palate.

Method error

To analyse the error of the method, 20 randomly selected lateral cephalograms were re-digitised and re-measured within a week by the same operator.

Statistical analysis

Data analysis was performed by using the Statistical Package for Social Sciences version 22.0 software (SPSS Inc., Chicago, III). The Mann-Whitney U-test was applied to comparisons between craniofacial starting forms in the treated group *vs* control group. Descriptive statistics were used for all the cephalometric measurements in the two groups for the T2-T1 changes (active treatment changes). Shapiro Wilks' test

revealed a normal distribution for the data. The two sample t-test was applied to compare T1-T2 changes in the treated sample vs the control group ($p < 0.05$). The males and females in each group were matched at T1 in terms of skeletal relationships and skeletal maturation and a student's T-test was applied at T2 to evaluate differences in skeletal relationships between the two subgroups.

Results

There were no significant differences between the treated group and the control group at study start (Table II).
Descriptive statistics and comparisons of the T2-T1 changes between treated and untreated control groups are given in Table III.
No significant differences between male and female subgroups were found at T2 for skeletal relationship.
Initial problems with use of MM include excessive salivation and discomfort at awakening after having a repositioning device in the mouth all night. These adverse effects were found to gradually diminish within a few days after beginning of treatment, and following this period all children and their parents reported good compliance with the MM. The appliances were, therefore, well tolerated. No dysfunctions of the dentition were noted.
The hyoid was located more anteriorly in treated patients: the value of AH-C3 horizontal presented a significant increase of + 3.6 mm in the T1-T2 period. In addition, the hyoid was found in a lower position at the end of treatment as determined from the increase of distance AH-SN and AH-FH (+ 7.2 mm and + 6.4 mm respectively).
The treated group exhibited a significant increase of the length of the soft palate (U-PNS: + 1.6 mm) and an increase of superior posterior and inferior airway space (Phw1-Psp: + 4.5 mm and Phw2-Tb: + 4.3 mm).
Middle pharyngeal width and upper airway thickness were significantly increased (MPW: + 2.1 mm; PNS-AD1: + 1.2 mm) and a reduction of upper and lower adenoid thickness (AD2-H, - 1.0 mm e AD1-Ba, - 0.4 mm) was observed.
The comparisons between the two groups showed that treated patients presented a more anterior position of the tongue (V-T, + 5.7 mm) and a reduction of its height (H perp VT, - 4,5 mm). Moreover, treated subjects presented a more lower position of the tongue as determined from the value of VT^FH (-2.2°).
When compared with controls, the treated group present-

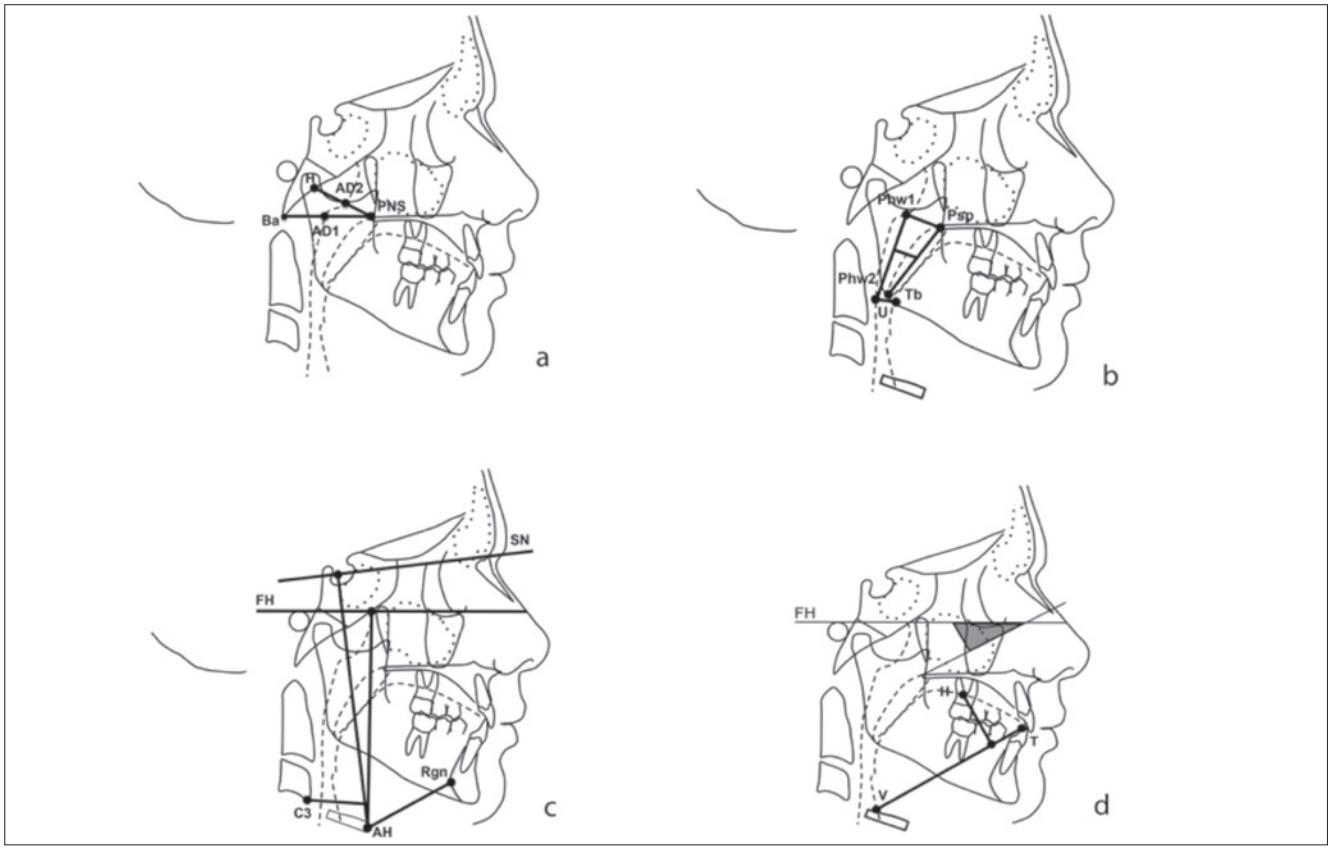


Fig. 1. a) Cephalometric analysis: Airway dimension [PNS-AD1(mm); AD1-Ba (mm); PNS-AD2 (mm); AD2-H (mm)]; b) Soft palate and oropharyngeal dimensions [MPW (mm); U-PNS; Phw1-Psp (mm); Phw2-Tb (mm)]; c) Hyoid bone [AH–C3 horizontal (mm), the horizontal distance from AH to C3; AH–C3 vertical (mm), the vertical distance from AH to C3; AH–FH (mm), the distance from AH perpendicular to Frankfort horizontal; AH–RGn (mm), the horizontal distance from AH to RGn; AH–AH1 (mm), the vertical distance from AH to the mandibular plane (AH1); AH–SN (mm), the vertical position from AH perpendicular to SN line]; d) Tongue [VT (mm); H perp VT (mm); VT^FH (degrees)].

Table II. Starting forms for the treated *vs* control group.

	Treated group		Control group		Mann-Whitney U test
Dentoskeletal measurements	Mean	SD	Mean	SD	
Sagittal analysis					
SNA (°)	82.5	3.0	81.5	2.6	ns
SNB (°)	75.7	2.8	74.8	2.1	ns
ANB (°)	6.8	1.8	6.7	1.0	ns
Co-Me (mm)	106.8	4.8	105.61	4.5	ns
Vertical analysis					
FMA (°)	23.3	4.0	21.6	1.5	ns
SN^GoGn	34.2	4.7	33.1	2.6	ns
CoGoMe (°)	124.8	6.3	122.1	5.0	ns
Dental analysis					
Overjet (mm)	7.0	3.9	6.1	1.8	ns
Overbite (mm)	5.5	2.3	5.6	1.2	ns
Hyoid					
AH-C3 hor (mm)	24.3	2.7	25.3	3.3	ns
AH-C3 vert (mm)	2.9	4.6	2.2	4.8	ns
AH-FH (mm)	64.0	5.2	66.0	5.4	ns
AH-RGn (mm)	33.2	4.1	34.7	5.9	ns
AH-AH1 (mm)	10.7	5.6	12.2	3.2	ns
AH-SN (mm)	78.2	5.4	80	5.8	ns
Soft palate and pharynx					
U-PNS (mm)	24.8	3.5	26.3	3.4	ns
Phw1-Psp (mm)	8.7	3.9	10.1	3.4	ns
Phw2-Tb (mm)	10.4	3.5	11.1	2.8	ns
MPW (mm)	5.4	2.3	6.4	2.5	ns
PNS-AD1 (mm)	16.1	4.4	18.0	5.5	ns
PNS-AD2 (mm)	12.1	5.9	13.2	4.1	ns
AD1-Ba (mm)	20.9	4.7	20.9	5.2	ns
AD2-H (mm)	13.2	4.4	14.1	3.4	ns
Tongue					
VT (mm)	60.8	2.5	59.5	4.1	ns
H perp VT (mm)	12.3	3.5	12.3	2.7	ns
VT^FH (°)	18.4	1.2	17.4	3.6	ns

Table III. Descriptive statistics and comparison of the pre-treatment and end of active treatment (T1-T2) changes between the treated and control group.

	Treated group		Control group		2 Sample T-test
Dentoskeletal measurements	Mean	SD	Mean	SD	
Sagittal analysis					
SNA (°)	0,0	2.3	0.5	1.5	ns
SNB (°)	1.5	1.6	1.2	0.3	ns
ANB (°)	-1.5	2.0	0.2	1.1	*
Co-Me (mm)	7.1	3.0	3.0	2.4	*
Vertical analysis					
FMA (°)	0.7	3.1	0.4	2.0	ns
SN^GoGn	-0.7	2.1	-0.5	1.3	ns
CoGoMe (°)	0.1	2.7	-1.1	2.0	ns
Dental analysis					
Overjet (mm)	-2.8	3.2	0.4	1.1	*
Overbite (mm)	-1.3	2.0	0.7	1.2	*
Hyoid					
AH-C3 hor (mm)	3.3	2.2	-0.2	2.4	*
AH-C3 vert (mm)	1.3	3.3	1.2	4.1	ns
AH-FH (mm)	8.7	3.2	2.3	4.4	*
AH-RGn (mm)	3.2	2.1	-0.5	4.2	*
AH-AH1 (mm)	0.6	1.2	0.2	4.3	ns
AH-SN (mm)	10.3	3.4	3.1	4.2	*
Soft palate and pharynx					
U-PNS (mm)	2.6	1.5	1.0	1.2	*
Phw1-Psp (mm)	5.6	1.4	1.1	1.4	*
Phw2-Tb (mm)	3.3	1.5	-1.0	1.2	*
MPW (mm)	2.6	1.9	0.5	1.6	*
PNS-AD1	2.8	1.6	2.0	1.5	*
PNS-AD2	2.5	1.5	2.3	1.8	ns
AD1-Ba	0.1	1.3	-1.1	1.7	*
AD2H	-0.1	1.7	-1.5	1.4	*
Tongue					
VT (mm)	6.8	2.5	1.1	2.1	*
H perp VT (mm)	-4.5	2.2	0.0	1.6	*
VT^FH (°)	-1.4	1.2	0.7	1.9	*

ed a significant decrease of -1.7° in ANB°, while mandibular length was significantly increased by + 4.0 mm (Co-Me). No significant differences were found between the two groups in vertical analysis.

Both overjet and overbite exhibited a significant improvement in treated subjects (-2.8 mm and - 1.3 mm respectively) compared to the control group.

After the trial, the children's parents again completed the same questionnaire and children underwent a new physical examination. After 1 year and 8 months of orthodontic treatment, a significant reduction in diurnal symptoms was observed in 45 of the participants who had received an oral appliance.

Thus, oral jaw positioning appliance is effective and well tolerated in the short term in children with SDB and malocclusions.

Discussion

The therapeutic effects of a mandibular advancement appliance in the treatment of obstructive sleep disorders is controversial and success rate varies substantially in

clinical investigations [23] [24]. This might be due to differences in study protocols, appliance design and subject selection.

The purpose of this clinical trial was to evaluate the therapeutic effects of mandibular advancement on pharyngeal dimension in children with sleep-disordered breathing, treated with a Modified Monoblock.

Sleep-disordered breathing, during childhood, is often associated with hypertrophic tonsils, adenoids, retrognathic mandibles and transverse maxillary deficiencies. The retroposition of the mandibular complex was associated with reduced retrobasilingual space, mouth breathing, nasopharyngeal airway obstructions and relative retrodisplacement of the tongue. In our study, orthopaedic treatment in SDB patients exerted a direct beneficial effect with anterior repositioning of the tongue (V-T, + 5.7 mm). The rationale for selecting the MM appliance in SDB children was to modify the occlusion and increase the intermaxillary space in which the tongue rests. Linked to this was the issue of correcting the underlying skeletal Class II pattern [10] [11].

According to our study, Schütz [25] observed that after mandibular advancement the anterior displacement of the mandible and hyoid bone caused an anterior traction of the tongue, which increased the posterior airway space, reduced airway resistance and facilitated nocturnal breathing. In the current study, the anterior displacement of the mandible by the functional appliances influenced the position of hyoid bone, thus improving the morphology of the upper airways [26]. Our results showed that the hyoid was located more anteriorly in treated patients (AH-C3 horizontal: +3.6 mm) and in a lower position (AH-SN, + 7.2 mm; AH-FH + 6.4 mm). Schutz in 2011 evaluated the hyoid position and confirmed that the hyoid moved anteriorly but maintained its vertical position.

In our study, the treated group presented a significant decrease of ANB° (-1.7°), while mandibular length was significantly increased (Co-Me: + 4.0 mm); both overjet and overbite values exhibited a significant improvement in treated subjects (OVJ: -2.8 mm; OVB: -1,3 mm). The absence of acrylic above the mandibular posterior teeth encouraged them to erupt. The lower molars tend to come out during the treatment time. The teeth intercuspidation stabilised and gradually improved the dental occlusion [27].

These results are in agreement with those reported by confirmed by Franchi [28]. He had observed, in fact, that the treated group presented with a significant increase in mandibular length and a decrease of both overjet and overbite, compared with controls.

Franchi et al. [29] in 2006 suggested that a small pre-treatment mandibular angle (Co-Go-Me angle < 125.5) was correlated with the evidence of an enhanced responsiveness to functional therapy; on the basis of this observation, we analysed the Co-Go-Me angle in our results and observed that all the Class II patients in this study are ''good responders'', confirming that these subjects are adequate for treatment with mandibular advancement.

The therapeutic benefit from early mandibular advancement may permanently modify nasal breathing and respiration, thereby preventing obstruction of the upper airway [18]. It is also able to enlarge the retrolingual space and at the same time promote lingual advancement [19].

Few studies have analysed the efficacy of functional appliance treatment on pharyngeal airway dimensions in growing patients with a Class II malocclusion and a retrognathic mandible. Ozbek et al. [30] compared pharyngeal airway dimensions in 26 Class II children treated with the Harvold-type activator to 15 controls. They found that pharyngeal airway dimensions significantly increased after functional orthopaedic treatment. According to Ozbek [30], we observed that the dimension of upper and lower airways increased significantly in the treatment group with an increase of the length of the soft palate and of superior posterior and inferior airway space (U-PNS, + 1.7 mm; Phw1-Psp, + 0.5 mm; Phw2-Tb, + 0.6 mm).

The middle pharyngeal width and upper and lower airway thickness showed a significant increase (MPW, + 2.1 mm; PNS-AD1, +1.2 mm; PNS-AD2, +1.2 mm) and we also found a reduction of upper and lower adenoid thickness (AD2-H, -1.0 mm; AD1-Ba, -0.4 mm). In a study published in 2011, Restrepo et al. [31] confirmed that when the measurements before and after treatment were compared, a statistically significant increase in the airway dimensions was found at the space where the adenoid tissue was located.

Orthodontic treatment based on oral appliances is considered to be a potential additional therapeutic approach for SDB in children. Orthodontists are playing an increasingly important role in managing snoring and respiratory problems by means of oral mandibular advancement devices [19] and rapid maxillary expansion [32].

Conclusions

Correction of mandibular retrusion by using mandibular advancement appliances in Class II malocclusion subjects with sleep disorder breathings increased the airway dimensions and improved nasal breathing. The hyoid bone was found to adopt a more anterior and lower position at the end of treatment. The dimension of upper and lower airways increased significantly in the treatment group subjects compared to the control group The tongue was found to adopt a more anterior and lower position at the end of treatment. In addition, treated patients presented a significant reduction in tongue height.

References

1 Carroll JL. *Obstructive sleep-disordered breathing in children: new controversies, new directions*. Clin Chest Med 2003;24:261-82.

[2] Nazarali N, Altalibi M, Nazarali S, et al. *Mandibular advancement appliances for the treatment of paediatric obstructive sleep apnea: a systematic review*. Eur J Orthod 2015;37:618-26.

[3] Sinha D, Guilleminault C. *Sleep disordered breathing in children*. Indian J Med Res 2010;131:311-20.

[4] Bianchini AP, Guedes ZC, Vieira MM. *A study on the relationship between mouth breathing and facial morphological pattern.* Rev Bras Otorrinolaringol 2007;73:500-5.

[5] Basheer B, Hegde KS, Bhat SS, et al. *Influence of mouth breathing on the dentofacial growth of children: a cephalometric study*. J Int Oral Health 2014;6:50-5.

[6] Grippaudo C, Paolantonio EG, Antonini G, et al. *Association between oral habits, mouth breathing and malocclusion.* Acta Otorhinolaryngol Ital 2016;36:386-94.

[7] Pracharktam N, Hans MG, Strohl KP, et al. *Upright and supine cephalometric evaluation of obstructive sleep apnea syndrome and snoring subjects*. Angle Orthod 1994;64:63-73.

[8] Mayer G, Meier-Ewert K. *Cephalometric predictors for orthopaedic mandibular advancement in obstructive sleep apnoea*. Eur J Orthod 1995;17:35-43.

[9] Tangugsorn V, Skatvedt O, Krogstad O, et al. *Obstructive sleep apnoea: a cephalometric study. Part II. Uvulo-glossopharyngeal morphology*. Eur J Orthod 1995;17:57-67.

[10] Cozza P, Ballanti F, Prete L. *A modified monoblock for treatment of young children with obstructive sleep apnea*. J Clin Orthod 2004;38:241-7.

[11] Lione R, Buongiorno M, Franchi L, et al. *Evaluation of maxillary arch dimensions and palatal morphology in mouth-breathing children by using digital dental casts*. Int J Peadiatr Otorhinolaryngol 2014;78:91-5.

[12] Cozza P, Polimeni A, Ballanti F. *A modified monobloc for the treatment of obstructive sleep apnoea in paediatric patients*. Eur J Orthod 2004;26:523-30.

[13] Iwasaki T, Takemoto Y, Inada E, et al. *Three-dimensional cone-beam computed tomography analysis of enlargement of the pharyngeal airway by the Herbst appliance*. Am J Orthod Dentofacial Orthop 2014;146:776-85.

[14] Baroni M, Ballanti F, Franchi L, et al. *Craniofacial features of subjects with adenoid, tonsillar, or adenotonsillar hypertrophy*. Prog Orthod 2011;12:38-44.

[15] Guilleminault C, Pelayo R, Leger D, et al. *Recognition of sleep-disordered breathing in children*. Pediatrics 1996;98:871-82.

[16] Kim YJ, Hong JS, Hwang YI, et al. *Three-dimensional analysis of pharyngeal airway in preadolescent children with different anteroposterior skeletal patterns*. Am J Orthod Dentofacial Orthop 2010;137:306.

[17] Villa MP, Bernkopf E, Pagani J, et al. *Randomized controlled study of an oral jaw-positioning appliance for the treatment of obstructive sleep apnea in children with malocclusion*. Am J Respir Crit Care Med 2002;165:123-7.

[18] Villa MP, Miano S, Rizzoli A. *Mandibular advancement devices are an alternative and valid treatment for pediatric obstructive sleep apnea syndrome*. Sleep Breath 2012;1:971-6.

[19] Ballanti F, Ranieri S, Baldini A, et al. *Long term therapeutic efficacy of a soft monobloc mandibular advancement device in adults with obstructive sleep apnea*. Scientific World Journal 2015;2015:408469.

[20] De Corso E, Bastanza G, Della Marca G, et al. *Drug-induced sleep endoscopy as a selection tool for mandibular advancement therapy by oral device in patients with mild to moderate obstructive sleep apnoea*. Acta Otorhinolaryngol Ital 2015;35:426-32.

[21] Baccetti T, Franchi L, McNamara JA Jr. *The cervical vertebral maturation (CVM) method for the assessment of optimal treatment timing in dentofacial orthopedics*. Sem Orthod 2005;11:119-29.

[22] Chervin RD, Hedger K, Dillon JE, et al. *Pediatric sleep questionnaire (PSQ): validity and reliability of scales for sleep-disordered breathing, snoring, sleepiness and behavioral problems*. Sleep Medicine 2000;1:21-32.

[23] O'Sullivan RA, Hillman DR, Mateljan R, et al. *Mandibular advancement splint: an appliance to treat snoring and obstructive sleep apnea.* Am J Respir Crit Care Med 1995;151:194-8.

[24] Rose EC, Staats R, Virchow C Jr, et al. *Occlusal and skeletal effects of an oral appliance in the treatment of obstructive sleep apnea*. Chest 2002;122:871-7.

[25] Schütz TC, Dominguez GC, Hallinan MP, et al. *Class II correction improves nocturnal breathing in adolescents*. Angle Orthod 2011;81:222-8.

[26] Jena AK, Singh SP, Utreja AK. *Effectiveness of twin-block and Mandibular Protraction Appliance-IV in the improvement of pharyngeal airway passage dimensions in Class II malocclusion subjects with a retrognathic mandible.* Angle Orthod 2013;83:728-34.

[27] Clark WJ. *Twin block functional therapy. applications* in *dentofacial orthopedics.* First Edition. London: Mosby-Wolfe; 1995.

[28] Franchi L, Pavoni C, Faltin K, et al. *Thin-plate spline analysis of mandibular shape changes induced by functional appliances in Class II malocclusion: A long-term evaluation.* J Orofac Orthop 2016;77:325-33.

[29] Franchi L, Baccetti T. *Prediction of individual mandibular changes induced by functional jaw orthopedics followed by fixed appliances in Class II patients*. Angle Orthod 2006;76:950-4.

[30] Ozbek MM, Memikoglu TU, Gögen H, et al. *Oropharyngeal airway dimensions and functional-orthopedic treatment in skeletal Class II cases*. Angle Orthod 1998;68:327-36.

[31] Restrepo C, Santamaría A, Peláez S, et al. *Oropharyngeal airway dimensions after treatment with functional appliances in class II retrognathic children*. J Oral Rehabil 2011;38:588-94.

[32] Lione R, Pavoni C, Laganà G, et al. *Rapid maxillary expansion: effects on palatal area investigated by computed tomography in growing subjects.* Eur J Paediatr Dent 2012;13:215-8.

Received: September 1, 2016 - Accepted: May 2, 2017

Address for correspondence: Chiara Pavoni, University of Rome Tor Vergata, Department of Clinical Sciences and Translational Medicine, viale Oxford 81, 00133 Rome, Italy. Tel. +39 06 20904813. E-mail: chiarapavoni@libero.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:486-492; doi: 10.14639/0392-100X-1313

Rhinology

Endoscopic repair of nasal septal perforation

Riparazione della perforazione del setto nasale con tecnica endoscopica

M. CASSANO
Department of Otorhinolaryngology, University of Foggia, Italy

SUMMARY

Surgical closure of nasal septal perforation is one of the most challenging procedures in nasal surgery. During the last decade, many endoscopic repair techniques have been described with a success of post-operative repair between 76.4% and 100%. The advantages of this approach are its minimal invasiveness (with no external scars), optimal exposure of the operative field (with better visibility of structures) and good control of perforation margins. The drawbacks are that it is time-consuming and can be difficult to perform, requiring years of endoscopic experience. In this review, all the relevant literature published in which repair was completely made endoscopically is overviewed, comparing the success rates, diameter of the perforation and materials used for the repair.

KEY WORDS: Nasal septal perforations • Nasal endoscopy • Septal perforations repair • Endoscopic techniques

RIASSUNTO

La riparazione della perforazione del setto nasale rappresenta delle più complesse procedure chirurgiche nasali. Nel corso dell'ultimo decennio, sono state descritte numerose tecniche endoscopiche di riparazione, con una percentuale di successo variabile fra il 76.4% e 100%. I vantaggi di questa tecnica sono la mini-invasività (nessuna cicatrice esterna), un'ottima esposizione del campo operatorio (con una migliore visualizzazione delle strutture anatomiche) e un buon controllo visivo dei margini della perforazione. Possibili svantaggi sono un maggior tempo operatorio ed una maggiore difficoltà di esecuzione che richiede curve di apprendimento di anni. In questa review riportiamo l'esperienza di numerosi lavori pubblicati sulla riparazione endoscopica delle perforazioni del setto nasale, mettendo a confronto la percentuale di successo, il diametro della perforazione e i materiali utilizzati per la riparazione.

PAROLE CHIAVE: *Perforazione del setto nasale • Endoscopia nasale • Riparazione di perforazioni settali • Tecnica endoscopica*

Acta Otorhinolaryngol Ital 2017;37:486-492

Introduction

Nasal septal perforation is an anatomical defect of the cartilaginous and/or bone nasal septum (Fig. 1). In many cases it is asymptomatic, but when symptomatic patients have recurrent epistaxis, nasal crusting, whistling, headache, dryness, and nasal obstruction. Anterior perforations are generally symptomatic, while posterior ones are not symptomatic as the inspired air is rapidly humidified by the nasal mucosa, preventing dryness [1].

Only symptomatic perforations require treatment to relieve symptoms. Medical treatment with nasal irrigation and ointments can only reduce crusting, dryness and nasal obstruction in mild symptomatic perforations.

A useful alternative is mechanical closure with a prosthesis such as the septal button [2]. Silicon buttons can alleviate epistaxis, whistling and nasal obstruction, but these prostheses cannot control the production of crusting around the margins of the button which causes discomfort for patients [3]. New silicon buttons [2] and magnet-based buttons [4] reduce the patient's discomfort and crusting.

If these treatments are unsuccessful, surgical treatment is recommended. Many surgical techniques for septal perforation repair have been reported, but most are technically difficult, require experienced surgeons and are associated with a relatively low rate of success, as demonstrated by the high number of re-perforations [5].

The major problems in the surgical approach are due to the tenuous nature of the tissues and to the limited surgical exposure of the area [6]. Moreover, many techniques proposed require graft harvesting, with a consequent morbidity of the donor site (temporalis fascia, conchal cartilage, mastoid periosteum, fascia lata, etc.), or an allograft (acellular human dermal graft, porcine small intestine mucosa) with possible rejection [7].

