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Review

Obstructive sleep apnoea syndrome: a new paradigm by chronic nocturnal intermittent hypoxia and sleep disruption

Ipossia cronica intermittente notturna e alterazioni dell'architettura del sonno: nuovo paradigma causale dell'aterosclerosi e cancro

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SUMMARY

Obstructive sleep apnoea syndrome (OSAS) is associated with severe cerebro-cardiovascular morbidity and mortality. It is an independent risk factor for atherosclerosis, arterial thrombosis and metabolic syndrome, and recently has been associated with an increased incidence of cancer and death. A causal link between OSAS and atherosclerosis has been partially established. Recent research on atherosclerosis in OSAS has focused on thrombotic tendency and blood viscosity, providing new insight into disease mechanisms. Hypoxia is a critical pathophysiological element in OSAS that leads to intensive sympathetic activity, in association with inflammation, oxidative stress and procoagulant activity. Hypoxia and the induction of oxidative stress can simultaneously represent an underlying mechanism in the pathogenesis of cancer development and progression. This mini-review will discuss the latest findings on the association and potential relationship between OSA and pathological vascular sequelae.

KEY WORDS: Atherosclerosis • Cancer • Chronic intermittent hypoxia • Obstructive sleep apnoea • Sleep disruption

RIASSUNTO

La sindrome delle apnee ostruttive durante il sonno è associata ad un aumento della morbilità e mortalità cerebro-cardiovascolare. Si tratta di un fattore di rischio indipendente per aterosclerosi precoce, trombosi vascolare e sindrome metabolica e di recente è stata anche associata ad un aumento dell'incidenza di cancro. Un nesso di causalità tra OSAS ed aterosclerosi è parzialmente fondata ma non completamente chiarita. Una recente ricerca su aterosclerosi precoce in OSAS ha messo in correlazione la tendenza alla trombosi e la viscosità del sangue, fornendo una nuova visione dei meccanismi della malattia. L'ipossia intermittente notturna cronica tipica dell'OSAS insieme alle alterazioni macro e micro strutturali del sonno e la conseguente induzione ematica di stress ossidativo infiammatorio cronico cellulare con alterazioni genetiche possono contemporaneamente allo sviluppo di aterosclerosi precoce, rappresentare anche un meccanismo sottostante a lungo termine che induce atipie cellulari e patogenesi e progressione del cancro

PAROLE CHIAVE: *Aterosclerosi • Cancro • Ipossia notturna intermittente • OSAS • Alterazioni dell'architettura del sonno*

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Introduction

Increased atherosclerosis with cardiovascular morbidity and mortality is associated with obstructive sleep apnoea syndrome (OSAS); the association persists after controlling for diabetes, hypertension, smoking and dyslipidaemia [1]. OSAS is characterised by recurrent episodes of complete or partial collapse of the upper airways during sleep, and can induce apnoea or hypopnoea, respectively, with recurrent episodes of intermittent hypoxia and higher carbon dioxide levels, leading to possible frequent interruption of sleep [2]. The association between OSAS, cardiovascular disease and coagulation abnormalities was suspected for many years and is supported by large scale epidemiological and prospective studies [3-5]. The mechanisms that mediate this association are not completely understood. The prognostic factors have not been determined, and studies to identify which patients are at higher risk have given controversial and disappointing results. Moreover, OSAS has been emerged as independently and strongly associated with cancer incidence and mortality [6]. Current data suggest that inflammatory processes leading to endothelial dysfunction play a central pathogenic role in OSAS-related complica-

tions. Chronic intermittent hypoxia (CIH) and sleep disruption are considered important causes of cerebro-cardio-vascular diseases in OSAS patients. Oxidative stress induced by CHI is an important physiological mechanism of the disease [7]. Increased blood clotting, caused by changes in the rheological properties (flow properties) of blood and plasma, seems to be an important factor linking OSAS to cardiovascular complications (CV) [8]. CIH may be responsible for increased blood hypercoagulability (platelet activation and decreased fibrinolytic activity) which predisposes patients to thrombotic events [9]. In addition, it is well known that chronic hypoxia plays an important role in regulating various stages of cancer formation and progression [6].

This review provides a critical analysis of the current evidence of an association between OSAS and haemostatic alterations, vascular remodelling/atherosclerosis and cancer. The aim is to discuss the contributing factors and potential mechanisms that may be responsible for this association.

Effects of chronic nocturnal intermittent hypoxia/sleep fragmentation: mechanisms of vascular remodelling in atherosclerosis

OSAS, via the chronic intermittent hypoxia and sleep disruption, can trigger the development of systemic inflammation [10], oxidative stress [11], endothelial dysfunction [12] and metabolic syndrome [13]. Atherosclerosis is a chronic inflammatory disease that maintains a silent course for several decades before reaching clinical significance. The majority of studies have demonstrated a considerable mortality rate in OSAS, which is associated with the severity of atherosclerosis. The incidence of CV events, i.e. stroke, myocardial infarction, or CV death, is very high [14], with an odds ratio (OR) of CV events or mortality varying in magnitude from 2 to 7 for moderate to severe OSAS. This finding was confirmed by a subsequent study on 202 consecutive patients who were investigated with electron-beam computer tomography [15]. Patients were asymptomatic in terms of their coronary artery disease (CAD) and were investigated by an overnight-sleep study, with a prevalence of OSAS of 76%. Coronary artery calcification (CAC) was found in 67% of OSAS patients and in 31% of non-OSAS patients ($p \leq 0.001$). The median CAC score was 9 in OSAS patients and 0 in non-OSAS patients ($p \leq 0.001$), strongly supporting the theory that OSAS is an independent risk factor for CAC.

CIH and sleep disruption leads to systemic hypertension due to the activation of the sympathetic system in patients on a high-cholesterol diet. The sympathetic hyperactivity determines haemodynamic alteration and high blood pressure, as well as cardiovascular inflammation (increased cell adhesion molecules, endothelial cell dysfunction, prothrombotic factor activation) and vascular remodelling [16]. OSAS is also associated with insulin resistance and glucose intolerance, which are known risk factors for atherosclerosis [17]. CIH can lead to insulin resistance and glucose intolerance in obese patients with dyslipidaemia [18]. The intermittent hypoxia is a major stimulus for oxidative stress with the production of reactive oxygen species (ROS) that contributes to the generation of systemic inflammation characterised by inflammatory cell proliferation and cytokine/chemokine production [19]<?> Moreover, CIH may trigger the activation of pro-inflammatory transcription factors including hypoxic inducible factor (HIF)-1α and nuclear factor (NF)-κB, which also share some gene products, such as inducible nitric oxide synthase [20]. However, the role of HIF-1α is still controversial in OSAS studies [21]. The transcriptional reprogramming induced by CIH includes the induction of cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and selectins, cytokines, such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), chemokines, such as IL-8, and C-reactive protein (CRP) [22]. Other researchers have reported that CIH increases lipid peroxidation in the myocardial tissue of rats [23], and activates inflammatory pathways in vitro [24]. Recently, it has been demonstrated that CIH significantly increased sizes of atherosclerotic lesions, and the mRNA levels of both cyclooxygenase (COX)-1 and thromboxane synthase (TXBS) [25]. Lesion size was correlated with levels of COX-1 and TXBS mRNA, and treatment with the selective COX-1 inhibitor SC-560 reduced lesion progression in intermittent hypoxia mice. This study has demonstrated, for the first time, that the activation of the COX-1 pathway in response to CIH is associated with increased atherosclerotic lesions in mice. In addition, in OSAS patients with cardiovascular risk factors an increase in urinary excretion of 11-dehydrothromboxane B_2 (11-dTXB_2) was observed, supporting the emerging role for COX-1 in CIH-induced vascular changes.

Detailed analysis of hypercoagulability in OSAS

Some studies have demonstrated considerable changes in haemostatic system components in OSAS patients, including platelet activation and increased plasma levels of tissue factor, von Willebrand factor (vWF) and fibrinogen [26 27]. CIH and sleep disruption are a critical pathophysiological issue triggering greater sympathetic nervous system activity in association with the levels of markers of inflammation, such as oxidative stress and procoagulant activity. Hypercoagulability has been shown to be a risk factor for cardiovascular morbidity and mortality in OSAS patients [28]. One possible explanation for the increase in blood coagulability is that during apnoea, desaturation triggers inflammatory factors, catecholamine and increased plasma levels of tissue factor as well as platelet aggregation, which alter capillary blood flow due to an increased sympathetic activity and a broader micro-endothelial damage, resulting in blood coagulability. Recently, one study has demonstrated increased pulmonary artery hypertension (PAH) in OSAS patients, which could be correlated with a genotypic heterogeneity with the plasminogen activator inhibitor-1 (PAI-1)

5G/5G polymorphism, possibly in relation with the severity of hypoxaemia and apnoea [29]. vWF, a glycoprotein that plays an important role in stopping the escape of blood from vessels (haemostasis) following vascular injury, works by mediating the adherence of platelets to one another and to sites of vascular damage, preventing factor-VIII degradation. At present, the relation between OSAS and vWF is controversial, with some data showing an increase of vWF in patients with OSAS, and other data showing no significant differences between controls and patients [30 31]. Increased levels of clotting factors XIIa (FXIIa) and VIIa (FVIIa), thrombin and antithrombin (TAT) has been shown in patients with OSAS. TAT is marker of thrombin turnover and indicates a tendency to blood coagulation disorders. TAT increased in patients with severe nocturnal desaturation, and FVIIa was found to be reduced in patients who practiced continuous positive airway pressure (CPAP) therapy [32].

Both FVIIa and FXIIa have been associated with increased mortality from CV diseases. The observed increase in the clotting factors in OSAS suggest that CIH may predispose to hypercoagulability [33]. Robinson et al. [34] have found that serum levels of these clotting factors were not improved after one month of treatment with optimal CPAP therapy. A possible explanation may be that the short-term treatment with CPAP therapy was not able to facilitate the improvement of sympathetic activity and CIH. Since there are no long-term studies, the role of CPAP therapy remains unclear. Although not consistently [35 36], some studies have found a correlation between platelet activation and OSAS. The cause of the increased platelet activation in OSAS patients remains unclear, but the severity of OSAS seems to influence platelet aggregation as a function of nocturnal hypoxic time. A possible interpretation is the release/activation of catecholamines during oxygen desaturation, triggering more intensive platelet activation. OSAS patients have indeed increased levels of urinary adrenaline/noradrenaline that correlate with the degree of oxygen desaturation and apnoea index during sleep [37]. The therapeutic use of CPAP treatment on platelet aggregation is controversial. CPAP therapy decreases urinary adrenaline/noradrenaline by enhancing its elimination from the blood. The study by Hui et al. [38] found that platelet aggregation decreases after one day for up to three months of treatment with CPAP therapy in patients with severe OSAS, without changes in the control group. Oga et al. [39] found that one month of treatment with CPAP is insufficient and only after 90 days of treatment with CPAP can a reduction in platelet activation be demonstrated. Therefore, data from the literature indicate that treatment with CPAP improves platelet aggregation, but the duration of therapy remains uncertain. Platelet activation results in the shedding of sub-microscopic membrane vesicles, known as platelet-derived microparticles (PDMPs), which are less than 1.5 μm in diameter and enriched in pro-coagulant platelet proteins. The membrane of PDMPs embraces all the properties of the activated platelet membrane, including the ability to bind to the components of pro-coagulant complexes, such as factor V (Va) and VIII (VIIIa). Plasma PDMPs have been shown to be amplified in patients with acute coronary syndrome, suggesting that PDMPs may play a role in the pathogenesis of arterial thrombosis in OSAS patients [40 41]. Increased levels of the pro-coagulation factors soluble CD40 ligand (sCD40L) and soluble P-selectin (sP-selectin) have been demonstrated in OSAS patients in correlation with the degree of nocturnal desaturation. CPAP therapy reduces their plasma levels. CD40L and sP-selectin appear in plasma during the early stages of blood coagulation and are well-known indicators of thrombogenic conditions, such as disseminated intravascular coagulation (DIC). Furthermore, levels of sCD40L and sP-selectin are increased in patients with hypertension, hyperlipidaemia and diabetes mellitus [42 43].

Blood viscosity is defined as the internal resistance of the blood to shear forces. Blood viscosity is determined by plasma viscosity, haematocrit (volume fraction of erythrocytes, which constitutes 99.9% of the cellular elements) and the mechanical behaviour of erythrocytes. Increased blood clotting caused by changes in the rheological properties of blood and plasma seems to be an important factor linking OSAS and cardiovascular complications [44]. Hyperviscosity is a potential mechanism for increased coagulability in OSAS [45 46], but the studies on this issue are limited by the small sample size and the absence of well-matched controls.

PAI-1, a member of the serine protease inhibitor family, inhibits fibrinolytic activity by binding to tissue type plasminogen activator (tPA). It was demonstrated that higher apnoea-hypopnoea index (AHI)/hs and Nadir SaO_2% were both associated with a higher concentration of circulating PAI-1 in a group of OSAS patients [47]. Increased concentrations of PAI-1 in OSAS predicted the occurrence of acute myocardial infarction in middle-aged men and women with a high prevalence of coronary heart disease [48 49]. However, many uncertainties still remain as to the independent effects of OSAS on increased blood coagulability, largely due to the common co-existence of other CV risk factors and the incomplete normalisation of coagulation after CPAP treatment [50].

Venous thromboembolism and OSAS

There are limited data in the literature that have shown a relationship between venous thromboembolism (VTE) and pulmonary embolism (PE) in association with a coagulation-related clinical problem. The above studies are not extensive nor do they have a control group [51 52]. These data suggest a high prevalence of OSAS in patients with PE. A retrospective study [53] showed a percentage higher than in the general population, accounting for 15.5% of OSAS in patients with VTE. The study is, however, limited due to incomplete instrumental diagnostics, since data on polysomnography is missing. A review [54] of the current considerable evidence demonstrates that OSAS is associated with a procoagulant state, although the relationship between OSAS and individual clotting factors are uncertain. More clinical

studies are needed in order to better control for confounding factors such as cardiovascular morbidity and mortality, demonstrating that a hypercoagulability state is induced by OSAS before the onset of cardiovascular disorders

CHI and cancer: future perspectives for research

CIH and sleep disruption are known to trigger pathophysiological pathways leading to systemic disease. In fact, OSAS has been associated with diseases with a high inflammatory potential, such as psoriasis and other autoimmune disorders [55]. The potential mechanisms involved in hypoxia-driven cancer development and progression have been previously investigated [56]. Moreover, it has been shown that nocturnal intermittent hypoxia can regulate different stages of cell differentiation and proliferation. Cellular hypoxia (also present in cancer) and the adaptive response is related to a family of transcription factors, the most significant being HIF-1, which activates the transcription of genes that play a fundamental role in angiogenesis and genetic modification, with the formation of cancer-related stem cells [57]. The HIF-1 pathway has been associated with a considerable increase in ROS generation during periods of hypoxia/reoxygenation, which may play an important role in modifying gene expression by regulating the activity of some redox-sensitive transcription factors. These include activator protein (AP)-1, which may play a key role in carcinogenesis through the induction of apoptotic inhibitory factors, matrix metalloproteases and pro-angiogenic factors including vascular endothelial growth factor (VEGF).

A recent animal model study has spurred an emerging pathophysiological hypothesis linking OSAS to cancer. The authors have demonstrated that when mice are exposed to intermittent hypoxia mimicking OSAS, the frequency of melanoma was twice high as in normal controls [58].

These promising results in experimental animals have encouraged studies on humans with the aim of evaluating the potential link between OSAS and cancer. Two independent large-scale clinical studies have recently investigated this possible relation. The first assessed the association between OSAS and cancer in a cohort of patients from Wisconsin (USA) (1,522 subjects), and showed a significant increase in the likelihood of cancer death in patients with severe OSAS [59]. Another database, called the Spanish Sleep Network, involved 5,000 patients with a median 5-year follow-up and found that OSA is associated with increased incidence of cancer. These two studies reported an increased cancer incidence and mortality in OSAS patients compared with those who do not have OSAS, also after adjustment for important confounders (age, sex, smoking, alcohol consumption and body mass index). In this context, it can be hypothesised that nocturnal intermittent hypoxia and consequent sleep disruption may play a key role in cancer development (Fig. 1). It would be important to determine whether the OSAS-cancer combination gives rise to a specific histological type of tumour, since different types of malignant cells have different adaptive responses to intermittent hypoxia. The identification of confounding factors will

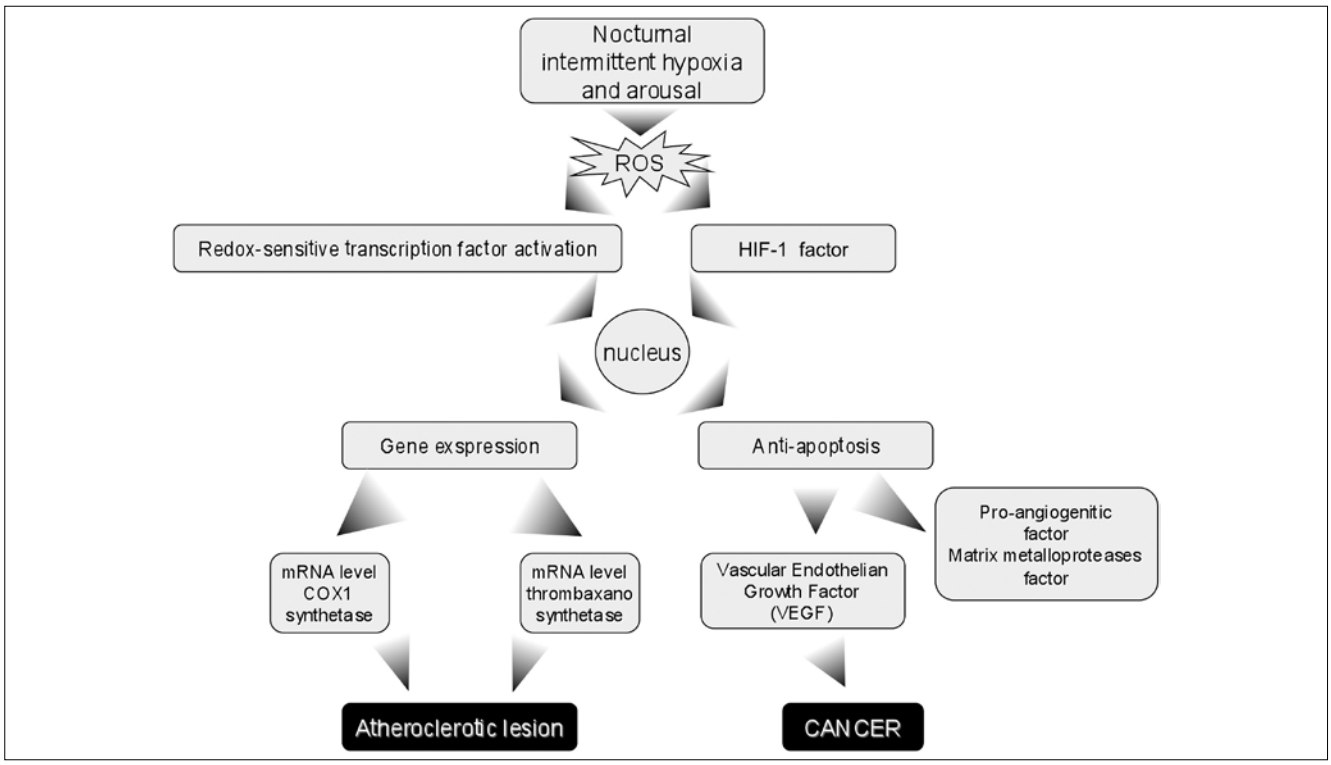

Fig. 1. Potential biological pathways mediating the development of atherosclerosis and cancer in sleep apnoea.

also be significant. A key issue that deserves further study is the potential role of CPAP therapy in cancer risk associated with OSA, and to investigate whether the effect of anti-cancer drugs is changed following treatment of CPAP.