The reported surgical approaches include external rhinoplasty, midfacial degloving, unilateral hemitransfixion and closed endonasal techniques [7]. The advantage of the latter is that they do not leave any external scar, but are more difficult to perform due to the narrow operating field. "Open" techniques offer a wider operating field, thus allowing better access to the superior and posterior margins of the perforation.

During the last decade, many studies have described optimal results with closed endoscopic techniques [1 8-21]. By

using the endoscope, excellent visualisation and exposure can be achieved without excessive dissection and with good control of septal perforation margins [15]. The drawback of these techniques is that they require good endoscopic skills (and so may be more difficult to achieve for less experienced surgeons) and a longer operating time [9].
Herein, the techniques of endoscopic repair of nasal septal perforation underlining the advantages and disadvantages of each technique, comparing the success rate, are reviewed. The review includes all studies published in which the repair was completely made endoscopically. Studies with endoscopice control of a single stage of surgery (harvesting of the flap or graft, control of haemostasis) were excluded.

Review of the literature

In 2002, Hier at al. first reported the use of nasal endoscope in a case of 27-year-old man with a 2x2 cm anterior nasal septal perforation that was repaired by a superiorly based rotation advancement flap associated with an interposition graft containing a mixture of bone and cartilage harvested from the septum. The graft was positioned in a pocket between the two mucosal flaps extending between the medial crura to hold the graft. The drawback of this technique was the presence of exposed bone/cartilage graft on the right side of the nose, which was covered by a Gelfilm splint. The authors reported only small amounts of crusting on the exposed cartilage, with a complete mucosalisation and healing of the septum after 7 months of follow-up. Hier underlined the excellent visualisation and exposure achieved by the endoscopic technique without excessive dissection and with excellent teaching capabilities [8].
The second report of an endoscopic repair of nasal septal perforation was by Ayshford et al. in 2003. The authors reported a series of 17 patients with symptomatic anterior perforation ranging from 1 cm to 2.5 cm in diameter who were submitted to endoscopic repair with an acellular human dermal allograft (alloderm) and an anteriorly based inferior turbinate flap. After excising the edges of the perforation and raising the mucopericondrial flaps around the margins of the perforation, unilateral (in small perforations) or bilateral (in large ones) inferior turbinate flaps were created endoscopically by incising the inferior turbinate posterior to the perforation and mobilising it on an anteriorly based pedicled flap. An acellular human dermal allograft was then positioned, after rehydratation, between the cartilage and mucoperichondrial flap and sutured with vicryl. Finally, the inferior turbinate flap was sutured to the anterior half of the perforation edge. The technique achieved successful closure of the perforation in 13 cases (76.5%). In two cases, a smaller residual perforation developed owing to persistent crust picking, in the other two cases the graft failed. The disadvantages of this technique are the cost of the alloderm and the need for a second stage surgery after 3 weeks, as the inferior turbinate flap needs to be divided and sutured to the posterior edge of the perforation [1].
In 2004, Meghachi et al. published in the French literature their technique of endoscopic repair of nasal perforation using a unilateral posterior pedicled mucosal rotation flap without interposition material, reporting a 75% success rate in a series of 11 cases. The case series also included perforations larger than 2 cm in diameter [9].
In 2007, Presutti et al. described their personal technique of nasal septal repair based on bilateral dissection of monopedicled mucosal flaps from nasal fossa floor sutured at the edge of the perforation without any interposition graft between the two mucosal layers. He prepared the flaps endoscopically (with a four-hand endoscopic approach), with an anterior caudal septal incision, extended laterally, to the floor of nasal fossa, and posteriorly, under the inferior turbinate proximal to the choana. The mucoperiostium and the mucopericondrium from the incision up to the perforation edge is elevated and the posteriorly based flap is transposed medially and pushed cranially to cover the perforation. The flap is then sutured to the mucosa of the upper edge of the perforation. This procedure is performed on the other side creating a double layer repair. This technique allowed complete repair in 28 (of 31) patients (90.3%) with better results in perforation smaller than 3 cm (26/27 patients; 96.3%). The authors underline the advantage of the endoscopic view in flap dissection and suturing, and the absence of donor site morbidity with their technique [10].
Lee et al. also reported in 2008 an endoscopic technique with unilateral advancement mucosal flaps and a temporalis fascia on the other side. The flaps were obtained by an hemitrasfixion incision extended laterally under the inferior turbinate and another horizontal incision made on the septal dorsum. The mucoperiostium and mucopericondrium were then elevated to create two flaps that advance (one upward and the other downward) to cover the perforation before suturing with 5.0 vicryl. The authors underline the advantages of the unilateral mucosal flap in avoiding the enlargement of the perforation and developing new perforations during surgery, as well as decreased operation time while performing a one stage procedure [11].
In 2011, four authors published personal techniques for endoscopic nasal septal repair.
Mansour treated 6 patients with a free graft harvested from the inferior turbinate and applied between the mucoperichondrium of both septum sides. At 2-year follow-up, 5 patients had complete repair (83%) and one partial repair, with a resolution of symptoms in all cases. This technique has the advantage to be very simple without the necessity of flap creation, but allows only a one-layer repair of the septal perforation [12].
In contrast, Giacomini et al. proposed a three-layer endoscopic reconstruction of the septum. They performed a hemitrasfix incision and elevation of the mucoperichondrial and mucoperiostal layers on both sides from the anterior septum

to the choana and until the nasal floor. After the scarification of perforation margins, the elevated flaps were advanced bilaterally (primarily in a horizontal plane) in an inverted sliding flap manner. Vertical or horizontal relaxation incision may be added to achieve a better mobilisation and to make vertical advancement. An interposition graft of auricular conchal autologous cartilage was inserted between the two flaps. With this approach, the authors reported complete repair in 10 large (2-4 cm) perforations with a success rate of 71.4% and symptom improvement in 12 patients (85.7%) [13].
In the same year, Kazkayasi and Yalcinozan described a case report in which the nasal perforation was endoscopically repaired using an over-projected uncinate process. Actually, the technique contemplated caudal septal incision with elevation of mucoperichondrium through the nasal floor up to the roof of the nasal cavity and suture of the margins of the perforation on one side. On the other side, the mucoperiostium of the resected uncinate process is sutured with the mucopericondrium of the perforation margin. In this way, the technique guarantees two-layer repair with the use of autologous flap and graft with a potential better integration due to the respiratory epithelium that they possess [14].
Optimal results with 100% of repair in 11 perforations ranged between 10 and 25 mm of diameter were reported by Castelnuovo et al. with their technique of unilateral superiorly based rotational-advancement flap, supplied by the anterior ethmoidal artery. The authors performed an incision vertically along the septum, until reaching the lateral wall of the posterior portion of the inferior meatus. Then, the incision turns horizontally following the inferior meatus until its anterior portion and finally turning upward perpendicular to the septum reaching the inferior border of the perforation. After the elevation, a large superiorly based flap is crated allowing comfortable advancement and sutured to the mucosa around the perimeter of the perforation. In this case, unilateral one-layer technique allowed repair in all cases at the first attempt [15].
In 2012, Tastan et al. described an endoscopic technique of septal perforation repair using an inferior turbinate composite graft. They harvested the graft from the middle part of the inferior turbinate (leaving the shape and volume of the inferior turbinate), obtaining a three-layer graft with bone attached with its mucosa on both surfaces. The graft must be slightly larger than perforation size so that it can be easily overlapped without tension to the perforation margins and is sutured to them with 5.0 absorbable sutures. In case of medium and large perforations (> 10 mm), a bipedicled mucosal advancement flap [22] can be added with flaps elevated from the nasal floor bilaterally and from the nasal roof unilaterally, and sutured to the composite inferior turbinate graft. Using this technique, the authors achieved complete repair in 24 (88.8%) of 27 patients with just two failures in medium sized perforations and one in a large perforation. The advantages of this technique are ease of development and insertion of the graft, three layer repair, single stage procedure and the possibility to combine with bilateral flaps to reduce mucosal tension [16].

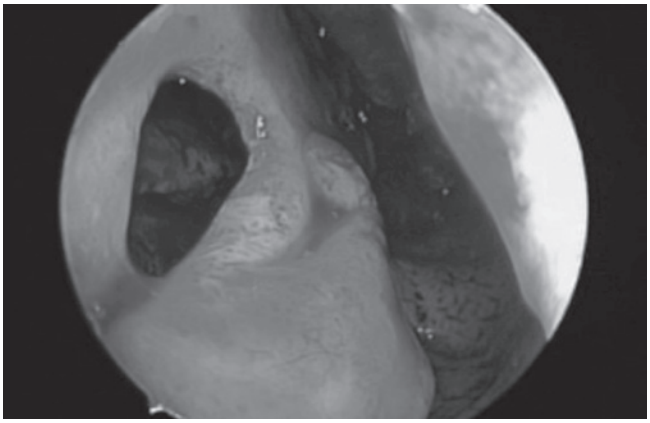


Fig. 1. A septal perforation of the cartilaginous part of the septum.

In the same year, Lee DH and colleagues reported on the usefulness of the anterior pedicled inferior turbinate flap for endoscopic repair of septal perforation and reconstruction of mucosal defects following excision of a septal tumor. In a small cohort of only 6 cases, the authors reported a success rate of 83.3% without any case of full-thickness necrosis of the flap, and no excessive crusting or empty nose syndrome. Finally, they underlined the considerable learning curve of endoscopic preparation of the flap, but the potential improvement of treatment outcomes of reconstruction when the familiarity with these flap will increase [17].
In 2012, Chen described an endoscopic sandwich technique for repair of septal perforation with diameters of 1-2 cm. The technique contemplates the use of three layers of interposition graft with cartilage (residual septum cartilage) or bone (vomer or perpendicular plate of ethmoid) in the middle with quadriceps fascia covering both sides of the graft. The size of the entire composite graft should be more than twice the size of the perforation. This triple-layer interposition graft is interposed between two mucopericondrium and mucoperiosteal flaps elevated from the residual septal mucosa starting from an anterior columellar incision. In cases of perforation about 2 cm, a middle turbinate mucoperiosteal graft can be added in an on-lay fashion on one side of the nasal septum to cover the perforation and the sandwich graft. Biological glue is then applied to increase the adhesiveness between the various components of the graft and the mucosa. Using this technique, the authors achieved a 92.3% success rate with just one case of partial repair (3x3 mm residual perforation) but without symptoms. The authors underlined the necessity of adding septal or nasal floor mucosal flaps in case of perforation larger than 2 cm because a simple graft could cause central necrosis in the absence of adequate blood supply [18].
In 2014, we described a new endoscopic technique called "slide and patch" because it combines a mucoperiosteal

Table I. Publications on endoscopic techniques of nasal septal perforation repair: A brief description of the technique , number of patients, dimension of the perforation and repair rate are reported.

Author, year	Technique	No. patients	Size of perforation	% success	Notes
Hier, 2002	Superiorly based rotation advancement flap associated with an interposition graft containing a mixture of bone and cartilage harvested from the septum	1	2 x 2 cm	100	Case report. Two layer repair Drawback: the presence of exposed bone/cartilage graft on the right side of the nose, that was covered just by a Gelfilm splint
Ayshford, 2003	Unilateral or bilateral anteriorly based inferior turbinate flap + alloderm	17	1-2.5 cm	76.4	Two - three layer repair. Drawback: cost of Aloderm and the need for second stage surgery to divide and suture inferior turbinate
Meghachi, 2004	Unilateral posterior pedicled mucosal rotation flap without interposition material	11	0.5- > 2 cm	75	One layer repair. Success also in large perforations (> 2 cm)
Presutti, 2007	Bilateral monopedicled mucosal flaps from nasal fossa floor sutured at the edge of the perforation without any interposiztion graft	31	< 3 cm	90.3	Two layer repair
Lee, 2008	Unilateral advancement mucosal flaps + temporalis fascia on the other side	14	0.7-2 cm	85.7	Two layer repair
Mansour, 2011	Inferior turbinate free graft	6	< 2 cm	83	Very simple. One layer repair.
Kazkayasi, 2011	Over-projected uncinate process graft + mucosal sutures	1	0.7 x 1 cm	100	Case report. Two layer repair
Giacomini, 2011	Bilateral bipedicled horizontal advancement flaps - choncha cartilage interposition graft.	14	2-4 cm	85.7	Three layer repair. Improvement of symptoms in 12 patients
Castelnuovo,2011	Unilateral superiorly based rotational-advancement flap , supplied by the anterior ethmoidal artery	11	1-2.5 cm	100	One layer repair.
Tastan, 2012	Inferior turbinate composite graft (bone + mucosa)	27	0.4-3.2 cm	88.8	Three layer graft. Can be combined with flaps elevated from the nasal floor bilaterally
Chen, 2012	Sandwich technique (interposition graft with cartilage or bone in the middle with quadriceps fascia covering both sides	13	1-2 cm	92	Three layer graft . A forth layer can be added with a middle turbinate mucoperiosteal graft
Lee, 2012	Unilateral or bilateral anterior pedicled inferior turbinate flap	6	1-3 cm	83.3	One-two layer repair
Cassano, 2014	"Slide and patch" technique : inferior turbinate mucoperiosteal free graft + mucosal rotational or advancement flap	22	< 0.5-3.5 cm	95.4	Two layer repair
Hanci, 2014	Unilateral superiorly based middle turbinate mucosa flap	31	< 2 cm	93.5	One layer repair
Kaya, 2015	Interposition graft made with a piece of conchal cartilage covered on both sides by temporalis fascia	22	< 2 cm	86.3	Three layer graft

free graft of the inferior turbinate with a mucosal rotational or advancement flap from nasal septum. The technique involves an initial bilateral trimming of perforation margins with wide dissection all around the perforation from the underlying cartilage or bone. Next, through a hemitransfix incision, the mucopericondrial and mucoperiosteal layers are extensively elevated on one side of the nasal septum, and a mucoperiosteal graft harvested from the inferior turbinate is inserted in the tunnel between the septal cartilage and the elevated septal mucoperichondrial flap and positioned un-

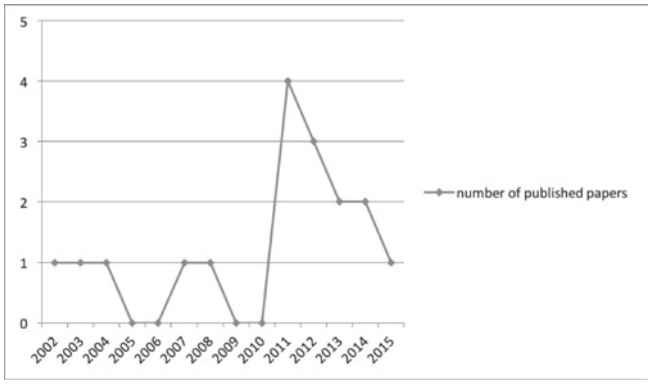


Fig. 2. Number of publications on endoscopic techniques of septal perforation repair from 2002 to 2015.

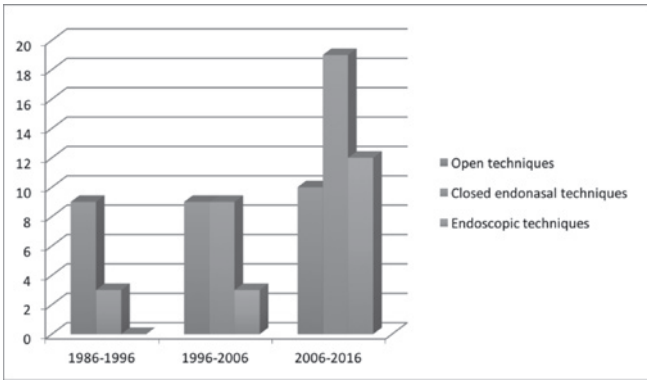


Fig. 3. Number of publications on open, closed and endoscopic techniques of septal perforation repair in the last three decades.

der the previously elevated perforation borders in underlay fashion for a minimum of 5 mm all around.

On the other side, in oval perforations with the horizontal large diameter, a horizontal incision the same length as the perforation large diameter is performed on nasal mucosa 1 cm from the dorsal border of septal cartilage. The mucopericondrial flap is then elevated from the perforation margin up to the incision, the flap is transposed downward and the borders of the perforation are sutured together with a 5.0 Vycril suture.

In the case of rounded perforations, a rotation/advancement mucoperiosteal flap is designed by a rounded incision based posteriorly and elevated up to the choana. Even in this case, the flap, based on the nasal-septal artery, is rotated in order to reach the inferior border of the perforation. In both cases the flap should advance to cover the perforation without tension. The use of a flap of native septal tissue (with the advantage of the rich vascular supply and proximity to the defect), with an interposition graft of inferior turbinate, provided optimal results with a success rate of 95.4% in 22 patients and just 1 case of partial closure [19].

In the same year, Hanci and Altun reported their experience with a unilateral middle turbinate mucosal flap. This was a monopedicled, superiorly-based bone included conchal flap, with which the authors achieved complete endoscopic repair in 29 of 31 patients without any other symptoms in the postoperative period [20].

The last reported technique of endoscopic nasal septal perforation repair was published in 2015 by Kaya et al. The authors repaired 19 of 22 septal perforations (success rate 86.3%) using an interposition graft made with a piece of conchal cartilage (at least 3 mm larger than perforation) covered on both sides by temporalis fascia. The graft was placed into the perforation endoscopically (after elevating the edges for 3-4 mm around the perforation) and stabilised with bioreadsorbable staples. The authors conclude that their technique is expected to allow better healing and mucosal resurfacing [21] (Table I).

Discussion

Although numerous surgical techniques have been described, the surgical closure of nasal septal perforations is still challenging for the surgeon and operating techniques are not yet standardised. In a review of various studies on nasal septal perforation repair, reporting an extensive range of surgical techniques, Goh found that the results were rarely statistically significant [7]. This can be explained by the scant experience of almost all surgeons with this surgery: in fact, very few authors have reported on a large number of operations in their study [15].

Numerous techniques have been proposed, such as external, intranasal, endoscopic, midfacial degloving or sublabial approach, with the use of various grafts (synthetic or autograft) and combined flaps (unilateral or bilateral), and each has its advantages and disadvantages.

The endoscopic endonasal approach has gained ground in the last decades with the studies of Hier and Ayshford [1 8]. From these first reports, many studies have been published, reporting a percentage of post-operative repair variables between 76.4% [1] and 100% [15].

The increasing interest on endoscopic techniques is certified by the increasing number of papers published in the last few years. In fact, 10 publications (of 15) were published in the last 5 years (from 2011) (Fig. 2).

As is shown in figure 3, the trend of septal perforation repair techniques has completely changed in the last three decades: from 1986 to 1996 an open approach (open rhinoplasty, midfacial degloving, etc.) was the favorite approach with just three publications about a closed approach. In the decade 1996-2006, open and closed approaches had the same numbers of publications and an endoscopic approach began to be described. In the last decade, closed approaches were prominent, together with a large number of publications (n = 12) on endoscopic approaches (Fig. 3)

The advantages of this approach are its minimal invasiveness (with no external scars), optimal exposure of the op-

erative field (with better visibility of the structures) and good control of perforation margins. The drawbacks are that it is time-consuming and can be quite difficult to perform, requiring some years of endoscopic experience.

The use of an endoscopic approach has allowed very high percentage of success even in cases of unilateral flap repair [9 12 15 20], which is classically considered insufficient by some authors [13 23]. In fact, Kridel stated that "a septal perforation is a hole in 3 distinct contiguous layers composed of both right and left septal mucoperichondrial flaps and the intervening cartilage, all 3 of which must be separated from each other and repaired individually" [24]. Nevertheless, Castelnuovo reported 100% of nasal perforation repair with only an anterior ethmoidal artery unilateral septal flap without any interposition graft [15]. The unilateral nasal flap has the advantage of avoiding enlargement of the perforation and development of any other perforations during the operation, as well as decreased surgical time since only a one stage procedure is performed [11].

In an evaluation of the predictive factors for the outcomes of nasal septal perforation repair, some authors reported that repair with bilateral flaps is the most important factor for successful closure [25 26]. Notwithstanding, with these techniques these authors did not reach the success rate of endoscopic techniques using only one flap and a graft [8 10 11 19].

However, the interposition graft could be useful because it serves not only as a scaffold for the migration of respiratory mucosa, but also provides a second layer of protection.

Although Moon stated that the kind of graft material does not dictate the success of surgery [26], the use of autologous nasal mucosa grafts has many advantages. First of all, it enables complete maintenance of normal nasal physiology since it integrates perfectly with the septal nasal mucosa. In fact, in a previous study, we used a mucoperiosteal graft harvested from the inferior turbinate and the side of this graft exposed during the repair is the respiratory mucosa of the inferior turbinate which perfectly integrates with the remaining septal mucosa [19]. Indeed, the most common failure of autogenous buccal mucosa or skin grafts is dry nose and crusting, since respiratory epithelium is not present [27]. Other endogenous tissues such as temporalis fascia or tragal cartilage can be difficult to handle and to mold into shape, whilst synthetic grafts may have problems of rejection by host tissues [15]. Actually, in most endoscopic techniques an allograft is applied. Only some authors report the use of autologous grafts harvested from the nose with optimal results [14 16 19].

The use of allograft, such as the Alloderm, has the advantage to eliminate donor site morbidity and to fit in all sizes of perforations, acting as an excellent scaffold for re-epithelialisation, but is associated with high costs [1].

Other authors report good results in nasal septal perforation repair only with inferior turbinate grafts, with a rate of success between 83% and 88% [12 16]. The disadvantage is that its bulk that may cause partial obstruction of the airway. At any rate, the addition of a septal mucopericondrial flap could help to sustain the graft. In fact, especially in larger defects, there is a limited amount of mucosa available to provide vascular supply to the graft. Thus, the process of integration becomes more difficult with larger grafts [15].

Some authors report that perforation size is one of the principal factors that can lead to failure of the repair technique. Both Moon and Kim reported a higher probability of developing re-perforation after surgery in patients with large perforation size, because size is inversely proportional to the amount of mucosa available for perforation closure [25 26]. In fact, in large perforations, approximating the mucosal flap almost always causes tension in the perforation site. The vertical height of a perforation has been shown to play a more important role in determining the surgical success than the horizontal length because tension between the floor of the nose and the the dorsum was found to be critical [22]. By "large perforation size", this is considered a perforation whose diameter is < 10 mm for Moon and < 20 mm for Kim [25 26]. In most cases, endoscopic techniques have been used in small-to-medium perforations (0.5-2 cm), but some report good results even in repair of perforations > 2 cm [13 16 19]. It is obvious that in large perforations the engraftment of the graft is more difficult, but an endoscopic approach enables the surgeon to achieve good precision in graft positioning, respecting the rule that the diameter of the grafted material must exceed that of perforation and all margins must be covered with nasal mucosa surrounding the perforation by at least 1 cm without tension [28].

Even for endoscopic techniques the racial criterion has proven to be critical for success in perforation repair. In fact, case series from Oriental authors have shown a lower percentage of success compared to case series by Western authors [11 17 18]. This can be explained by the smaller nasal cavity and septum of Asians and therefore by the consequent insufficient viable tissue to cover the perforation site [26].

Conclusions

Endoscopic techniques can nowadays be considered the gold standard for septal perforation repair. They cause less trauma and provide a better surgical view, helping the surgeon to close various size perforations even in the posterior part of the septum with a high percentage of success. Notwithstanding the high outcome rates, it is not possible to compare the results with other approaches because the number of patients is usually small, leading to statistically insignificant results. Larger series with multicentre studies are desirable to demonstrate the superiority of endoscopic techniques.

References

1 Ayshford CA, Shykhon M, Uppal HS, Et al. *Endoscopic repair of nasal septal perforation with acellular human dermal allograft and an inferior turbinate flap*. Clin Otolaryngol Allied Sci 2003;28:29-33.

2 Mullace M, Gorini E, Sbrocca M, et al. *Management of nasal septal perforation using silicone nasal septal button*. Acta Otorhinolaryngol Ital 2006;26:216-8.

3 Osma U, Cüreoðlu S, Akbulut N, et al. *The results of septal button insertion in the management of nasal septal perforation*. J Laryngol Otol 1999;113:823-4.

4 Teschner M, Willenborg K, Lenarz T. *Preliminary results of the new individual made magnet-based nasal septal button*. Eur Arch Otorhinolaryngol 2012; 269:861-5.

5 Dosen LK, Haye R. *Surgical closure of nasal septal perforation. Early and long term observations.* Rhinology 2011;49:486-91.

6 Watson D, Barkdull G. *Surgical management of the septal perforation*. Otolaryngol Clin North Am 2009;42:483-93.

7 Goh AY, Hussain SS. *Different surgical treatments for nasal septal perforation and their outcomes*. J Laryngol Otol 2007;121:419-26.

8 Hier MP, Yoskovitch A, Panje WR. *Endoscopic repair of a nasal septal perforation*. J Otolaryngol 2002;31:323-6.

9 Meghachi AS, Jankowski R, Védrine PO, et al. *Endoscopic closure of septal perforations by mucosal rotation flaps*. Ann Otolaryngol Chir Cervicofac 2004;121:222-8.

10 Presutti L, Alicandri Ciufelli M, et al. *Nasal septal perforations: our surgical technique*. Otolaryngol Head Neck Surg 2007;136:369-72.

11 Lee HR, Ahn DB, Park JH, et al. *Endoscopic repairment of septal perforation with using a unilateral nasal mucosal flap*. Clin Exp Otorhinolaryngol 2008;1:154-7.

12 Mansour HA. *Repair of nasal septal perforation using inferior turbinate graft*. J Laryngol Otol 2011;125:474-8.

13 Giacomini PG, Ferraro S, Di Girolamo S, et al. *Large nasal septal perforation repair by closed endoscopically assisted approach*. Ann Plast Surg 2011;66:633-6.

14 Kazkayasi M, Yalcinozan ET. *Uncinate process in the repair of nasoseptal perforation*. Aesthetic Plast Surg 2011;35:878-81.

15 Castelnuovo P, Ferreli F, Khodaei I, et al. *Anterior ethmoidal artery septal flap for the management of septal perforation*. Arch Facial Plast Surg 2011;13:411-4.

16 Tastan E, Aydogan F, Aydin E, et al. *Inferior turbinate composite graft for repair of nasal septal perforation*. Am J Rhinol Allergy 2012;26:237-42.

17 Lee DH, Yoon TM, Lee JK, et al. *Clinical utility of the inferior turbinate flaps in the reconstruction of the nasal septum and skull base*. J Craniofac Surg 2012;23:322-6.

18 Chen FH, Rui X, Deng J, et al. *Endoscopic sandwich technique for moderate nasal septal perforations*. Laryngoscope 2012;122:2367-72.

19 Cassano M. *Endoscopic repair of nasal septal perforation with "slide and patch" technique*. Otolaryngol Head Neck Surg 2014;151:176-8.

20 Hanci D, Altun H. *Repair of nasal septal perforation using middle turbinate flap (monopedicled superiory based bone included conchal flap): a new unilateral middle turbinate mucosal flap technique*. Eur Arch Otorhinolaryngol 2015;272:1707-12.