Conclusions and future guidelines

The available data highlight that patients with OSAS experience a pro-coagulant condition that may represent a contributing factor in the development and progression of vascular diseases. The potential for anticoagulant and antiplatelet drugs to decrease morbidity/mortality is worth further investigation. Broad randomised studies will be necessary to provide greater statistic power in order to determine whether the treatment of OSAS with CPAP/drugs can stop or even reverse vascular remodelling, atherosclerosis progression and, ultimately, reduce the rate of cardiovascular disease. The possible relationship between OSAS and cancer is a good gateway for further research and an opportunity to perform international clinical studies that can answer the many open questions still remaining.

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HEAD AND NECK

Long-term treatment outcomes of juvenile nasopharyngeal angiofibroma treated with radiotherapy

Risultati a lungo termine del trattamento radioterapico dell'angiofibroma nasofaringeo giovanile

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SUMMARY

Juvenile nasopharyngeal angiofibroma (JNA) is a disease of adolescent males characterised by high vascularity with local aggressiveness. This analysis was intended to see the effectiveness of radiation in locally advanced JNA. We included patients treated from 1990-2012. A total of 31 patients met study criteria. Median age was 16 years (range: 12-33 years). Radiation was used for refractory, residual or unresectable locally advanced disease. The median radiation dose was 30 Gy (range: 30-45 Gy). Median follow-up was 36 months (Range: 1-271 months). The median progression-free survival [PFS] was not reached. PFS at 3, 5 and 10 years was 91.7, 70.7 and 70.7% respectively. Three patients progressed at 38, 43 and 58 months after completion of treatment and opted for alternative therapy. One patient developed squamous cell carcinoma of the nasal ale 15 years after radiation.

KEY WORDS: Juvenile • Nasopharyngeal • Angio-fibroma • Radiotherapy

RIASSUNTO

L'angiofibroma nasofaringeo giovanile (Juvenile Nasopharyngeal angiofibroma o JNA) è una patologia tipica dei ragazzi in età adolescenziale nota per essere riccamente vascolarizzata e localmente aggressiva. L'obiettivo dello studio è stato quello di valutare l'efficacia del trattamento radioterapico nell'angiofibroma nasofaringeo localmente avanzato. Abbiamo arruolato pazienti trattati in un periodo compreso tra il 1990 ed il 2012. 31 pazienti sono risultati confacenti ai criteri di inclusione dello studio, con un'età media di 16 anni (range: 12-33 anni). Il trattamento radioterapico è stato effettuato in pazienti con malattia localmente avanzata residua, refrattaria ai precedenti trattamenti o non resecabile. La dose media somministrata era di 30 Gray (range: 30-45 Gray) con un follow-up medio di 36 mesi (range: 1-271mesi). A 3, 5 e 10 anni si è registrata una percentuale di sopravvivenza libera da progressione del 91.7, 70.7 e 70.7%, rispettivamente. La progressione è avvenuta in tre pazienti rispettivamente 38, 43 e 58 mesi dopo aver completato il trattamento e gli stessi hanno dunque optato per un trattamento alternativo. Un paziente ha sviluppato un carcinoma squamo cellulare dell'ala nasale 15 anni dopo il trattamento radioterapico.

PAROLE CHIAVE: *Giovanile • Nasofaringeo • Angio-fibroma • Radioterapia*

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Introduction

Juvenile nasopharyngeal angiofibroma (JNA) is a benign, locally aggressive tumour found commonly in young boys [1 2]. The internal maxillary artery is the most common vascular source of JNAs followed by the ascending pharyngeal artery. Intracranial involvement has been reported to occur in 10% to 37% of all cases [3 4]. Staging is based on anatomic tumour extension, and the Radkowski system of staging is used most frequently. Over the years surgical excision has remained standard treatment [5]. Transnasal endoscopic resection is generally used in early stages, while advanced cases require craniofacial approaches [6]. The availably of pre-operative embolisation has helped surgeons in reducing bleeding during surgery and improve outcomes of surgery [7]. However, surgery alone in advanced stages has reported high recurrence rates especially if resection is not complete, and there is little consensus regarding the management of such patients. The high rate of recurrence in cases has paved way for adjuvant radiotherapy, especially in patients with residual disease after surgery. There is also much debate regarding early postoperative radiotherapy vs. delayed radiotherapy on progression. There is also a group of patients in whom surgery is not feasible where an option of radical radiotherapy is an option. The long term morbidity associated with

radiotherapy has remained a concern [8]. In recent years the introduction of conformal radiotherapy has made it possible to deliver radiation with precision and minimal morbidity [9 10]. Herein, we present data on patients with stage III JNA treated with radiotherapy at our institute by conformal techniques.

Materials and methods

We performed a retrospective review of patients with JNA undergoing radiotherapy in our institute from 1990 to 2012. Data regarding clinical features, treatment details and outcomes of 31 patients were recorded in a predesigned proforma. Data was analysed and categorical variables were summarised by frequency (%) and quantitative variables were summarised by median and range. The survival analysis of the data was done using Kaplan-Meier test.

Radiotherapy

A customised thermoplastic cast was used for immobilisation. A CECT simulation was done using a Philips™ CT scanner with 3 mm slice thickness following intravenous contrast injection [1 mg/kg body weight]. Whole cranium and the paranasal sinus were scanned. In the pre-ICRU era, radiotherapy was delivered to residual disease with 1 cm expansion. Thereafter, the GTV was delineated as evident on the planning CT scan. An isotropic expansion of 5 mm was added to form the PTV. The planning was done using the Eclipse treatment planning system version 6.5 (Varian Medical Systems, Palo Alto, CA) or Pinnacle treatment planning system version 9.0. Treatment was delivered by 3D conformal radiotherapy [3D-CRT] in 30 patients, while for one it was by conventional technique. During radiotherapy planning first priority was given to achieve a conformal dose distribution to the PTV followed by maximal sparing of the optic structures.

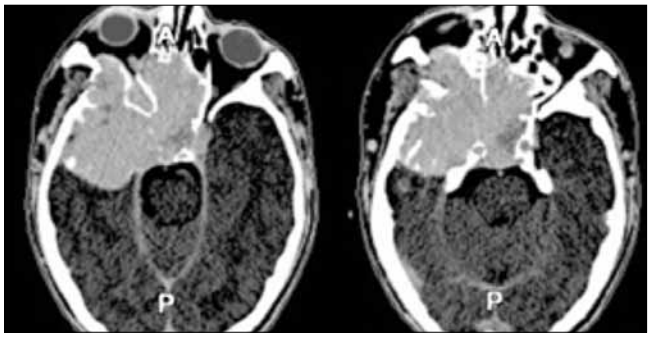

Fig. 1. Axial CECT image of a case of JNA with intracranial extension.

Response assessment and follow-up

The patients were followed at one month after completion of radiation and subsequently every three months with clinical examination and endoscopy. A contrast-enhanced CECT of the head and face was ordered once in a year.

Results

Patient characteristics

Median age of this cohort of patients was 16 years (range: 12-33 years). Epistaxis was the commonest presenting symptom found in 24 [77.4%] patients followed by nasal blockade (n = 19) and proptosis (n = 12). Ophthalmic evaluation revealed vision abnormalities in 14 (45.1%) patients. All patients were thoroughly evaluated with contrast enhancing CT scan or contrast enhanced MRI. Imaging revealed gross intracranial extension in 19 (61.2%), orbital involvement in eight [25.8%] and cavernous sinus involvement in 12 (38.7%) patients. All the patients were in Radkowski stage III (Table I). The clinical characteristics are summarised in Table II.

Surgery

Surgery was done in 22 patients (70.9%). Lateral rhinotomy was the most common surgical approach. Since complete excision of the tumour was difficult in most cases,

Table I. Radowski's modification of Sessions' classification.

Stage	Tumour extent
Ia	Limited to the posterior nares and/or nasopharyngeal vault
Ib	Involving the posterior nares and/or nasopharyngeal vault with involvement of at least one paranasal sinus
IIa	Minimal lateral extension to the pterygomaxillary fossa
IIb	Full occupation of the pterygomaxillary fossa with or without superior erosion orbital bones
IIc	Extension into the infratemporal fossa or extension posterior to the pterygoid plates
IIIa	Erosion of the base of the skull (middle cranial fossa/base of pterygoids) – minimal intracranial extension
IIIb	Extensive intracranial extension with or without extension into the cavernous sinus

Table II. Patient characteristics.

Characteristics	(N = 31)
Age	
Median	16 years
Range	12-33 years
Symptoms	
Epistaxis	24
Nasal blockade	19
Proptosis	12
Loss of vision	14
Stage	
III	31
Surgery	
Yes	22
No	9

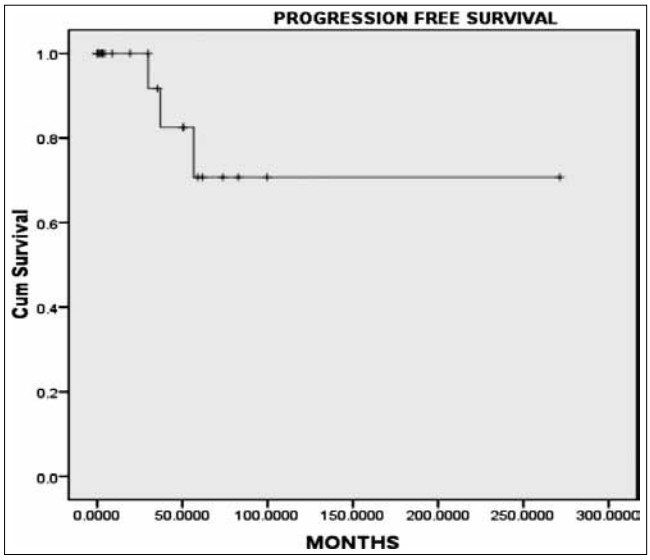

Fig. 2. Kaplan-Meier survival curve (progression-free survival).

decompression of the tumour was done in the majority of cases undergoing surgery.

Radiotherapy

Radiotherapy was given with radical intent in 10 patients and postoperative radiotherapy was given in 10 patients. It was given in a salvage or recurrent setting in 11 patients. The radiation dose of 30-50 Gy (median 32.5 Gy) was prescribed at 1.8-2 Gy per fraction. A two field technique (anterior + lateral) or a three field technique was used for treatment delivery. Since the dose required was less that optimal, sparing of organs at risk without compromising coverage of PTV could be achieved in most of cases using 3D-CRT. The dosimetric characteristics are summarised in Table III.

All patients tolerated the prescribed dose without grade III or Grade IV acute toxicity. Grade II mucosal reaction was the most common acute toxicity followed by Grade I skin reaction and grade II conjunctival reaction.

Survival outcomes

Median follow-up was 36 months (range 1-271 months). Three patients were found to have progressive disease, while three patients showed features of slow regression documented in MRI/CECT scan done every three months suggestive of disease control. Hence, disease control could be achieved in 28 patients (90.3%). The median progression-free survival (PFS) was not reached. PFS at 3, 5 and 10 years was 91.7%, 70.7% and 70.7% respectively. The survival curve is shown in Fig. 2. One patient developed squamous cell carcinoma of the nasal ale 9 years after diagnosis of JNA in the radiation field likely due to radiation-induced second malignancy. The patient underwent surgical salvage and was disease-free at seven years after the salvage treatment.

Table III. Dosimetry of 3D-conformal plan [OAR-Organ at risk].

OAR	Dmax Gy (Median) and Range
Brainstem	31.06 [10.40-49.84]
Eye	30.67 [4.56-47.00]
Optic nerve	32.50 [2.71-52.21]
Optic chiasm	31.13 [20.47-50.06]
Spinal Cord	20.68 [3.99-40.01]

Discussion

Management of locally advanced JNA has long remained a challenge. Gross total excision is always attempted whenever possible for long term disease control. However, the presence of skull base involvement or intracranial extension makes most patients not amenable for complete surgical resection [12]. In advanced JNA with intracranial extension, even after surgical resection, recurrence is high due to incomplete excision. Tyagi et al., in a review of 10 patients, reported residual disease in 80% patients after surgical excision with 30% subsequent recurrence [13]. Fagan et al. reported 37.5% recurrence after surgical excision for JNA with intracranial extension [14]. In the present series, 32.25% patients were deemed unresectable. The remaining 32.25% of patients underwent surgery but developed recurrence during follow-up. The remaining patients had R1 resection. This shows the difficulty of surgical excision in locally advanced JNA and therefore, the importance of adjuvant/salvage local therapy. Preoperative embolisation is generally undertaken 24-72 hours prior to resection and rationale is that occluding the responsible artery will decrease intraoperative bleeding and decrease the tumour size. Endoscopic resection may be associated with significantly lower intraoperative blood loss and lower recurrence rate compared to open resection [15].

The use of adjuvant/salvage/radical radiation has remained controversial owing to its anticipated long term morbidity and second primary neoplasm. In the last two decades the introduction of sophisticated radiation delivery technique has allowed investigators to use radiation and limit morbidity. In a review, Reddy et al. reported the efficacy of radiation in 10 patients with intracranial extension [10]. A total of 30 Gy was delivered with customised field design with megavoltage X rays. After a median follow-up of 2.5 years, the authors reported 85% local control. The authors reported the occurrence of basal cell carcinoma in one patient 14 years after radiation. The authors concluded that a moderate dose of radiation can confer long term disease control for such cases. In our study, the local control rate was 90.3% at a median after a median follow-up of 12 months. Lee et al., in a retrospective review, analysed treatment outcomes of 27 patients treated with radiotherapy and reported 85% local control [8]. In this study, the radiation dose ranged from 30-55 Gy. However, the radiation dose

Table IV. Summary of the published data of JNA treated with radiation.

Author/Year	Number/Age	Stage IIIB	Dose (Gy)	Setting of RT	Result/Implication or late toxicity
Cummings et al., 1984	55	17	30-35	Inoperable disease	LC 80%; 1 thyroid CA, 1 basal cell carcinoma 2 cataracts, Hypo-pituitarism
Robinson et al., 1989	10	30	30-40	NA	4-year LC of 100%, 1 -Cataract
McGahan et al., 1989	15	100	32-46	Radical: 10 Salvage: 5	2-year LC of 73.33% Dose greater than 40 Gy allow improved LC
Fields et al., 1990	13	15	36.6-52	Salvage RT	11-year LC of 85%, xerostomia and caries
Reddy et al., 2001	15	67	30-35	Inoperable disease	5-year LC of 85% 3-cataracts, delayed transient CNS syndrome- 1 patient, a basal cell carcinoma of the skin - 1
Lee et al., 2002	27. Mean 8 years (8-22)	85	30-55	Inoperable disease	85, Long term complication 15%
Chakraborty et al., 2010	8	100	30-46	Inoperable disease	2-year LC of 87.5%; minimal acute and late side effects
Present series, 2014	31, Median age 16 (12-330)	100	30-50 (median 32.5)	Salvage:11 PORT (R1 resection): 10 Radical: 10	5-year PFS were 91.7%; SCC nasal ale-1

was restricted to 36-40 Gy for patients treated after 1999. The authors reported a 15% long term complication rate including cataract, hypopituitarism and temporal lobe necrosis (TLN) and growth retardation in one case each. However, the patient with TLN received a cumulative dose of 75 Gy over three years. In our study, no grade III or IV radiation-induced toxicity was noted.

Long term disease control has reached a new horizon, but the long term morbidity remains an area of concern (Table IV). The introduction of conformal radiotherapy has evolved as a promising treatment approach for locally advanced cohorts of JNA. Beriwal et al., in a report of two cases, showed the promising results in JNA treated with intensity modulated radiotherapy (IMRT) [9]. Subsequently, two large series published by Chakraborty et al. and Kuppersmith et al. investigated the effectiveness of radiation when delivered with the conformal technique [16,17]. Chakraborty et al., in a larger series of eight patients treated by conformal radiotherapy (7 planned with IMRT, one 3DCRT) reported progressive resolution in seven cases after a median follow-up of 17 months [16]. The authors reported 87.5% actual control at two years with excellent sparing of organs at risk. Another series reported long term disease control in excess of 70-100%. Kuppersmith et al. reported on in a series of three patients treated with highly conformal IMRT aimed at limiting radiation doses to the optic nerves, optic chiasm, brainstem, brain, spinal cord, lens, retina, mandible and parotid [17]. The dose delivered ranged from 34-45 Gy. The authors highlighted excellent sparing of critical normal organs as well as excellent disease control.

Reddy et al. reported on basal cell carcinoma in one patient 14 years after radiation [10]. In our study, we also found one patient with squamous cell carcinoma (SCC) of the nasal ale. However, this patient was disease free after successful salvage surgery.

The major limitations of the present study are the retrospective nature of the analysis and short follow-up. However, we achieved good local control with very limited toxicity in these advanced cases of JNA.

Conclusions

Radiotherapy can be considered as a feasible option in stage III JNA with a local control rate of 90.3% at 1 year. The estimated 3- and 5-year median PFS were 91.7 and 70.7%, respectively. Moderate doses of radiation (30-45 Gy) delivered by the conformal technique can achieve durable disease control with limited morbidity.

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Head and neck

Benign tumours affecting the deep lobe of the parotid gland: how to select the optimal surgical approach

Tumori benigni del lobo profondo della parotide: come scegliere il più adeguato approccio chirurgico

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SUMMARY

Many types of approaches allow extra-capsular dissection in the deep parotid parenchyma in the treatment of benign tumours. A transcervical approach (TCA), transparotid approach (TPA) and a combined transcervical-transparotid approach (TPTCA) are the three main procedures performed to expose the deep parenchyma. We conducted a retrospective chart review enrolling 24 consecutive patients treated for benign tumours affecting the deep lobe of the parotid. Review of the surgical data was accompanied by careful follow-up to establish surgical morbidity, functional (Frey's Syndrome and first-bite syndrome) and aesthetical outcomes. A TPA was performed in the majority of cases; in 26% superficial parotidectomy was not required (selective deep parotidectomy). Minor's test showed a low rate of Frey's syndrome (3 cases of 23, 13%). No long-lasting first-bite syndrome was reported. Some additional procedures were easily performed in order to improve aesthetical results (rotational flap of sternocleidomastoid muscle, free abdominal fat transfer); these had the same results as selective deep parotidectomy. TCA (or TPTCA) ensures the best control of the facial nerve, providing good exposure and good functional and aesthetical results (without sparing the superficial parenchyma if additional techniques are performed with the aim of reducing skin depression in the treated area). The choice of the approach should have only the aim of safe resection and should not be influenced by aesthetical outcome; the craniocaudal level of the tumour seems to be the best indicator of the feasibility of the procedure also considering the branches of the facial nerve. In our experience, mandibulotomy can always be avoided.