21 Kaya E, Cingi C, Olgun Y, et al. *Three layer interlocking: a novel technique for repairing a nasal septum perforation*. Ann Otol Rhinol Laryngol 2015;124:212-5.

22 Kridel RW. *Septal perforation repair*. Otolaryngol Clin North Am 1999; 32:695-724.

23 Schulz-Coulon HJ. *Three-layer repair of naso-septal repair*. Eur Arch Otorhinolaryngol 2007;264:S265, ric 9.

24 Kridel RW. *Considerations in the etiology, treatment, and repair of septal perforations*. Facial Plast Surg Clin North Am 2004;12:435-50.

25 Kim SW, Rhee CS. *Nasal septal perforation repair: predictive factors and systematic review of the literature*. Curr Opin Otolaryngol Head Neck Surg 2012; 20:58-65.

26 Moon IJ, Kim SW, Han DH, et al. *Predictive factors for the outcome of nasal septal perforation repair*. Auris Nasus Larynx 2011; 38:52-7.

27 Ceylan A, Ileri F, Celenk F, et al. *Upper lateral cartilage inner mucoperichondrial flap technique for the repair of nasal septal perforation*. ORL J Otorhinolaryngol Relat Spec 2007;69:245-50.

28 Re M, Paolucci L, Romeo R, et al. *Surgical treatment of nasal septal perforations. Our experience.* Acta Otorhinolaryngol Ital 2006;26:102-9.

Received: June 22, 2016 - Accepted: November 30, 2016

Address for correspondence: Michele Cassano, via Guerrieri 2, 71100 Foggia, Italy. Tel. +39 0881 736031. Fax +39 0881 736031. E-mail: michele.cassano@unifg.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:493-499; doi: 10.14639/0392-100X-1252

Rhinology

Clinical study of extrapulmonary head and neck tuberculosis in an urban setting

Studio clinico riguardo la tubercolosi extrapolmonare in ambiente urbano

S. MONGA[1], J.N. MALIK[1], S. JAN[1], S. BAHADUR[1], S. JETLEY[2], H. KAUR[3]

[1] Department of Otorhinolaryngology and Head & Neck Surgery, Hamdard Institute of Medical Sciences & Research and HAHC Hospital, Jamia Hamdard University, Delhi, India; [2] Department of Pathology, Hamdard Institute of Medical Sciences & Research and HAHC Hospital, Jamia Hamdard University, Delhi, India; [3] Department of Computer Science & Engineering, Jamia Hamdard University, Delhi, India

SUMMARY

Tuberculosis (TB) of the head and neck region is quite common in endemic countries, but is still misdiagnosed due to its varied presentation and different sites of involvement. The aims of the present study were to present the diversities of presentation of head and neck tuberculosis with the diagnostic predicaments faced during evaluation and to assess treatment response to anti-tubercular treatment (ATT). We analysed 48 patients with head and neck tuberculosis who presented to the Department of Otorhinolaryngology in our tertiary care urban hospital over a period of two years from 2013 to 2015 and recorded their data, which included presenting complaints, local and systemic examination findings, investigation results and treatment outcomes. The results showed that majority (64.5%) of cases were female and none of the patients were HIV positive. The most common manifestation was cervical lymphadenopathy (81.25%) with level II being the most commonly affected (31.3%). Three of the 48 patients had coexisting pulmonary TB. Fine needle aspiration cytology (FNAC), histopathological diagnosis and acid fast bacilli (AFB) staining were used to confirm diagnosis. All patients were treated with Category I ATT, which achieved cure in 96.8% of cases. Though cervical lymphadenitis is the most common presentation of head and neck TB, isolated involvement of the sinonasal region, larynx, oral cavity and other sub-sites are not solely unknown entities. It is, therefore, important for clinicians to be aware of atypical and misleading presentations and consider TB as a major differential diagnosis in the head and neck region, even in non-immunocompromised individuals.

KEY WORDS: Extrapulmonary tuberculosis • Cervical lymphadenopathy • Head and neck • Anti-tubercular therapy

RIASSUNTO

La tubercolosi del distretto testa collo è abbastanza comune nei paesi endemici, ma è ancora sottostimata a causa della presentazione clinica assai variabile e a causa dei differenti siti coinvolti. Pertanto, gli obiettivi di questo studio sono stati quelli di voler descrivere la variabilità di presentazione clinica dei pazienti affetti da tubercolosi del distretto testa-collo, durante la cui valutazione ci si scontra con notevoli difficoltà diagnostiche, e stimare la risposta di questi pazienti al trattamento anti-tubercolare (ATT). Sono stati reclutati 48 pazienti affetti da tubercolosi del distretto testa-collo, i quali si sono presentati tra il 2013 e il 2015 presso il dipartimento di Otorinolaringoiatria del nostro centro di III livello; per ciascuno di essi sono stati raccolti sintomi, reperti obiettivi locali e sistemici, risultati diagnostici e risultati del trattamento. Dai dati è emerso che la maggioranza dei casi (64,5%) erano femmine, e nessuno dei pazienti era HIV positivo. Le modalità di presentazione più comuni sono state le linfoadenopatie cervicali (81,25%), e in particolare quelle coinvolgenti il livello IIB (31,3%). 3 pazienti su 48 erano affetti contemporaneamente da tubercolosi polmonare. Per confermare la diagnosi sono stati utilizzati l'esame citologico su agoaspirato con ago sottile, l'esame istopatologico e la colorazione per evidenziare l'alcol-acido resistenza. Tutti i pazienti sono stati trattati con antitubercolari di prima scelta, i quali hanno permesso di raggiungere la guarigione nel 96,8%. Nonostante la linfoadenite cervicale è la più comune forma di presentazione della tubercolosi del distretto testa-collo, i coinvolgimenti isolati della regione naso-sinusale, della laringe, della cavità orale o di altre sotto-sedi non sono entità sconosciute. Ciononostante, è importante prestare attenzione a queste presentazioni atipiche e misconosciute e considerarle nella diagnosi differenziale del testa-collo, anche in individui non immunocompromessi.

PAROLE CHIAVE: *Tubercolosi extrapolmonare • Linfoadnopatie cervicali • Testa-collo • Terapia anti-tubercolare*

Acta Otorhinolaryngol Ital 2017;37:493-499

Introduction

Tuberculosis (TB) remains a major problem for mankind worldwide, especially in Asia and Africa. There are around 9 million new cases and 2 million deaths from TB worldwide. India has 23% of world's share. As per the WHO TB report, the incidence in India was 167/100,000 population with a mortality of 17/100,000 population for the year 2014. Among the total 1.68 million cases of TB notified in the year 2014, 275,000 cases were of extrapulmonary tuberculosis (EPTB) [1].

Despite introduction and improvements in anti-tubercular chemotherapy, TB (pulmonary and extrapulmonary) is still a leading cause of morbidity and mortality in India. Among the factors associated with the rising incidence of TB in India are increased prevalence of immunodeficiency through HIV, drug addiction, increasingly more clusters of poverty and overcrowding.

While pulmonary TB is the most common presentation, EPTB is also a significant disease entity. In the head and neck region, TB affects the lymph nodes, larynx, middle ear, sinonasal region, oral cavity and pharynx.

The incidence of TB in the head and neck region is quite frequent and provide an interesting area of study because of diverse presentations and due to changing clinical scenarios that have occurred over the past years [2]. These days, gradually evolving patterns of TB that do not manifest characteristic clinical symptoms are frequent, while acute fulminant rapidly progressive patterns are sporadically observed [3].

The aims and objectives of this study were to analyze the clinical profile of head and neck TB patients with radiological, haematological and microbiological investigations and to assess response of these patients to ATT.

Materials and methods

This was a prospective study done in the Department of Otorhinolaryngology and Head Neck Surgery at the Hamdard Institute of Medical Sciences & Research and HAHC Hospital over a period of two years, from 2013 to 2015. All new patients with EPTB of the head and neck region were included in the study. Cases of previous failure, relapse and defaulters were excluded from the study as they are to be managed differently in accordance with the existing Revised National Tuberculosis Control Programme (RNTCP) and WHO guidelines.

Data was obtained from each patient included in the study regarding age, gender, socioeconomic status, tobacco or alcohol addiction, co-morbidities, contact history of TB and presenting and constitutional symptoms. After complete ENT and systemic examination, relevant investigations including haemogram with ESR (Erythrocyte Sedimentation Rate), blood sugar (random), liver and kidney function tests, Mantoux test, HIV and HBsAg were done. Presence/absence of pulmonary TB was diagnosed based on radiological and microbiological (sputum smear) examinations. Fine needle lymph node cytology and/or histopathological examination (HPE) of biopsy of the involved tissue was done.

Treatment was done as per the RNTCP (Revised National Tuberculosis Control Programme) and WHO guidelines. Patients were started on six months of TB drug treatment consisting of a two month "intensive" treatment phase followed by a four month "continuation" phase. For the two month "intensive" TB drug treatment phase, patients received: isoniazid (5 mg/kg body wt), rifampicin (10 mg/kg body wt), pyrazinamide (25 mg/kg body wt) and ethambutol (15 mg/kg body wt) followed by isoniazid with rifampicin at the same dosage for the "continuation" phase. These patients were then followed up during the treatment period and outcomes analysed over a six month period [4].

Results

A total of 48 patients were diagnosed with Head and Neck EPTB in our institute during the period of study. Out of these, 17 were males and 31 females with a male to female ratio of 1:1.8, with most patients being in the adult age group, Figure 1. All patients were HIV-I & II and HBsAg negative. Routine baseline investigations like haemogram, random blood sugar, LFT and KFT were normal in all. None of the patients had any major medical co-morbidities. Out of the total, only 3 were smokers (6.2%).

The most common site of EPTB in the head and neck was cervical lymph nodes (39 patients) followed by sinonasal TB (three patients) - Figure 2. In addition, one had laryngeal TB, two lip TB, one cheek TB, one cutaneous TB of the head and neck region and one was diagnosed as a case of parapharyngeal tubercular abscess. Detailed data of all patients is presented in Table I.

Patients with tubercular cervical lymphadenopathy accounted for 81.25% (39/48) of all cases. The chief complaint with cervical lymph node TB was neck swelling (unilateral 37 and bilateral two cases) - Figure 3. The most

Table I. Selected data of patients with head and neck TB.

Site/ diagnosis	Number	Mantoux (induration ≥ 10 mm diameter)	ESR	Concomittant pulmonary TB	H/o contact with TB	Response
Cervical lymph node TB	39	37	27	3 (7.6%)	5	97.4% (38/39)
Sinonasal TB	3	2	2	0	0	100%
Lip TB	2	1	1	0	1	100%
Laryngeal TB	1	1	1	0	0	100%
Parapharyngeal TB	1	1	1	0	0	100%
Cutaneous TB	1	1	1	0	0	100%
Cheek TB	1	1	1	0	0	100%
TOTAL	48	44 (91.6%)	34 (70.8%)	3 (6.2%)	6 (12.5%)	97.9%

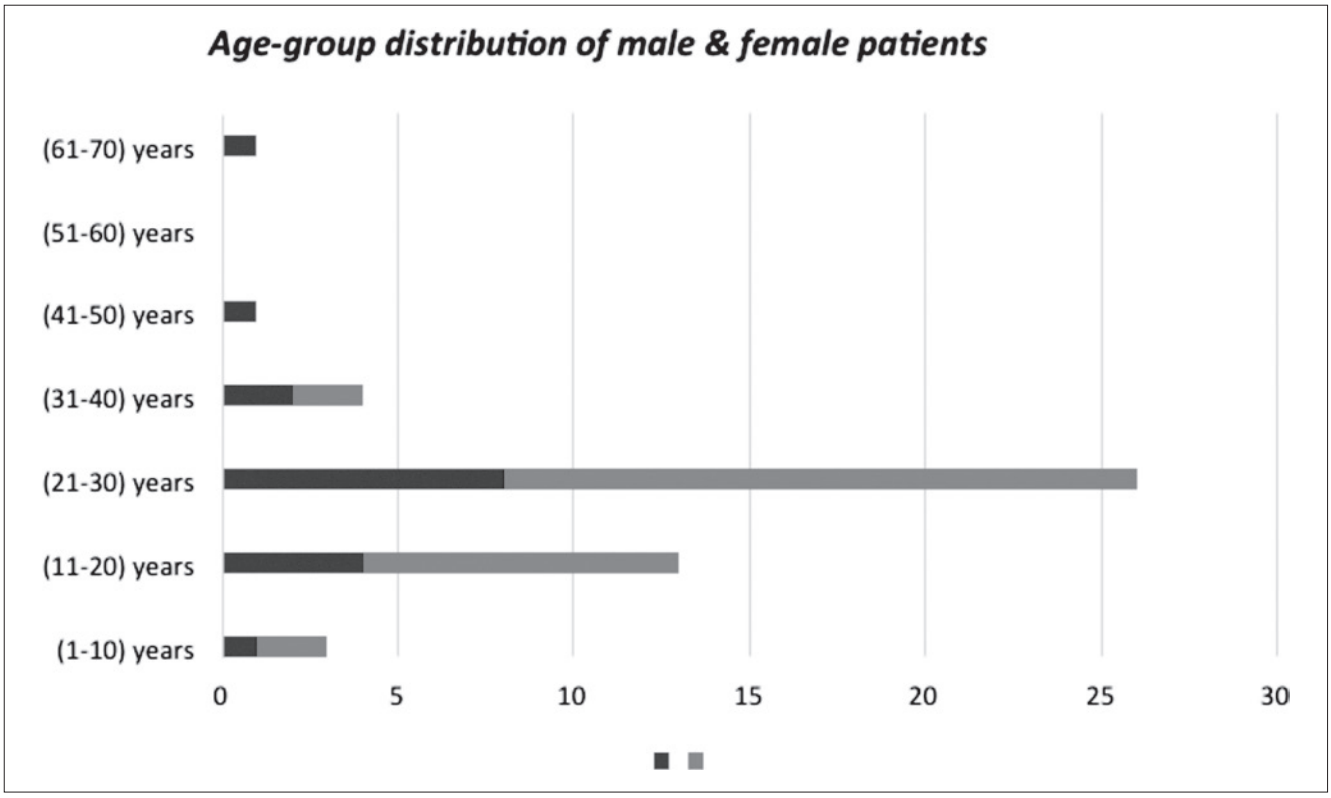


Fig. 1. Age and gender distribution of the patients.

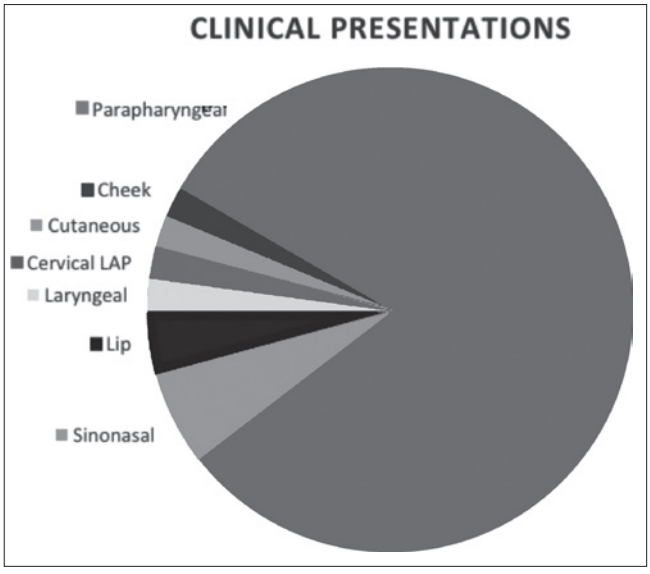


Fig. 2. Site of various EPTB head and neck cases.

commonly affected lymph node was level II (31.3%), followed by level V (25.4%). Further information is shown in Table II.

The commonest age group affected was 21-30 years. There were 11 males and 28 females. These patients also had other complaints including fever 22 (56.4%), weight loss 8 (20.5%) and cough with expectoration 3(7.6%). All patients were sent for FNAC. All except two exhibited granulomatous lymphadenitis of tubercular origin on cytopathology and only 8 were ZN staining positive for AFB. Those two cases had to undergo lymph node excision biopsy for confirmation. Further ancillary investigations for TB were carried out. In majority of cases (n = 37), Mantoux was positive (induration ≥ 10 mm diameter). ESR was raised in 27 patients. Three (7.6%) of these 39 patients had concomitant pulmonary TB. All patients were started on Category I ATT as per RNTCP guidelines. Patients were followed every two weeks for the first two months and monthly thereafter until the completion of treatment or as per need. All responded well to the prescribed ATT with the resolution of symptoms except one patient who had to be given treatment for MDR-TB to which he responded. Routine investigations of all patients remained within normal limits except one patient who suffered from derangement of LFT with the onset of ATT for which further evaluation and dose adjustments were required.

Three patients (6.2%) with nasal TB presented with symptoms of blood-tinged nasal discharge and obstruction. Out of these, two were males and one female. They were diagnosed by nasal endoscopy and biopsy of the inflammatory nasal tissue (Fig. 4), which showed granulomatous lesions of tubercular origin on HPE. None had pulmonary TB. All three responded well to Category I ATT with resolution of disease.

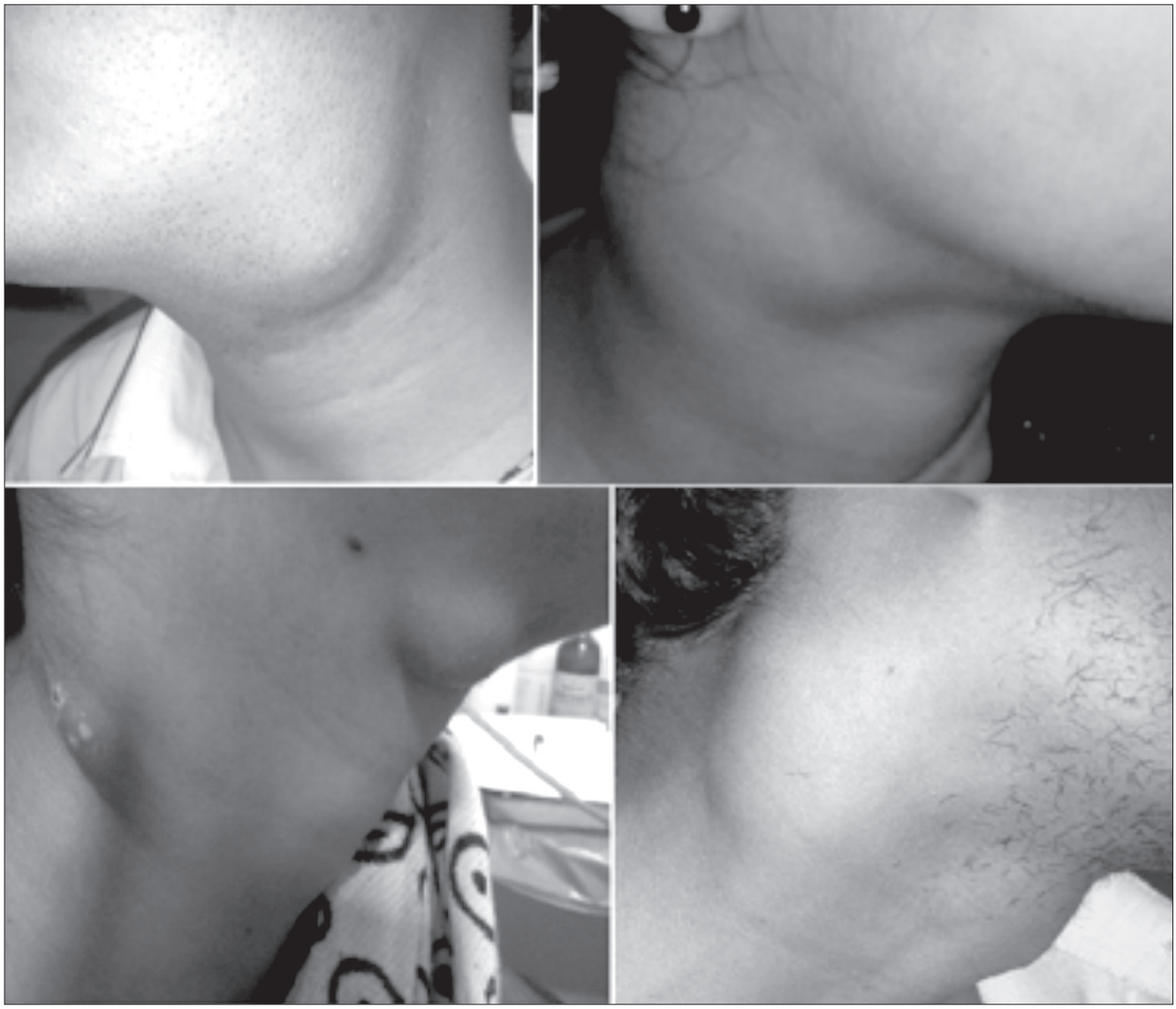


Fig. 3. Different presentations of cervical tuberculosis.

Two patients (4.1%), one male and other female, presented with complaints of lower lip swelling which were positive for TB on cytopathology.

One patient, a young male, presented with a short history of pain and swelling in front of the neck. CECT scan of neck demonstrated involvement of the larynx with erosion of the thyroid cartilage. He was diagnosed on open biopsy by HPE to be laryngeal TB.

One case, a young male, presented with pain throat and dysphagia with fever of 2 weeks' duration. On examination he had a posterior pharyngeal wall bulge which on needle aspiration came out to be pus that was positive for AFB bacilli. CT imaging revealed retropharyngeal abscess with right parapharyngeal extension without associated caries cervical spine (Fig. 5).

One teenage female presented with multiple refractory ulcerations of the facial skin (Fig. 6) which on HPE of biopsies revealed chronic granulomatous lesion consistent with TB.

One patient, a teenage male presented with right cheek swelling which on FNAC showed findings suggestive of buccal space tubercular abscess.

All these patients were Mantoux positive and had raised ESR. None had pulmonary TB. All were started

Table II. Distribution by level of cervical lymph node involvement.

Levels of lymph nodes involved		No. of cases	Percentage
Level I	IA	2	3.9%
	IB	6	11.7%
Level II		16	31.3%
Level III		11	21.5%
Level IV		3	5.8%
Level V		13	25.4%
Level VI		0	0

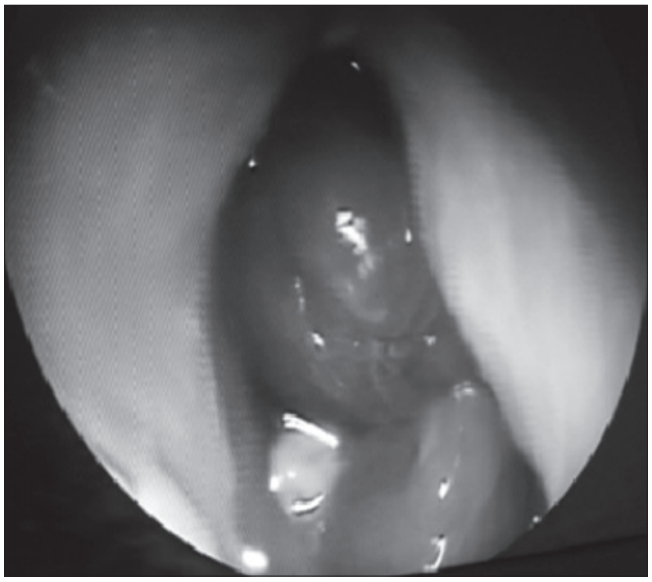


Fig. 4. Endoscopic picture showing polypoidal mucosa and caseous material in sinonasal tuberculosis.

on Category I ATT and were followed for six months. All responded to treatment with resolution of disease. None had any derangements of routine baseline investigations.

Discussion

The female predominance in our study group (64.5% females) is somewhat different from data reported in other Indian studies, which have shown a clear-cut male dominance [5 6].

The mean age of subjects was 23.4 years and most patients were in the age group of 21-30 years. This was similar to the finding observed in the studies Arora et al.(7) and Soumyajit Das et al. [8] in which the most common age group affected was 15-24 years.

Association of socioeconomic status and TB is not well studied, but a study from China by Liu et al. [9] indicated that it is more prevalent in the lower socioeconomic strata. This could be attributed to various factors such as malnutrition, overcrowding, etc. In our study, the economic profile was taken into account as per revised Modified B G Prasad socioeconomic scale [10], and it was observed that most patients were in scale III i.e. middle class (54.1%) - Table III. This is probably due to factors like increasing health awareness in this class and also to the demographics of the hospital catchment area.

In the present study, the most common site of EPTB in the head and neck region was in the cervical lymph nodes (81.25%) followed by sinonasal TB (6.2%), oral cavity (4.1%), laryngeal TB, retropharyngeal region, buccal space and cutaneous TB of the head and neck region (one case each representing 2.05%). In a study by Akkara et al. [5], the most common site in their 211 patients was the cervical lymph node (201 patients, 95.3%), followed by the middle ear (2.8%), larynx (1.4%) and nasal cavity (0.5%). In the study by Ricciardiello et al. [3], the most common site was the cervical lymph nodes (94.12%), followed by the larynx (4.33%), palatine tonsil (0.62%), oral cavity (0.31%), middle ear (0.31%) and nasal cavity (0.31%). In another study by Choudhary [11] in a South London hospital on 33 patients with head and neck TB, they found that 58% (n = 19) had cervical LAP, 9 (27%) salivary gland TB, 2 (6%) laryngeal T, and one (3%) each of nasopharyngeal, hypopharyngeal and ear TB. Thus, while most Indian and International studies reported that the cervical lymph node was the most commonly affected, it is noteworthy that the present study also shows a relatively high proportion of sinonasal TB in the study group.

In the present study, the pattern of lymphadenopathy showed multiple lymph node group involvement in 14 (35.8%) cases, and the commonest involved group was level II (31.3%), either alone or in association with other levels. This corresponds to the findings of other studies (Soumyajit Das et al. [8], BC Jha et al. [12]). On the other hand, Akkara et al. [5] and Dharma Baskota et al. [13] noted that the

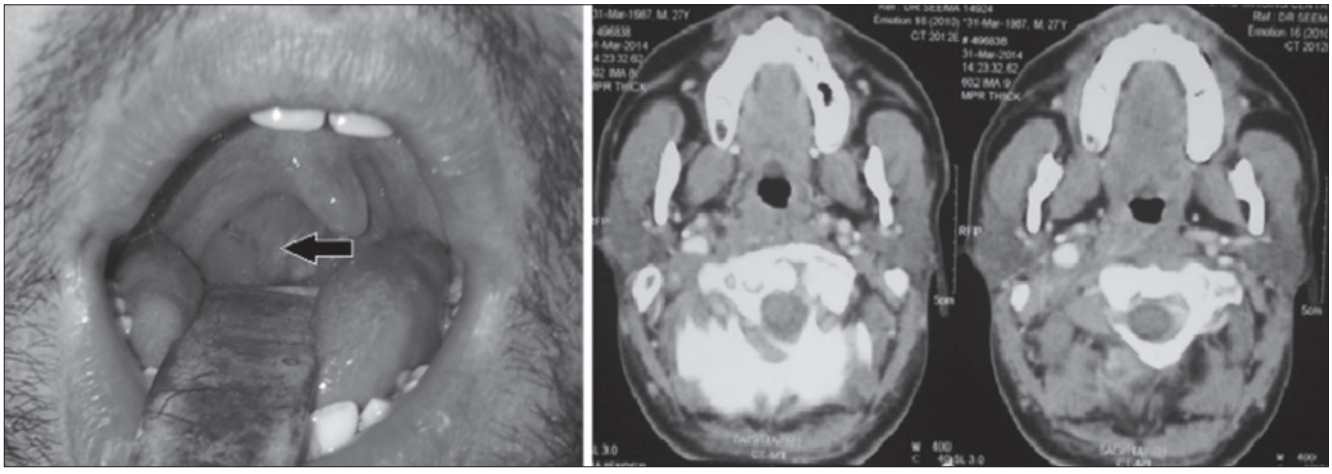


Fig. 5. Clinical presentation and radiological findings of parapharyngeal tuberculosis.

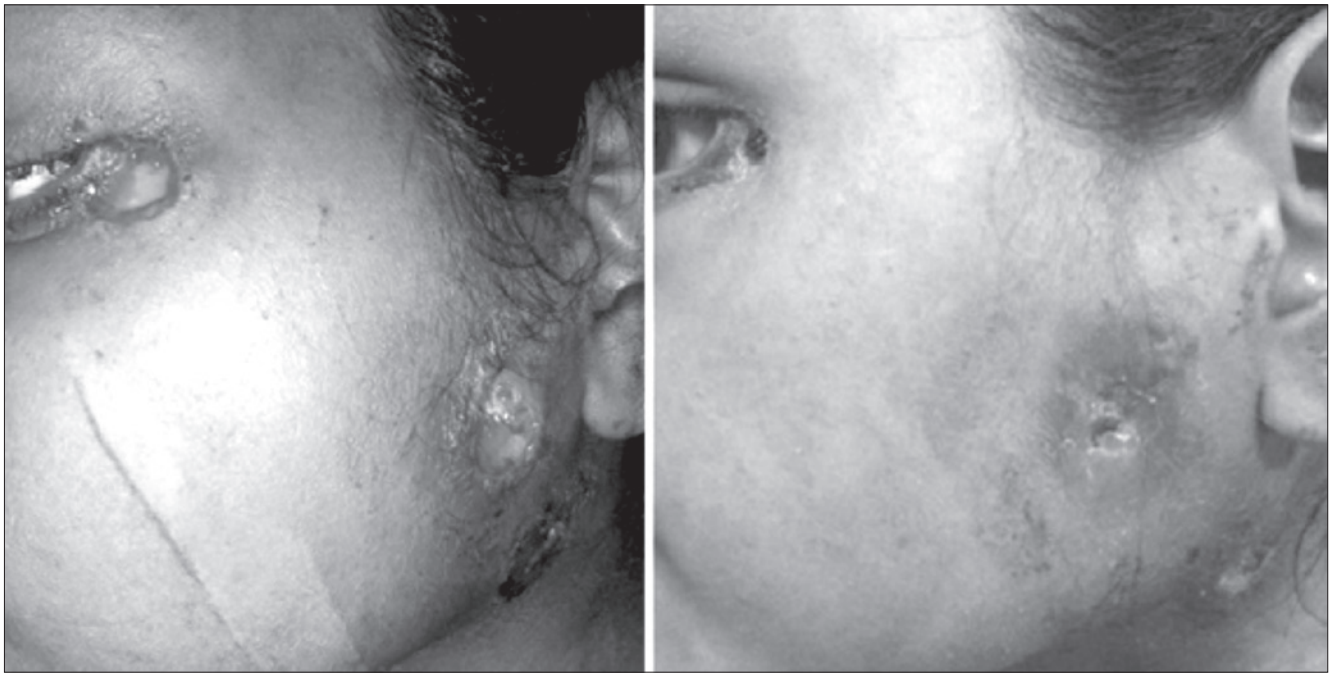


Fig. 6. Cutaneous tuberculosis before and after 2 months of ATT.

posterior triangle was the most commonly affected lymph node. In our study, level V was next, in 25.4% of cases, followed by level III (21.5%), level IB (11.7%), level IV (5.8%) and level IA (3.9%). Level VI and VII involvement was not observed. In the majority of these cases (94.8%), diagnosis was established based on FNAC. The most consistent feature was that of epitheloid granuloma with Langhan's giant cell, acute neutrophillic infiltration with or without evidence of necrosis. AFB by ZN staining could be demonstrated in 8 of 39 patients who underwent FNAC. However, in 2 (5.1%) cases surgical excision biopsy was needed to confirm the diagnosis. Malakar et al. [14] also found that FNAC was sensitive to detect tubercular lymphadenopathy in 79.1% of cases. However, in their study, in 41.6% of patients with tubercular lymphadenopathy, the aspirates were positive for AFB. It has been suggested by Chakravarty et al. [6] that the paucibacillary nature of the tissue other than sputum compromises the diagnosis rate in TB. Three of these cases in our study also had concomitant pulmonary TB (7.7%).

Table III. Patient distribution as per Revised modified BG Prasad socioeconomic classification scale (2014).

Socioeconomic class (with per capita monthly income in Rupees)	Number of patients (%)
Upper class (5357 & above)	2 (4.1%)
Upper middle class (2652-5356)	8 (16.6%)
Middle class (1570-2651)	26 (54.1%)
Lower middle class (812-1569)	10 (20.8%)
Lower class (< 811)	2 (4.1%)

Although culture of mycobacteria in special media is the gold standard for diagnosis of tuberculosis, cultivation has limited yield and is a slow technique that often takes weeks, so that the results are generally received when the patient is already on treatment. Alternatively, the tissue may be subjected to molecular diagnostic methods like polymerase chain reaction or even DNA specific probes for rapid diagnosis and subtyping of mycobacteria, especially in paucibacillary samples. The GeneXpert platform based *Xpert MTB/RIF* is commonly used, which has also been advocated by the WHO especially for diagnosis in HIV patients [1]. However, these techniques are not only expensive and technically demanding, but also not routinely available in many centres in India.

Although nasal TB is a very rare entity even in countries with high disease load, we observed three cases over the two year period. Primary TB of the nasal cavity or oral cavity are seen rarely in countries even with high disease load like India. However, we observed three cases of primary sinonasal and two cases of lip TB without pulmonary involvement over a period of two years. Most common symptom in sinonasal TB was blood tinged nasal discharge, and in the oral cavity the commonest presentation was lower lip cystic swelling. All these five cases were diagnosed by HPE of the biopsy taken from the involved site. Akkara et al. [5] found only one case of nasal TB and no oral TB in their study of 211 head and neck EPTB, while Soumyajit Das et al. [8] did not report any case.

Though hoarseness is the commonest presenting complaints in laryngeal TB due to spread from associated

pulmonary involvement, our patient was a young male who presented with chief complaints of painful swelling over the anterior aspect of neck, which on CT showed necrosis of the thyroid cartilage; HPE of the biopsy confirmed it as TB. He had no associated pulmonary involvement.

We did not observe any case of tubercular otitis media or thyroid gland TB in our two year study.

Contact history in a diagnosed case of TB was present in 5 (12.8%) of cases, while in the majority of cases (87.2%) it was absent. It is similar to the trend seen in the study by Soumyajit Das et al. [8], which reported 34.9% of cases with positive contact history; history of contact was absent in 65% cases.

Constitutional symptoms were present in 29 (59%) of cases, while it was absent in 41% of cases similar to the study by Soumyajit Das et al. [8], where they were present in 38.1% of cases.

Category I treatment (as per present RNTCP) was found to be effective in all except one case showing favourable response at the end of 6 months of treatment. This result is similar to the that observed in study by Soumyajit Das et al. [8], which showed 96.8% of treatment success. In addition, there were no significant derangements in baseline investigations during treatment except in one case.

Conclusions

TB of head and neck region can have atypical and varied manifestations in an endemic country such as India. Otolaryngologists should be aware of the diagnostic dilemmas presented in these patients in order to avoid misdiagnosis and unnecessary delay in treatment, especially in cases without pulmonary involvement. A team approach of surgeons, chest physicians, microbiologists and pathologists for management is highly recommended. Category I ATT as per RNTCP is quite effective for EPTB of the head and neck region.

References

1 Global tuberculosis report 2015 [Internet]. *Geneva World Health Organization 2015*. Available at: http://www.who.int/tb/publications/ global_report/en/. Accessed March 01, 2016.

2 Aisenberg GM, Jacobson K, Chemaly RF, et al. *Extrapulmonary tuberculosis active infection misdiagnosed as cancer: Mycobacterium tuberculosis disease in patients at a comprehensive cancer center (2001-2005)*. Cancer 2005;104: 2882-7.

3 Ricciardiello F, Martufi S, Cardone M, et al. *Otorhinolaryngology-related tuberculosis*. Acta Otorhinolaryngol Ital 2006;26:38-42.

4 *Treatment of Tuberculosis: Guidelines. 4th edition*. Geneva: World Health Organization; 2010. 3, Standard treatment regimens.

5 Akkara SA, Singhania A, Akkara AG, et al. *A study of manifestations of extrapulmonary tuberculosis in the ENT region*. Indian J Otolaryngol Head Neck Surg 2014;66:46-50.

6 Chakravorty S, Sen MK, Tyagi JS. *Diagnosis of extrapulmonary tuberculosis by smear, culture, and PCR using universal sample processing technology*. J Clin Microbiol 2005;43:4357-62.

7 Arora VK, Gupta R. *Trends of extra-pulmonary tuberculosis under Revised National Tuberculosis Control Programme: A study from South Delhi*. Indian J Tuberc 2006;53:77-83.

8 Das S, Das D, Bhuyan UT, et al. *Head and neck tuberculosis: scenario in a tertiary care hospital of North Eastern India*. J Clin Diagn Res 2016;10:MC04-7.

9 JJ Liu, HY Yao, EY Liu. *Analysis of factors affecting the epidemiology of tuberculosis in China*. Int J Tuberc Lung Dis 2005;9:450-4.

10 Mangal A, Kumar V, Panesar S, et al. *Updated BG Prasad socioeconomic classification, 2014: a commentary*. Indian J Public Health 2015;59:42-4.

11 Choudhury N, Bruch G, Kothari P, et al. *4 years' experience of head and neck tuberculosis in a south London hospital*. J R Soc Med 2005;98:267-9.

12 Jha BC, Dass A, Nagarkar NM, et al. *Cervical tuberculous lymphadenopathy: changing clinical pattern and concepts in management*. Postgrad Med J 2001;77:185-7.

13 Baskota DK, Prasad R, Sinha BK, et al. *Distribution of lymph nodes in the neck in cases of tuberculous cervical lymphadenitis*. Acta Otolaryngol 2004;124:1095-8.

14 Malakar D, Jajoo I, Swarup K, et al. *A clinical evaluation of fine needle aspiration cytology in the diagnosis of lymphadenopathy*. Ind J Tub 1991;38:17-9.

Received: May 27, 2016 - Accepted: January 15, 2017

Address for correspondence: Junaid Nasim Malik, Department of Otorhinolaryngology and Head & Neck Surgery, Hamdard Institute of Medical Sciences & Research and HAHC Hospital, Jamia Hamdard University, 10062 Delhi, India. Tel. +91 9811710433. E-mail: drjunaidnasim@yahoo.co.in

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:500-508; doi: 10.14639/0392-100X-1574

AUDIOLOGY

Acquired sensorineural hearing loss in children: current research and therapeutic perspectives

Sordità infantile acquisita: stato dell'arte della ricerca e prospettive terapeutiche

M. RALLI[1], R. ROLESI[2], R. ANZIVINO[2], R. TURCHETTA[3], A.R. FETONI[2]

[1] Department of Oral and Maxillofacial Sciences, Sapienza University of Rome, Italy; [2] Department of Otolaryngology, Catholic University of Sacred Heart, Rome, Italy; [3] Department of Sense Organs, Sapienza University of Rome, Italy

SUMMARY

The knowledge of mechanisms responsible for acquired sensorineural hearing loss in children, such as viral and bacterial infections, noise exposure, aminoglycoside and cisplatin ototoxicity, is increasing and progressively changing the clinical management of affected patients. Viral infections are by far the most relevant cause of acquired hearing loss, followed by aminoglycoside and platinum derivative ototoxicity; moreover, cochlear damage induced by noise overexposure, mainly in adolescents, is an emerging topic. Pharmacological approaches are still challenging to develop a truly effective cochlear protection; however, the use of steroids, antioxidants, antiviral drugs and other small molecules is encouraging for clinical practice. Most of evidence on the effectiveness of antioxidants is still limited to experimental models, while the use of corticosteroids and antiviral drugs has a wide correspondence in literature but with controversial safety. Future therapeutic perspectives include innovative strategies to transport drugs into the cochlea, such as molecules incorporated in nanoparticles that can be delivered to a specific target. Innovative approaches also include the gene therapy designed to compensate for abnormal genes or to make proteins by introducing genetic material into cells; finally, regenerative medicine (including stem cell approaches) may play a central role in the upcoming years in hearing preservation and restoration even if its role in the inner ear is still debated.

KEY WORDS: Acquired hearing loss • Pediatric otolaryngology • Genetic diagnosis • Cochlear implant

RIASSUNTO

La conoscenza dei meccanismi fisiopatologici delle condizioni responsabili dell'ipoacusia acquisita nei bambini, tra cui le infezioni virali e batteriche, l'esposizione al rumore, l'ototossicità da chemioterapici ed antibiotici aminoglicosidici, è in costante aumento e sta portando ad un progressivo cambiamento della gestione diagnostica e clinica del bambino ipoacusico. Le infezioni virali rappresentano la causa più frequente di sordità infantile acquisita, seguita dalla tossicità di antibiotici e chemioterapici; mentre l'esposizione al rumore, soprattutto negli adolescenti, rappresenta un fattore emergente. Le terapie farmacologiche protettive attualmente in uso includono steroidi, antiossidanti, antivirali; l'efficacia degli antiossidanti è ancora in fase di conferma clinica anche se vi sono significative evidenze sperimentali, mentre i farmaci steroidei ed antivirali sono certamente validi seppur la loro tossicità sistemica rappresenti ancora un problema non chiarito per i quali la somministrazione locale potrebbe rappresentare una possibile evoluzione. Le prospettive di ricerca future includono l'uso di nanoparticelle per veicolare molecole direttamente nel sito di danno; inoltre, la terapia genica con l'inserimento di materiale genetico all'interno delle cellule per la cura di condizioni da alterazione del patrimonio genetico con la produzione di proteine normali, potrebbe svolgere un ruolo rilevante nella cura e soprattutto nella prevenzione delle sordità acquisite; infine, la terapia rigenerativa e l'impianto delle cellule staminali, nonostante il loro ruolo nell'orecchio interno sia ancora dibattuto, per le notevole limitazioni del loro impiego, potrebbe trovare un ruolo nei processi riparativi più che nella differenziazione in cellule sensoriali.

PAROLE CHIAVE: *Ipoacusie acquisite • Otorinolaringoiatria pediatrica • Diagnosi genetica • Impianto cocleare*

Acta Otorhinolaryngol Ital 2017;37:500-508

Introduction

Advances in the development of diagnostic tools, neuroimaging techniques and molecular biology are rapidly changing the landscape of management in children affected by sensorineural hearing loss (SNHL) [1]; new cases of idiopathic hearing loss (HL) are significantly decreasing thanks to the introduction of diagnostic and therapeutic tools that go well beyond the traditional audiological examination and contribute to more accurate diagnosis and selective therapeutic approaches [2]. In the past, treatment of SNHL in children was performed exclusively with the use of hearing aids; in recent years, cochlear implants have radically changed the prognosis of deafness, and now play a central role in treatment of SNHL [3].

The better understanding of the mechanisms involved in the acquired HL in children, such as viral and bacterial infections, noise exposure, aminoglycoside and cisplatin ototoxicity, gives way to innovative therapeutic approaches. It is becoming evident that oxidative stress is a final common endpoint for complex converging events, some genetically determined and some triggered by different stressors. These

pathways of oxidative stress are the major causes of most types of SNHL, including hereditary drug-induced- and noise-induced HL [4-7]. At the same time, advances in molecular analysis and identification of new genetic alterations play a significant role in the improvement of aetiological classification [8 9] and identification of different sensitivity to exogenous factors of specific genotypes and how they influence susceptibility to ototoxicity in children exposed to chemotherapeutic agents and antibiotics [10-14].

Therapeutic approaches targeting the mechanism of cochlear damage are challenging, while hearing research is focusing on new strategies for treatment including the regeneration of neural epithelium and ganglion neurons through gene therapy, implantation of stem cells, and reactivation of the processes of cell differentiation [15]. In this paper, current pharmacological approaches will be reviewed.

Current pharmacological approaches

Corticosteroids

Cochlear inflammation in children has been observed in several pathophysiological conditions, such as electrode insertion during cochlear implant surgery, bacterial meningitis, labyrinthitis, otitis media, cisplatin treatment and autoimmune diseases [16]. TNF-alpha is a key pro-inflammatory and pro-apoptotic molecule in the cochlea, released both by fibroblasts in the spiral ligament and by outer hair cells and supporting cells in response to stress factors [17]. As seen in conditions such as HL following meningitis, inflammatory mechanisms can induce damage of the spiral ligament, death of hair cells, ossification of the cochlea and disruption of cochlear homeostasis [18].

On this basis, it is well known that systemic administration of steroids, such as dexamethasone, methylprednisolone and triamcinolone, is the most common clinical approach for cochlear inflammation in children. This approach is established on the empirical data and experimental observations of anti-inflammatory and immune suppressive activity of these molecules in the cochlea [19 20]. However, the serious side effects of systemic administration can be avoided by local intratympanic administration or intracochlear application of polymers through the round window or by a micro-osmotic pump [21 22].

As previously described, protection from electrode insertion trauma in cochlear implantation represents a common condition in which steroid therapy is used [23]. Damage can be immediate or delayed, due to elevated noise and vibrations during surgery, insertion of the array, or activation of immunological and inflammatory mechanisms [24]. Prolonged treatment with steroids seems prevent both early and late damage [25]. These observations support the clinical use of dexamethasone administered through an array to guarantee the preservation of residual hearing, an important target in cochlear implant surgery. Experimental observations show that dexamethasone administered through a cochleostomy before implantation reduces signs of inflammation and improves the density of ganglion neurons; in addition, the availability of the drug increases from 2% to 20% if the infusion was performed 1 hour to 30 minutes before surgery [26]. An electrode (Nucleus 24 Contour, by Cochlear®) having a built-in channel for the administration of steroids has been proposed [27], and the reduction of electrode impedance values in patients treated with triamcinolone has been shown [28 29]. However, it is questionable whether a single application of steroids can interfere with a mechanism of continued damage and if the application of topical steroids may favour chronic problems such as infections. In a recent study in an animal model, dexamethasone (4 mg/ml) was parenterally delivered via a mini-osmotic pump for either 3 or 7 days; the delayed administration was more effective in preserving hearing than a 3-day delivery [30].

A major use for steroids in children is treatment, in addition to antibiotic therapy, of bacterial meningitis. Community-acquired bacterial meningitis continues to be a heavy toll even in developed countries, despite the implementation of childhood vaccination programs and effective antimicrobial agents. The most common aetiological agents are *Streptococcus pneumoniae* and *Neisseria meningitidis*. Today about two-thirds of cases in Europe and United States are pneumococcal meningitis, and despite advances in medical care mortality is about 15-30% with neurological sequelae, including HL, occurring in about 30-50% of surviving patients. It is known that bacteria cause the release of pro-inflammatory factors, breakdown of the blood-labyrinth barrier and loss of ganglion neurons which lead to fibrosis and ossification of the cochlear turns. A recent experimental study demonstrated that administration of betamethasone in a model of pneumococcal meningitis could reduce SNHL and loss of ganglion neurons [31]. However, in humans, a large series of studies involving nearly 600 cases in a population from Malawi did not support the effectiveness of treatment with dexamethasone [32]. In 2010, an epidemiological Cochrane analysis concluded that the effectiveness of an adjuvant treatment with steroids in children in developing countries is quite dissimilar [33]. A more recent Cochrane review examined the effect of adjuvant corticosteroid therapy versus placebo on mortality, HL and neurological sequelae in people, providing evidence that corticosteroids significantly reduced HL and neurological sequelae, but not overall mortality. Surprisingly, corticosteroids reduced severe HL in children with *Haemophilus influenzae* meningitis [34].

Over 30 years ago glycerol, a diuretic and hyperosmotic agent, was used to reduce the HL sequelae of *Haemophilus meningitis* [35]. These results were corroborated by another study conducted in Finland, demonstrating equal efficacy of glycerol and dexamethasone [36]. On the contrary, a recent study showed that both intravenous administration of dexamethasone and oral administration of glycerol (or the combination of the two) could not prevent HL, suggesting

that the children's general conditions and age are effective predictors for appearance of HL sequelae [37]. Despite the extensive use of steroids in treatments of conditions including ischaemia, viral infection and reactivation and microtrauma, better knowledge on the immuno-system of inner ear and the mechanism of immuno-mediated inner ear disease are still under needed, and therefore the precise mechanism of corticosteroids remains unclear. The inner ear was considered to be "immune-privileged" and to exclude all immunocompetent cells except in the endolymphatic sac; however, recent studies have demonstrated the presence of immunoreactive cells placed in other parts of the cochlea, mainly macrophages residing in the spiral ligament and spiral limbus. Recent advances in inner ear immunology are promising for a more rational and effective use of steroids in inner ear diseases.

Antioxidants

Recently, many antioxidant-based protocols have been introduced for multiorgan dysfunction in newborns who need intensive care, including hypoxia and reperfusion damage, sepsis and ototoxic drug administration. Newborn-preterm infants are susceptible to oxidative damage because of the immature antioxidant system and an environment much richer in oxygen; in addition, the preterm infant has increased susceptibility to infection and inflammation, which increases oxidative stress. In clinical practice, early markers of oxidative stress indicate that prenatal or peri-natal prophylactic use of antioxidants could help to prevent or at least reduce oxidative stress related diseases in newborns. Several protocols with lutein, melatonin, oxygen and magnesium and calcium channel blockers such as flunarizine have been introduced. Lutein administration in newborns, at 12 and 36 hours after birth, increases the levels of physiological antioxidant activity decreasing total hydroperoxides [38]. Hypothermia, a phenomenon which notoriously slows oxidative metabolism, has been proposed for prevention and treatment of hypoxic-ischaemic encephalopathy [39]. Interestingly, bilirubin showed a protective effect against oxidative stress at low levels, but was severely toxic at higher levels. We demonstrate that a polyphenol, ferulic acid, in a noise induced damage model, upregulates the expression of the haemeoxygenase 1 (HO-1) gene (Fig. 1), which is a phase two endogenous antioxidant enzyme with the ability to: (i) degrade haeme, which in the presence of ROS generates lipid peroxidation, (ii) produce biliverdin, which is the precursor of bilirubin with antioxidant properties [40].

Numerous studies have documented that continuous noise exposure in infants in newborn ICUs induces cochlear damage that can be incremented by the synergic effects of other stressors including hypoxia/reperfusion and aminoglycoside treatment. Paediatricians are encouraged to monitor sound in the newborn ICU and within incubators, and a noise levels > 45 dB should be avoided. Furthermore,

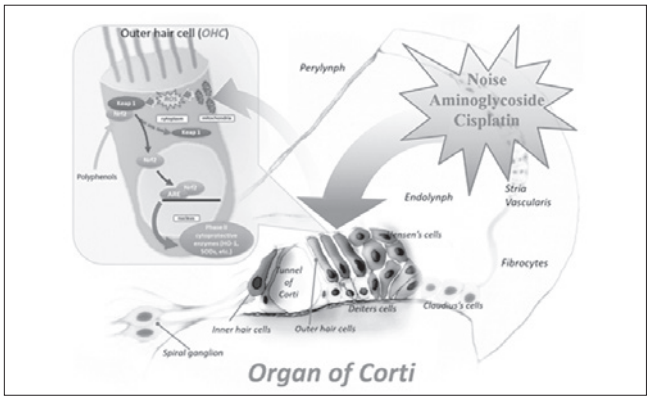


Fig. 1. Polyphenols can activate the Nrf-2/HO-1 pathway. We have demonstrated that many polyphenols (ferulic acid, curcumin) can potentiate endogenous antioxidant defenses both in outer hair cells and spiral ganglion neurons, promoting inactivation of the Nrf2-Keap1 complex. As a consequence, the enhancement of Nrf-2 nuclear translocation appears to upregulate the expression of many Phase-II cytoprotective enzymes (HO-1, SODs).

an estimated 12.5% of teenagers and adolescents aged 6-19 years have suffered permanent hearing damage from excessive exposure to recreational noise. Thus, strategies for hearing prevention and protection are desirable. Unfortunately, even if several experimental studies have shown the beneficial effects of antioxidant supplementation, limited results have been reported in clinical studies. Experimentally, the effectiveness of coenzyme Q10 and N-acetylcysteine has been extensively demonstrated. In a recent paper, Fetoni et al. demonstrated that a soluble formulation of CoQ10, called Q-ter, significantly reduces mitochondrial damage, lipid peroxidation and oxidative damage of cellular proteins induced by oxidative stress and decreases signs of apoptosis [41]; these data were confirmed by the same authors in which they also evaluated systemic versus transtympanic modality [42,43]. In addition, in a pilot prospective study, randomised and double-blinded, significant hearing protection was shown in a group of young volunteers that received Qter 200 mg once a day for 7 days before exposure to white noise at 90 dB HL for 15 minutes [44].

N-acetylcysteine (NAC) is a derivative of cysteine that acts as an augmenter of the antioxidant glutathione reserves in the body. NAC is used as a mucolytic agent and in the treatment of diseases and conditions caused by oxidative stress, such as paracetamol toxicity and nephropathy induced by contrast agents [45]. In vivo studies have shown that NAC works as a scavenger of ROS, but its main antioxidant activity derives from being a precursor of cysteine, itself a precursor in the formation of glutathione. In addition, NAC protects against mitochondrial damage and reduces glutamate excitotoxicity both in outer hair cells and dendrites of afferent neurons. NAC is one of the few molecules tested in clinical trials involving children affected by acoustic trauma. In a randomised placebo-controlled double-blind study including a population of adolescents exposed to loud music, oral NAC

(900 mg single dose) or placebo were given 1 hour before exposure to loud music. This study did not demonstrate a significant difference on the temporary threshold shift in hearing between the treated and placebo groups [46].