KEY WORDS: Parotid • Deep lobe • Parapharyngeal space • Salivary gland tumours • Frey's syndrome

RIASSUNTO

Numerose sono le modalità di approccio chirurgico al parenchima parotideo profondo che permettono di trattare neoplasie benigne secondo il principio della dissezione extracapsulare. L'approccio transcervicale (TCA), il transparotideo (TPA) e una combinazione fra i due (transcervicale-transparotideo, TPTCA) sono le 3 principali procedure effettuate per esporre il parenchima profondo. Il nostro studio retrospettivo include 24 pazienti trattati per patologia neoplastica benigna del lobo profondo. La revisione dei dati chirurgici è stata associata ad una mirata visita di follow-up atta a stabilire vantaggi e svantaggi di ciascun approccio in termini di morbilità chirurgica sugli aspetti funzionali (sindrome di Frey e First-Bite Syndrome) ed estetici. TPA è stato eseguito nella maggioranza dei casi e nel 26% dei casi non ha previsto l'asportazione del lobo superficiale (parotidectomia profonda selettiva). Una sindrome di Frey clinicamente evidente (test di Minor) è stata documentata in 3 casi su 23 (13%) mentre nessuna first-bite syndrome di lunga durata è mai stata riportata. Procedure chirurgiche aggiuntive allo scopo di migliorare il risultato estetico (lembo di rotazione di sternocleidomastoideo, riempimento del minus chirurgico con grasso libero addominale) sono state facilmente eseguite e senza complicanze maggiori, assicurando un risultato estetico sovrapponibile a quello di una parotidectomia profonda selettiva. L'approccio transparotideo al lobo profondo assicura il controllo ideale sulle branche del nervo facciale e garantisce comunque buoni risultati estetici e funzionali (anche senza risparmio del parenchima superficiale a patto di eseguire semplici procedure aggiuntive allo scopo di minimizzare la depressione della pelle della zona trattata). La scelta del tipo di approccio non deve essere influenzata dal risultato estetico ma solo dalla fattibilità di una rimozione oncologicamente sicura; il livello cranio-caudale al quale si trova il tumore è il miglior indicatore delle difficoltà tecniche che possono presentarsi e delle branche nervose da dover preservare. Nella nostra esperienza la mandibulotomia può essere sempre evitata.

PAROLE CHIAVE: *Parotide • Lobo profondo • Spazio parafaringeo • Tumori delle ghiandole salivary • Syndrome di Frey*

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Introduction

The deep lobe of the parotid gland occupies the pre-styloid compartment of the parapharyngeal space (PPS). It has been estimated that almost half of all parapharyngeal tumours originate from a salivary gland (40-50%), while the remaining arise from lymphatic or nervous structures [1]. Pleomorphic adenoma (PA) is the most common neoplasm of the PPS [2].

Many surgical approaches have been described to expose the deep parenchyma and allow a safe and appropriate removal of a tumour, usually by performing an extracapsular dissection (ECD) [3-5]. A transoral approach is limited to

small and benign lesions [6]. A transcervical approach (TCA) (without mandibulotomy) is correlated with lower morbidity [7 8], but cannot be performed in all patients, especially in patients with large tumours, very poor exposure, or malignancy. Transoral robotic surgery (TORS) approach is an interesting and new technique; the experience of O'Malley and colleagues has confirmed the safety and feasibility of a TORS approach for PPS tumours in terms of local control and the low surgical complication rate [9].

A transparotid approach (TPA) is the most widely used, and can be combined with a TCA procedure. Mandibulotomy can be necessary in the presence of anatomical limitations which in most cases are represented by the ascending mandibular branch and posteriorly by the temporal bone and styloid apophysis.

Parotid surgery for benign pathologies can lead to several complications, such as Frey's syndrome (FS), first-bite syndrome (FBS), facial nerve deficits, or great auricular nerve anaesthesia. It also can lead to a poor aesthetical result due to severe asymmetry of the face.

The aim of this report is, after providing a brief description of surgical techniques, to evaluate the advantages and disadvantages of each approach to the deep lobe. These features have been investigated in terms of morbidity, aesthetic outcomes and oncologic results after long and careful follow-up.

Materials and methods

Patients

With the aim of examining a series of patients with long-term follow-up treated for a benign tumour of the parotid gland arising from the deep lobe, we conducted a retrospective study by searching through our digital archive (Ormaweb, Avelco s.r.l.) using specific numerical codes employed for salivary gland tumours. After exclusion of surgical procedures performed on the submandibulary gland, we reviewed 135 medical records starting from January 2009 to December 2011 to identify surgical procedures on the parotid gland. From the entire group, we excluded biopsies and surgical procedures performed for malignancy (19 cases, 14.07%) or for benign tumours affecting only the superficial lobe (92 cases, 79.14%). Of 135 procedures, we selected 24 records that identified 24 consecutive resections of benign tumours arising from the deep lobe of the parotid gland (17.7% of all procedures, 20.4% of benign tumours); MRI was available in all 24 cases. The study protocol was approved by the local Ethics committee and adhered to the principles outlined in the Declaration of Helsinki.

Surgical techniques

TPA: we perform a modified Blair incision, starting from the posterior edge of the tragus (or on a skin crease anteriorly to the tragus). A skin flap in the subplatysmal and sub-superficial musculoaponeurotic system (SMAS) plane is then elevated. The parotid capsule is separated from the external auditory canal and from the anterior aspect of the sternocleidomastoid muscle by sharp dissection. The posterior branch of the greater auricular nerve can be preserved, while the anterior branch must be sectioned. After exposing the posterior belly of the digastric muscle, we are able to identify the main trunk of the facial nerve and perform a superficial parotidectomy with an anterograde technique. We always employ a neural stimulator to identify and preserve the branches of the facial nerve until the deep lobe is completely exposed. At this point, we are able to perform extra-capsular dissection (ECD) of the deep lobe tumour by gently retracting the overlying neural branches of the facial nerve (Fig. 1). In order to improve the exposure (and to reduce the risk of damaging the structures of the PPS), fracture (or sectioning) of the styloid process is sometimes required.

TCA: we perform a small vertical cervical incision and subsequently elevate a skin flap in the subplatysmal plane, including the capsule of the submandibulary gland. The parotid is retracted posteriorly and superiorly; the submandibulary gland is retracted anteriorly. In some cases a section of the posterior belly of the digastric muscle is useful. This procedure allows to access the PPS and to identify the tumour. At this point, we are able to perform an ECD with blunt dissection and thus preserve any potential vascular structures surrounding the lesion.

We consider a transparotid-transcervical approach (TPTCA) as a combination of both TPA and TCA, and it is employed for large tumours or in cases of very poor exposure. TPA and TPTCA can lead to a depression over the mandibular angle or the pre-auricular region, causing poor aesthetical outcome due to facial asymmetry. In order to avoid this (the indication was given by the volume of the contralateral parotid lodge on the MRI), two types of additional surgical techniques are performed: sternocleidomastoid muscle (SCM) rotational flap and free abdominal fat transfer. An SCM muscle rotational flap is performed by sharply sectioning the most superficial aspect of the upper third of SCM muscle. A neural stimulator is employed to prevent damage to cranial nerve XI.

The transfer of free abdominal fat is performed by making a small horizontal skin incision 8-10 cm under the belly and removing an amount of abdominal fat tissue by sharp dissection. Alternatively, the skin incision can be carried out along the inferior aspect of the belly.

In selected cases, it is also possible to perform a selective deep lobe parotidectomy (SDP) by dividing the superficial lobe into two halves (upper and lower); this allows quick access to the deep lobe by leaving the superficial lobe pedicled anteriorly. The two halves are repositioned and sutured, preserving the symmetry of the face [10].

Three surgeons performed all surgical procedures as first operator.

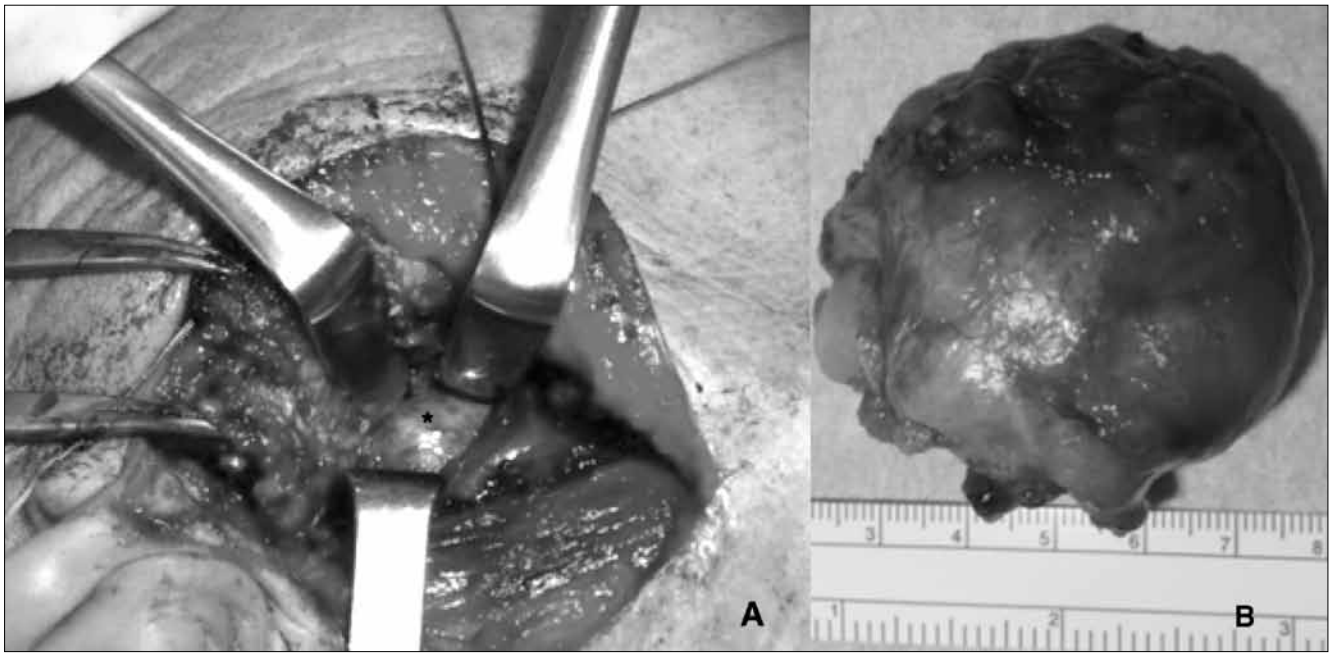

Fig. 1. (A) tumour of the deep lobe (asterisk) under the superficial parenchyma, removed performing an ECD after TPA (a portion arising from the deep tumour occupies part of the superficial lobe and is surrounded by some branches of the facial nerve); (B) the tumour (pleomorphic adenoma) after the procedure.

All procedures were performed using an 0.6 mA direct nerve stimulator (Neuropacer®, FIAB S.p.A., Vicchio, Florence, Italy) that was also employed during preparation of SCM flaps.
All specimens were sent for histological examination where the dimensions of the lesion were measured, separating the neoplasm from the surrounding salivary tissue. On the basis of this data, we calculated the average size of the resected tumour.

Preoperative evaluation
Pre-operative scans were reviewed using an open-source DICOM file reader software (Osirix Imaging Software, Pixmeo, Geneve, Switzerland). Each scan was compared to identify a radiological pattern that could suggest one type of surgical approach over another.
In particular, after overlapping and comparing each MRI scan, a T1-weighted MRI sequence (enhanced with gadolinium) on the coronal plane was selected and employed to detect the tumour. The lateral pterygoid muscle (LPM) was detected as well, with the aim to assess the presence of healthy salivary tissue between the muscle and the lesion.

Follow-up and post-operative evaluation
Clinical examination, represented by a careful palpation of the treated area and critical inspection to detect facial asymmetry or facial nerve weakness, was initiated after administering a series of questionnaires.
The onset of Frey's syndrome (FS) was investigated by the Luna-Ortiz Questionnaire [11]. Patients were asked to rate their aesthetic satisfaction on a simple scale from 1 to 5 (1: very poor satisfaction; 2: poor satisfaction; 3: sufficient; 4: good result; 5: excellent result).
Once a diagnosis of FBS was made, time of onset since surgery, location, duration and frequency of symptoms, and associating symptoms were noted.
The last MRI scan was examined to identify recurrences.
All patients underwent the Minor test (starch-iodine test) by applying an iodine solution (Lugol's Iodine) to the skin. Once air-dried, the area was dusted with cornstarch. Sweating was encouraged with the aid of lemon-flavoured candy. We considered the test as positive when the sweat reached the surface of the skin and the starch and iodine combined causing an evident colour change (from white to dark blue).

Results

The subjects in the present analysis included 11 males and 13 females, from 30 to 78 years old (mean 52.7 years). In most of patients, the tumour presented as a slow-growing, painless, non-ulcerated, deep nodule affecting the parotid region and not easily appreciable on palpation. In contrast, in 4 patients the tumour mass was well appreciable: this occurred in the 2 cases of multifocal involvement of the gland and in the 2 cases of massive involvement of the residual parenchyma (also superficial) by recurrence of disease. The neoplasm presented as a swelling of the oropharynx that displaced the tonsil in only one patient (Fig. 2). All patients underwent MRI imaging of the region with gadolinium (3 patients also underwent CT).

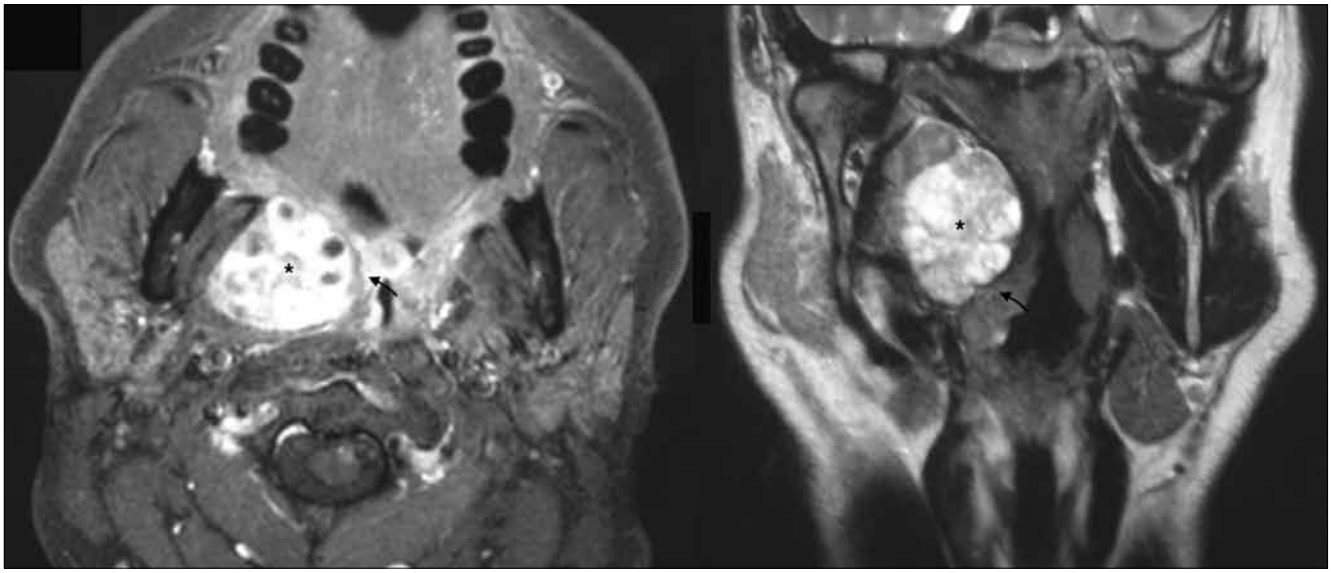

Fig. 2. Large tumour of the deep lobe widely occupying the parapharyngeal space (black asterisk) and displacing the tonsil lodge (black arrow).

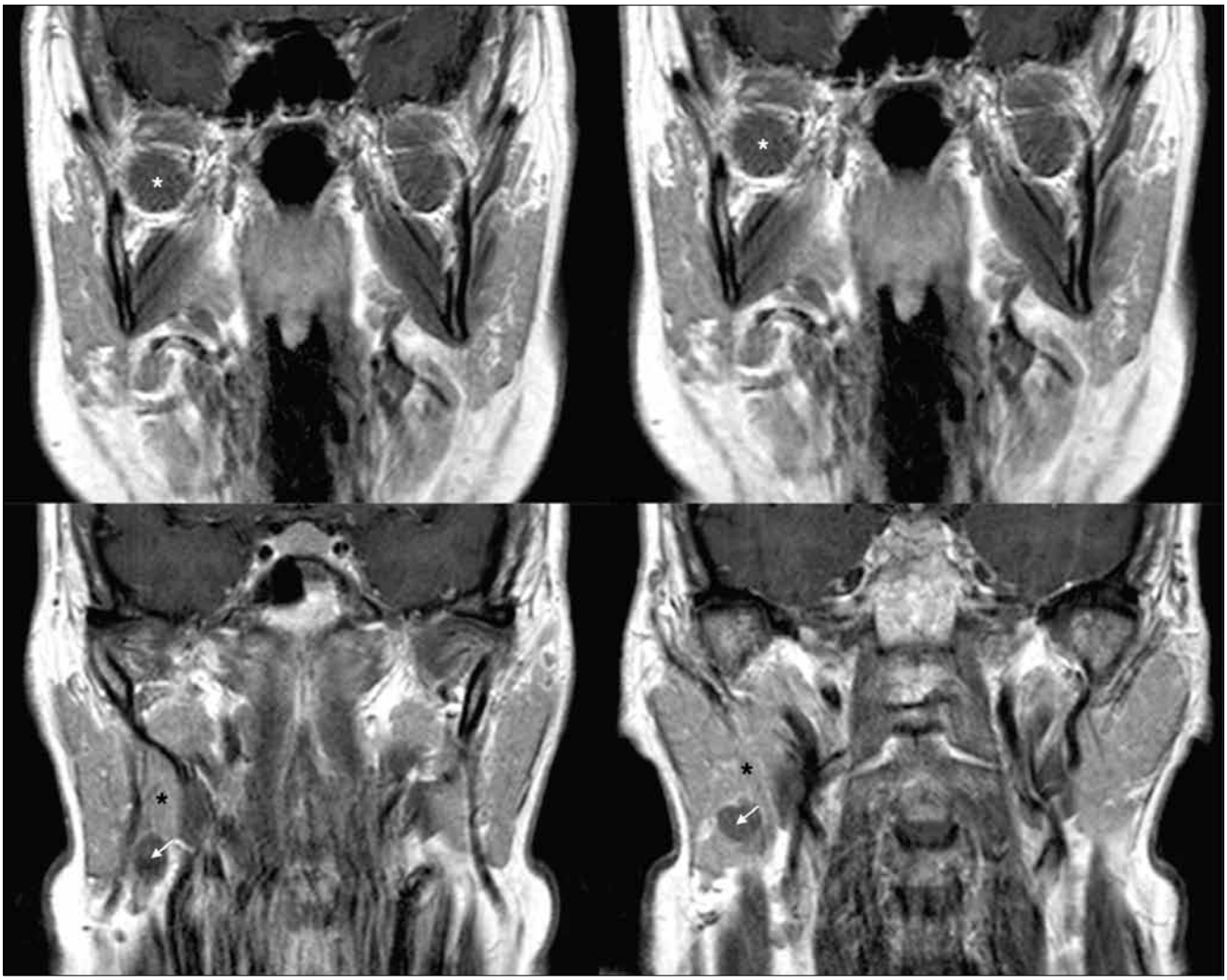

Fig. 3. Four consecutive coronal preoperative MRI scans in a TCA; the images show the presence of healthy salivary tissue (black asterisk) between the tumour (white arrow) and the LPM (white asterisk).