Sodium salicylate is a potent antioxidant and anti-inflammatory drug with several effects on hearing function [47]. Salicylate hydroxyl groups destroy free radicals and promote translocation of the anti-apoptotic transcription factor NF-kB, preventing HL and cochlear damage especially when induced by aminoglycosides [48] and cisplatin [49]. In a double-blind controlled study involving patients treated with gentamicin for acute infections, aspirin at a dose of 3 g/day for 14 days showed a significant level of hearing protection compared with placebo [50]. However, long term treatment with salicylate has been reported to impair auditory neural activity [51] and to induce tinnitus and HL [52 53]. Antioxidants represent the most rational and safe approach for treatment of SNHL, although more data from clinical trials is needed for better knowledge of doses and timing of treatment.

Protective agents against cisplatin-induced ototoxicity

Platinum compounds form the mainstay of currently used chemotherapeutic regimens for several malignancies in paediatric patients, such as neuroblastoma, germ-cells tumours, osteosarcomas, hepatoblastomas, brain tumours and relapsed and refractory lymphomas. Debilitating dose dependent side effects include nephrotoxicity, myelosuppression, neurotoxicity and HL. Although deafness is not a life-threatening condition and its degree is highly variable, HL causes communicative disorders in affected children that can result in a poorer quality of life. Thus, the development of effective strategies with protective molecules acting on the side effects without affecting or decreasing antitumour activity is recommended.

Several studies have demonstrated the effectiveness of sodium thiosulphate (STS) protection against cisplatin cytotoxicity [54 55]. This molecule acts through its thiolic groups as a chelator of cisplatin, forming an inactive complex that prevents the absorption of cisplatin into the cell; unfortunately, differently from animal study results, the drug has been reported to reduce the effectiveness of systemic cisplatin therapy [56]. However, it has been shown that treatment with a delayed high dose of intravenous STS in children with malignant brain tumours a few hours after administration of carboplatin results in hearing protection without affecting the antineoplastic effect of cisplatin through alteration of the blood-brain barrier system [57].

WR1065, the active metabolite of amifostine, has shown remarkable radio- and chemoprotective effects both in vitro and in vivo [58]. The conversion of amifostine to WR1065 is catalysed by alkaline phosphatase and depends on the presence of alkaline pH. Differences in the concentration of alkaline phosphatase of normal versus cancer tissues can result in greater conversion of amifostine in normal tissues, providing relatively selective cytoprotection. The timing of the drug administration is an important factor for its protective efficacy and only limited pharmacokinetic data are available for children [59 60]. Data from literature are often contradictory and several clinical reports focus on the peripheral neurotoxicity without studying HL.

Other protective drugs have been experimented in animal and human studies during the past few years; these include alpha-lipoic acid [61], trichostatin A [62], oxytocin [63], resveratol [64], hesperetin [65] and lutein [66]. However, data are preliminary and have not been confirmed. From a critical reading of the literature, a lack of knowledge of the pharmacokinetic mechanisms, particularly in children, and a lack of data on dosage and ways of administration appear to be the main obstacles. Interestingly, attention has been more recently focused on the opportunity of molecular targeted approaches for cancer prevention and therapy and the use of adjuvant chemotherapeutics to overcome the limitations of cisplatin. Owing to their safe use, some polyphenols, such as curcumin, might modulate important pathways or molecular targets in cancers. We evaluated curcumin as an adjuvant molecule to cisplatin, demonstrating that curcumin attenuated all stages of tumour progression (survival, proliferation) and, by targeting pSTAT3 and Nrf-2 signalling pathways, provided chemosensitisation to cisplatin in vitro and protection from its ototoxic adverse effects in vivo. In the perspective of a personalised approach of cancer therapy, the beneficial effects of curcumin as an adjuvant agent to cisplatin offer strong preliminary data for clinical studies in humans [67]. Innovative approaches modulating the chemoresistence to cisplatin and acting in the prevention of its side effects is challenging for the future of antineoplastic therapy, especially in children who are more sensitive than adults to adverse effects such as ototoxicity.

Antiviral therapy for CMV infection

CMV is also a major cause of morbidity and occasional mortality in newborn infants. In recent years, it has become evident that CMV is the most important cause of congenital infection in the developed world, and that it frequently leads to mental retardation and developmental disability including HL and neuro-developmental delay. The incidence of congenital CMV infection ranges from 0.5% to 2% of all live births. Maternal CMV seroprevalence varies widely, ranging from 45% to 100%, with higher prevalence and earlier CMV acquisition associated with lower socioeconomic status. Transmission can occur in mothers with no evidence of CMV immunity (primary infection) and in women with preexisting antibodies either by reactivation of previous maternal infection or by acquisition of a different viral strain during pregnancy. Primary maternal CMV infection during pregnancy is associated with a greater risk of in utero transmission, although about two-thirds of infants with congenital CMV infection are born from mothers with preexisting antibodies against CMV. Among congenitally CMV infected infants,

approximately 10-15% are symptomatic at birth. Clinical manifestations range from mild and transient symptoms to severe multi-system dysfunction including intrauterine growth restriction, petechiae, jaundice, hepatosplenomegaly, microcephaly, chorioretinitis and SNHL, which represents the most common non-genetic cause of SNHL. It has been estimated that HL occurs in nearly 50% of infants with symptomatic congenital CMV infection, while 7% of asymptomatically infected infants develop HL with delayed onset, or a progressive or fluctuating course [68].

The measure of viral load, as determined by polymerase chain reaction (PCR) assay in blood, is a biomarker and a predictor of sequelae development. The effectiveness of control of viral infection during pregnancy and in the perinatal period is essential in decreasing early and late disorders and mortality. At present, there are no approved treatments during pregnancy to prevent or ameliorate the severity of foetal CMV infection. However, ongoing trials are studying treatment with hyperimmune globulin (HIG), pooled intravenous immunoglobulin and antiviral drugs to prevent mother-to-child transmission and ameliorate foetal sequelae. Currently, four antiviral drugs are available against CMV: ganciclovir, valganciclovir, foscarnet and cidofovir. Treatment with antiviral drugs cannot be recommended for use during pregnancy because of the limited evidence on their safety and efficacy [69]. Due to the increasing incidence of CMV infection, the need for antiviral therapy in infants and children is growing, and progresses in vaccine therapy will probably lead to the eradication of the infection [70]. Although there are limited data on the dosage, pharmacokinetics, safety and risk of adverse effects for some of these antiviral agents, the systemic administration of ganciclovir, due to its low oral availability, and its oral prodrug valganciclovir, have been extensively studied in the newborn [71]. Among adverse effects, it is important to highlight renal toxicity, which is irreversible for cidofovir and foscarnet, and neutropenia, more frequently associated with ganciclovir and valganciclovir. In addition, these drugs have other potentially serious toxic effects such as carcinogenicity, teratogenicity and azoospermia and deposition in bone or teeth (foscarnet) that may have significant implications when used in children [72].

For these reasons, antiviral treatment should be reserved to severe cases. The most common treatment is intravenous ganciclovir (12 mg/kg/d in 2 doses), whereas oral administration of valganciclovir (32 mg/kg/d in 2 doses) could be an alternative in some cases [73]. However, there is a lack of clinical experience in the post-natal period; there is little evidence of efficacy and pharmacokinetic studies are lacking in premature infants [74]. Duration of therapy is a controversial factor, as it should be continued for at least 2 to 6 weeks for intravenous therapy and up to 6 months for oral therapy; duration of treatment should be correlated with PCR monitoring [75]. Prophylaxis or treatment with acquired infection immunoglobulin in preterm infants showed no benefit to justify its use in all children. The treatment of children with early infection is effective in reducing HL in case of brain infection manifestations, but has been shown to be ineffective in asymptomatic infections. In a retrospective paper, it appears that about 90% of children maintain or enhance normal hearing after treatment, while the data on the efficacy of treatment in children with late diagnosis of HL are still limited [76]. Serological testing in at-risk pregnant women is recommended in prevention of conceptional infection, and identification in early pregnancy by serological testing may improve outcomes for CMV prevention [69]. Trials are needed to demonstrate the effectiveness of the use of antiviral molecules in less severe disease. The absence of guidelines is a major problem for management of CMV infection; however, the International Congenital Cytomegalovirus Recommendations Group has recently provided recommendations for prevention, diagnosis and treatment that are summarised in Figure 2 [77].

New perspectives in HL in children

Nanoparticles

The use of nanoparticles has rapidly increased in hearing research in the past decade and probably represents the experimental approach that is closest to clinical applications for drug, peptide or gene therapy. In the inner ear, the most studied nanoparticles are biodegradable liposomes, nanocapsules and lipid micelles. The history of nanoparticles is quite recent and there are still many doubts concerning the type of nanoparticles, their stabilisation, their ability to reach and bind the target and their cochlear degradation. Recent experimental studies focused on the use of polyethyleneimine (PEI) and poly lactic-glycolic acid (PLGA); however, PEI was highly toxic and PLGA is still undergoing safety studies [78]. Lipid nanocapsules (LNCS), thanks to their lipoprotein-like structure with an oily core, show good distribution in the inner ear after application to the round window membrane [79] and prolonged stability that permits close control on the dynamic release of the carried molecules. Interestingly, it has been demonstrated that lipid nanoparticles conjugated with a neurotrophic peptide focus selectively on the cells of the inner ear of mice [80]. However, the main concern about nanoparticles is their biocompatibility and intracochlear toxicity: in a recent study, it was shown that in vivo administration of lipid nanoparticles did not cause hearing damage or morphological changes in the inner ear [81]. In conclusion, nanoparticles can be equipped with targetability, immuno-transparency, although their application in clinical practice is still limited by possible side effects.

Gene therapy

Gene therapy has the goal to introduce a new or a regulatory gene in a target cell to replace or repair the defective gene [82]. Due to the risk of nucleic acid degradation related to the action of nucleases that recognise a foreign gene, gene therapy

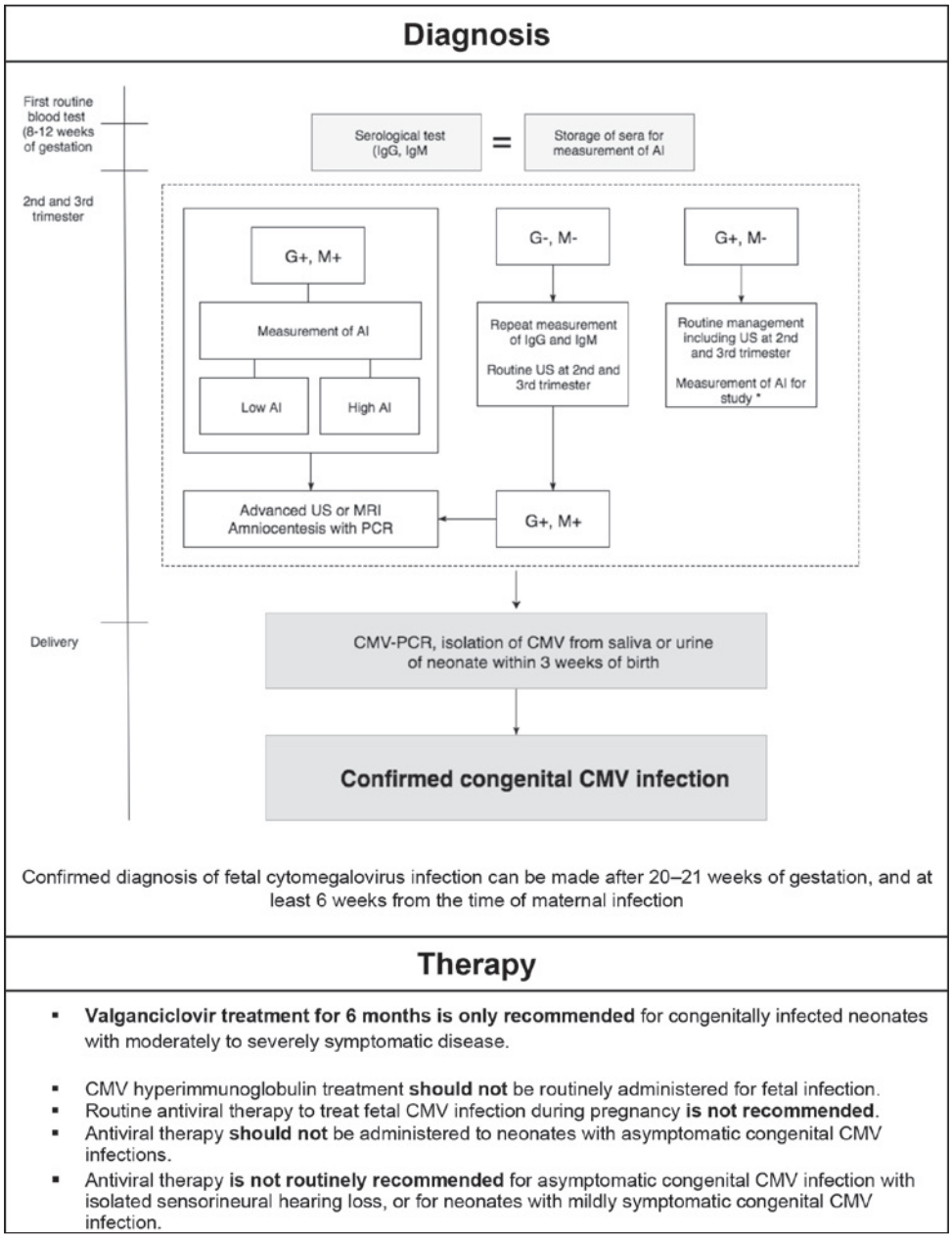


Fig. 2. Flow chart to identify an intrauterine cytomegalovirus-infected infant and recommendations for appropriate treatment (modified from Rawlinson et al, Lancet 2017).
AI, avidity index; ABR, auditory brain stem response; IgG, immunoglobulin G; IgM, immunoglobulin M; US, ultrasonography.

**When AIs were measured, maternal sera collected at first trimester were used*

should be administered in a protected environment by an appropriate vehicle leading itself to the target cell.

Two basic types of approaches have been proposed: the introduction of protective genes and the activation of transdifferentiation genes. Many investigations have recently shown the success of transfection and expression of neurotrophic factors in the inner ear by viral and non-viral vectors administered through cochlear implants. Neurotrophic factors play a crucial role in differentiation, proliferation, development, neuronal plasticity and cell survival in embryonic development and throughout life. About 20 neurotrophic factors have been studied; among these, the neurotrophin family (NT-3 and 7), glial growth factor (GDNF glial cell line-derived neurotrophic factor), brain (brain-derived neurotrophic factor BDNF), platelet (platelet-derived growth factor PDGF) and insulin neurotrophic factors [(IGF, insulin-like growth factor (IGF)] have been extensively studied in improving spiral ganglion neurons survival after cochlear implantation [83 84].

The success of gene therapy depends on the carrier of the gene and both viral or non-viral carriers have been used. Adenovirus [4 5], Adeno-associated viruses [6-8], Lentivirus [9], type 1 Herpes simplex virus and Vaccinia virus [10 11] have all shown promising results. However, their use is coupled with a high potential for toxicity, immunogenicity and/or mutagenicity. Non-viral vectors are safer and offer greater possibilities for manipulation and more flexibility for the transfected gene size; among these, the most important candidates as carriers are nanoparticles and nanolipidic-capsules that could be applied to the round window. However, their poor nuclear localisation in cochlear cells limits their application in gene therapy. Although gene therapy is a promising treatment option, its application is currently limited by the risk of side effects and is still under study to ensure that it will be safe and effective.

Stem cells

Transplantation of progenitor cells capable of differentiation into functional hair and/or spiral ganglion cells is undoubtedly a fascinating strategy. Embryonic, foetal, cord blood, central nervous system, placental stem cells or adult cells of the inner ear have been shown to differentiate into cells such as hair cells with mechano-sensitive functioning

cilia in vitro [85]. More recently, Koehler et al. have published the generation in 3D culture of functioning inner ear sensory epithelia from pluripotent stem cells; they reported that these stem-cell-derived hair cells exhibit functional properties of native mechanosensitive hair cells and form specialised synapses with sensory neurons that have also arisen from mouse embryonic stem cells in the culture [86].

Several laboratories have begun to study the implantation of these cells in models of deafness. At present, only limited survival has been shown for implanted cells in vivo; in rare cases, these cells became host integrated. Despite much evidence for differentiation into mature cell types, there has been no clear demonstration that hair cells can have functional recovery [87]. However, it remains to be accurately determined if stem cells can differentiate into hair cells or rather, more likely, they can promote reparative and trophic effects on sensorineural epithelia, stria vascularis and spiral ganglion neurons, limiting the regenerative effects on the supporting cells [15 87 88].

Conclusions

Although considerable progresses have been made in recent years, pathways for hearing preservation and restoration in children are still controversial. The progressively increasing knowledge about the pathophysiological mechanisms underlying cell death and reparation, inner ear genetics and development of new technologies to deliver therapies into a specific target represents a solid basis for research in this field.

The literature shows more than 110 molecules proposed to date in the prevention or repair of cochlear damage by exogenous factors; however, for most of these drugs, their use is not supported by clinical data. Corticosteroids represent a milestone for treatment of SNHL in children, even if there are no guidelines or indications for their use in children. Antiviral therapy for CMV infection may reduce the incidence of the most common cause of acquired sensorineural hearing loss, while research for a CMV vaccine is continues to be challenging. The possibility of combining nanotechnologies with cochlear implants for the application of targeted molecules or gene is probably the most attractive perspective for the near future. Finally, the role of stem cells in the inner ear is still debated, as they appear to be more involved in the reparative processes rather than differentiating into functioning hair cells.

References

1 Kral A, O'Donoghue GM. *Profound deafness in childhood*. N Engl J Med 2010;363:1438-50.

2 Prosser JD, Cohen AP, Greinwald JH. *Diagnostic evaluation of children with sensorineural hearing loss*. Otolaryngol Clin North Am 2015;48:975-82.

3 Iseli C, Buchman CA. *Management of children with severe, severe-profound, and profound sensorineural hearing loss*. Otolaryngol Clin North Am 2015;48:995-1010.

4 Bass JK, Bhagat SP. *Challenges in ototoxicity monitoring in the pediatric oncology population*. J Am Acad Audiol 2014;25:760-74.

5 Smith RJ, Bale JF Jr, White KR. *Sensorineural HL in children*. Lancet 2005;365:879-90.

6 Elziere M, Roman S, Nicollas R, et al. *Value of systematic aetiological investigation in children with sensorineural hearing loss*. Eur Ann Otorhinolaryngol Head Neck Dis 2012;129:185-9.

7 Fetoni AR, Ralli M, Sergi B, et al. *Protective effects of N-acetylcysteine on noise-induced HL in guinea pigs*. Acta Otorhinolaryngol Ital 2009;29:70-5.

8 Carey JC, Palumbos JC. *Advances in the understanding of the genetic causes of HL in children inform a rational approach to evaluation*. Indian J Pediatr 2016;83:1150-6.

9 Xia W, Liu F, Ma D. *Research progress in pathogenic genes of hereditary non-syndromic mid-frequency deafness*. Front Med 2016;10:137-42.

10 Spracklen TF, Vorster AA, Ramma L, et al. *Promoter region variation in NFE2L2 influences susceptibility to ototoxicity in patients exposed to high cumulative doses of cisplatin*. Pharmacogenomics J 2016;doi:10.1038/tpj.2016.52.

11 Rainey RN, Ng SY, Llamas J, et al. *Mutations in Cockayne syndrome-associated genes (Csa and Csb) predispose to cisplatin-induced HL in mice*. J Neurosci 2016;36:4758-70.

12 Brown AL, Lupo PJ, Okcu MF, et al. *SOD2 genetic variant associated with treatment-related ototoxicity in cisplatin-treated pediatric medulloblastoma*. Cancer Med 2015;4:1679-86.

13 Brock PR, Knight KR, Freyer DR, et al. *Platinum-induced ototoxicity in children: a consensus review on mechanisms, predisposition, and protection, including a new International Society of Pediatric Oncology Boston ototoxicity scale*. J Clin Oncol 2012;30:2408-17.

14 Fetoni AR, Ruggiero A, Lucidi D, et al. *Audiological monitoring in children treated with platinum chemotherapy*. Audiol Neurootol 2016;21:203-11.

15 Brigande JV, Heller S. *Quo vadis, hair cell regeneration?* Nat Neurosci 2009;12:679-85.

16 Abi-Hachem RN, Zine A, Van De Water TR. *The injured cochlea as a target for inflammatory processes, initiation of cell death pathways and application of related otoprotectives strategies*. Recent Pat CNS Drug Discov 2010;5:147-63.

17 Keithley EM, Wang X, Barkdull GC. *Tumor necrosis factor alpha can induce recruitment of inflammatory cells to the cochlea*. Otol Neurotol 2008;29:854-9.

18 Dinh CT, Haake S, Chen S, et al. *Dexamethasone protects organ of corti explants against tumor necrosis factor-alpha-induced loss of auditory hair cells and alters the expression levels of apoptosis-related genes*. Neuroscience 2008;157:405-13.

19 Lawrence R, Thevasagayam R. *Controversies in the management of sudden sensorineural hearing loss: an evidence-based review*. Clin Otolaryngol 2015;40:176-82.

20 Wei BP, Stathopoulos D, O'Leary S. *Steroids for idiopathic sudden sensorineural hearing loss*. Cochrane Database Syst Rev 2013;(7):CD003998.

21 Ng JH, Ho RC, Cheong CS, et al. *Intratympanic steroids as*

a salvage treatment for sudden sensorineural hearing loss? A meta-analysis. Eur Arch Otorhinolaryngol 2015;272:2777-82.

[22] Bear ZW, Mikulec AA. *Intratympanic steroid therapy for treatment of idiopathic sudden sensorineural hearing loss*. Mo Med 2014;111:352-6.

[23] Eshraghi AA, Lang DM, Roell J, et al. *Mechanisms of programmed cell death signaling in hair cells and support cells post-electrode insertion trauma*. Acta Otolaryngol 2015;135:328-34.

[24] Eastwood H, Chang A, Kel G, et al. *Round window delivery of dexamethasone ameliorates local and remote HL produced by cochlear implantation into the second turn of the guinea pig cochlea*. Hear Res 2010;265:25-9.

[25] Vivero RJ, Joseph DE, Angeli S, et al. *Dexamethasone base conserves hearing from electrode trauma-induced hearing loss*. Laryngoscope 2008;118:2028-35.

[26] Maini S, Lisnichuk H, Eastwood H, et al. *Targeted therapy of the inner ear*. Audiol Neurootol 2009;14:402-10.

[27] Paasche G, Bockel F, Tasche C, et al. *Changes of postoperative impedances in cochlear implant patients: the short-term effects of modified electrode surfaces and intracochlear corticosteroids*. Otol Neurotol 2006;27:639-47.

[28] Ye Q, Tillein J, Hartmann R, et al. *Application of a corticosteroid (Triamcinolon) protects inner ear function after surgical intervention*. Ear Hear 2007;28:361-9.

[29] Paasche G, Tasche C, Stöver T, et al. *The long-term effects of modified electrode surfaces and intracochlear corticosteroids on postoperative impedances in cochlear implant patients*. Otol Neurotol 2009;30:592-8.

[30] Rah YC, Lee MY, Kim SH, et al. *Extended use of systemic steroid is beneficial in preserving hearing in guinea pigs after cochlear implant*. Acta Otolaryngol 2016;136:1213-9.

[31] Worsøe L, Brandt CT, Lund SP, et al. *Intratympanic steroid prevents long-term spiral ganglion neuron loss in experimental meningitis*. Otol Neurotol 2010;31:394-403.

[32] Molyneux EM, Walsh AL, Forsyth H, et al. *Dexamethasone treatment in childhood bacterial meningitis in Malawi: a randomised controlled trial*. Lancet 2002;360:211-8.

[33] Brouwer MC, Tunkel AR, van de Beek D. *Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis*. Clin Microbiol Rev 2010;23:467-92.

[34] Brouwer MC, McIntyre P, Prasad K, et al. *Corticosteroids for acute bacterial meningitis*. Cochrane Database Syst Rev 2015:CD004405.

[35] Herson VC, Todd JK. *Prediction of morbidity in Hemophilusinfluenzae meningitis*. Pediatrics 1977;59:35-9.

[36] Kilpi T, Peltola H, Jauhiainen T, et al. *Oral glycerol and intravenous dexamethasone in preventing neurologic and audiologic sequelae of childhood bacterial meningitis*. The Finnish Study Group. Pediatr Infect Dis J 1995;14:270-8.

[37] Peltola H, Roine I, Fernández J, et al. *Hearing impairment in childhood bacterial meningitis is little relieved by dexamethasone or glycerol*. Pediatrics 2010;125:e1-8.

[38] Azzopardi D. *Hypoxic ischaemic encephalopathy in newborn infants*. Early Hum Dev 2010;86:327.

[39] Perrone S, Szabó M, Bellieni CV, et al. *Whole body hypothermia and oxidative stress in babies with hypoxic-ischemic brain injury*. Pediatr Neurol 2010;43:236-40.

[40] Fetoni AR, Mancuso C, Eramo SL, et al. *In vivo protective effect of ferulic acid against noise-induced HL in the guinea-pig*. Neuroscience 2010;169:1575-88.

[41] Fetoni AR, Piacentini R, Fiorita A, et al. *Water-soluble Coenzyme Q10 formulation (Q-ter) promotes outer hair cell survival in a guinea pig model of noise induced HL(NIHL)*. Brain Res 2009;1257:108-16.

[42] Fetoni AR, Troiani D, Eramo SL, et al. *Efficacy of different routes of administration for Coenzyme Q10 formulation in noise-induced hearing loss: systemic versus transtympanic modality*. Acta Otolaryngol 2012;132:391-9

[43] Fetoni AR, Eramo SL, Rolesi R, et al. *Antioxidant treatment with coenzyme Q-ter in prevention of gentamycin ototoxicity in an animal model*. Acta Otorhinolaryngol Ital 2012;32:103-10.

[44] Fetoni AR, Garzaro M, Ralli M, et al. *The monitoring role of otoacoustic emissions and oxidative stress markers in the protective effects of antioxidant administration in noise-exposed subjects: a pilot study*. Med Sci Monit 2009;15:PR1-8.

[45] Poirrier AL, Van den Ackerveken P, Kim TS, et al. *Ototoxic drugs: difference in sensitivity between mice and guinea pigs*. Toxicol Lett 2010;193:41-9.

[46] Gilles A, Ihtijarevic B, Wouters K, et al. *Using prophylactic antioxidants to prevent noise-induced hearing damage in young adults: a protocol for a double-blind, randomized controlled trial*. Trials 2014;15:110.

[47] Sheppard A, Hayes SH, Chen GD, et al. *Review of salicylate-induced hearing loss, neurotoxicity, tinnitus and neuropathophysiology*. Acta Otorhinolaryngol Ital 2014;34:79-93.

[48] Sha SH, Schacht J. *Salicylate attenuates gentamicin-induced ototoxicity*. Lab Invest 1999;79:807-13.

[49] Li G, Sha SH, Zotova E, et al. *Salicylate protects hearing and kidney function from cisplatin toxicity without compromising its oncolytic action*. Lab Invest 2002;82:585-96.

[50] Chen Y, Huang WG, Zha DJ, et al. *Aspirin attenuates gentamicin ototoxicity: from the laboratory to the clinic*. Hear Res 2007;226:178-82.