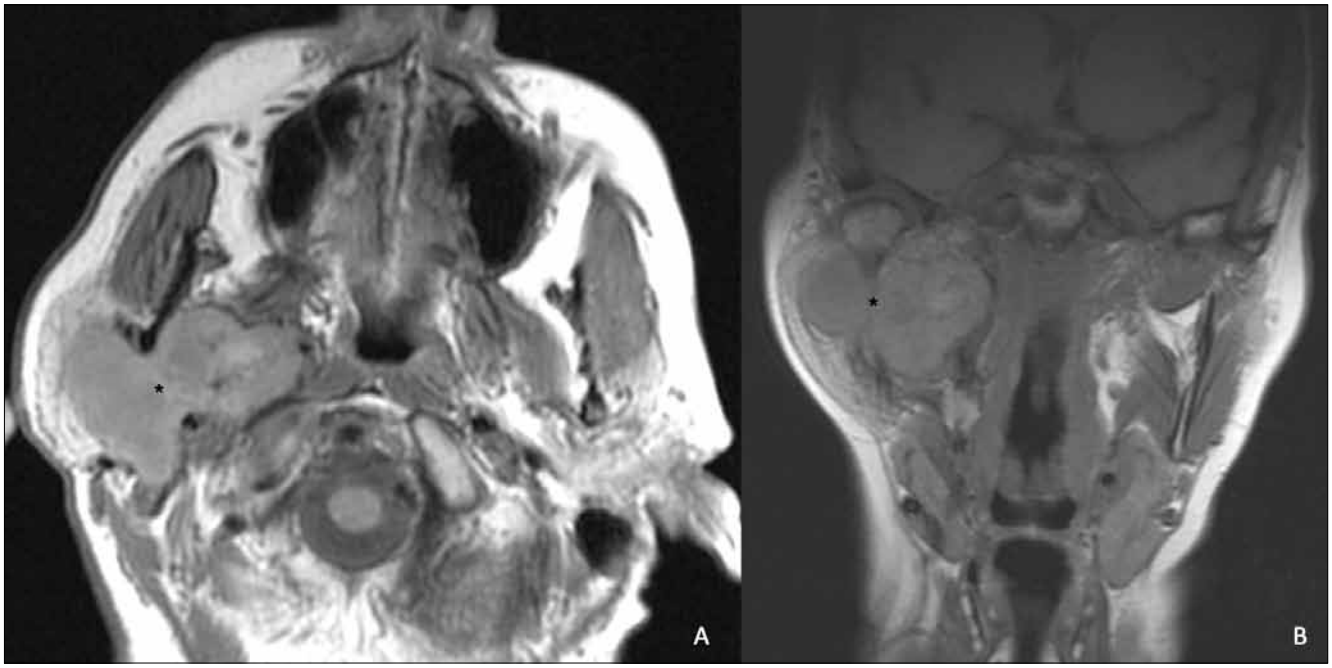

Fig. 4. Pleomorphic adenoma (black asterisk), occupying both the superficial and the deep lobe treated via a TPTCA.

TCA was performed in 1 patient (4.2%) a; the tumour was 1.5 cm in diameter and completely extra-glandular, located in the prestyloid compartment of the PPS, with a clear manifestation of healthy salivary tissue between the tumour and the LPM on MRI (Fig. 3).

TPA was performed in 19 patients (79.2%), of which 17 (70.8%) as first surgical procedure (in 5 an SDP was also feasible) and 2 (4.2%) as surgical revision after recurrence. Of 19 patients treated via a TPA, in 5 cases (20.8%) the tumour occupied both the superficial and the deep lobe, while in 14 cases (58.3%) the tumour was limited to the deep lobe, but no salivary tissue between the tumour and the LPM was detectable.

In 4 patients combined TPTCA was necessary (first operation in 3 cases and surgery for recurrence in 1 case); in all these cases, MRI revealed that the tumour widely occupied the entire deep lobe of the gland (in 1 case part of the superficial lobe as well) (Fig. 4), with a clear point of contact between the tumour and the LPM.

Mandibulotomy was not needed in any of the 24 patients. Fracture of the styloid process was performed in 2 of the 4 cases undergoing TPTCA to assure better exposure of all the edges of the tumour and allow a safer ECD procedure from the parapharyngeal space.

In 9 cases, during the same surgical session (7 TPA and 2 TPTCA), an additional procedure was performed to prevent important skin depression over and posterior to the mandibular angle and in the pre-auricular region. This prove minimised the asymmetry of the operated side compared to the contralateral side. In 3 cases we placed a rotational flap of sternocleidomastoid muscle. In 6 cases, we performed free abdominal fat grafting.

The mean size of the resected tumour was 3.4 x 2.9 x 2.2 cm (the superficial parenchyma removed to access the deep lobe, when not involved by neoplasm, was not considered). The results of pathological examination are shown in Table I.

An evident intraoperative complication was apparent in only one case, consisting in damage by stretching to the marginalis mandibulae branch of the facial nerve, also due to the large dimensions of the tumour. Intraoperative bleeding was controllable in all patients. No other major complications occurred during surgical procedures. In 3 patients a deficit of the marginalis mandibulae branch of the facial nerve was documented postoperatively; in 2 patients it recovered in a maximum of 12 weeks, while in the other (4.1%) patients it was permanent.

Regarding long-term follow-up, all 24 patients underwent yearly MRI scan. The mean period of follow-up was 2 years and 3 months after surgery. In 2 of 22 cases the onset of a new lesion was reported: in one case it was a new neoplasm in a patient affected by a multifocal Warthin's tumour; in the other case, recurrence was a salivary duct cyst (diagnosis by fine needle aspiration). In only 3 cases was onset of an FBS documented, although it completely disappeared within a few months. An FS was reported in 6 (25%) patients, but Minor's test was positive in only 3 cases (12.5%) (Luna-Ortiz questionnaire scoring respectively 1, 5 and 6). As such, no clinically silent FS was documented in our series.

Table I. Clinical features and results of the surgical approach in the 24 patients.

ID	SEX	Age	Year	Localisation of the tumour	Approach	Superficial lobe removed	Additional procedure	Pathology	Clinical and radiological follow-up	FBS	Positive minor's test	Luna-Ortiz scoring	Aesthetic outcome (1-5)
1	F	65	2011	DL	TPA	Yes	Abdominal fat transfer	Basal Cell Adenoma	NED	No	No	0	5
2	F	50	2010	DL	TPA	Yes	Abdominal fat transfer	Pleomorphic Adenoma, Lipoma	NED	No	Yes	1	4
3	F	30	2011	DL	TPA	No (selective deep parotidectomy)	No	Pleomorphic Adenoma	NED	No	No	0	5
4	M	61	2011	DL	TPA	No (selective deep parotidectomy)	No	Salivary Duct Cyst	NED	No	No	0	4
5	M	62	2009	DL	TPA	Yes	SCM flap	Warthin's Tumour	NED	No	No	0	5
6	F	67	2011	DL	TPA	Yes	Abdominal fat transfer	Pleomorphic Adenoma	NED	No	No	0	5
7	M	45	2011	SLDL	TPA	Yes	No	Multifocal Warthin's Tumour	Recurrence	No	No	0	3
8	F	58	2011	DL	TPTCA	Yes	Abdominal fat transfer	Pleomorphic Adenoma	NED	No	No	0	5
9	M	49	2009	DL	TPTCA	Yes	No	Pleomorphic Adenoma	NED	No	Yes	6	4
10	F	45	2010	DL	TPTCA	Yes	SCM flap	Pleomorphic Adenoma	NED	Yes	Yes	5	5
11	F	47	2010	DL	TPA	Yes	Abdominal fat transfer	Pleomorphic Adenoma	NED	No	No	0	5
12	F	56	2011	DL	TPA	Yes	No	Pleomorphic Adenoma	NED	No	No	0	3
13	F	61	2010	DL	TPA	No (selective deep parotidectomy)	No	Pleomorphic Adenoma	NED	No	No	0	5
14	F	42	2011	SLDL	TPA	Yes	No	Pleomorphic Adenoma	NED	No	No	0	1
15	M	33	2009	DL	TPA	No (selective deep parotidectomy)	No	Pleomorphic Adenoma	NED	No	No	0	5
16	F	78	2010	DL	TPA	Yes	SCM flap	Pleomorphic Adenoma	NED	No	No	0	4
17	M	46	2011	DL	TPA	Yes	Abdominal fat transfer	Warthin's Tumour	NED	No	No	0	5
18	F	40	2011	DL	TPA	No (selective deep parotidectomy)	No	Cystadenoma	NED	No	No	0	5
19	M	58	2010	DL	TPA	Yes	No	Schwannoma	NED	No	No	0	3
20	M	52	2010	SLDL	TPA	Yes	No	Multifocal Warthin's Tumour	NED	No	No	0	4
21	M	66	2011	SLDL	TPA	No (revision)	No	Pleomorphic Adenoma	NED	No	No	0	4
22	M	38	2010	DL	TPA	No (revision)	No	Salivary Duct Cyst	Recurrence	No	No	0	2
23	F	51	2011	SLDL	TPTCA	No (revision)	No	Oncocytoma	NED	Yes	No	0	2
24	M	65	2011	DL	TCA	No (TCA)	No	Pleomorphic Adenoma	NED	Yes	No	0	5

DL: Deep Lobe; SL: Superficial Lobe; TPA: Trans Parotid Approach; TPTCA: combined Trans Parotid and Trans Cervical Approach; TCA: Trans Cervical Approach; SCM: Sternocleidomastoid muscle; NED: No Evidence of Disease.

Regarding aesthetic outcomes, the average rating was: 4.8 in the 6 patients (25%) who underwent the abdominal fat transfer; 4.6 in the 3 patients (12.5%) with the rotational SCM flap; 4.8 where an SDP was possible (5 patients, 20.8%) and 3.3 where the superficial lobe was removed with no additional procedures (10 patients, 41.6%). A summary of these results is reported in Table I.

Discussion

In parotid surgery, ECD, first described by Gleave [12], has been established to be the most appropriate treatment for benign tumours [3]. The procedure is performed by carefully dissecting around the neoplasm without violating its capsule. This procedure allows sparing a sufficient amount of surrounding (healthy) salivary tissue around the capsule of the lesion. The technique appears to provide an acceptable rate of recurrence with low morbidity [4 5 13], especially where an extensive dissection could increase the risk of surgical complications (tumour surrounded by the facial nerve or involving the PPS).

Among recent studies, TCA (without mandibulotomy) has been correlated with lower morbidity but cannot be performed in all patients, especially in cases of large tumours, patients with very poor exposure, or with suspicion of malignancy [7 8]. TPA is more invasive because it requires facial incision (Blair incision) extending to the upper part of the neck that can remain visible; alternatively, a standard face-lift incision can be employed in order to minimise the scar. Furthermore, the procedure may lead to a skin depression in the area of the parotid region where the superficial lobe has been removed. In addition, a traditional TPA can more easily cause the onset of Frey's syndrome [14]. On the other hand, in our surgical experience, traditional TPA allows best control on the branches of the facial nerve and can be easily extended to a combined TPTCA, for example in the cases of poor exposure, large tumours, intra-operative suspicion of malignancy, or intra-operative complications such as uncontrollable bleeding. Regarding the choice of surgical approach, we retain that the possibility to perform a TCA must be considered only after accurate radiological study: in our experience, the presence of healthy salivary tissue between the LPM and the lesion is the best indicator of the feasibility of a TCA because it suggests a lower cranial-caudal level and a peripheral localisation within the deep lobe. Furthermore, our experience suggests that a preoperatively established high cranial-caudal level of the tumour can also provide some indications regarding the neural branches of the facial nerve that may require management: in this circumstance, direct control over the middle and the higher branches via a TPA (or TPTCA) is mandatory, also because these branches are often multiple with a relevant number of anastomoses [15]. In summary, we believe that the choice of TCA should be limited to small or medium-sized benign tumours, located in the lower part of the deep lobe (and preferably characterised by an extra-glandular pattern of growth towards the PPS). In our experience, mandibulotomy can be avoided in the majority of cases.

As shown in the literature, some techniques can be easily added to a TPA (or TPTCA) in order to improve aesthetical outcomes, such as a standard face-lift skin incision, SDP and the filling of the lodge with abdominal fat or other tissues (SCM muscle or superficial muscle-aponeurotic system – SMAS - application) [16]. These methods, in our experience, do not produce major complications and are able to minimise the scar, skin depression and facial asymmetry in the region with the same results as a selective deep parotidectomy.

This study has several limitations. Firstly, each type of lesion (similar tumours regarding histopathology, dimensions and location) was treated with the same type of approach, without a control group. Furthermore, the range of cases is heterogeneous, including both primary diagnosis and recurrences. The other important limitation is that the mean period of follow-up (2 years) is sufficient for assessment of surgical complications, but too short to obtain reliable oncological outcomes (PA needs at least 10 years of follow-up). The main aim of this study was to provide indications and short-term outcomes related to each surgical approach to the deep lobe, and not to establish the superiority of one approach in terms of oncological results. Additional studies are required to establish whether the employment of fat tissue or SMAS can effectively reduce the onset of an FS, especially correlating data with volume and localisation of the removed parenchyma.

The radiological parameters considered in our series have not been validated: further studies also involving radiologists are required to obtain a standardised method that allows the surgeon to stratify the choice of the right approach.

Conclusions

TPA (or TPTCA) ensures best control of the facial nerve providing good exposure and good functional and aesthetical results. In selected cases, the superficial parenchyma can be spared by performing selective deep parotidectomy with good functional outcomes. Aesthetic outcomes can be improved by adding simple additional techniques such as SCM flap and abdominal fat transfer. In our experience, mandibulotomy can always be avoided.

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Head and neck

Cervical paragangliomas: single centre experience with 44 cases

Paragangliomi cervicali: esperienza monocentrica in 44 casi

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SUMMARY

Paragangliomas (PGL) are rare lesions of the neuroendocrine system; in the neck, they usually affect the carotid glomus (carotid body tumours-CBT). This retrospective analysis reports our experience in management of these lesions in patients treated by surgical resection. Between 2000 and 2014, 33 patients were surgically treated at our institution, obtaining a series of 44 cervical PGLs. Tumour characteristics, family history, diagnostic procedures, surgical treatment, short- and long-term outcomes were reviewed. A female prevalence was found (76% of cases). Familial cases occurred in 9 patients (20%); 6 presented with bilateral lesions and 1 had multiple paragangliomas. Lymph node metastasis was not found in any patient. All lesions were classified into three groups according to the latero-lateral diameter. Complete resection of the PGL was performed in all patients. Mortality was null; transitory cranial nerve deficit occurred in 20% of cases with permanent palsy in 6.7%. No perioperative stroke/TIA were observed. Surgical resection of PGL should be considered as the only therapeutic option because it can ensure complete removal of the disease. Patients with bilateral lesions and positive family history should be referred for genetic analysis. Preoperative planning of the surgical procedure by integrated diagnostic imaging and a full mastery of vascular surgery techniques are mandatory to minimise the risk of the most common postoperative complications. Long-term follow-up is recommended, particularly in patients with familial disease or sporadic lesions treated in an advanced stage.

KEY WORDS: Paraganglioma • Carotid body tumour

RIASSUNTO

I paragangliomi (PGL) sono tumori rari del sistema neuroendocrino; a livello cervicale, originano nella maggior parte dei casi dal glomo carotideo (Carotid Body Tumor, CBT). Scopo del presente lavoro è illustrare l'esperienza personale maturata dagli Autori sul trattamento chirurgico di queste lesioni. Tra il 2000 e il 2014, 33 pazienti sono stati sottoposti a exeresi di complessivi 44 PGL presso la nostra Unità Operativa. Sono state analizzate retrospettivamente le caratteristiche epidemiologiche, strumentali, operatorie nonché i risultati immediati e lungo termine. È risultata evidente una prevalenza di pazienti di sesso femminile (76%); forme familiari sono state osservate in 9 casi (20%), nei quali abbiamo osservato 3 lesioni bilaterali e, in un paziente, lesioni multifocali. Non sono mai state rilevate metastasi ai linfonodi regionali. I PGL sono stati classificati secondo criteri dimensionali utilizzando il massimo diametro latero-laterale. La rimozione della lesione è stata completa in tutti i pazienti. La mortalità operatoria è stata nulla; deficit transitori a livello dei nervi cranici sono stati osservati nel 20% dei casi ma una paralisi permanente è risultata evidente solo nel 6,7%. Non abbiamo osservato perioperatoriamente alcun caso di ictus/TIA. L'exeresi chirurgica del PGL è da considerare, a nostra opinione, l'unica reale opzione terapeutica in quanto permette la completa rimozione della neoplasia. L'analisi genetica è consigliabile nelle lesioni bilaterali e nei casi di familiarità. Una strategia operatoria pianificata mediante l'integrazione delle tecniche di imaging, e una piena padronanza della tecnica chirurgica vascolare sono indispensabili per ridurre al minimo i rischi delle complicanze periperatorie più comuni. Nei pazienti operati è indispensabile un follow-up a lungo termine, soprattutto nei casi di familiarità o di lesioni sporadiche trattate in stadio avanzato.

PAROLE CHIAVE: *Paraganglioma • Tumore del glomo carotideo*

Acta Otorhinolaryngol Ital 2015;35:88-92

Introduction

Paragangliomas (PGLs) are rare lesions, with an incidence of 1:30.000 but are the most common tumours of extra-adrenal chromaffin tissue and represent more than 50% of head and neck paragangliomas [1]. These tumours are benign in the majority of the cases, but clinical malignant behaviour is described in 5-30% of cases. Most CBTs occur in adults between 30 and 60 years [2], although cases with earlier outset due to genetic inheritance have been reported [3]. CBTs have been associated with nine susceptibility genes: NF1, RET, VHL, SDHA, SDHB, SDHC, SDHD, SDHAF2 (SDH5) and TMEM127; hereditary tumours are mostly caused by mutations of the SDHD gene, but SDHB and SDHC mutations are not uncommon in such patients; moreover, they are rarely associated with

mutations of VHL, RET, or NF1. Further investigations on SDHA, SDHAF2 and TMEM127 are on-going [4]. Sex prevalence is controversial.

CBTs are space-occupying tumours, and therefore signs and symptoms are induced by compression on the surrounding anatomical structures. Due to its slow growth, the most common clinical presentation of CBT is a painless mass in the latero-cervical region. Surgical excision is the only treatment that can provide complete eradication of the disease, considering that histological evaluation is unable to provide a definitive diagnosis of malignancy [5 6].

Materials and methods

Between 1988 and the time of writing, 33 patients with 44 CBTs have been treated by surgical excision in our unit: 7 men and 26 women with mean age of 55 years. In 16 (48%) patients, the lesion was asymptomatic and diagnosis was incidentally made by ultrasound scan performed for another reason; 15 (45%) patients presented a painless mass in the latero-cervical region; only 2 (7%) patients reported symptoms such as headache, palpitations and local pain. First evaluation was performed, in all patients, by duplex ultrasound. All patients admitted were previously diagnosed by second level examination such as angioCT scan (Fig. 1) or angio MRI scan (Fig. 2) and ^{131}I-MIBG scintigraphy. A detailed list of imaging procedures performed in all patients is given in Table I. Familial cases occurred in 9 patients (20%); of these, 3 patients had bilateral lesions and 1 patient had multiple paragangliomas. No lymph node metastasis was found at preoperative imaging in any case. The lesions were classified into three groups according to the latero-lateral diameter: 17 tumours (40%) in Group I (< 3 cm), 19 (44%) in Group II (3-5 cm), and 8 (16%) in Group III (> 5 cm). Tumours that extended to the skull base were not included in this series. In 22 cases (group II-III), preoperative embolisation of the CBT feeding vessels was performed by superselective carotid angiography (Fig. 3).

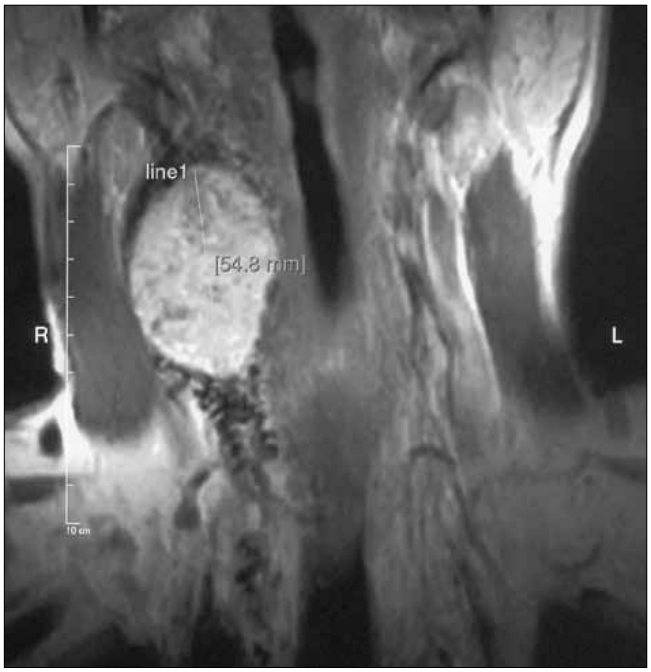

Fig. 2. MIP reconstruction from high-resolution angio-CT scan of a CBT: in addition to the peculiar contrast uptake by CBT and the "cup" appearance of the carotid bifurcation, the detailed view of vascular anatomy is comparable to a conventional angiographic study.

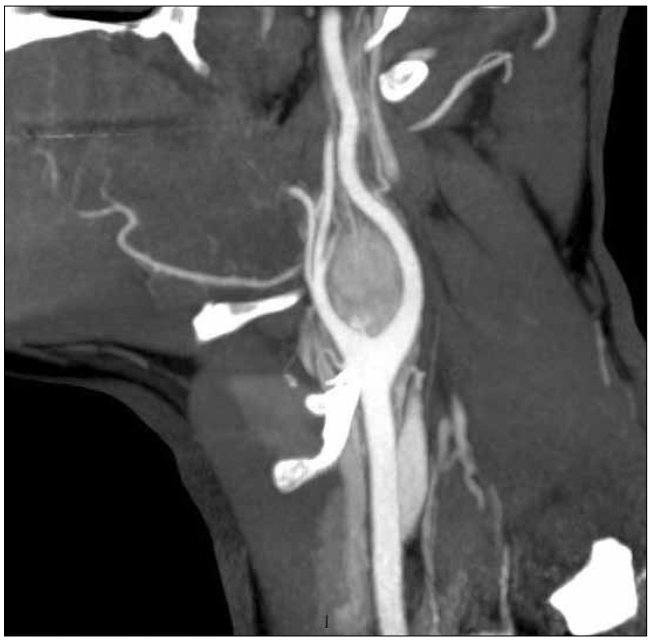

Fig. 1. Coronal view with angio-MRI showing the typical "salt-pepper" pattern of a CBT.

Table I. Preoperative imaging techniques in 33 patients.

Duplex ultrasound	44 (100%)
Angio-CT	23 (52%)
Angio-MRI	14 (32%)
Angiography	22 (50%)
PET-CT	1 (2.3%)
MIBG	1 (2.3%)

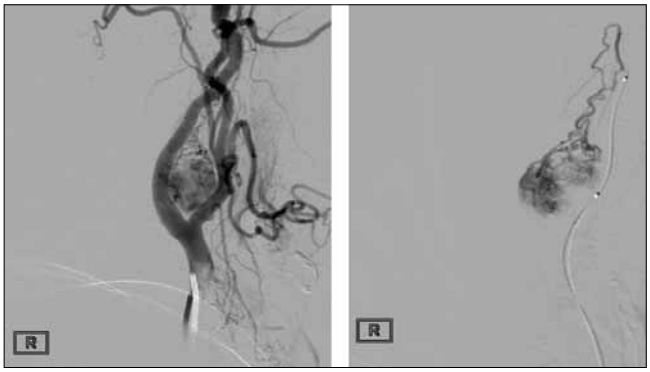

Fig. 3. Diagnostic selective carotid angiography (on the left) and superselective embolisation (on the right) of feeding vessels of a CBT.

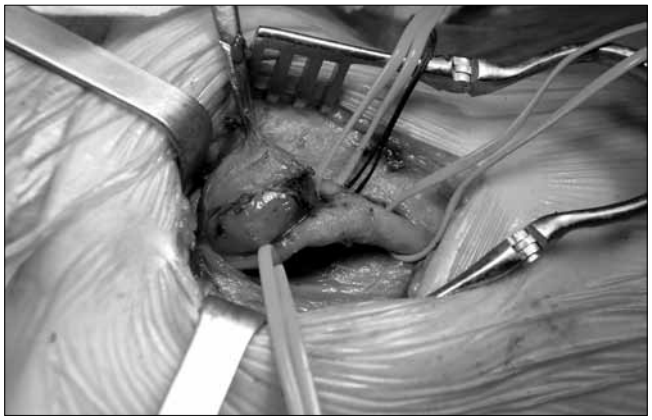

Fig. 4. Intraoperative view of a CBT: the carotid bifurcation is exposed and its branches secured on silicone loops before starting tumour resection.

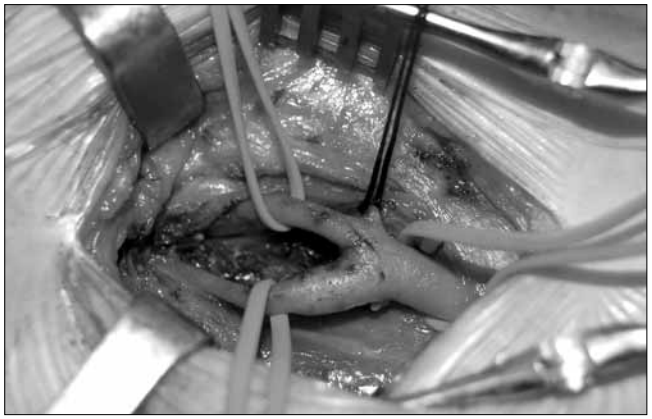

Fig. 5. Intraoperative view of the cleared carotid bifurcation.

Preoperative and postoperative laryngoscopy as well as phoniatric evaluation were performed in all patients. All lesions were treated by the same surgeon (F.S.) by complete surgical resection through a conventional approach to the carotid artery and its bifurcation (Figs. 4, 5); all interventions were performed under general anaesthesia.
The follow-up protocol included yearly clinical evaluation and duplex ultrasound of the neck.

Results

Operative mortality was null; no strokes were observed prior surgery or in the immediate postoperative period. Surgical revision was needed for postoperative bleeding in 1 case. The incidence of transient peripheral neurologic complications was 16% (7 cases). In 6 cases partial or complete encasement of the vagus nerve was found at surgical exploration; although this increased the difficulty of the procedure, the nerve was preserved by a meticulous dissection from the tumour. We observed only 2 (4.5%) permanent lesions, both affecting the X cranial nerve: in one case in group II, dysphonia from recurrent laryngeal nerve palsy was observed after *en bloc* resection of a paraganglioma arising from the vagus (the same patient – already underwent bilateral removal of CBT – presented postoperative arterial hypertension treated with clonidine and β-blockers); in the other case, a type III CBT tightly adherent to the vagus was successfully resected sparing the nerve, but postoperatively complained of permanent dysgeusia. Detailed complications are listed in Table II. In 1 of 44 (2.3%) interventions, a tight adhesion of the tumour to the posterior wall of the carotid bulb was found, requiring a resection-anastomosis of the first portion of the internal carotid artery. One of the two cases of suspected recurrent CBT was found intraoperatively to be a metachronous primary paraganglioma of the vagus nerve, which was treated by *en bloc* resection of the nerve containing the tumour. The other case was a local recurrence found during follow-up, in a case with family history of disease, 2 months after resection of the primary lesion. All resected lymph nodes were negative for metastasis at histologic examination.

Table II. Postoperative complications following 44 CBT resections.

	Group 1	Group 2	Group 3
Permanent cranial nerve palsy	0	1	1
Horner's syndrome	0	1	0
Hypertension	1	1	0
Stroke	0	0	0
Dysphagia / transient sore throat	3	1	2
Bleeding	0	1	0

Discussion

CBT are rare lesions and their optimal treatment deserves some considerations that we have gained from our experience in the last 14 years. The incidence of familial CBTs in our series (20%) is similar to other series [6-8], and the finding of painless mass as the most common clinical presentation is also in agreement with other authors [9 10]. A female prevalence has been reported in some series, but has not been observed in other reports [11]. Preoperative diagnostic imaging is crucial; differential diagnosis is with thyroid nodule, lymphadenopathy and brachial cysts. Fine-needle aspiration biopsy (FNAB) as preoperative diagnostic tool is absolutely not indicated because of the hypervascularisation of the tumour, and its proximity to nervous and vascular structures makes the procedure extremely dangerous; moreover, the risk of dissemination is not negligible and – last but not least – cytologic evaluation cannot differentiate benign from malignant lesions. In our series, 3 of 33 patients had previous FNAB in other hospitals.
The use of different types of non-invasive imaging techniques can provide correct diagnosis in most cases with satisfactory sensitivity and specificity. Duplex ultrasound scan is usually the first diagnostic exam; it can provide information on tumour size and location, relationships with the vascular structures and intralesional flow patterns. Angio-CT

scan and angio-NMR provide further details about regional extension of the tumour and eventual vascular encasement; hypervascularised PGL shows characteristic features that are helpful for differential diagnosis. Selective carotid angiography can identify the feeding vessels of the tumour, usually rising from the pharyngeal and external carotid artery: however, this information can be provided by latest generation multilayer CT scanners, and the use of an invasive procedure is advisable only to perform a superselective embolisation aimed at obtaining preoperative PGL shrinkage, reduced vascularisation of the mass and contained risk of intraoperative bleeding [12-14]. Preoperative embolisation is therefore particularly useful in large and hypervascularised tumours (group II-III) [8 12]; in our practice, we prefer to perform embolisation the day before the surgical procedure to avoid any perilesional inflammatory reaction and to achieve the best reduction of intraoperative bleeding; re-adsorbable material (shredded gelatine sponge) was used in our early experience, while we currently prefer non-reabsorbable agents such as PVA (polyvinyl alcohol) particles.

In our series, 22 patients (all with tumour size > 3 cm) underwent the procedure because of suspected vagus nerve encasement at CT (1 case) or hypervascularisation signal at duplex ultrasound scan. However, in a large series [15], the procedure does not seem to affect the rate of cranial nerve injuries – although most are temporary; some authors do not use embolisation at all [11] and others [16] recommend it in a few selected cases (size > 5 cm, Shamblin's type III, or significant cranial extension). Recently, the insertion of a covered stent in the external carotid artery has been sporadically reported as an alternative method to embolisation [17]; assuming a young age of most patients and the risk of early and late complications, we consider the choice of permanently stenting a normal vessel highly questionable.

Unlike extraadrenal chromaffin tissue tumours, CBTs rarely secrete vasoactive substances; we have no clinical nor laboratory findings suggestive of endocrine activity in any of our patients. However, according to the literature [18 19], the use of a nuclear medicine imaging technique such as MIBG, PET-CT, or Octreoscan is highly recommended as complementary study for total body investigation in familial cases or multifocal disease.

In our series, we did not adopt the classification criteria proposed by Shamblin [20], because we consider this classification useful for predicting vascular morbidity but not neurological morbidity [21]. The risk of intraoperative cranial nerve injury proportionally increases with the size and extension of tumour, with higher rates observed in bilateral and/or large lesions [8 22-24]; for this reason, also considering potential forensic implications, it is mandatory to assess the preoperative status of cranial nerves (VII, X, XI, XII) that are potentially at risk of intraoperative injury.

General anaesthesia is routine for safe PGL surgery; in our experience, sevoflurane is the hypnotic of choice since it assures haemodynamic steadiness through a lower vasodilatory action compared to other halogenated agents; moreover, it preserves the microcirculatory self-regulation. Remifentanyl provides optimal analgesia during the surgical procedure; due to its very fast kinetics, awakening is almost immediate when its administration is stopped [25].

The surgical procedure must include regional lymph nodes with enlarged size, suspicious morphology, or closely adherent to the tumour. Accurate dissection of the principal regional nerves from the PGL is needed. Careful excision of the tumour using microsurgical instruments and bipolar cautery can be performed along a sub adventitial plane or "white line" as suggested by Gordon Taylor [24]; only in this way it is possible to separate the lesion from the surrounding vessels by slipping on a relatively avascular plane [26 27]. In the present series vascular reconstruction was necessary in only one case; this is a particularly good result compared with the higher rates reported in the literature [12]. In our opinion, solid experience in carotid surgery is crucial to minimise arterial and nerve lesions, as well to perform eventual vessel reconstructions.

When faced with large PGLs that extend cranially to the skull base, a multidisciplinary team is recommended, including an otolaryngologist or maxillofacial surgeons, to obtain the best exposure through a surgical approach including standard or modified mandibulotomy [28 29].

Conclusions

The development of imaging techniques improved the opportunity to correctly diagnose CBTs, but an integrated multidisciplinary approach is essential for planning the best treatment and providing the best outcome [28]. On the basis of our personal experience and of the reported experiences, we can draw some conclusions:

- Surgical resection of cervical paragangliomas is the only curative treatment and should be considered as the first therapeutic option [5 6].
- PGL removal is a challenging surgery because of the tumour's location near large vascular structures and cranial nerves. Therefore, an experienced vascular surgical team is recommended, also for correct management of eventual vascular complications. Excision should be as conservative as possible in preserving main vessels and adjacent nerves; extensive resections should be limited to cases of actual locoregional invasion to minimise the risk of complications, with particular reference to neurologic ones (transitory and/or permanent).
- Tumours associated with succinate dehydrogenase (SDH) mutations show more aggressive behaviour. For this reason, some authors recommend family screening as routine in patients with hereditary paraganglioma carotid for early diagnosis and treatment [30-32].
- Histological criteria of malignancy do not apply to CBTs; for this reason, lifelong follow-up is crucial, particularly in patients with familial disease or sporadic lesions in an advanced stage.

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Rhinology

Maxillary fungus ball: zinc-oxide endodontic materials as a risk factor

Il Fungus Ball mascellare: il materiale endodontico a base di ossido di zinco come fattore di rischio

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SUMMARY

The objective of this study was to demonstrate the correlation between endodontic treatment on maxillary teeth and fungus ball with inductively coupled plasma mass spectrometry measurement of zinc and other metals (barium, lead and copper) in fungus ball samples. Samples of normal maxillary mucosa were used as comparison. Metal concentration was also measured in several endodontic materials. A significant difference was found between the concentration of zinc and copper in fungus ball compared to normal mucosa. Metal distribution was more similar in fungus ball and in the endodontic materials tested than normal mucosa. The similar metal concentration in the endodontic materials and fungus ball suggests that endodontic materials play a role in the pathogenesis of fungus ball. Endodontic materials accidentally pushed into the maxillary sinus during endodontic treatments may play a crucial role. Dentists should be as careful as possible when treating maxillary teeth to avoid perforating the maxillary sinus floor; the use of zinc-free endodontic materials, as zinc is a metal that plays a pivotal role in fungus growth, should be encouraged.

KEY WORDS: Maxillary fungus ball • Zinc-oxide materials • Endodontic treatment • Inductively coupled plasma mass spectrometry

RIASSUNTO

L'obiettivo di questo studio è stato dimostrare la correlazione tra il trattamento endodontico su denti mascellari antrali e il Fungus Ball mascellare. mediante L'utilizzo della ICP-MS (Inductively Coupled Plasma Mass Spectrometry) è stata utilizzata per la misurazione della concentrazione di zinco e altri metalli (bario, piombo e rame) in campioni di Fungus Ball. Per il confronto sono stati utilizzati campioni di mucosa sana del seno mascellare. La concentrazione di questi degli stessi metalli è stata misurata anche in diversi materiali utilizzati per le terapie endodontiche. Vi era è una differenza significativa tra la concentrazione di zinco e rame nei campioni di Fungs Ball rispetto alla mucosa sana. La concentrazione del dei metallo metalli nei fungus ball e nei materiali endodontici testati è invece sovrapponibile è stata sovrapponibile tra il Fungus Ball e i materiali endodontici testati. Tale dato La concentrazione simile nei materiali endodontici e nel Fungus Ball suggerisce che il materiale endodontico svolga un ruolo nella patogenesi del Fungus Ball. Ciò suggerisce che i materiali endodontici inavvertitamente spinti nel seno mascellare durante i trattamenti canalari possano svolgere un ruolo fondamentale. I dentisti dovrebbero essere il più attenti possibile durante il trattamento di denti dell'arcata superiore per evitare di forare il pavimento del seno mascellare. e forse L'uso di materiali endodontici privi di zinco dovrebbe essere incoraggiato, essendo lo zinco un metallo che gioca un ruolo fondamentale nella crescita del fungo.

PAROLE CHIAVE: *Fungus ball mascellare • Materiali a base di ossido di zinco • Trattamento endodontico • ICP-MS (Inductively Coupled Plasma Mass Spectrometry)*

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Introduction

Continuity or contiguity of the upper teeth apical dental root foramina (molars and premolars, more rarely canines) with the maxillary sinus floor [1-3] may cause spread of odontogenic inflammatory and/or infective processes to the maxillary sinus. The most frequent infective process is the maxillary sinusitis and a less frequent form of possible odontogenic origin is a non-invasive mycotic form: fungus ball (FB) [4-8]. The most credible aetiology of FB is endodontic treatment of the upper teeth [2-12]. The time interval between endodontic therapy and diagnosis of FB is several years (6 on average) [12]. Usually, symptoms appear when the fungal mass fills the entire maxillary sinus. At this point, surgery becomes the only viable option.

It is well-known that endodontic sealers, long used in dental practice, are mostly made of zinc oxide, and it has been demonstrated [13 14] that zinc promotes fungal growth as it is an essential microelement for helping some fungi such as *Aspergillus sp*. (which is the main pathogen leading to FB) survive and proliferate [7 8].

Over a series of 102 patents with FB, clinical and radiologic diagnosis showed the presence of endodontically-

treated teeth or post-extraction sites of previously endodontically-treated teeth in 99% of cases [9].
This is, however, only a clinical observation. Thus, is it sufficient to justify the odontogenic origin of FB? The objective of this prospective study was to evaluate the presence of zinc and other metals contained in the most important endodontic sealers in FB samples and in the adjacent mucosa in order to determine if there is a statistically significant difference.