[51] Chen GD, Kermany MH, D'Elia A, et al. *Too much of a good thing: long-term treatment with salicylate strengthens outer hair cell function but impairs auditory neural activity*. Hear Res 2010;265:63-9.

[52] Ralli M, Lobarinas E, Fetoni AR, et al. *Comparison of salicylate- and quinine-induced tinnitus in rats: development, time course, and evaluation of audiologic correlates*. Otol-Neurotol 2010;31:823-31.

[53] Ralli M, Troiani D, Podda MV, et al. *The effect of the NMDA channel blocker memantine on salicylate-induced tinnitus in rats*. Acta Otorhinolaryngol Ital 2014;34:198-204.

[54] Dickey DT, Wu YJ, Muldoon LL, et al. *Protection against cisplatin-induced toxicities by N-acetylcysteine and sodium thiosulfate as assessed at the molecular, cellular, and in vivo levels*. J Pharmacol Exp Ther 2005;314:1052-8.

[55] Wang J, Lloyd Faulconbridge RV, Fetoni A, et al. *Local application of sodium thiosulfate prevents cisplatin-induced HLin the guinea pig*. Neuropharmacology 2003;45:380-93.

[56] Harned TM, Kalous O, Neuwelt A, et al. *Sodium thiosulfate administered six hours after cisplatin does not compromise antineuroblastoma activity*. Clin Cancer Res 2008;14:533-40.

[57] Neuwelt EA, Gilmer-Knight K, Lacy C et al. *Toxicity profile of delayed high dose sodium thiosulfate in children treated*

with carboplatin in conjunction with blood-brain-barrier disruption. Pediatr Blood Cancer 2006;47:174-82.

58 Gurney JG, Bass JK, Onar-Thomas A, et al. *Evaluation of amifostine for protection against cisplatin-induced serious HL in children treated for average-risk or high-risk medulloblastoma*. Neuro Oncol 2014;16:848-55.

59 Fouladi M, Chintagumpala M, Ashley D, et al. *Amifostine protects against cisplatin-induced ototoxicity in children with average-risk medulloblastoma*. J Clin Oncol 2008;26:3749-55.

60 Marina N, Chang KW, Malogolowkin M, et al. *Children's Oncology Group. Amifostine does not protect against the ototoxicity of high-dose cisplatin combined with etoposide and bleomycin in pediatric germ-cell tumors: a Children's Oncology Group study.* Cancer 2005;104:841-7.

61 Ozkul Y, Songu M, Basoglu MS, et al. *Evaluation of the protective effect of α-lipoic acid on cisplatin ototoxicity using distortion-product otoacoustic emission measurements: an experimental animal study*. J Craniofac Surg 2014;25:1515-8.

62 Huang J, Wang P, Li M et al. *Trichostatin A reduces cisplatin-induced ototoxicity through the STAT6 signaling pathway*. Int J Mol Med 2015;36:493-500.

63 BekmezBilmez ZE, Aydin S, Şanli A, et al. *Oxytocin as a protective agent in cisplatin-induced ototoxicity*. Cancer Chemother Pharmacol 2016;77:875-9.

64 Lee SH, Kim HS, An YS, et al. *Protective effect of resveratrol against cisplatin-induced ototoxicity in HEI-OC1 auditory cells*. Int J Pediatr Otorhinolaryngol 2015;79:58-62.

65 Kara M, Türkön H, Karaca T, et al. *Evaluation of the protective effects of hesperetin against cisplatin-induced ototoxicity in a rat animal model*. Int J Pediatr Otorhinolaryngol 2016;85:12-8.

66 Roldán-Fidalgo A, Martín Saldaña S, Trinidad A, et al. *In vitro and in vivo effects of lutein against cisplatin-induced ototoxicity*. ExpToxicol Pathol 2016;68:197-204.

67 Fetoni AR, Paciello F, Mezzogori D, et al. *Molecular targets for anticancer redox chemotherapy and cisplatin-induced ototoxicity: the role of curcumin on pSTAT3 and Nrf-2 signalling*. Br J Cancer 2015;113:1434-44.

68 James SH, Kimberlin DW. *Advances in the prevention and treatment of congenital cytomegalovirus infection*. Curr Opin Pediatr 2016;28:81-5.

69 Kimberlin DW, Jester PM, Sánchez PJ, et al. *Valganciclovir for symptomatic congenital cytomegalovirus disease*. N Engl J Med 2015;372:933-43.

70 Boeckh M, Murphy WJ, Peggs KS. *Recent advances in cytomegalovirus: an update on pharmacologic and cellular therapies*. Biol Blood Marrow Transplant 2015;21:24-9.

71 Stockmann C, Roberts JK, Knackstedt ED, et al. *Clinical pharmacokinetics and pharmacodynamics of ganciclovir and valganciclovir in children with cytomegalovirus infection*. Expert Opin Drug MetabToxicol 2015;11:205-19.

72 Marshall BC, Koch WC. *Antivirals for cytomegalovirus infection in neonates and infants: focus on pharmacokinetics, formulations, dosing, and adverse events*. Paediatr Drugs 2009;11:309-21.

73 Vora SB, Englund JA. *Cytomegalovirus in immunocompromised children*. Curr Opin Infect Dis 2015;28:323-9.

74 Gunkel J, Wolfs TF, de Vries LS, et al. *Predictors of severity for postnatal cytomegalovirus infection in preterm infants and implications for treatment*. Expert Rev Anti Infect Ther 2014;12:1345-55.

75 Dioverti MV, Lahr B, Razonable RR. *Treatment of cytomegalovirus infection and disease pre- and post-quantitative nucleic acid test standardization: does use of a more sensitive assay lead to longer treatment duration?* Clin Transplant 2016;30:154-60.

76 Shin JJ, Keamy DG Jr, Steinberg EA. *Medical and surgical interventions for HL associated with congenital cytomegalovirus: a systematic review*. Otolaryngol Head Neck Surg 2011;144:662-75.

77 Rawlinson WD, Boppana SB, Fowler KB, et al. *Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy*. Lancet Infect Dis 2017;17:e177-e188.

78 Pyykkö I, Zou J, Schrott-Fischer A, et al. *An overview of nanoparticle based delivery for treatment of inner ear disorders*. Methods Mol Biol 2016;1427:363-415.

79 Zou J, Saulnier P, Perrier T, et al. *Distribution of lipid nanocapsules in different cochlear cell populations after round window membrane permeation*. J Biomed Mater Res B Appl Biomater 2008;87:10-8.

80 Roy S, Johnston AH, Newman TA, et al. *Cell-specific targeting in the mouse inner ear using nanoparticles conjugated with a neurotrophin-derived peptide ligand: potential tool for drug delivery*. Int J Pharm 2010;390:214-24.

81 Zhang Y, Zhang W, Löbler M, et al. *Inner ear biocompatibility of lipid nanocapsules after round window membrane application*. Int J Pharm 2011;404:211-9.

82 Mulligan RC. *The basic science of gene therapy*. Science 1993;260:926-32.

83 Wise AK, Tu T, Atkinson PJ, et al. *The effect of deafness duration on neurotrophin gene therapy for spiral ganglion neuron protection*. Hear Res 2011;278:69-76.

84 Taecker H, Garnham C. *Neurotrophin therapy and cochlear implantation: translating animal models to human therapy*. Exp Neurol 2010;226:1-5.

85 Oshima K, Shin K, Diensthuber M, et al. *Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells*. Cell 2010;141:704-16.

86 Koehler KR, Mikosz AM, Molosh AI, et al. *Generation of inner ear sensory epithelia from pluripotent stem cells in 3D culture*. Nature 2013;500:217-21.

87 Santaolalla F, Salvador C, Martínez A, et al. *Inner ear hair cell regeneration: A look from the past to the future*. Neural Regen Res 2013;8:2284-9.

88 Park YH. *Stem cell therapy for sensorineural hearing loss, still alive?* J Audiol Otol 2015;19:63-7.

Received: January 11, 2017 - Accepted: May 2, 2017

Address for correspondence: Massimo Ralli, Department of Oral and Maxillofacial Sciences, Sapienza University of Rome. viale del Policlinico 155, 00186 Rome, Italy. E-mail: massimo.ralli@uniroma1.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:509-512; doi: 10.14639/0392-100X-1690

Otology

Balloon dilation of the Eustachian tube: clinical experience in the management of 126 children

Dilatazione tubarica con balloon: nostra esperienza nella gestione di 126 bambini

M. TISCH, H. MAIER, H. SUDHOFF

Department of Otolaryngology, Head and Neck Surgery, German Armed Forces Hospital of Ulm, Germany;
Department of Otolaryngology, Head and Neck Surgery, Bielefeld Academic Teaching Hospital, Bielefeld, Germany

SUMMARY

Balloon dilation of the Eustachian tube has been recently introduced as a novel and minimally invasive method for treating chronic obstructive Eustachian tube dysfunction. For the first time worldwide, we assessed the role of this technique in the treatment of children with Eustachian tube dysfunction who did not respond to other treatments. We retrospectively analysed the medical records of 60 children (mean age: 6.3 years, range: 28 months to 12 years) who underwent balloon dilation of the Eustachian tube using the Bielefeld balloon catheter. In addition, the parents of a further 66 children who underwent balloon dilation (mean age: 8 years, range: 4 to 13 years) were asked to complete a standardised written questionnaire and were interviewed by telephone about the postoperative course of their children. There were no complications during surgery. Clinical symptoms improved in more than 80% of patients. No patient reported a deterioration of symptoms. Of the participating parents, 81.3% were very satisfied or satisfied with the outcome of treatment. Balloon dilation is a rapid, simple and safe method for the treatment of both adults and children with Eustachian tube dysfunction that does not respond to other treatments. Further studies, ideally multicentre studies, are required in order to optimise the definition of existing and potential new indications for this treatment approach and to establish this treatment in the management of children with refractory chronic Eustachian tube dysfunction.

KEY WORDS: Eustachian tube • Eustachian tube dysfunction • Balloon dilation • Children

RIASSUNTO

La dilatazione tubarica con balloon è stata recentemente annoverata com nuovo metodo minimamente invasive per il trattmento della disfunzione cronica ostruttiva della tuba di Eustachio. Per la prima volta nel mondo, abbiamo definito il ruolo della suddetta tecnica nel trattamento della disfunzione tubarica cronica non rispondente ad altri trattamenti. Sono stati analizzati i dati clinici di 60 bambini (età media: 6,3 anni; range: da 28 mesi a 12 anni) sottoposti a dilatazione della Tuba di Eustachio con il balloon di Bielefeld. In aggiunta, sono stati reclutati i genitori di altri 66 bambini sottoposti a dilatazione con balloon, ed è stato chiesto loro di compilare un questionario standardizzato, e di rispondere ad alcune domande riguardo il decorso postoperatorio dei loro bambini. Non ci sono state complicanze durante gli interventi chirurgici. I sintomi clinici sono migliorati in più dell'80% dei casi. Nessun paziente ha riferito un peggioramento sintomatologico. L'83% dei partecipanti è rimasto notevolmente soddisfatto dei risultati derivanti dal trattamento. La dilatazione con balloon è una tecnica semplice, rapida e sicura per il trattamento della disfunzione tubarica non rispondente ad altri trattamenti sia negli adulti, sia nei bambini. Ulteriori studi, preferibilmente multicentrici, sarebbero utili per definire al meglio le indicazioni già esistenti e potenziali nuovi indicazioni per questa tipologia di trattamento, e per stabilire definitivamente il ruolo di questa tecnica nella gestione della disfunzione tubarica cronica refrattaria.

PAROLE CHIAVE: *Tuba di Eustachio • Disfunzione tubarica • Dilatazione con balloon • Bambini*

Acta Otorhinolaryngol Ital 2017;37:509-512

Introduction

Balloon dilation of the Eustachian tube has been recently introduced as a novel and minimally invasive method for treating chronic obstructive Eustachian tube dysfunction [1-3]. Success rates of 63% to 92.3% have been reported in studies with follow-up periods from 3 to 12 months [1-4]. Although children are most commonly affected by Eustachian tube dysfunction, balloon dilation was initially used only in adult patients because it was believed that the design of commercially available balloon catheters did not meet the specific anatomical conditions of the Eustachian tube in children. On closer examination, however, this argument was unconvincing. In 2010, the Department of Otolaryngology at the German Armed Forces Hospital of Ulm therefore used balloon dilation for the first time worldwide in the management of children with chronic obstructive tube dysfunction [5].

More than 250 children with refractory chronic Eustachian tube dysfunction have now undergone this treatment in our department.

In this article, we retrospectively analyse our patient population and present two cases in order to illustrate our treatment approach and first results.

Materials and methods

Patients

In a retrospective analysis, 60 children (first group of patients) were identified who underwent balloon dilation of the Eustachian tube for refractory chronic Eustachian tube dysfunction at the Department of Otolaryngology of the German Armed Forces Hospital in Ulm from October 2010 to December 2012.

In addition, the parents of a further 66 children (second group of patients) who underwent treatment between December 2012 and October 2013 were asked to complete a standardised written questionnaire (Fig. 1) and were interviewed by telephone about the postoperative course of their children.

The study was approved by the ethics committee of the University of Ulm.

Diagnostic assessment and treatment

Data on preoperative diagnostic procedures, previous treatment, surgical intervention, and postoperative course were obtained and documented from medical records of the first group of patients.

The parents of the patients from the second group were asked to complete a written questionnaire and were interviewed by telephone about the postoperative course of their children and about their satisfaction with outcome. Indications for balloon dilation were the presence of chronic obstructive Eustachian tube dysfunction that was not managed satisfactorily with medical treatments (i.e. anti-inflammatory agents, decongestants, anti-allergic agents, autoinflation) and surgical treatments (i.e. adenoidectomy, turbinoplasty, myringotomy and tympanostomy tube placement).

Eustachian tube function was assessed on the basis of the following tests:

- Valsalva maneuver and Toynbee test (if possible).
- Tympanogram (intact tympanic membrane).
- Otomicroscopy.

These examinations were performed before surgery and at 6 to 8 weeks after surgery.

1. When was balloon dilation performed?
2. How many middle ear infections has your child had since balloon dilation of the Eustachian tube was performed?
3. How many times has your child required antibiotic treatment?
4. How many times has your child had otitis media with effusion?
5. How many times has your child undergone myringotomy or tympanostomy tube placement since balloon dilation of the Eustachian tube was performed?
6. Has your child's hearing improved after balloon dilation?
7. Has your child had any problems after balloon dilation?
   Yes/no
   If yes, what problems?
   Severe – moderate – mild
8. How satisfied are you with the treatment result?
   Very satisfied – satisfied – fairly satisfied – not satisfied

Fig. 1. Parent questionnaire.

Balloon dilation

We used a modification of the technique that was originally described by Sudhoff et al. [3 6]. Laryngeal mask airways were generally used during anaesthesia. A xylometazoline solution was used for nasal decongestion. A 30° Hopkins rod was then inserted on the side to be treated. An insertion instrument was carefully introduced on the contralateral side. A balloon catheter was passed through the insertion instrument and pushed 2 cm into the Eustachian tube under endoscopic vision. The balloon was inflated to a pressure of 10 bar for 2 min. Routine postoperative care consisted of the application of nasal drops containing xylometazoline and panthenol ointment for three days. Some patients also received antihistamines or topical corticosteroids. Starting on the third day following surgery, patients were instructed, if possible, to autoinflate the Eustachian tube by performing Valsalva maneuvers or using the Otovent device.

Results

The patient group that was investigated in the first part of the study consisted of 60 children with a mean age of 6.3 years and a range from 28 months to 12 years. Of these patients, 20 had undergone at least one previous tympanoplasty. The mean follow-up was 13 months.

Before surgery, 91.6% of the children were unable to equalise middle ear pressure, as indicated by the absence of a positive Valsalva test on microscopy or the absence of a definable peak on a tympanogram ("flat" tympanogram). Complete adhesion was observed in 22 children (37%). Preoperative computed tomography (CT) scans were obtained from 28 patients with chronic otitis media and suspected cholesteatoma. The other 32 children did not undergo computed tomography of the petrous portion of the temporal bone before surgical intervention.

A total of 30 unilateral and 30 bilateral dilations were performed.

There were no complications during surgery. Immediately after surgery, one patient (1%) had a nose bleed, which

was easily managed with bipolar coagulation and anterior nasal packing.

No further treatment-associated complications were reported.

After surgery, only 11 children (18.3%) were objectively unable to equalise middle ear pressure, as indicated by the absence of a positive Valsalva test on microscopy or the absence of a definable peak on a tympanogram. In 18 of the 22 patients, adhesions had been separated so that there was no further contact between the tympanic membrane and the promontory or between the tympanic membrane and the long process of the incus.

Of the 66 parents who were contacted during the second part of the study, 34 (51.5%) agreed to take part. The mean age of the children in this group was 8 years with a range from 4 to 13 years. The mean follow-up period was 9.5 months.

Hearing improved in 76.5% of children. Of the participating parents, 55.9% were very satisfied and 25.4% were satisfied with the results of surgery. The parents of only one child stated that they were not satisfied with the outcome. No serious complications following balloon dilation were reported. Immediately after the procedure, one child experienced mild epistaxis. Another child had postoperative pain. Three children developed otitis media in the days following balloon dilation. In the further postoperative course, otitis media with effusion occurred in three cases. Balloon dilation of the Eustachian tube had to be repeated in 9 of the 34 patients.

Discussion

Obstructive Eustachian tube dysfunction is most commonly seen in children. It is currently assumed that at least 80% of all preschool children are affected at least temporarily by this condition. The greater predisposition of children for Eustachian tube dysfunction can be primarily explained by the anatomy of the Eustachian tube in children [7].

1. The tube is considerably shorter in infants and young children than in older children or adults. This impairs the protective function of the middle ear system.
2. In young children, the Eustachian tube lies at a relatively flat angle in relation to the horizontal plane, which adversely affects the role of the tensor veli palatini muscle in opening the tube. This can lead to limited ventilation of the middle ear during upper respiratory tract infections.
3. The mass of Ostmann's fat pad is relatively greater in children than in adults. This limits the opening of the tubal lumen when the tensor veli palatini muscle contracts.
4. There are considerable differences in cartilage structure between children and adults. Cartilage in children has less volume and is softer. In addition, the elastin in the cartilage is less dense and cartilage cell density is greater in young children. This results in a lack of stiffness, increased compliance, and limited efficiency of the opening mechanism of the Eustachian tube.
5. Moreover, the mucous membrane of the cartilaginous portion of the Eustachian tube is thicker and contains more folds in children. It is possible that this tissue, which is known as the tubal tonsil, quickly reacts with inflammatory responses to exposure to microbial agents and allergens.

As a result, post-inflammatory adhesions are likely to occur in this region of the Eustachian tube and impair tubal opening. Sheer et al. [8] developed finite element models of Eustachian tube function and found that compliance of the periluminal mucosa was a significant predictor of the effectiveness of the tensor veli palatini muscle.

Differential diagnosis includes a variety of conditions that can contribute to the development of obstructive Eustachian tube dysfunction in children. The first to be mentioned is adenoid hyperplasia. Other conditions that can play a causative role are acute and chronic infections, allergic disease, hyperplasia of the nasal conchae, immune defects, impairments in ciliary function and reflux.

Initial treatment should generally focus on the management of the aforementioned causes or contributors.

Until recently, the options available for restoring middle ear aeration have been limited when treatments fail and chronic obstructive Eustachian tube dysfunction develops. Apart from pressure equalization manoeuvers (e.g. Valsalva, Otovent), repeated myringotomies and tympanostomy tube insertions were the only possible treatment modalities.

With the advent of balloon dilation, a novel and minimally invasive method for treating chronic obstructive Eustachian tube dysfunction has been established and successfully used for the management of adult patients in recent years [1-4 9].

The results of the present study suggest that children appear to benefit from this intervention in a similar fashion. When all established treatment modalities were unsuccessful in an 18-month-old girl, we decided to perform balloon dilation of the Eustachian tube for the first time in a young child. The excellent treatment result encouraged us to use this technique in an increasing number of children at our department. This decision is supported by an analysis of the treatment results we obtained for 60 children with complex and chronic obstructive Eustachian tube dysfunction that was unresponsive to other treatments. The percentage of children who were unable to equalise middle ear pressure decreased from 91.6% of the children before balloon dilation to 18.3% after the intervention. In 18 of 22 children (81.8%), we were even able to successfully separate adhesions.

These positive findings are all the more remarkable as the method is minimally invasive and causes only mild dis-

comfort. No patient experienced serious complications.
The parents' evaluation of the treatment results, too, is convincing. With a percentage as high as 81.3%, the vast majority of participating parents were very satisfied or satisfied with the outcome of balloon dilation.
The reasons why balloon dilation was not recommended for children until recently are difficult to understand. A major concern was that the procedure could be associated with serious complications such as the risk of injury to the internal carotid artery, especially when the artery follows an aberrant course in the osseous region of the Eustachian tube or when there is a dehiscence of the bony carotid canal. Such concerns had been expressed even before the technique was used in the treatment of adults and were raised even more when the use of balloon dilation was considered in children who have thinner and more vulnerable vessel walls. This argument would be understandable if the osseous portion of the Eustachian tube were dilated, as some authors mistakenly believed in the past. It is, however, not the osseous portion but the cartilaginous portion of the Eustachian tube that is dilated and which is located at a safe distance from the carotid canal. This is one of the reasons why we do not necessarily obtain preoperative CT scans of the skull in all cases, as demanded by some authors [4]. Other critics of the use of balloon dilation in children argue that the balloon length (20 mm) of commercially available catheters makes these devices unsuitable in children for anatomical reasons. In our opinion, this argument is also unconvincing. The Eustachian tube lengthens during childhood and by 7 years of age is approximately as long as in adults. Although the tube is shorter in young children, the ratio of the length of the cartilaginous portion to the osseous portion is 8:1 in the infant compared to 4:1 in the adult. As a result, the length of the balloon appears not to be a critical factor in the risk of placing the balloon in the osseous portion instead of the cartilaginous portion of the Eustachian tube. Moreover, catheters with a balloon length of 1.5 cm or 1.0 cm are today available.
There is still some controversy regarding the mechanism of action of balloon dilation. A possible explanation is that microruptures in the region of the tubal cartilage lead to a permanent widening of the cartilaginous Eustachian tube [10 11]. Another possible explanation is that the stimulation of proprioceptors may influence the tensor veli palatini and levator veli palatini muscles [11]. Apart from these possible explanations, we have established the hypothesis that dilation can separate scars or adhesions of the folded mucosa of the Eustachian tube, which can develop after inflammation.

Conclusions

In our opinion, balloon dilation of the Eustachian tube is a safe and reliable alternative in the management of adults and especially children with chronic Eustachian tube dysfunction that does not respond to conservative/established therapies.

References

1 Poe D. *In reference to Balloon dilatation Eustachian tuboplasty: a clinical study*. Laryngoscope 2011;121:908.

2 Poe DS, Hanna BM. *Balloon dilation of the cartilaginous portion of the eustachian tube: initial safety and feasibility analysis in a cadaver model*. Am J Otolaryngol 2011;32:115-23.

3 Schröder S, Reineke U, Lehmann M, et al. *Chronic obstructive Eustachian tube dysfunction in adults: long-term results of balloon Eustachian tuboplasty*. HNO 2013; 61:142-51.

4 Tisch M, Maier S, Maier H. *Eustachian tube dilation using the Bielefeld balloon catheter: clinical experience with 320 interventions*. HNO 2013;61:483-7.

5 Tisch M, Maier S, Hecht P, et al. *Bilateral Eustachian tube dilation in infants: an alternative treatment for persistent middle ear functional dysfunction*. HNO 2013;61:492-3.

6 Sudhoff H, Ockermann T, Mikolajczyk R, et al. *[Clinical and experimental considerations for evaluation of Eustachian tube physiology]*. HNO 2009;57:428-35.

7 Bluestone CD, Doyle WJ. *Anatomy and physiology of eustachian tube and middle ear related to otitis media*. J Allergy Clin Immunol 1988;81:997-1003.

8 Sheer FJ, Swarts JD, Ghadiali SN. *Three-dimensional finite element analysis of Eustachian tube function under normal and pathological conditions*. Med Eng Phys 2012;34:605-16.

9 Jurkiewicz D, Bień D, Szczygielski K, et al. *Clinical evaluation of balloon dilation Eustachian tuboplasty in the Eustachian tube dysfunction*. Eur Arch Otorhinolaryngol 2013;270:1157-60.

10 Ockermann T, Reineke U, Upile T, et al. *Balloon dilation eustachian tuboplasty: a feasibility study*. Otol Neurotol 2010; 31:1100-3.

11 Ockermann T, Reineke U, Upile T, et al. *Balloon dilatation eustachian tuboplasty: a clinical study*. Laryngoscope 2010;120:1411-6.

Received: March 22 , 2017 - Accepted: March 28, 2017

Address for correspondence: Holger Sudhoff, Department of Otolaryngology, Head and Neck Surgery, Bielefeld Academic Teaching Hospital, Teutoburger Str. 50, 33604 Bielefeld, Germany. Tel. +49 521/581-3301. Fax +49 521/581-3399. E-mail: holger.sudhoff@rub.de

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:513-518; doi: 10.14639/0392-100X-1403

VESTIBOLOGY

Uphill/downhill nystagmus

Nistagmo in salita e nistagmo in discesa

M. GUFONI

Department of Otorhinolaryngology, Spedali Riuniti Livorno, Italy

SUMMARY

Differential diagnosis between peripheral and central spontaneous nystagmus can be difficult to classify (as peripheral or central) even on the basis of criteria recommended in the recent literature. The aim of this paper is to use the combination of spontaneous nystagmus and ocular tilt reaction to determine the site of origin of the disease that causes nystagmus. We propose to classify the nystagmus in: 1) "Uphill" nystagmus in which the nystagmus takes on an inclined plane and the direction of the fast phase is towards the hypertropic eye (this type of nystagmus is likely peripheral); 2) "Downhill" nystagmus when the nystagmus beats toward the hypotropic eye (this type of nystagmus is likely central); 3) "Flat" nystagmus when the plane on which nystagmus beats is perfectly horizontal: in this case, we cannot say anything about the site of lesion (it was only detected in 15% of cases). The spatial position of nystagmus vector has to be considered as an intrinsic characteristic of the nystagmus itself (as direction, frequency, angular velocity etc.) and must be reported in the description, possibly giving an indication of the site of damage (peripheral or central). In particular, similar results are obtained by comparing the inclination of the nystagmus with the head impulse test (HIT, considered the best bedside test now available). It seems that this sign may confirm HIT for safer diagnosis or replace it in case of doubt. In contrast, in case of "Flat" nystagmus (probably attributable to the fact that the utricular maculae are spared), HIT can replace observation of the plane of the nystagmus. Thus, the two signs confirm and integrate each other. The test does not require additional time and is not tedious for the patient. It is proposed that it be included in the evaluation of spontaneous nystagmus in everyday clinical practice.