Materials and methods

From October 2002 to September 2007, 53 patients affected by FB were surgically treated by endoscopic surgery at the Department of Otorhinolaryngology, University of Brescia (Italy). All patients were also examined in the Dental Clinic before undergoing surgical treatment. Demographics and historical data were collected along with radiographic findings (panoramic, intraoral periapical radiographs and CT of the sinuses).
The treatment consisted in the complete removal of the fungal debris with reaeration of the sinus through an endoscopic minimally-invasive approach [15]. During the surgery a sample of normal mucosa was taken from the maxillary sinus.
After the surgical procedure, FB and maxillary mucosa samples were sent to the Pathology Department to confirm diagnosis and to the Industrial Toxicology Laboratory of the Section of Occupational Medicine and Industrial Hygiene of the Department of Experimental and Applied Medicine to perform inductively coupled plasma mass spectrometry (ICP-MS).
Using the same method, the following endodontic materials were also examined in the Occupational Medicine Laboratory using the ICP-MS method :
PCS-Pulp Canal Sealer (Kerr, Romulus - MI 48174, USA); N2-Sargenti Cement (Hager & Werken GmbH & Co KG, Duisburg 47006, Germany); Endometasone (Septodont, Saint-Maur-Des-Fossès, Cedex 94107, France); Standardised gutta-percha (Kumapan, Dia Dent Group Internetional, Choongehong Buk Do. Korea); Non-standardised gutta-percha (Dentsply Maillefer 1338 Ballaigues Switzerland) [16].
Two samples of each material were analysed and the average value of each metal was kept as a reference.
Moreover, 5 cultures of different fungi (*Aspergillus fumigatus*, A*spergillus flavus, Aspergillus niger*, *Alternaria, bipolaris*) were analysed with the same methods.

Measuring instruments and procedures

Tissue specimens were oven dried at 70°C for 2 h, left to cool at room temperature in a desiccator, and finally weighed. Samples were then dissolved in HNO_3 (ACS reagent, Sigma) 70% (v/v) for 2 h at 70°C. The digested samples (0.1 ml) were diluted with deionised water to 5 ml.
Prepared samples underwent ICP-MS analysis on a Perkin Elmer ELAN DRC II instrument (Perkin Elmer, Woodbridge, ON, Canada) using the analytical technique total quant with external calibration.
The method's accuracy was determined in natural water reference materials (NIST 1640, National Institute of Standard and Technology, Gaithersburg, MD).
The coefficients of variation (CV) ranged from 4 to 8% among series and from 6 to 12% between series. The instrument was calibrated using a standard solution at a concentration of 10 µg/l (Multielement ICP-MS Calibration Standard 3, Matrix per Volume: 5% HNO_3 per 100 ml, Perkin Elmer Plus).
The limits of detection (LOD) were determined on the basis of three standard deviations (SD) of the background signal, and the following values (as µg/l) were obtained: 0.006 for Bi and Ba; 0.005 for Hg; 0.004 for Cu, Sr and Pb; 0.003 for Ag; 0.002 for Zn and Mn [17].

Statistical analysis

The data were considered as average values (Standard Deviation, SD). The non-parametric Mann-Whitney test was used to compare metal (Zn, Cu) concentrations in the FB and maxillary mucosa. SPSS was used to analyse the data. A $p < 0.005$ was considered as statistically significant.

Results

Anamnestic and radiographic data showed that 99% of patients had undergone at least one endodontic treatment in the maxillary sector where the FB was found. The results of ICP-MS of elements in endodontic materials are listed in Table I. Since zinc and copper are widely present in endodontic materials, they may be used as a reliable finger-printing of those materials. In Table II, the results of FB, mucosa and cultures of fungi are compared to endodontic material.
It can be clearly seen that zinc and copper are the elements most widely used in endodontic materials. These metals were considered in statistical analysis to sustain the evidence for the presence of endodontic materials in FB samples compared to their absence in normal mucosa.
Various metals may be traced [18] in all biological tissues, but this objective of this study was to show a difference between the amounts of zinc and copperin the FB (and therefore of exogenous origin) and that of those to be found in mucosa.
FBs were found to contain a quantity of zinc and copper that was more similar to endodontic materials tested than the mucosa samples, while lower average values were detected in fungi cultures.
The Mann-Whitney test is significant with $p < 0.05$ for metals tested comparing samples of FB and mucosa (Table II).

Discussion

ICP-MS is a highly sensitive, multielement analytical method that has increasingly become affordable to many labs. It is being applied to biological samples and has sev-

Table I. Average values by ICP-MS of elements in endodontic sealers.

Metals in endodontic sealers									
Metal [μg/g]	**Cu**	**Zn**	**Pb**	**Ba**	**Mn**	**Ag**	**Bi**	**Sr**	**Hg**
Endometasone	50	395000	17500	22500	3	4,5	35	160	8
N2	75	620500	8	0	1.5	160	193000	0	0
PCS	70	601000	15	25	7.5	421000	10	8.5	45
Non-standardised guttaperca	115	719500	5	2	0	1	0	0.1	0
Standardised guttaperca	100	627500	5	1600	7	2.5	0	30	0

Cu: copper, Zn: zinc, Pb: lead, Ba: barium, Mn: manganese, Ag: silver, Bi: bismuth, Sr: strontium, Hg: mercury.

eral advantages: multielement stimulation determinations, excellent detection limits, a wide linear dynamic range and a high sample throughput [18 19].

The results obtained with ICP-MS and statistical analysis show that metals present in the most widely used endodontic materials are found in significantly higher amounts in FBs compared to mucosa.

Traces of these metals, as found in nasal mucosa, are however compatible with those found in lung tissues and in the upper respiratory tract as a result of atmospheric contamination by microelements such as lead, zinc and barium [20].

The results obtained strengthen the odontogenic aetiologic theory of FB, as there seem to be no other plausible causes for such high concentrations of these metals.

It was also held that these metals resulted from fungal spores, but the ICP-MS on mycological cultures showed average concentrations of the above mentioned metals similar to those found in mucosa. This supports the assumption that the high amounts found in FBs can only come from contamination of the maxillary sinus from endodontic sealers that have been accidentally pushed into it during endodontic treatment or carried beyond the apex by an endodontic over-instrumentation, and therefore brought into contact with the sinus mucosa by an acute or chronic inflammatory process causing the surrounding bone to undergo an erosive process.

Endomethasone has been the most widely used sealer over the last 50 years and it used to be pushed into root canals up until 15 years ago using Lentulo placed on low-speed handpieces without any precise check of the work length, thus easily causing iatrogenic perforation of the Schneiderian membrane and passage of the sealer into the sinus.

The fact that the concentrations of the zinc found in sealers are clearly higher than those found in FBs might be due to the very slight diffusion of that metal through a sinus mucosa only partially allowing the passage into the sinus and therefore the FB. Furthermore, the samples of FB analysed do not correspond to the whole FB taken from the maxillary sinus. The amounts of metal found might therefore be lower than their actual concentration in FBs.

Moreover, to validated more our theory about the aetiopathogenesis of FB, it would be interesting to gather data on metal content in non-maxillary FBs and in non-fungal odontogenic sinusitis.

INn the only case in this series, the data on metal content in μg are: FB: Cu 26, Zn 170, Mn 0, Pb 19, Ba 14, Ag 0,02, Bi 0.08, Sr 47, and Hg 0.01, and in normal mucosa: Cu 2.3, Zn 72, Mn 0.01, Pb 0.4, Ba 0.7, Ag 0.6, Bi 0.2, Sr 16 and Hg 0.

Conclusions

FB is a non-invasive mycotic sinusitis. While the incidence of FB is not well known (3.7%) [21], it is certainly of substantial relevance as it cannot be treated through medical therapy. Therefore, a surgical procedure is needed [22 23], which represents an invasive intervention for the patient.

The correlation between endodontic therapies and FB as shown in a case control study [9] and supported by different case series [2 6 11 24], together with the demonstration, through this study, of the presence of metal contents in endodontic material in samples of FB, contributes to strongly support the odontogenic aetiologic hypothesis. Moreover, Tung-Lung Tsai et al. [25] have demonstrated that there is no relation between obstruction of the ostiomeatal complex and FB, thus confirming a different aetiopathogenesis of FB.

Despite the fact that research is progressing in this direction, it is crucial that dentists take some simple precautions to prevent this problem from occurring. It is advisable to execute root canal therapies on antral teeth (molars, premolars, canines of upper jaw) with the utmost attention to work length (electronic apex locator and intraoral peri-

Table II. Average values and SD of ICP-MS for normal mucosa, fungus ball, endodontic materials and a culture of *Aspergillus* spores and Mann-Whitney test results.

Metal [μg/g]	Cu	Zn
Endodontic sealers	82.00 (25.64)	592700 (119599)
Fungus ball	98.84 (128.02)	3199 (15961.58)
Mucosa	15.73 (26.09)	220.75 (965.91)
Fungl	7.8 (5.11)	7.37 (3.97)
Fungus ball vs. mucosa	p = 0.000	p = 0.000

Cu = copper, Zn = zinc.

apical radiographs [26]) to avoid over-instrumentation, to use warm canal obturation techniques which need low amounts of sealers and possibly endodontic sealers not based on zinc oxide [27]. The follow-up is present beyond the apex after endodontic treatment should include a radiological check-up and evaluation of possible symptoms at least once a year in order to precociously identify any pathologic signs and to subsequently refer the patient to an ENT specialist.

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Rhinology

Magnetic nanoparticles: a new tool for antibiotic delivery to sinonasal tissues. Results of preliminary studies

Nanoparticelle magnetiche: un nuovo strumento per la diffusione degli antibiotici nei tessuti naso-sinusali. Risultati degli studi preliminari

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SUMMARY

Herein we examined the toxicity, penetration properties and ability of $Fe_2O_3 \cdot nH_2O$ magnetic nanoparticles extracted from silt of the Borovoye Lake (Krasnoyarsk, Russia) to bind an antibiotic. Experimental studies were carried out using magnetic nanoparticles alone and after antibiotic exposure in tissue samples from nasal mucosa, cartilage and bone (*in vitro).* Toxicity of particles was studied in laboratory animals (*in vivo)*. Tissues removed at endonasal surgery (nasal mucosa, cartilage and bone of the nasal septum) were placed in solution containing nanoparticles and exposed to a magnetic field. Distribution of nanoparticles was determined by Perls' reaction. After intravenous injection, possible toxic effects of injected nanoparticles on the organs and tissues of rats were evaluated by histological examination. Binding between the nanoparticles and antibiotic (amoxicillin clavulanate) was studied using infrared spectroscopy. In 30 *in vitro* experiments, magnetisation of $Fe_2O_3 \cdot nH_2O$ nanoparticles resulted in their diffuse infiltration into the mucosa, cartilage and bone tissue of the nose and paranasal sinuses. Intravenous injection of 0.2 ml of magnetic nanoparticles into the rat's tail vein did not result in any changes in parenchymatous organs, and the nanoparticles were completely eliminated from the body within 24 hours. The interaction of nanoparticles with amoxicillin clavulanate was demonstrated by infrared spectroscopy. Positive results of experimental studies provide a basis for further clinical investigations of these magnetic nanoparticles and their use in otorhinolaryngology.

KEY WORDS: Nanomedicine • Ferrihydrite nanoparticles • Magnetic field • Antibiotics • Chronic rhinosinusitis

RIASSUNTO

Lo studio ha esaminato la tossicità, le proprietà di penetrazione e la capacità di legarsi con un antibiotico delle nanoparticelle magnetiche $Fe_2O_3 \cdot nH_2O$ estratte dal limo del lago Borovoye (Krasnoyarsk, Russia). Studi sperimentali sono stati effettuati utilizzando nanoparticelle magnetiche da sole e dopo esposizione ad antibiotico su campioni di tessuto di mucosa nasale, cartilagine ed osso (in vitro). La tossicità delle particelle è stata studiata su animali da laboratorio (in vivo). Tessuti prelevati durante interventi di chirurgia endonasale (mucosa nasale, cartilagine ed osso del setto nasale) sono stati immersi in una soluzione contenente nanoparticelle e poi esposti ad un campo magnetico. La distribuzione delle nanoparticelle è stata determinata attraverso la reazione di Perls. Dopo l'iniezione endovenosa, sono stati valutati possibili effetti tossici delle nanoparticelle su organi e tessuti nei ratti mediante esame istologico. Il legame tra nanoparticelle ed antibiotico (amoxicillina clavulanato) è stato studiato utilizzando la spettroscopia ad infrarossi. In 30 esperimenti in vitro, la magnetizzazione di nanoparticelle $Fe_2O_3 \cdot nH_2O$ ha portato alla loro infiltrazione diffusa nel tessuto mucoso, cartilagineo ed osseo del naso e dei seni paranasali. Con gli esperimenti, si è scoperto che l'iniezione endovenosa di 0.2 ml di nanoparticelle magnetiche nella vena della coda del ratto non determinava cambiamenti a livello degli organi parenchimatosi, e le nanoparticelle venivano completamente eliminate dall'organismo entro 24 h. L'interazione delle nanoparticelle con amoxicillina clavulanato è stata dimostrata mediante spettroscopia ad infrarossi. I risultati positivi degli studi sperimentali forniscono una base per ulteriori indagini cliniche sulle nanoparticelle magnetiche ed il loro utilizzo in otorinolaringoiatria.

PAROLE CHIAVE: *Nanomedicine • Ferrihydrite nanoparticelle • Campo magnetico • Antibiotici • Rinosinusite cronica*

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Introduction

The use of new drug formulations is especially important in the treatment of diseases where conventional therapy is not effective. One such example is chronic rhinosinusitis (CRS). According to the European Position Paper on Rhinosinusitis and Nasal Polyp (EPOS, 2012) [1], the prevalence of CRS is increasing annually, and is now one of the most common chronic diseases in humans. For example, in Canada, the prevalence of CRS is 3.4% among men and 5.7% among women [2], in Belgium it is 6% [3] and in Scotland reaches 9.6% of the population [4].

The ineffectiveness of standard courses of systemic antibiotic therapy [5-7], coupled with the doubtful efficacy of corticosteroids, especially in patients with CRS without polyps and concomitant allergy [8-10], and the inability to achieve 100% success with surgery [11], indicate the need to develop newer and more effective treatment modalities. Magnetic nanoparticles as a targeted drug delivery tool for the tissues of nose and paranasal sinuses in CRS are a promising research direction.

Materials and methods

To determine penetration of magnetic nanoparticles in tissues, samples of nasal mucosa were taken from the ethmoidal labyrinth cells during functional endoscopic surgery, while cartilage and bone of the nasal septum were obtained during nasal septal surgery. The indications for nasal surgery were nasal obstruction secondary to CRS and significant nasal septal deformity.

For controlled delivery of nanoparticles in tissues, an external magnetic field device for low-frequency magnetic therapy "Pole 101" (EMA Factory, Yekaterinburg, Russia) ensuring continuous operation of one inductor with a magnetic field gradient of 4.6 mT/mm and magnetic induction 10.14-19.56 mT was used.

The study drug was prepared as follows: 0.125 grams of magnetic nanoparticles were diluted in 1 ml of normal saline (sterile 0.9% NaCl). The material was equally divided into three groups (20 experiments in each group).

In Group 1, mucosa, cartilage and bone tissue samples were placed in a flask with dispersed nanoparticles in normal saline for 20 minutes, washed in saline and sent for histological examination. In Groups 2 and 3, the same tissue samples were immersed in a flask with dispersed nanoparticles in normal saline and kept in a magnetic field produced by the device "Pole-101" for 20 and 40 min, respectively, before rinsing in saline and sent for histological examination.

For identification of the presence of nanoparticles in tissue samples, a specific reaction for detecting iron (reaction to Berlin blue or Perls' reaction) was used. Therefore, tissue sections remained in 1% hydrochloric acid solution and after rinsing in distilled water they were stained again with aluminous carmine, rinsed in water and carried through ethanol, xylene and carbol-xylene. Iron deposits were coloured dark blue. After staining, tissue samples were studied under a light microscope at magnifications of ×100 and ×200.

To investigate the potential toxicity of magnetic nanoparticles, parenchymatous organs of laboratory animals (rats) were selected. Over a 10 day period, 0.2 ml of magnetic nanoparticles dissolved in distilled water were injected into the tail vein of rats (n = 10) every other day. Weight changes during drug administration as well as the animals' eating habits were controlled daily. After 12 days, all rats were euthanised under general anaesthesia in accordance with GLP principles of euthanasia. To determine changes in investigated organs, morphological examination assessing macroindicators was carried out. Tissue biopsies were fixed in 10% formalin solution. Slices of 5-7 μm thickness were stained with haematoxylin followed by microscopic examination of the material at magnifications of ×100 and ×200. All animal experiments were carried out in accordance with the requirements and provisions outlined in the Declaration of Helsinki of the World Medical Association (2000) and the local ethics committee approved the final study protocol.

Given the fact that magnetic nanoparticles of ferrihydrite are biologically neutral and may bind drugs by either covalent bonding or simple adsorption, nanoparticles were mixed with the antibiotic amoxicillin clavulanate. To confirm binding, infrared spectroscopy was used. For this purpose, powder of 1.2 g of amoxicillin clavulanate was added to 5 ml of a solution of ferrihydrite nanoparticles and magnetised in the magnetic therapy machine "Magofon-01" (Elatomsk Instrument Plant, Elatma, Russia) with a magnetic induction of 30 ± 9 mT and a frequency of acoustic range 0.02-20 kHz for

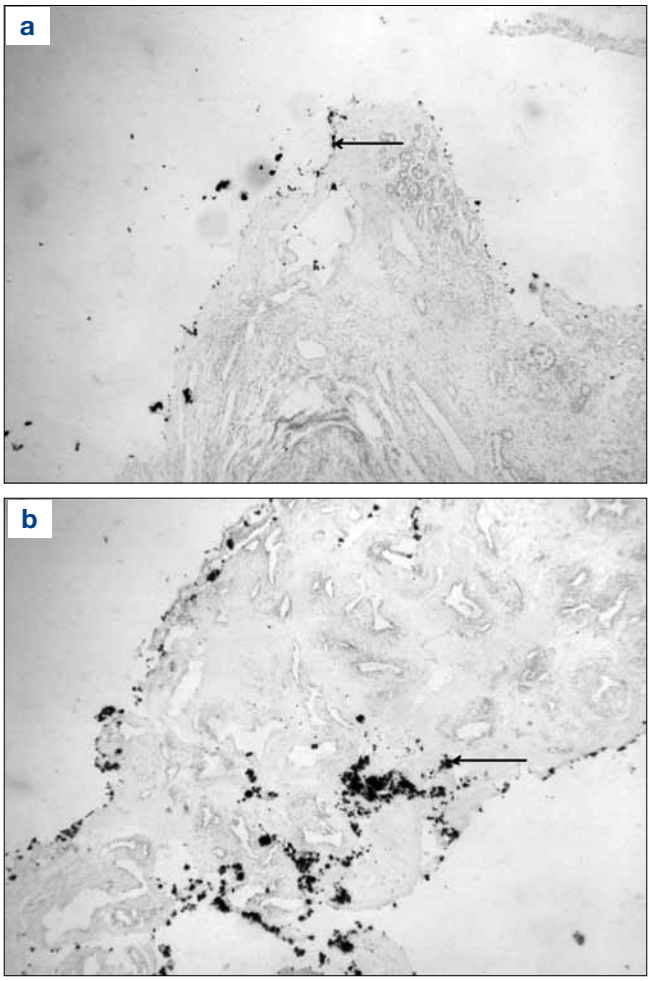

Fig. 1. Histological picture of the respiratory epithelium: a) nanoparticles (indicated by arrow) are located on the mucous membrane (Group 1); b) nanoparticles (indicated by arrows) after magnetic action, located in the nasal mucosal and submucosal layers of epithelium. Perls' reaction. ×100.

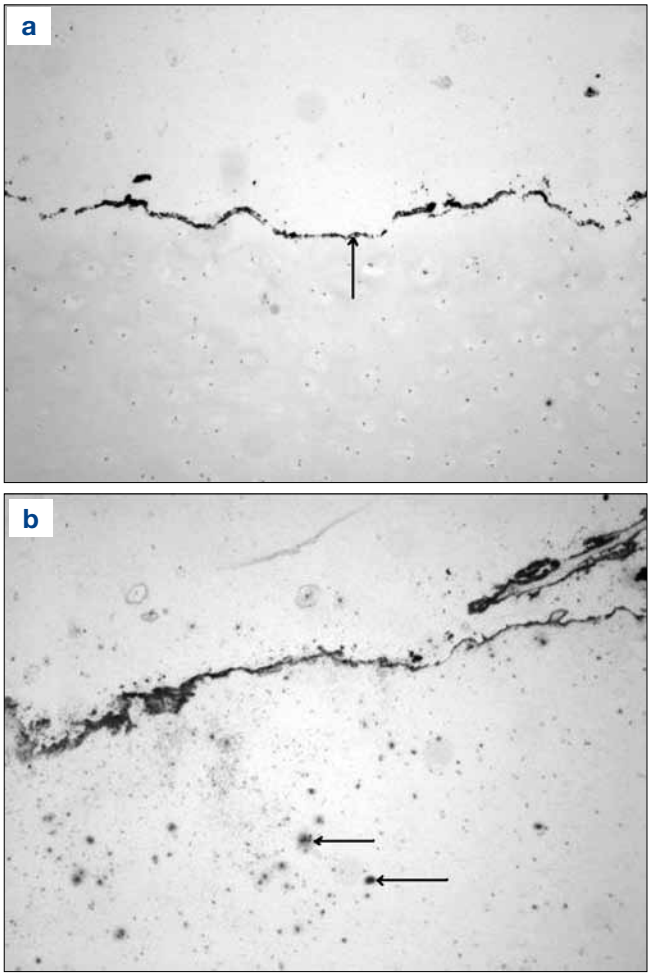

Fig. 2. Histological picture of nasal septum cartilage tissue: a) iron nanoparticles (indicated by arrow) are present only on the surface of cartilage (Group 1); b) iron-containing nanoparticles (arrows) after exposure to magnetic field are defined in the thickness of the cartilage. Perls' reaction. ×100.

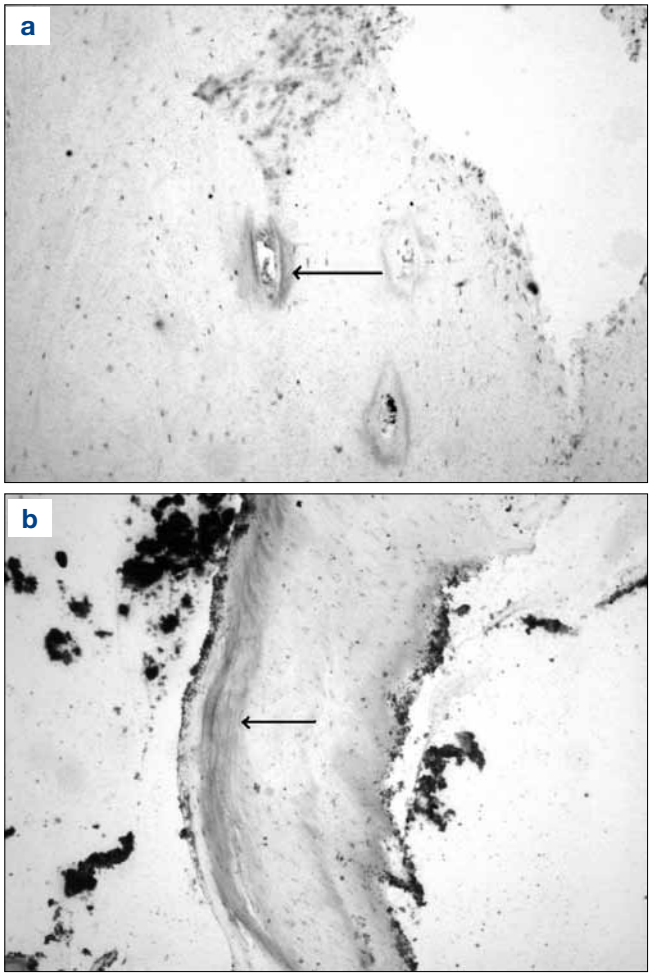

Fig. 3. Histological picture of bone tissue: a) iron nanoparticles (indicated by arrow) located on the edge of the bone channels (Group 1); b) nanoparticles (indicated by arrow) after magnetic action determined throughout the bone thickness. Perls' reaction. ×100.

5 min, and placed in a liquid reservoir with $BaFe_2$ glasses of 0.1 mm thickness. Infrared spectrum was recorded at room temperature using Nicolet-6700 (Thermo Scientific, USA) in the range from 1100 to 2800 cm^{-1} (Group 1). A similar study was conducted with 1.2 g of amoxicillin clavulanate powder dissolved in 5 ml of ferrihydrite nanoparticles without magnetic action (Group 2) as well as with 1.2 g of amoxicillin clavulanate powder dissolved in 5 ml of water without nanoparticles (Group 3). Each group was studied with 10 identical experiments.

Results

Tissue penetration of nanoparticles (nasal mucosa and nasal septum cartilage and bone morphology)

The study of penetration of magnetic nanoparticles in tissues of the nose showed that in Group 1 nanoparticles did not penetrate deeply into the nasal mucosa, but were found only on the surface of epithelium (Fig. 1a). In addition, despite the relatively loose structure of the nasal septal cartilage, without exposure to a magnetic field, nanoparticles were located on the edge of cartilage (Fig. 2a). A similar pattern was also observed in the bone tissue of the nasal septum. Coloured nanomaterial was located mainly in the lumen and around the bony canals. Ferruginous substance did not penetrate into the bone tissue (Fig. 3a). Following the exposure of tissues to a magnetic field for 20 min (Group 2), the activity of iron-containing nanoparticles increased substantially. As a result, magnetic nanoparticles were found in the epithelium and subepithelial layer of the nasal mucosa and also sporadically dispersed (Fig. 1b). In addition, under the influence of a magnetic field gradient, nanoparticles freely penetrated into the body of the nasal septal cartilage (Fig. 2b) and were diffusely distributed. The same pattern was observed in bone tissue of the nasal septum where the nanoparticles penetrated throughout bone fragments (Fig. 3b). After doubling exposure time to the magnetic field (Group 3), there was no significant increase in the concentration of iron compounds in tissues.

In vivo analysis of nanoparticle injection in a rat model

To assess toxicity of the magnetic nanoparticles, laboratory animals (rats, n = 10) treated with nanoparticles were followed for 10 days and then examined histologically. Global vital parameters of the experimental animals, i.e. medium body weight, did not change significantly and eating habits remained the same over the time of observation.

Animal liver tissue. Microscopic examination revealed that the lobular-girder structure of the liver was preserved. Hepatic lobule, sinusoidal capillaries, blood vessels and bile ducts displayed normal structure in all animals. Moderate polymorphism of hepatocytes and their nuclei was observed. The cytoplasm of investigated cells was stained uniformly. Kupffer and endothelial cells had normal shape; their hyperchromic nuclei were properly located. Perls' reaction did not demonstrate any presence of iron nanoparticles in liver tissue.

Animal kidney tissue. Renal glomeruli demonstrated normal structure. Capillary loops of some glomeruli were collapsed, and nuclei were elongated. Epithelium of renal tubules was structurally normal. Clear brush rim was observed in the proximal tubules, and the lumen had normal width. Collecting glomeruli had a normal shape and contained dark and light epithelial cells. Magnetic nanoparticles in tissues of the kidney, as well as in the liver, were not detected.

Animal lung tissue. No pathological changes were observed in lung tissue. Bronchi were lined with ciliated epithelium. Peribronchial accumulations of lymphoid cells with the formation of solitary lymphoid follicles were noted. The pulmonary vascular bed was anaemic. Alveoli were well inflated; small foci of atelectases were found. In Perls' reaction, nanoparticles in the lung tissue were not present.

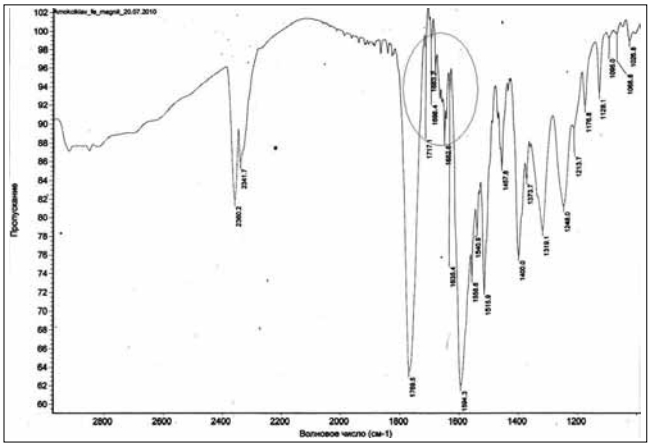

Fig. 4. Infrared spectrometry of amoxicillin clavulanate: a) dissolved in distilled water; b) associated with nanoparticles after magnetic action (changes in the spectrum are circled). Axis of abscissas, wave number (sm-1); ordinate, %.

Conjugation of nanoparticle to antibiotic

When comparing the results of infrared spectroscopy of the complex nanoparticle/amoxicillin clavulanate without magnetic action and conventional aqueous amoxicillin clavulanate, no significant differences were found. However, after introduction of the complex into magnetic field, there was sharp decrease in absorption at 1650-1670 cm^{-1} with the appearance of additional bands at 1558.6 and 1540.9 cm^{-1} (Fig. 4a-b).

Results of all experimental studies conducted are summarised in Table I.

Discussion

One of the most rapidly developing and promising fields of scientific research to date is nanotechnology [12 13]. This technology explores the properties and practical applications of nanoparticles which are extremely small in size

Table I. Experimental studies on magnetic nanoparticles Fe_2O_3-nH_2O.

	Purpose	Methods	Result	Conclusion
I	Investigation of penetration ability of Fe_2O_3-nH_2O nanoparticles into human tissues	Tissue samples (mucosa, cartilage and bone tissue of the human nose) were kept in a flask with dispersed nanoparticles in normal saline under the action of a magnetic field or without it	Magnetisation of nanoparticles for 20 min results in their diffuse infiltration into the mucous membrane, cartilage and bone tissue	Ability to control distribution of the nanoparticles in the humans nasal mucosa, bone and cartilage by an external magnetic field
II	Assessment of the toxicity of magnetic nanoparticles in laboratory animals	Injection of magnetic nanoparticles dissolved in distilled water into the tail vein of rats (N = 10) over a 10-day period followed by morphological examination of tissue biopsies	No pathological changes in the parenchymatous tissues (liver, kidney, and lung). Complete clearance of nanoparticles from the body within days	Magnetic nanoparticles are nontoxic
III	Investigation of the ability of magnetic nanoparticles to interact and bind with an antibiotic	Infrared spectroscopy: a magnetised solution of amoxicillin clavulanate mixed with ferrihydrite nanoparticles (Group 1), an amoxicillin clavulanate + nanoparticles solution without previous magnetic action (Group 2) and an amoxicillin clavulanate solution alone (Group 3)	The change of the absorption spectrum of magnetised complex ferrihydrite/amoxicillin clavulanate at the range of 1650-1670 cm^{-1}	Formation of a complex nanoparticles + antibiotic by hydrogen bonds or weak induction forces

(a few nm); a variety of physical and chemical substances have been examined: silicon, gold, zinc, iron, aluminosilicates, ferromagnetic and diamagnetic materials [14 15].

The most promising direction for nanotechnology in medicine is the use of magnetic nanoparticles as carriers of drugs (vehicles). This is due to the ability of drugs immobilised on the surface of magnetic nanoparticles to selectively concentrate and move under the influence of an external magnetic field in a specific body area [16-18].

In 2006, magnetic nanoparticles of bacterial origin were developed in the Institute of Biophysics (Siberian Branch of the Russian Academy of Science, Krasnoyarsk, Russia). They were synthesised by cultivating gram-negative bacilli from the Enterobacteriaceae family (genus *Klebsiella*, type *Klebsiella oxytoca*) isolated from silt (sapropel) of the lake Borovoye, Krasnoyarsk District, Russia. Based on comparison of the results of magnetic and direct structural methods, the resulting magnetic nanoparticles are ferrihydrite Fe_2O_3-nH_2O as seen in Figure 5. It was also demonstrated that the ferrihydrite nanoparticles 2-5 nm in size produced by *Klebsiella oxytoca* have an effective magnetic charge enabling magnetic control of these nanoparticles [13].

Ferrihydrite nanoparticles produced by *Klebsiella oxytoca* possess unique magnetic properties in the biomineralisation of natural iron salt solutions. Antiferromagnetic order inherent in the massive ferrihydrite and spontaneous magnetic moment due to decompensation of the spins in the sublattices coexists in these nanoparticles. Enhanced by the effect of superantiferromagnetism, the magnetic susceptibility enabled magnetic control of these materials. Intravenous injection of magnetic nanoparticles in laboratory animals (rats) for 10 days caused no pathological changes in the parenchymatous tissues (liver, kidney, and lung). Absence of changes in the kidney histological architecture of these animals demonstrates that these magnetic nanoparticles were nontoxic. In addition, the study showed complete clearance of nanoparticles from the animals' body within days, characterised by negative Perls' reaction in all animal tissues studied.

The study of magnetic nanoparticles penetration into nasal tissues *in vitro* showed that the effect of an external magnetic field for 20 min resulted in a diffuse distribution of nanoparticles in mucosa, cartilage and bone tissue of the human nose. Therefore, the possibility of targeted delivery of nanoparticles into the lamina propria of the nasal respiratory epithelium is especially important in the treatment of CRS when chronic inflammation affects primarily the mucous membrane of the nose and paranasal sinuses. Penetration of iron nanoparticles into tissues confirmed their magnetic reactivity and strongly suggests that these iron nanoparticles can be controlled by an external magnetic field.

Although there is an absence of evidence-based literature that topical application of antibiotics is effective in the treatment of CRS [7], bacteria are believed to universally present in CRS. Certain bacteria likely influence mucosal inflammation in the paranasal sinuses contributing to CRS persistence and severity [19 20]. Under certain circumstances, at least in case of acute exacerbation, antibiotics can ameliorate the severity of CRS. Dependence of treatment outcome on contamination of the sinuses with bacteria like *S. aureus*, and the need for pathogen eradication has been also stressed [21]. However, the current options in topical antimicrobial therapy and methods for delivery of antimicrobial agents to the paranasal sinuses are few at best [22-24]. Therefore, our first step toward future clinical implications of the new method of drug delivery was investigation of possible conjugation of a nanoparticle to antibiotic.

Preliminary results of our study have shown that the antibiotic molecule readily links with nanoparticles. Infrared spectrometry revealed the change of the absorption spectrum of magnetised complex ferrihydrite/amoxicillin clavulanate, which reliably indicates the formation of bonding between the initial reagents with participation of either hydrogen bonds or weak induction forces.

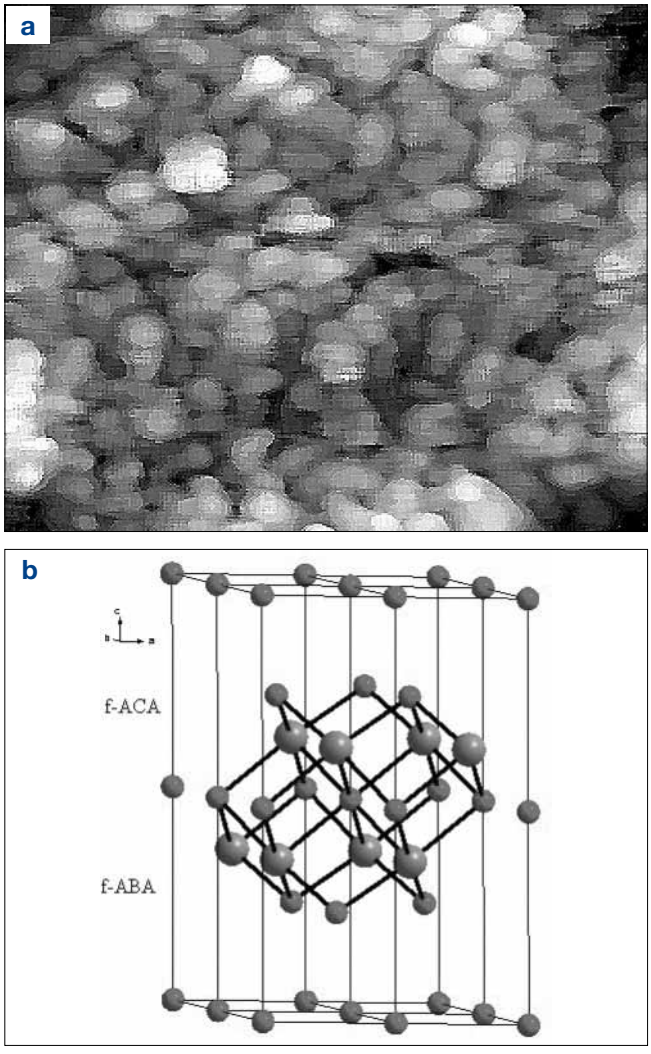

Fig. 5. Magnetic nanoparticles Fe_2O_3: a) scanning tunnelling microscopy, ×20 nm; b) schematic drawing of Fe-O-Fe links in a non-defect phase: smaller grey balls, O and OH ligands; larger grey balls, Fe^{3+}.

Conclusions

We believe that the initial results of these experimental studies provide a rational basis for development of a new method of targeted drug delivery to tissues of the nose and paranasal sinuses. Further experimental and clinical investigations of magnetic Fe_2O_3 nanoparticles linked with an antibiotic, glucocorticosteroids, or some other agents are needed, as they can considerably improve our therapeutic options in treatment of chronic inflammation of the sino-nasal mucosa.

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Audiology

Idiopathic sudden sensorineural hearing loss: cardiovascular risk factors do not influence hearing threshold recovery

Ipoacusia improvvisa idiopatica: i fattori di rischio cardiovascolare non influenzano il recupero di soglia uditiva

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SUMMARY

Previous studies have suggested that risk factors for ischaemic vascular disease, such as cigarette smoking, hypertension and hyperlipidaemia, can also be considered risk factors for the development of idiopathic sudden sensorineural hearing loss (ISSNHL). In this study, we have evaluated the hypothesis that these factors can influence hearing threshold recovery in patients affected by ISSNHL. A total of 141 subjects who suffered an episode of ISSNHL were included. All subjects were assessed with tonal audiometry, auditory brainstem responses and MRI to exclude retrocochlear pathology. Hearing tests were conducted at ISSNHL onset (t = 0) and after 30 days. Patients were divided into three classes according to the presence/absence of one or more cardiovascular risk factors including: history of smoking, total serum cholesterol/triglycerides, history of hypertension and diabetes mellitus. Values of hearing threshold recovery were estimated and comparisons were conducted across the three risk factor classes. 75% of patients affected by ISSNHL showed a threshold recovery. However, the threshold recovery was found to be class-independent (average recovery value of 18 dB HL per classes) and also independent of age and gender. Even if cardiovascular risk factors have been found to be involved in the pathogenesis of ISSNHL, the present study suggests that these factors do not have any significant influence on the threshold recovery in ISSNHL.