KEY WORDS: Vertigo • Nystagmus • Ocular tilt reaction • Utriculus • Semicircular canal

RIASSUNTO

Capita spesso che un paziente con vertigini sia difficilmente inquadrabile (se periferico o centrale), anche affidandosi ai criteri consigliati nella recente letteratura. In questo lavoro si propone di utilizzare la valutazione della combinazione tra nistagmo spontaneo ed "Ocular Tilt Reaction" per dare un giudizio sulla sede della patologia che provoca il nistagmo. Si propone di dividere il nistagmo in : 1) nistagmo "in salita" in cui il nistagmo batte su un piano inclinato e il verso della fase rapida è verso l'occhio ipertropico (questo tipo di nistagmo è verosimilmente periferico); 2) nistagmo " in discesa" " in cui il nistagmo batte verso l'occhio ipotropico (questo tipo di nistagmo è verosimilmente centrale); 3) nistagmo "in piano" " in cui il piano su cui batte il nistagmo è perfettamente orizzontale e sul quale non si può dire nulla (è stato rilevato solo nel 15% dei casi). La posizione nello spazio del vettore del nistagmo è da considerare una caratteristica intrinseca del nistagmo stesso (come direzione, verso ,frequenza, velocità angolare ecc.) e va riportata nella descrizione del nistagmo, potendo dare un'indicazione sulla sua natura (periferico o centrale). In particolare, confrontando l'inclinazione del nistagmo con l'Head Impulse Test (HIT), si ottengono risultati simili nella valutazione topodiagnostica di un nistagmo spontaneo. Sembra dunque che questo segno possa confermare l'HIT per una diagnosi più sicura o sostituirlo in casi dubbi. Al contrario, in caso di nistagmo che batte in piano (né in salita né in discesa, attribuibile probabilmente al fatto che le macule utriculari sono risparmiate) l'HIT può sostituire l'osservazione del piano del nistagmo. In questo modo i due segni si confermano e si integrano a vicenda nei casi dubbi. Il test non richiede tempi aggiuntivi e non è in alcun modo causa di disturbo per il paziente, per cui se ne propone l'inserimento nella valutazione di ogni nistagmo spontaneo.

PAROLE CHIAVE: *Vertigine • Nistagmo • Ocular tilt reaction • Utriculo • Canale semicircolare*

Acta Otorhinolaryngol Ital 2017;37:513-518

Introduction

Vertigo is a widespread problem and one of the most common reasons for medical consultation in Emergency Departments. Even in Italy, in a recent study, 40.3% subjects reported at least one episode of vertigo/dizziness during their lifetime; 71.3% were females, and 28.7% were males, confirming the high prevalence of these symptoms in the general population.

In emergency rooms a diagnosis is requested as rapid and accurate as possible, but sometimes it can be difficult to distinguish central (and possibly dangerous) from peripheral cases [1].

Anyway, as far as possible, a spontaneous peripheral nystagmus should not be confused with a central one.

For this purpose, the recent literature suggests few signs which seem more accurate than imaging [2]:

1. Head impulse test. A pathological test toward the af-

fected side (at least one correction saccadic movement) addresses toward a peripheral problem. A normal test (no corrective saccades at all) may be related to central damage [3 4].
2. Romberg test. A peripheral patient is able to keep standing without support, while a central patient needs help to avoid falling down [5].
3. Associated neurological signs (gaze evoked, direction changing or positional nystagmus, specific cerebellar or medulla signs) aim towards a diagnosis of centrality [6].
4. Quick recovery: a peripheral patient is expected to recover soon after the event.
5. Unilateral weakness seems suggestive of a peripheral pathology [7].
6. Pathologic cover test is considered a sign of central vestibular disorder as associated with ocular tilt reaction.
Unfortunately, none of these signs taken alone allows certain diagnosis:
1. HIT may be difficult to perform in acute vertigo and covert saccades can be misleading [8].
2. Imbalance grade is not highly specific and has rather low sensitivity [7].
3. In early stages, a spontaneous nystagmus after a cerebellar stroke may mimic a peripheral nystagmus with no other central sign.
4. The recovery trend is evident after some days, which is too late: diagnosis is expected as soon as possible and clinical evolution is (hopefully) only a confirmation of the previous diagnosis.
5. Bithermal caloric test may not be available for everyone and everywhere.
6. Ocular tilt reaction can be present even with normal cover test.
Thus, the efficiency of this standard can depend on a single sign.
The aim of this work is to evaluate ocular tilt reaction (OTR), and not just cover test, as a means of diagnosis for a spontaneous nystagmus.
In case of utricular macula damage an OTR is generated, with three components:
1. Head tilted toward the injured ear.
2. Hypotropia of the eye on the side of the damaged ear.
3. Eye balls torsion (counterclockwise in case of right utriculus deficit, by the examiner point of view) [9].
The utricular afferent pathways cross the midline in the pons. Consequently, three different conditions are expected:
1. Utricular peripheral dysfunction: in this case, the hypotropic eyeball is homolateral to the lesion.
2. Central pathway dysfunction, before the crossing of fibres: even in this case the hypotropic eye is homolateral to the lesion.
3. Central pathway lesion after fibre crossing: in this case, the hypotropic eye is contralateral to the lesion because the injured fibres are coming from the opposite side and are to be referred to the opposite utricle.

The cerebellum is thought to apply an ipsilateral inhibition, so the result of cerebellar suffering would be an OTR toward the intact side (as a consequence of lack of inhibition) [10].
Thus, two types of OTR can be expected:
1. A peripheral form (the lower eye is ipsilateral to the lesion).
2. A central form (the lower eye is contralateral to the lesion).
A peripheral OTR is associated with lesions of the utricular receptor and afferent fibres until crossing.
Indeed, a lesion of the central pathway until crossing is impossible to distinguish from a peripheral lesion of the utricle, if no other signs of brain stem suffering are clinically appreciable.
This situation is reminiscent of the case in which a lesion occurs in the root entry zone of the vestibular nerve: the lesion is central from an anatomical point of view, but it is functionally peripheral.
With regards to the vestibular patient in the acute phase, three possibilities may be considered:
1. Labyrinthine deficit, including lateral semicircular canal and utricular macula.
2. Central utricular pathways injury (including medulla and/or cerebellum) associated with a central deficit sustaining spontaneous nystagmus.
3. No utricular (or utricular pathways) lesion.
In case 1), a spontaneous nystagmus would be expected towards the healthy side and an OTR on the affected side. The eye on the side of the affected ear is undermost with respect to the contralateral one: as a consequence, nystagmus is directed towards the uppermost ear ("Uphill nystagmus", Fig. 1).
In case 2), a spontaneous nystagmus would be expected towards the healthy side, but an OTR on the right side. The eye on the side of the damage is uppermost with respect

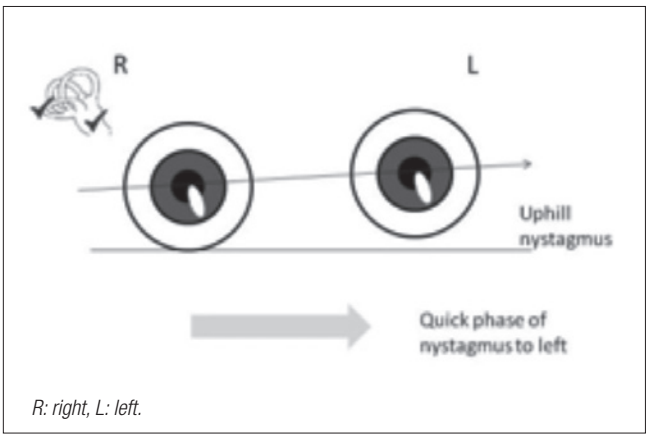


Fig. 1. The right lateral semicircular canal is damaged and a spontaneous horizontal nystagmus arises to the left. At the same time, right utriculus is injured (by the same disease) and an ocular tilt reaction is present, with hypotropia of the right eye. As a result, the plane on which nystagmus beats is inclined upward ("uphill").

of that of the other side and nystagmus is directed towards the undermost eye ("Downhill nystagmus", Fig. 2).
In case 3), an OTR cannot be expected. Eyes stand on the same line and nystagmus maintains in the horizontal plane (possibly with a rotatory component not due to the maculae, "Flat nystagmus", Fig. 3).
This aspect can be useful for the differential diagnosis between peripheral and central spontaneous nystagmus (Fig. 4).
To evaluate this sign, the direction (uphill, downhill, horizontal) of nystagmus was confronted with the presence/absence of a corrective saccade in the HIT, which is considered the best bedside evaluation test now available.

Materials and methods

65 patients were included after randomisation and exclusion of unsuitable cases (mean age 59, range 21 to 91, 32 males and 33 females) sent from the Emergency Department for acute rotatory vertigo in the period from 1/1/2012 to 31/12/2013.
Apart from the nystagmus, no signs were found by neurological examination performed in emergency room.
After careful history, patients were examined by otoscopy, pure tone audiometry (if this was impossible for the conditions of the patient, a bedside screening was obtained [11]) and evaluation of spontaneous, gaze, rebound, positional and positioning nystagmus.
Furthermore, HIT and the ability to remain standing alone were observed.
Finally, patients were examined for neurological (and especially cerebellar) signs.
A second researcher was asked to evaluate the skew deviation and to report the possible presence of an hypotropic eye. For this task, the patient, wearing Frenzel glasses, was sitting without leaning.
Patients diagnosed as central were referred back to the Emergency Department to complete examinations, the others were treated with symptomatic drugs (usually antiemetics, antihistamines, or benzodiazepines) and rechecked after one day and three days.

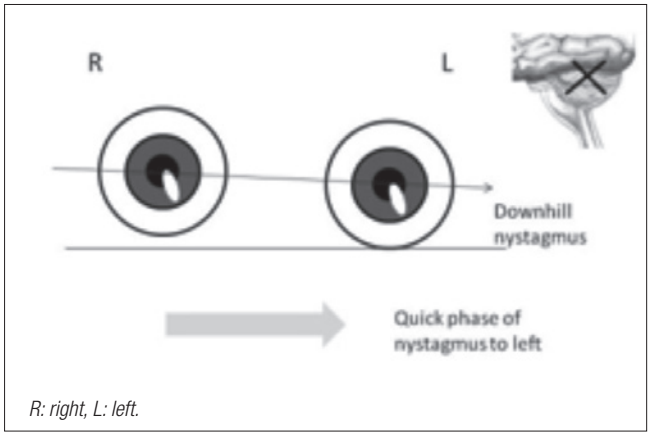


Fig. 2. A lesion is present after decussation of the utricular afferent fibres. Ocular tilt reaction is referred to the opposite side. The (central) resulting nystagmus is tilted "downhill".

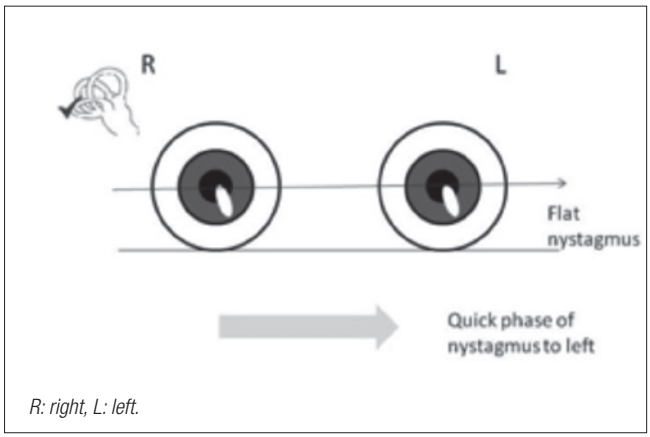


Fig. 3. The right lateral semicircular canal is damaged and a spontaneous horizontal nystagmus arises directed to the left, but the right utriculus is spared. As a result, the plane on which nystagmus beats is horizontal ("flat" nystagmus).

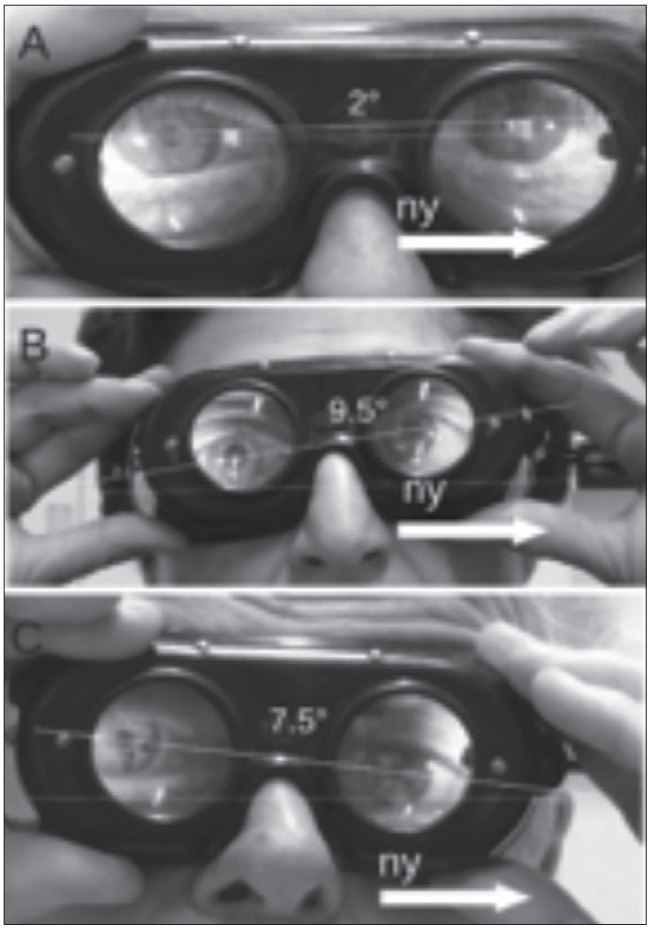


Fig. 4. "Flat" (unknown) nystagmus (A) , "Uphill" (peripheral) nystagmus (B), "Downhill" (central) nystagmus (C).

Exclusion criteria were:
- previous medical treatment;
- known eye motility disorders;
- stiff neck;
- presence of previous audiological, vestibular, neurological diseases.

Only patients with horizontal spontaneous nystagmus were considered.

Excluded patients underwent the same tests and treatments as the others, but were not considered for statistical purposes.

At day 1 some improvement, even if slight, was expected, otherwise the patient was reconsidered as central.

On the third day, a bithermal caloric test was performed (after Fitzgerald-Hallpike), and evaluation of unilateral weakness and directional preponderance were obtained (applying Jongkees formulas).

Results

The direction of nystagmus (beating on a plane tilted toward the hypotropic or hypertropic eyeball) was compared with the HIT, considered as the gold standard in patients with spontaneous nystagmus. The rate of congruence of the two tests is reported in Table I and Table II.

In 32 cases, the quick phase of nystagmus was directed to the right, and in 33 cases to the left. In 10 of 65 cases (about 15%), a tilt direction of nystagmus (with respect to the horizon) was not detected.

In 10 of 65 cases (about 15%), a bedside evaluation of the HIT test was considered questionable (difficult to understand). In one case, neither skew deviation nor HIT were clearly detectable.

Table I reports the cases in which a correlation between nystagmus direction and HIT were impossible, either for a "flat" nystagmus or for a HIT that was difficult to interpret by bedside examination. In total, 19 of 65 cases were not comparable.

Table II reports the 46 cases in which a correlation between nystagmus direction and HIT were possible.

In 43 of the remaining 46 cases, nystagmus tilt was congruent with HIT: 8 cases were of the central type (downhill nystagmus and normal HIT), and 35 of the peripheral type (uphill nystagmus and pathological HIT) (Table II).

In three cases, there was no congruence between the two tests.

In the two cases of negativity of HIT, the presence of "covert saccades" must be taken into account. In the case in which the HIT was abnormal, vertigo had been lasting for about two weeks, and thus it can be argued that a compensation of skew deviation was present. Keeping HIT as the gold standard, testing for nystagmus tilt allowed to obtain 80% sensitivity and 97 % specificity.

Discussion

Overall, the results are suggestive for a close relationship between the OTR test and HIT.

Table I. If HIT is ambiguous (difficult to understand) or the spontaneous nystagmus beats strictly on the horizontal plane (or both) the results are not comparable. In our series, this occurred in 19 of 65 cases.

Case N	Gender	Age	Direction of nystagmus	Hypertropic eye	HIT	Congruence
1	M	41	Right	Right	?	?
2	M	58	Right	Right	?	?
3	F	58	Right	Left	?	?
4	F	46	Right	Right	?	?
5	M	55	Right	Right	?	?
6	M	72	Right	=	-	?
7	F	51	Left	=	-	?
8	M	48	Left	=	+	?
9	M	77	Right	=	+	?
10	M	58	Right	=	?	?
11	F	46	Left	Left	?	?
12	M	55	Right	Right	?	?
13	F	58	Right	Right	?	?
14	F	49	Right	=	-	?
15	F	44	Left	=	-	?
16	F	21	Left	=	+	?
17	M	79	Left	=	-	?
18	M	78	Left	Left	?	?
19	F	66	Right	=	+	?

Table II. Comparing the plane of nystagmus (directed toward the hypertropic eye, "Uphill",or directed to the hypotropic eye, "Downhill") with HIT, chosen as the "gold standard", a close relation appears between the results of the two tests. Only in three cases was equivalency lacking (n = 1; n = 17; n = 37). In the first two cases, this was attributed to "covert" saccades or a bias in execution of HIT. In the last case, a compensation of macular deficit seems possible since nystagmus was present for about two weeks.

Case N	Gender	Age	Direction of nystagmus	Hypertropic eye	HIT	Congruence
1	**F**	**66**	**Left**	**Right**	**+**	**No**
2	F	67	Left	Left	+	Yes
3	M	56	Left	Left	+	Yes
4	M	62	Left	Left	+	Yes
5	M	42	Left	Left	+	Yes
6	F	72	Left	Right	-	Yes
7	F	52	Right	Right	+	Yes
8	F	74	Right	Right	+	Yes
9	F		Right	Left	-	Yes
10	M	72	Left	Right	-	Yes
11	F	59	Right	Right	+	Yes
12	F	85	Right	Right	+	Yes
13	M	41	Left	Left	+	Yes
14	M	21	Left	Left	+	Yes
15	F	40	Right	Right	+	Yes
16	F	53	Right	Right	+	Yes
17	**M**	**72**	**Left**	**Left**	**-**	**No**
18	F	69	Left	Left	+	Yes
19	M	53	Left	Left	+	Yes
20	F	66	Left	Left	+	Yes
21	M	55	Right	Right	+	Yes
22	M	41	Right	Right	+	Yes
23	M	35	Left	Left	+	Yes
24	M	56	Left	Left	+	Yes
25	M	91	Right	Right	+	Yes
26	M	42	Left	Left	+	Yes
27	F	52	Right	Right	+	Yes
28	F	79	Right	Right	+	Yes
29	F	74	Right	Right	+	Yes
30	M	39	Right	Right	+	Yes
31	M	62	Left	Left	+	Yes
32	F	69	Right	Left	-	Yes
33	M	62	Left	Left	+	Yes
34	M	42	Left	Left	+	Yes
35	F	72	Left	Right	-	Yes
36	F	42	Right	Right	+	Yes
37	**M**	**64**	**Left**	**Left**	**-**	**No**
38	F	73	Left	Left	+	Yes
39	F	70	Left	Right	-	Yes
40	F	57	Left	Left	+	Yes
41	F	65	Right	Right	+	Yes
42	M	84	Right	Right	+	Yes
43	F	62	Right	Left	-	Yes
44	F	65	Right	Right	+	Yes
45	M	60	Left	Right	-	Yes
46	M	66	Left	Left	+	Yes

A nystagmus directed toward the healthy side associated with a peripheral skew deviation beats slightly uphill, as a consequence of hypotropia of the unwell side eye.
A nystagmus directed toward the healthy side associated with a central skew deviation beats slightly downhill, as a consequence of hypotropia of the healthy side eye.
A nystagmus originated by a disfacilitating cerebellar mechanism beats slightly downhill as a consequence of the presence of a central skew deviation.
If otherwise the utricular component is lacking (utricular maculae are spared), the nystagmus direction is fairly horizontal and no information can be argued about nystagmus origin: in our series, this is a fairly uncommon situation.
In case of doubt, the inter-pupillary plane can be obtained from a photograph and measured. In a previous work, the normal values of deviation were not over 2.2 degrees [12]. The examiner most often has the exact perception of the situation just by observing the patient who is sitting and holding glasses alone: thus, this test is actually a bedside test, confirming HIT or replacing it in cases of ambiguity (not collaborating patients, covert saccades, etc.).
Frenzel glasses are preferable to videooculoscopy because cameras are not likely to be perfectly horizontal, but inspection without any kind of device is possible if, of course, nystagmus is not completely inhibited by fixation.
On the basis of our results, it would appear that in the majority of cases utriculus is damaged along with the canal: only in 15 % of cases did we find spared utricular maculae in spontaneous nystagmus. This is associated with a strictly horizontal nystagmus (not uphill, not downhill).

Conclusions

At present, no bedside test alone is able to diagnose the lesion site in every case of spontaneous nystagmus. HIT is a resource, but not always it is easy to perform and to interpret. Nevertheless, a prompt diagnosis is required in case of acute vertigo and spontaneous nystagmus: in these cases, a decision is taken on the base of clinical examination (magnetic resonance imaging is not always available and not entirely reliable at the early stages).
As each clue can be useful, observation of the plane on which the nystagmus beats can be convenient as not time consuming or tedious for the patient.
It might be suggested that nystagmus be classified in three groups:

1. uphill nystagmus (to be considered as peripheral);
2. downhill nystagmus (to be considered as central);
3. plane nystagmus (nystagmus is beating right in plane, not rising nor falling), not meaningful, as utricular macula is likely not concerned.

References

[1] Teggi R, Manfrin M, Balzanelli C, et al. *Point prevalence of vertigo and dizziness in a sample of 2672 subjects and correlation with headaches*. Acta Otorhinolaryngol Ital 2016;36:215-9.

[2] Kattah JC, Talkad AV, Wang DZ, et al. *Newman-Toker DE HINTS to diagnose stroke in the acute vestibular syndrome: three-step bedside oculomotor examination more sensitive than early mri diffusion-weighted imaging.* Stroke 2009;40:3504-10.

[3] Halmagyi GM, Curthoys IS. *A clinical sign of canal paresis.* Arch Neurol 1988;45:737-9.

[4] Vanni S, Pecci R, Casati C, et al. *STANDING, a four-step bedside algorithm for differential diagnosis of acute vertigo in the Emergency Department*. Acta Otorhinolaryngol Ital 2014;34:419-26.

[5] Nelson JA, Viirre E. *The clinical differentiation of cerebellar infarction from common vertigo syndromes.* West J Emerg Med 2009;10:273-7.

[6] Cnyrim CD, Newman-Toker D, Karch C, et al. *Bedside differentiation of vestibular neuritis from central "vestibular pseudoneuritis"*. Neurol Neurosurg Psychiatry 2008;79:458-60.

[7] Lee H, Sohn SI,Cho JW, et al. *Cerebellar infarction presenting isolated vertigo.* Neurology 2006;67:1178-83.

[8] Tjernström F, Nyström A, Magnusson M. *How to uncover the covert saccade during the head impulse test.* Otol Neurotol 2012;33:1583-5.

[9] Westheimer G, Blair SM. *The ocular tilt reaction - a brainstem oculomotor routine*. Invest Ophtal 1975;14: 833-9.

[10] Mossman S, Halmagyi GM. *Partial ocular tilt reaction due to unilateral cerebellar lesion.* Neurology 1997;49:491-3.

[11] Eekhof JA, de Bock GH, de Laat JA, et al. *The whispered voice: The best test for screening for hearing impairment in general practice?* Br J Gen Pract 1996;46:473-4.

[12] Gufoni M. *Le manifestazioni cliniche della VPPB del canale laterale*. In: *La Vertigine Parossistica Posizionale (Benigna): Stato dell'arte.* LV Raduno del Gruppo Alta Italia di Otorinolaringoiatria e Chirurgia Cervico-Facciale. Presidente Prof. D. Nuti. Siena 5 Dicembre 2009.

Received: August 20, 2016 - Accepted: May 4, 2017

Address for correspondence: Mauro Gufoni, Department of Otorhinolaryngology, Spedali Riuniti Livorno, viale Alfieri 36, 57100 Livorno, Italy. Tel. +39 0586 223195. E-mail: mgufoni@alice.it

ACTA OTORHINOLARYNGOLOGICA ITALICA 2017;37:519-522; doi: 10.14639/0392-100X-1043

CASE SERIES AND REPORTS

Can the onset of orbital cancer be the result of a prosthetic eye?

L'insorgenza di un tumore orbitario può risultare dall'utilizzo di una protesi oculare?

A. CROCE, V. MASTRONARDI, M. LAUS, E. FESTA KOTELNIKOVA
ENT Department, University "G. d'Annunzio" of Chieti-Pescara, Hospital "SS Annunziata", Chieti, Italy

SUMMARY

Orbital exenteration is a disfiguring procedure performed for unresponsive orbital infections and control of recurrent benign tumours and malignancies arising from the eyelids (basal cell carcinoma, squamous cell carcinoma, conjunctival malignant melanoma), lachrymal glands (adenoid cystic carcinoma) or surrounding sinuses. In extremely rare cases the use of a prosthetic eye after enucleation can lead to anophthalmic socket tumours. We report the case of a 54-year-old man who had left eye enucleation due to recurring events of retinal detachment and who developed an invasive fast growing epidermoid carcinoma 30 years later. We review the literature to evaluate the rarity of the occurrence, time of onset after enucleation, treatments and outcomes. Our case illustrates the management of the pathology and emphasises the necessity of careful examination of the anophthalmic socket and the ocular prosthesis to identify any irregularities or damage on its surface even after exenteration that is not performed for malignant disease. Long-term follow up is necessary because this tumour could occur at long time periods after enucleation.

KEY WORDS: Orbital exenteration • Eyelid carcinoma • Anophthalmic socket

RIASSUNTO

L'exenteratio orbitae è un intervento deturpante che si pratica in caso di infezioni orbitarie non responsive a terapia medica e in caso di tumori benigni ricorrenti e tumori maligni che insorgono dalle palpebre (carcinoma basocellulare, carcinoma squamocellulare, melanoma maligno della congiuntiva), dalle ghiandole lacrimali (carcinoma adenoideo cistico) o dalle strutture circostanti. In casi estremamente rari l'uso di protesi oculari dopo l'enucleazione può causare l'insorgenza di tumori orbitari. In questo articolo riportiamo il caso di un uomo di 54 anni che è stato sottoposto ad enucleazione dell'occhio sinistro in seguito a ricorrenti distacchi di retina e che ha sviluppato, solo 30 anni dopo, un carcinoma epidermoidale infiltrante a rapida crescita. Abbiamo esaminato gli articoli in letteratura per valutare la rarità di tale occorrenza, i tempi d'insorgenza in seguito all'enucleazione, i trattamenti e i risultati. Il nostro caso illustra il trattamento della patologia ed enfatizza la necessità di un attento esame della cavità orbitaria e della protesi oculare per identificare anche le irregolarità e i primi danni sulla superficie orbitaria anche nei casi in cui l'exenteratio orbitae è stata eseguita per patologie benigne. Il nostro caso dimostra che il follow-up a lungo termine è fondamentale perché il tumore può insorgere a lunga distanza dopo l'enucleazione.

PAROLE CHIAVE: *Exenteratio orbitae • Carcinoma della palpebra • Cavità orbitaria*

Acta Otorhinolaryngol Ital 2017;37:519-522

Introduction

Orbital exenteration is a procedure performed for unresponsive orbital infections as well as local control of recurrent benign tumours and malignancies arising from the eyelids (basal cell carcinoma, squamous cell carcinoma, conjunctival malignant melanoma), lachrymal glands (adenoid cystic carcinoma), or the surrounding sinuses.

In 1971 Meyer and Zaoli classified the exenteration procedure into four groups [1]:

- Type I: the palpebral skin and the conjunctiva are left intact;
- Type II: only the palpebral skin is left intact;
- Type III: both eyelids are removed with orbital contents;
- Type IV: the eyeball, eyelids and appendages of the eye are removed together with the involved bone structures.

From 1999, according to Mouriaux F et al. [2], orbital exenterations may be subdivided into three categories:

- sub-total: orbital tissues are partially removed with sacrifice of the globe;
- total: removal of all tissues within the orbit including the globe and periorbital;
- extended: resection of adjacent bone.

A simpler classification was proposed by Yeatts (2005) [3], who divided exenterations in only two categories:

- subtotal: partial removal of orbital tissues;
- total exenteration: removal of the entire orbital content.

Orbital exenteration is a disfiguring procedure but if combined with immediate reconstruction treatment, it improves the quality of life of patients [4].
To reconstruct an exenteration defect, there are a large number of options: spontaneous granulation (often takes several months but provides better colour), skin grafts (the process is faster), temporalis muscle flap associated with skin graft, cervicofacial flap, temporoparietal fascial flap, frontal flap, free tissue transfer using the rectus abdominis, latissimus dorsi, radial forearm, lateral arm and anterolateral thigh flap. Reconstruction methods by free tissue transfer are often used when the exenteration is associated with maxillectomy. Attempts have been made with a fibular osteocutaneous flap, an iliac crest internal oblique free flap and latissimus dorsi-scapular/parascapular flap. Orbital exenteration and reconstruction with prosthesis can lead to other pathologies on rare occasions, as we demonstrate in the following case.

Description of clinical case

A 54-year-old man presented to the ENT Clinic of SS Annunziata of Chieti with a fast growing mass from his left orbit that completely occluding the left eye socket (Fig. 1-A).
From the patient's history, it was noticed that the left eye had been enucleated 30 years before, due to recurring events of retinal detachment with complications that made left eye exenteration necessary. Since that time he had used a smooth ocular prosthesis made of artificial resin in the anophthalmic socket (Fig. 1-B). He referred he had no discomfort, irritation, or discharge in the eye socket or need for maintenance of the prosthesis over the years.
Apart from enucleation, the patient had an unremarkable medical history and no family history of malignancy.
In 2014 the patient had surgery for right retinal detachment. At that time no difficulty in wearing the left eye prosthesis was noticed.
At admission time, the patient had a large mass covering the whole left ocular surface with several bleeding sites on it. The mass was reported to be growing fast in the previous few months before presentation at our eye clinic. On examination, the mass was arising from his left lower eyelid, and cilia were present in upper left eyelid. Regional lymph nodes were not palpable.
Gadolinium-enhanced magnetic resonance imaging revealed a contrast enhanced mass occupying the anterior part of the left orbit and spreading outside, measuring 3.6 cm x 2 cm x 2 cm. There were no signs of invasion of the surrounding bony orbit.
A biopsy was performed and histological examination revealed invasive, keratinised, moderately differentiated epidermoid carcinoma.
Surgical treatment consisted of excision of the entire tumour mass, removing the orbital content and the left lower eyelid (Fig. 1-C). The eye socket was reconstructed using a Thiersch skin graft taken from the abdomen of the patient (Fig. 1-D).
The mass measured 5.5 x 4.8 x 3 cm. Histological examination confirmed invasive, ulcerated, keratinised, moderately differentiated epidermoid carcinoma and margins free of disease.
The patient recovered well. After 6 months free of surgical complications, he started the procedure to fit an epithesis.

Discussion

Malignant tumours of the orbit and the orbitomaxillary region constitute 4-8% of head and neck malignancies. In particular, eyelid malignancies represent approximately 5% to 10% of all skin cancers and more than 90% of all ophthalmic tumours. Incidence studies report that basal cell carcinoma is the most common malignant ocular neoplasia, counting for 80-90% of cases, followed by squamous cell carcinoma (5-10%), retinoblastoma, sebaceous gland carcinoma, malignant melanoma [4], rhabdomyosarcoma and Merckel cell carcinoma, even if it is demonstrated that the histopathological spectrum and relative frequencies vary markedly among different countries. The regional differences may be due to different skin types (high rate in fair-skinned populations), sunlight exposure and disease awareness.
Ultraviolet light exposure, smocking, genetic mutation, radiation, HPV type 16 and 18 infection, HIV infection and chronic inflammation of the ocular surface are the main risk factors for ocular surface epithelial neoplasia. Although sun exposure is widely accepted as a risk factor for developing basal cell carcinoma, squamous cell carcinoma and melanoma, the mechanism of carcinogenesis in these lesions is not clear.
Basal cell carcinoma is a neoplasm of epithelial origin caused mainly by ultraviolet radiation.
Squamous neoplasms can involve the conjunctiva or the cornea individually, but they probably arise from the corneal epithelial stem cells of the limbal region, which represent a transitional zone between the conjunctival epithelium and the stratified squamous corneal epithelium, and then extend across the limbus to involve the adjacent cornea.
A malignant tumour growing in an anophthalmic orbit, without a history of previous malignant tumours, is extremely rare. A review of literature identified only a few cases of carcinoma developing in an anophthalmic socket. Espana et al., in 2011, reported a detailed review of eight previous published cases reporting some similar characteristics [5].
Like our case, all patients were male and all developed a squamous cell carcinoma of the eyelid in an average time of 44.8 years after prosthesis use. The present case, however, would represent the shortest interval post-exentera-

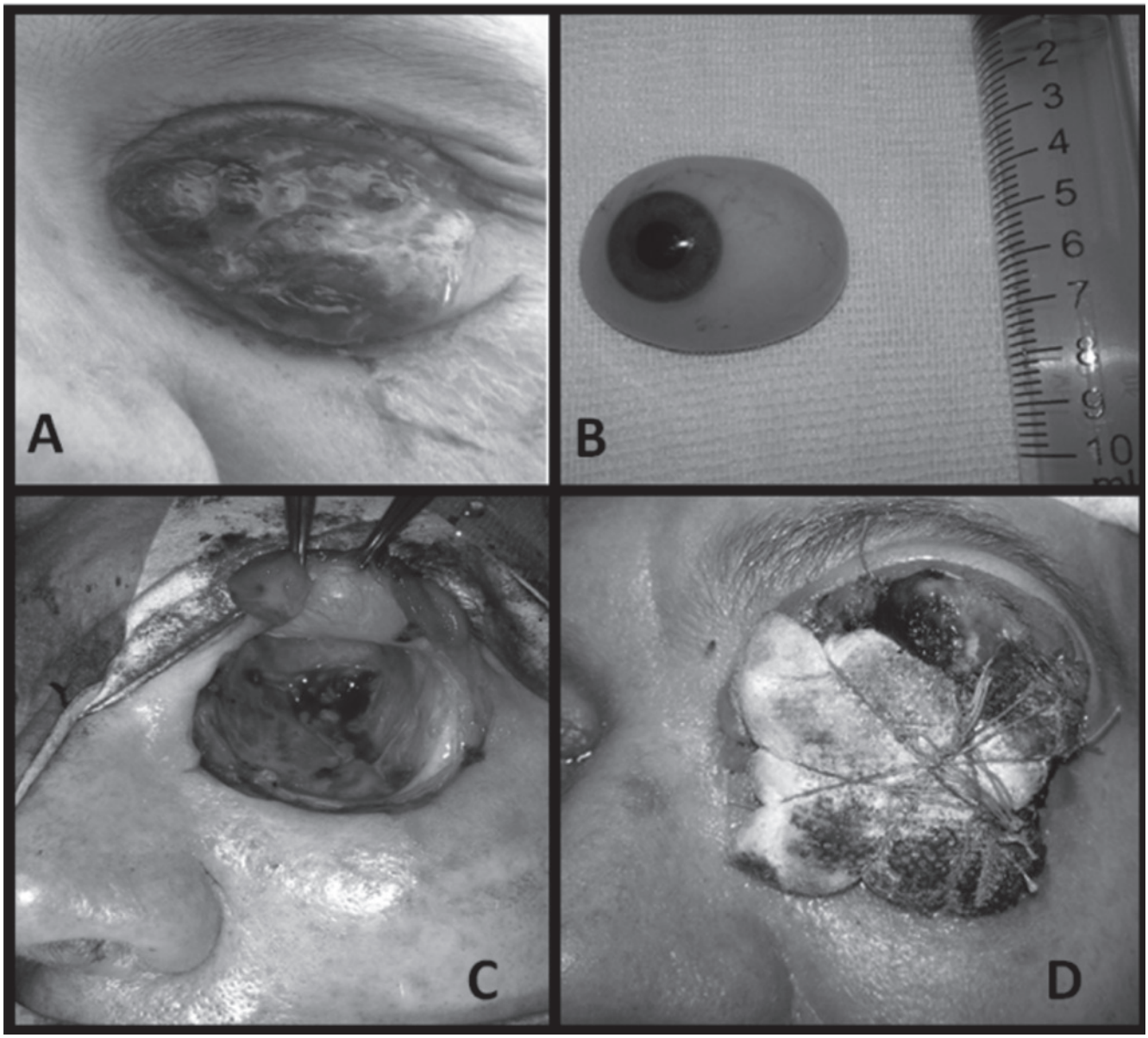


Fig. 1. A 54-year-old man presented in ENT Clinic of SS Annunziata of Chieti with a mass fast growing from his left orbit and completely occluding the left eye socket (Fig. 1-A). For 30 years he had used a smooth ocular prosthesis made of artificial resin in the anophthalmic socket (Figure 1-B). The surgical treatment consisted of excision of the entire tumour mass removing the orbital content and the left lower eyelid (Fig. 1-C). The eye socket was reconstructed using a Thiersch skin graft taken from the abdomen of the patient (Fig. 1-D).

tion reported, at 30 years after exenteration and diagnosis of squamous cell carcinoma. The upper eyelid was, according to Espana review, the most common site, while in our case the lower eyelid was involved [5]. Our patient also presented an advanced local stage of the tumour at diagnosis with a large, rapidly-growing cauliflower-like mass covering the entire ocular surface, but without regional and distant metastasis.

Previous studies hypothesised that chronic irritation from wearing an eye prosthesis for more than 40 years was suspected to be associated with the development of conjunctival squamous cell carcinoma and melanoma. Our patient did not present any signs or symptoms of prosthetic discomfort before tumour growth, and his ocular prosthesis did not present any irregularities or damage that could lead to chronic inflammation. Despite this, after 30 years of wearing a good-fitting prosthesis a squamous cell ocular carcinoma developed in an anophthalmic socket. Kim et al. (2008) demonstrated that squamous metaplasia with decreased goblet cell density and increased nucleus-to-cytoplasm ratio can occur in anophthalmic sockets without any particular aspects of prosthesis care [6]. There were probably other risk factors involved that remain unknown in this carcinogenesis. For example, a viral origin cannot be excluded in the pathogenic mechanism because we did not perform molecular testing to identify a viral cause.

Our patient is a rare case of ocular surface squamous neoplasia that developed in an anophthalmic socket 30 years after exenteration not performed for malignant disease. It arose from the lower palpebral conjunctival surface as a large mass occupying all the ocular surface. We performed an exenteration revision. Our patient has exceeded the postoperative period without any complications and currently rehabilitation is planned with a new aesthetic orbital prosthesis considering his young age. In the literature it is reported that only 10.8% of patients received ocular prosthetic rehabilitation after surgical treatment, which is associated with lower age and enucleation surgery [7].

The onset of discharge, swelling, recent poor fitting of prosthesis, or a palpable orbital mass in a patient wearing an ocular prosthesis for many years should suggest a possible malignant neoplasm. Accordingly, careful ophthalmic examination of the anophthalmic socket and the ocular prosthesis should be performed to identify possible irregularities or damages on its surface. Long-term follow up is necessary because this tumour can occur after long time period following enucleation.

Acknowledgements

The authors would like to thank the patient for providing consent to use his photograph in this article.

References

1 Radici M, Bicciolo G, Palma O, et al. *Il massicciofaciale*. In: De Campora E, Marzetti F, editors. *La chirurgia oncologica della testa e del collo*. Pisa, Pacini Editore; 1996. ,p. 345-381.

2 Mouriaux F, Martinot V, Pellerin P, et al. *Survival after malignant tumors of the orbit and periorbit treated by exenteration*. Acta Ophthalmol Scand 1999;77:326-30.

3 Yeatts RP. *The esthetics of orbital exenteration*. Am J Ophthalmol 2005;139:152-3.

4 Croce A, Moretti A, D'Agostino L, et al. *Orbital exenteration in elderly patients: personal experience*. Acta Otorhinolaryngol Ital 2008;28:193-9.

5 Espana EM, Levine M, Schoenfield L, et al. *Ocular surface squamous neoplasia in an anophthalmic socket 60 years after enucleation*. Surv Ophthalmol 2011;56:539-43.

6 Kim JH, Lee MJ, Choung HK, et al. *Conjunctival cytologic features in anophthalmic patients wearing an ocular prosthesis*. Ophthal Plast Reconstr Surg 2008;24:290-5.

7 Sirianni D, Rodriguez Leles C, Mendonça EF. *A 12-years retrospective survey of management of patients with malignant neoplasm in the orbital cavity in a Brazilian cancer hospital*. Open Dent J 2013;7:140-5.

Received: January 13, 2016 - Accepted: October 12, 2016

Address for correspondence: Melissa Laus, ENT Department, University of Chieti, via dei Vestini 5, 66013, Chieti, Italy. Tel. + 39 0871 552033. Fax +39 0871 358375. E-mail: melissa.laus@hotmail.it

Acknowledgements to our reviewers

Acta Otorhinolaryngol Ital 2017;37:523-524

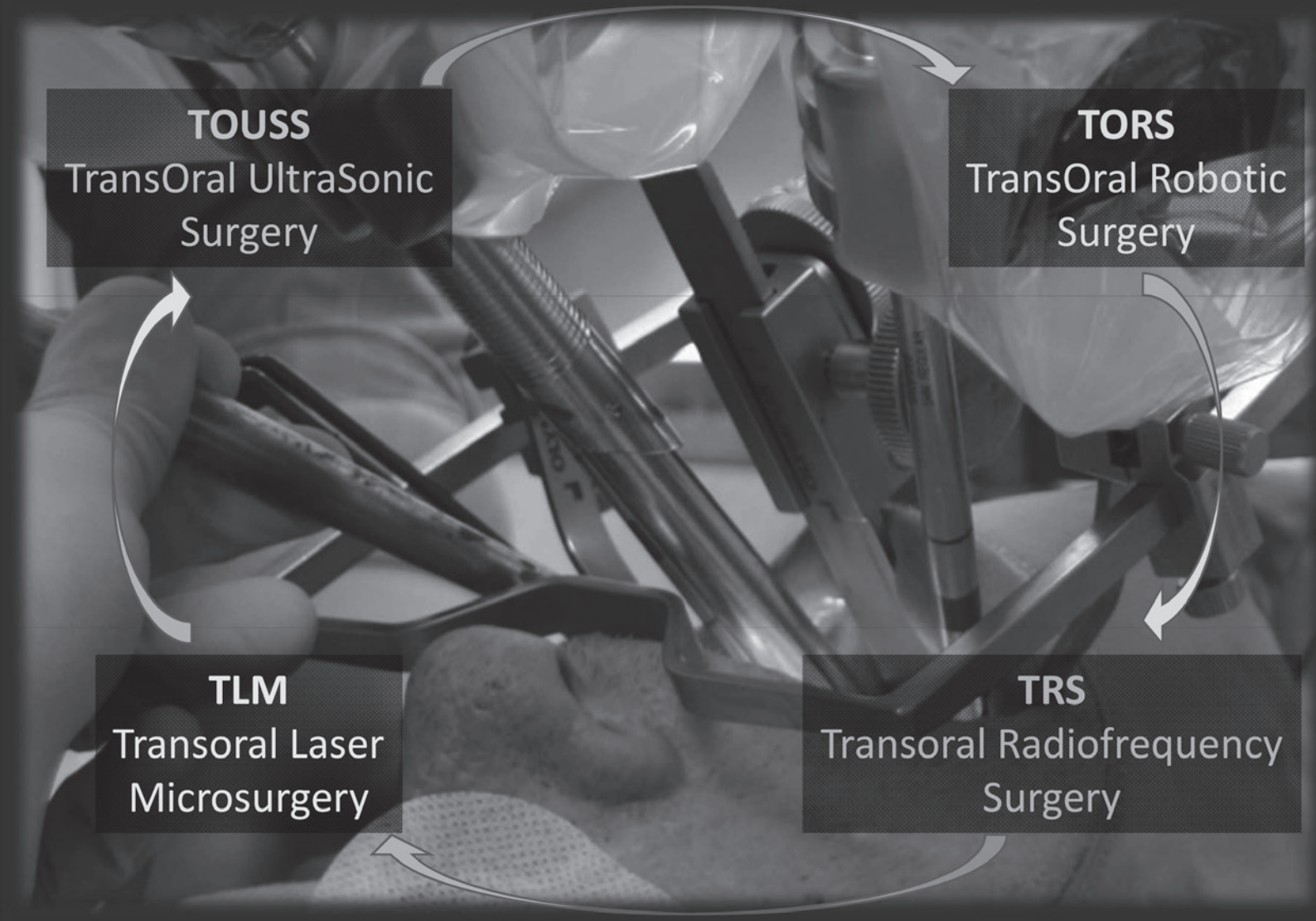
The Editor-in-Chief and the Editorial Board of *Acta Otorhinolaryngologica Italica* are extremely grateful to all the colleagues who, during 2017, together with the Scientific Committee, kindly reviewed the scientific papers submitted for publication, contributing to a meticulous selection of the material and appropriate re-elaboration of the manuscripts:

Albera Roberto
Alicandri Ciufelli Matteo
Almadori Giovanni
Ambrosetti Umberto
Andreano Anita
Anile Carmelo
Antonelli Massimo
Asprella Libonati Giacinto
Attanasio Giuseppe
Bacciu Salvatore
Baj Alessandro
Barbara Maurizio
Barillari Umberto
Barzan Luigi
Bellantone Rocco
Bellocchi Gianluca
Bellussi Luisa
Benazzo Marco
Berrettini Stefano
Berrone Mattia
Bertolin Andy
Bignami Maurizio
Boccieri Armando
Bolzoni Villaret Andrea
Boscolo Rizzo Paolo
Bottero Sergio
Bovo Roberto
Bruschini Roberto
Buonomo Alessandro
Burdo Sandro
Bussi Mario
Bussu Francesco
Cadoni Gabriella
Calabrese Luca
Califano Luigi
Calo' Lea
Canale Andrea
Canevari Frank Rikki
Cantone Elena
Cantù Giulio
Canzi Pietro
Capaccio Pasquale
Carey Thomas E.
Carrau Ricardo
Casani Augusto Pietro
Cassandro Ettore
Cassano Alessandra
Cassano Michele
Castagnola Massimo
Castelnuovo Paolo
Cattaneo Augusto
Cavaliere Matteo
Cavalot Andrea
Cesarani Antonio
Chiesa Fausto
Chiocca Susanna
Chu Francesco
Cianfrone Giancarlo
Cianfrone Francesca
Cingi Cemal
Ciorba Andrea
Ciprandi Giorgio
Colletti Vittorio
Colosimo Cesare
Congedo Maria Teresa
Conti Guido
Copelli Chiara
Costamagna Guido
Cristofari Eliana
Croce Adelchi
Crosetti Erika
Cuda Domenico
Da Mosto Maria Cristina
D'Alatri Lucia
D'Ambrosio Ferdinando
Damiani Valerio
Danesi Giovanni
Danilo Patrocinio
De Benedetto Luigi
De Benedetto Michele
De Bree Remco
De Campora Luca
De Corso Eugenio
De Crea Carmela
De Cristofaro Raimondo
De Vincentiis Marco
De Vincentiis Giovanni Carlo
De Virgilio Armando
Deganello Alberto
Deli Giorgio
Deli Roberto
Della Marca Giacomo
Della Vecchia Laura
Delogu Giovanni
Desrosiers Martin
Di Gennaro Leonardo
Di Nardo Walter
Dispenza Francesco
Doglietto Francesco
Dragonetti Alberto
Eisele David W.
Emanuelli Enzo
Fadda Guido
Fadda Gianluca
Falcioni Maurizio
Faralli Mario
Fariselli Laura
Farneti Daniele
Fasanella Luigi
Fattori Bruno
Felisati Giovanni
Ferreli Fabio
Fetoni Annarita
Filipo Roberto
Fiorino Francesco
Fiorita Antonella
Forli Francesca
Galli Jacopo
Gallo Oreste
Gallo Andrea
Garavello Werner
Gaudino Simona
Gelardi Matteo
Genovese Elisabetta
Genuardi Maurizio
Georgalas Christos
Georgios Giourgos
Giacalone Andrea
Gilbert Ralph W.
Giugliano Gioacchino
Greco Antonio
Grippaudo Cristina
Gufoni Mauro
Guidetti Giorgio
Halmagyi Gabor M.
Hatzopoulos Stavros
Hellings Peter W.
Hilgers Frans J.M.
Hummel Thomas
Iannetti Giorgio
Iro Heinrich
Karligkiotis Apostolos
Kawauchi Hideyuki John
Laszig Roland
Leemans René
Lenzi Riccardo
Leone Carlo Antonio

Licitra Lisa
Lopatin Andrey S.
Lucidi Daniela
Lucioni Marco
Macchi Alberto
Magnani Massimo
Maiolino Luigi
Mallardi Vito
Mandalà Marco
Manea Claudiu
Manfrin Marco
Mansi Nicola
Marcelli Vincenzo
Marchal Francis
Marchese Maria Raffaella
Marchese Ragona Rosario
Marchisio Paola
Marciano Elio
Marioni Gino
Maroldi Roberto
Marsella Pasquale
Martina Bianca Maria
Martini Alessandro
Marzetti Andrea
Mastrapasqua Rodolfo Francesco
Meacci Elisa
Meglio Mario
Mele Maria Cristina
Mele Vincenzo
Melendez Amarillis
Mennuni Gioacchino
Mesolella Massimo
Miani Cesare
Miccichè Francesco
Milano Francesca
Mladina Ranko
Mocella Stelio
Moesges Ralph
Mohssen Ansarin
Montevecchi Filippo
Moretti Antonio
Motta Sergio
Mullol Joaquim
Murri Alessandra
Musacchio Angela
Navach Valeria
Navarra Pier Luigi
Neri Giampiero
Nicolai Piero
Nosengo Stefano
Novelli Giuseppe
Nuti Daniele
Odone Anna
O'Donoghue Gerard
Olivi Alessandro
Orzan Eva
Ottaviani Fabrizio
Ottaviano Giancarlo
Pagella Fabio Giuseppe
Pagnini Paolo
Pagnoni Mario
Palma Pietro
Palma Stefano
Panatta Maria Laura
Pani Giovambattista
Parrilla Claudio
Pascali Vincenzo Lorenzo
Pasquini Ernesto
Passali Desiderio
Passali Giulio Cesare
Passali Francesco Maria
Patrizi Mario
Pecci Rudi
Pellini Raul
Pelo Sandro
Pelucchi Claudio
Peretti Giorgio
Petrone Gianluigi
Petrone Paolo
Pezzoli Matteo
Pia Francesco
Piazza Cesare
Piazza Fabio
Piccin Ottavio
Picciotti Pasqualina Maria
Piccirillo Enrico
Piemonte Marco
Pignataro Lorenzo
Pirodda Antonio
Pittiruti Mauro
Pomponi Massimiliano
Pontecorvi Alfredo
Portincasa Aurelio
Presutti Livio
Principi Nicola
Prosser Silvano
Pusateri Alessandro
Puxeddu Roberto
Quaranta Nicola
Raffaelli Marco
Ralli Giovanni
Ranelletti Franco Oreste
Redaelli De Zinis Luca
Remacle Marc
Ricci Giampietro
Ricci Maccarini Andrea
Rigante Mario
Rigante Donato
Rinaldi Ceroni Alberto
Rindi Guido
Riva Francesco
Rivolta Marcelo N.
Rizzotto Giuseppe
Roland Peter
Rolesi Rolando
Romagnoli Costantino
Rossi Marco
Rufini Vittoria
Ruggiero Antonio
Rugiu Maria Gabriella
Ruoppolo Giovanni
Ruscito Paolo
Russi Elvio G.
Saccomanno Sabina
Salerni Lorenzo
Salgarello Marzia
Sali Michela
Salvi Richard
Santarelli Rosamaria
Sarafoleanu Codrut
Savastano Vincenzo
Savino Gustavo
Sbarra Morelli Gloria
Scarano Emanuele
Scelsi Daniele
Schiavino Domenico
Schindler Antonio
Scimemi Pietro
Sciuto Sebastiano
Scola Yurrita Bartolome`
Scorpecci Alessandro
Sellari Franceschini Stefano
Sergi Bruno
Serra Agostino
Sesenna Enrico
Sgambato Giovanni
Shah Jatin P.
Siegert Ralf
Speciale Riccardo
Spriano Giuseppe
Stabile Achille
Staibano Stefania
Stammberger Heinz
Sterkers Olivier
Succo Giovanni
Tagliabue Marta
Tarsitano Achille
Tartaglione Tommaso
Tasca Ignazio
Teggi Roberto
Testa Domenico
Tirelli Giancarlo
Tombolini Vincenzo
Trabalzini Franco
Troiani Diana
Turri Zanoni Mario
Ursino Francesco
Valentini Vincenzo
Valentini Valentino
Van der Putten Lisa
Vannucchi Paolo
Varini Alessandro
Vetrugno Giuseppe
Vicini Claudio
Vigili Maurizio Giovanni
Villa Maria Pia
Visocchi Massimiliano
Zampino Giuseppe
Zanetti Diego
Zanoletti Elisabetta
Zenner Peter

International Workshop

TRANSORAL MINI-INVASIVE SURGERY IN HEAD AND NECK CANCER



President
GIANCARLO TIRELLI

15th - 16th - 17th March 2018

TRIESTE (ITALY)