KEY WORDS: Idiopathic sudden sensorineural hearing loss • Cardiovascular risk factors • Threshold recovery

RIASSUNTO

Diversi studi in letteratura hanno già suggerito che i fattori di rischio per la patologia ischemica vascolare, tra cui il fumo di sigaretta, l'ipertensione e l'iperlipidemia, sono anche fattori di rischio per lo sviluppo dell'ipoacusia improvvisa idiopatica (ISSNHL). In questo studio, abbiamo valutato l'ipotesi che questi fattori possano anche avere un ruolo nel recupero della soglia uditiva, in un gruppo di pazienti affetti da ISSNHL. 141 soggetti affetti da ISSNHL sono stati inclusi in questo studio. Tutti i pazienti sono stati valutati con audiometria tonale, potenziali evocati uditivi (ABR) e risonanza magnetica per escludere patologia retrococleare. L'audiometria tonale è stata eseguita all'esordio dell'ISSNHL e quindi dopo 30 giorni. I pazienti sono stati suddivisi in tre classi in base alla presenza/assenza di uno o più fattori di rischio cardiovascolare. I fattori di rischio cardiovascolare esaminati sono stati: storia di fumo, colesterolo totale e trigliceridi, ipertensione e diabete mellito. Il recupero di soglia uditiva è stata valutato e poi confrontato attraverso le tre classi dei fattori di rischio. Nelle tre diverse classi di rischio cardiovascolare, il 75% dei pazienti affetti da ISSNHL ha mostrato un recupero di soglia. Il recupero di soglia è risultato essere indipendente dalla classe di rischio di appartenenza (valore di recupero medio di 18 dB HL per classe) e anche indipendente da età e sesso. Anche se i fattori di rischio cardiovascolare sono risultati essere coinvolti nella patogenesi dell'ISSNHL, i dati del presente studio hanno mostrato che questi fattori non hanno però influenza significativa sul recupero soglia in un gruppo di soggetti affetti da ISSNHL.

PAROLE CHIAVE: *Ipoacusia improvvisa idiopatica • ISSNHL • Fattori di rischio cardiovascolare • recupero di soglia uditiva*

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Introduction

Idiopathic sudden sensorineural hearing loss (ISSNHL) is an acute inner ear disorder, mostly unilateral. It has been reported that ISSNHL has an overall incidence of 5-20 per 100,000 and that it is more frequent in western countries [1-7]. The differential diagnosis for ISSNHL includes more than 100 potential aetiologies [1 3]. Cases with a potentially discoverable aetiology fall into one of several categories including infectious, autoimmune, traumatic, vascular, neoplastic, metabolic and neurologic diseases. In a meta-analysis of 23 studies of ISSNHL, the most frequent causes were identified as infectious, vascular, haematologic, otologic, traumatic and neoplastic factors [1 3].

In support of the vascular occlusion thesis, impaired cochlear perfusion is the most widely reported hypothesis [7]. The cochlea has a terminal capillary bed and is not supplied by collateral vessels that can restore blood flow in ischaemic regions. Moreover, since cochlear hair cells have a high metabolic activity, they are particularly vulnerable to hypoxic or ischaemic damage [7]. A number of studies in the literature have found that risk factors for ischaemic vascular disease, including cigarette smoking, hypertension, and hyperlipidaemia, are also risk factors for the development of ISSNHL [8]. Cochlear microvascular disorders can be related to increased plasma viscosity, to microembolic and/or thrombotic events [9].

In a previous publication [7], we studied the relation between the occurrence of ISSNHL and cardiovascular risk factors within this same group of subjects affected by ISSNHL; we have found a significant correlation between the risk of ISSNHL and diabetes when matching ISSNHL subjects with a group from the general population. However, we did not study the relationship between audiological features of ISSNHL and cardiovascular risk factors, which is the topic of this investigation. In this study, we analysed cardiovascular risk factors in a group of patients affected by ISSNHL; we then evaluated the hypothesis that these factors could influence hearing threshold recovery.

Materials and methods

This study analysed data retrospectively. A total of 141 subjects (75 M and 66 F), aged between 16 and 89 years (mean age 54 ± 15.8) who suffered an episode of ISSNHL were included in the analysis. Cases presenting other causes of hearing loss (either progressive or fluctuating) were excluded.

All patients were queried about medical history and were assessed by tonal audiometry and auditory brainstem responses (ABR). Medical resonance imaging (MRI) was used to exclude a retrocochlear pathology. Pure tone audiometry was performed within a sound-proof cabin (model E2X2, roll 01008 220V 10A; Mercury, Milan, Italy) using an Amplaid audiometer (Amplaid, Milan, Italy) calibrated to ISO 9001 standards. The audiometric procedure was performed using headphones to assess air conduction and a bone vibrator for bone conduction. The better ear was evaluated first. An ascending method using 5 dB steps was utilised to calculate hearing threshold. Air conduction hearing thresholds were assessed at 125, 250, 500, 1000, 2000, 4000 and 8000 Hz. Bone conduction hearing thresholds were assessed with the use of a masking, white, contralateral noise, at 250, 500, 1000, 2000 and 4000 Hz. Data on the ABR procedures are presented in the Appendix (section A1).

Hearing assessment was performed at the onset of ISSNHL (t = 0) and after 30 days (t = 30d). Each patient was classified into a hearing loss (HL) group, according to a pure tone average (PTA) value, estimated from hearing threshold levels at 0.5, 1, 2, 4 kHz. The HL groups were defined according to a modified method from Clark [10] as follows: normal hearing (-10-15 dB HL); slight hearing loss (16-25 dB HL); moderate hearing loss (26-55 dB HL); severe hearing loss (56-90 dB HL); and profound hearing loss (> 90 dB HL).

All subjects were treated with: (i) betametasone 4 mg IM for 3 days and then betametasone 1.5 mg IM for 3 days with gastroprotection; and (ii) glycerol 10% in 250 ml per day for 5 days. Steroid therapy (either system or intratympanic) is considered the best treatment option for ISSNHL by the American Academy of Otolaryngology [11]. Alternatively, osmotic diuretics have been also proposed for treatment of ISSNHL [12], but are not considered as standard therapy by the American Academy of Otolaryngology [11].

The cardiovascular risk factors considered in this study, included: (i) history of smoking; (ii) total serum cholesterol and triglycerides; (iii) history of hypertension and (iv) diabetes mellitus. Subjects with a history of diagnosed diabetes or who were receiving oral hypoglycaemic drugs or insulin, were defined as diabetic. For those who were not diagnosed with diabetes and who were not taking any antidiabetic medications, the value of fasting blood glucose was used to assess the presence of diabetes, according to American Diabetes Association guidelines (> 126 mg/dl) [Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997]. Patients with a history of diagnosed hypertension or who were taking medications for high blood pressure were considered as hypertensive. Total cholesterol and triglyceride values were determined using standardised commercial enzymatic tests after overnight fasting.

In order to assess the effects of cardiovascular risk factors on hearing threshold recovery, patients were divided into the following three classes according to the presence/absence of one or more cardiovascular risk factors (i.e. smoking, hypertension, cholesterol, diabetes, previous ischaemia, triglycerides, LDL):

- *Risk class 1*: subjects with no risk factors (no smoking history, cholesterol < 190 mg/dl, LDL < 120 mg/dl, TG < 180 mg/dl, no diabetes, no previous ischaemic episodes, regular blood pressure values).
- *Risk class 2*: subjects with a single risk factor (cholesterol > 190 mg/dl, TG > 180 mg/dl, or smoking history, hypertension, diabetes).
- *Risk class 3*: subjects with two or more risk factors (cholesterol > 190 mg/dl, TG > 180 mg/dl and/or smoking history, diabetes, hypertension).

The definition of "hearing recovery" criteria represents a critical problem that has been acknowledged in previous publications. Several criteria have been proposed in the literature, such as: (i) PTA improvement of 30 dB HL or 50% recovery [11]; and (ii) a less conservative criterion of 10 dB HL PTA improvement [11]. In this study, the threshold-recovery was calculated by PTA (t = 30d) – PTA

(t = 0). This value was considered important if it was ≥ 10 dB HL.

Regarding the hearing threshold levels prior to the onset of ISSNHL, since an audiogram prior to the onset of ISSNHL was not available, the initial hearing level values refer to the un-affected ear. The decision to use these data was based on multiple observations from patients during the first audiometric evaluation. patients have stated that before the ISSNHL incident, they could not perceive hearing differences between the two ears. In this context, the degree of recovery was based on the hearing threshold values of the un-affected ear.

Auditory brainstem responses (ABR) procedure

Abrasive paste was used to clean the skin and electrodes were placed using an electrolytic paste and adhesive tape on the vertex and right and left mastoids. Stimuli were given monoaurally by an earphone and consisted of 0.1 ms clicks with alternating polarity starting at the maximum intensity of 90 dB nHL (120 dB SPL), starting from the better ear. Signals were differentially amplified (50000), filtered (5000–8000 Hz), and sent to a computer for analogue-to-digital conversion, displaying and averaging. In presence of doubtful results two or more registrations were performed in order to obtain a well recognisable wave V, in order to exclude a retrocochlear pathology.

The clinical protocol for this study dictated that whenever the ABR traces were not reliable (i.e. no detectable responses), the patient should assessed with a cerebral MRI. As a safe guard and following the guidelines of the Academy of Otolaryngology-Head and Neck Surgery [11] all the patients participating in this study, underwent cerebral MRI to exclude retrocochlear pathology.

Statistical analysis

The characteristics of the sample were assessed using descriptive statistics by age, gender and degree of hearing loss with SPSS (version 16 for Windows). The presence of significant differences in hearing loss, or functional recovery, was investigated with the analysis of variance.

Moreover, a logistic regression model was used to assess the probability of threshold recovery, on the basis of the analysed variables and the presence of one or more cardiovascular risk factors.

Statistical significance was considered at $p < 0.05$.

Results

From the analysed variables (age, gender, PTA, threshold recovery), significant differences were observed only for age. Different patterns of threshold recovery were observed in the three risk-factor classes, but these differences were not statistically significant. The latter was also confirmed by a logistic regression model.

Descriptive statistics of the studied sample

Figure 1 shows the distribution of age, gender and cardiovascular risk factors of the 141 patients. The data show a

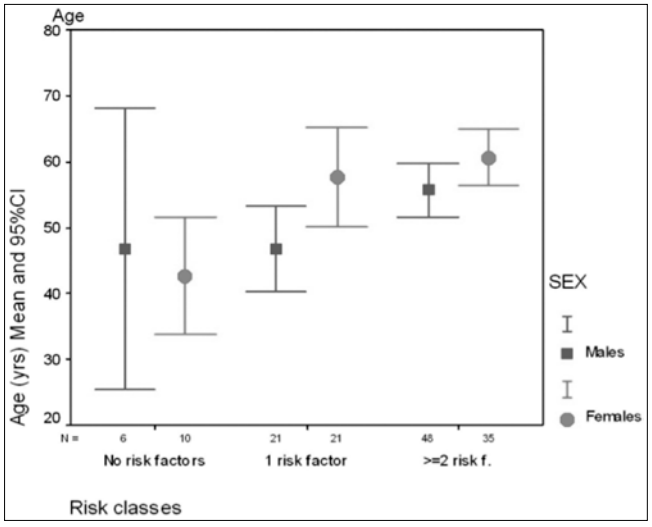

Fig. 1. Distribution of cardiovascular risk factors by age and gender. There is a statistically significant difference between the average age of class 1 (no risk factors) and class 3 (two or more risk factors).

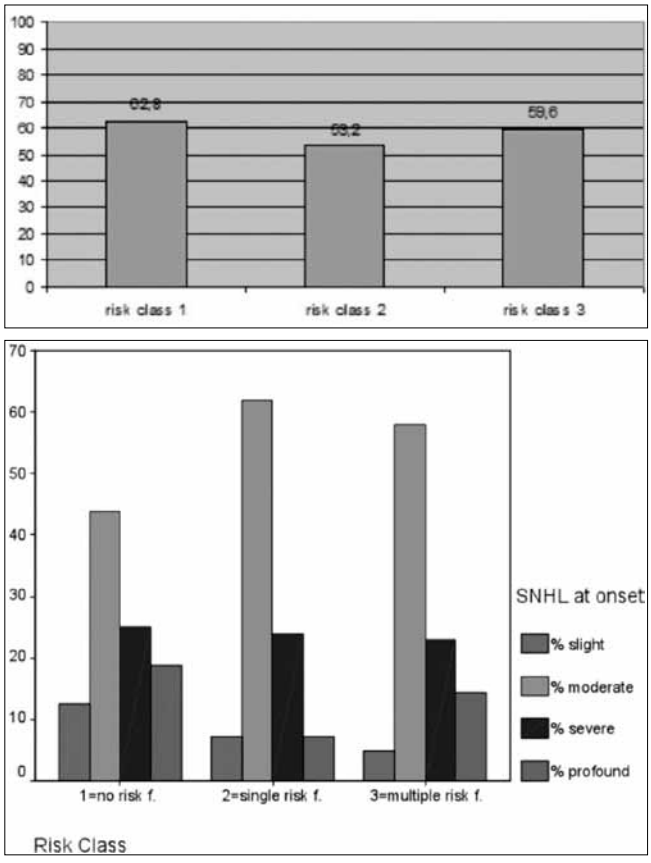

Fig. 2. A. PTA values (PTA = average of 0.5-4 kHz) for the three risk classes at onset (t = 0). B. Distribution of PTA values (PTA = average of 0.5-4 kHz) at t = 0 by risk-class and grade of hearing loss (slight, moderate, severe, profound).

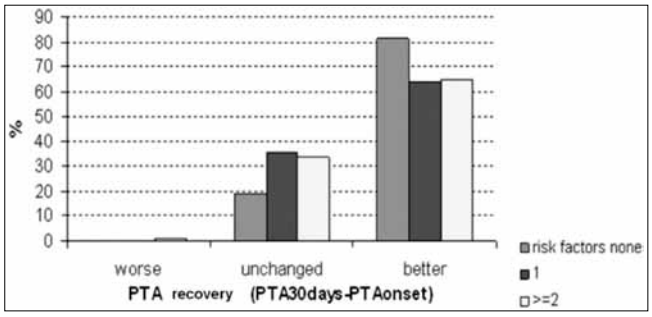

Fig. 3. Percentage of PTA (PTA = average of 0.5-4 kHz) threshold recovery at 30 days across the three risk classes. The data are presented as cases with improved threshold (better); cases with no threshold improvement (unchanged); and cases presenting a threshold deterioration. The classification outcome for cases belonging to risk-classes 2 and 3 is very similar.

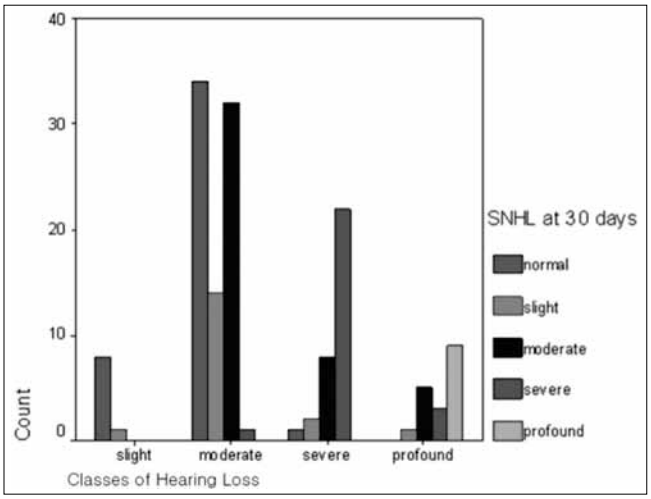

Fig. 4. Distribution of the PTA (PTA = average of 0.5-4 kHz) threshold recovery at 30 days. The data suggest that better recovery is a function of the initial hearing-loss classification. In this context, it can be hypothesised that better-hearing ears recover more of the lost threshold than worse-hearing ears.

slight gender effect on age. The average age of female subjects was higher than it males and is affected by ISSNHL. ANOVA analyses on age and cardiovascular risk factors show that there is a statistically significant difference between the average age of class 1 (no risk factors) and class 3 (two or more risk factors). The latter was significantly higher.

Figure 2A depicts the pure tone average values (1-4.0 kHz) at onset across the three risk classes. As expected, no significant differences were observed. Figure 2B expands the hearing loss data (at t = 0) in terms of slight, moderate, severe and profound losses.

Threshold recovery across risk classes

Figure 3 shows hearing loss distribution data at 30 days. For visual clarity, cases are classified according to the value of the threshold-recovery as worse, unchanged or better. Risk classes 2 and 3 show almost identical values in the "unchanged" and "better" groups.

Considering all patients, there was threshold recovery in 75% of cases; in about the 20% of the patients, no PTA changes were observed across the 30 day period; there was only one case, in risk class 3, where the PTA showed deterioration. Analytically, in risk class 1 (no risk factors), 80% of subjects presented changes in their hearing threshold, while in risk classes 2 and 3 only 65% of patients presented PTA changes.

The estimated amount of threshold-recovery was very similar across the three risk classes, with average values of: 18.75 ± 16.9 (class 1), 18.9 ± 19.1 (class 2) and 18.85 ± 19.4 (class 3). No statistically significant differences were found across these three threshold recovery values.

Patterns of threshold recovery

The patterns of threshold recovery across the three risk-classes were different. In particular:

1. 50% of subjects affected by profound ISSNHL (9 of 18), improved their hearing and moved to a lower

Table I. Cross-tabulation of ISSNHL grade at onset (t = 0) and after 30 days. The table shows the overall classification changes over the observed 30-day period. The rows of the table provide information on the re-distribution of patients (slight, moderate, etc.). The columns show data on the composition of a certain group, that is where its members are coming from at 30 days.

For example, the distribution of the 33 severe cases at onset (group total under the "Total" right-hand column) changes at 30 days (read data from row 3 in the table) as follows; 1 case moves to the normal category; 2 cases move to the slight HL category; 8 cases move to the moderate HL category; and 22 cases remain in the severe category.

The 33 severe HL cases at t = 0 became 26 (see total under the "severe" column) at 30 days. This number is composed from contributions of the following groups: 1 case from the moderate group showed a threshold deterioration and was moved to the severe group; 22 cases from the severe group were classified into the same category; 3 cases from the profound group moved to the severe HL category.

		ISSNHL grade at 30 days: all subjects					
		Normal	Slight	Moderate	Severe	Profound	Total
ISSNHL grade at onset	Slight	88.9% (8)	11.1% (1)	–	–	–	6.4% (9)
	Moderate	42.0% (34)	17.3% (14)	39.5% (32)	1.2% (1)	–	57.5% (81)
	Severe	3.0% (1)	6.1% (2)	24.2% (8)	66.7% (22)	–	23.4% (33)
	Profound	–	5.6% (1)	27.8% (5)	16.7% (3)	50.0% (9)	12.7% (18)
Total ISSNHL at 30 days		30.5% (43)	12.8% (18)	31.9% (45)	18.4% (26)	6.4% (9)	100% (141)

Table II. Cross-tabulation of ISSNHL grade considering only the subjects in risk class 2.

		ISSNHL grade at 30 days: Class 2 only					
		Normal	Slight	Moderate	Severe	Profound	Total
ISSNHL grade at onset	Slight	100.0%(3)	0% (0)	–	–	–	7.1% (3)
	Moderate	34.6%(9)	11.5% (3)	53.8% (14)	–	–	61.9% (26)
	Severe	–	–	40% (4)	60% (6)	–	23.9% (10)
	Profound	–	33.3% (1)	33.3% (1)	–	33.3% (1)	7.1% (3)
Total ISSNHL at 30 days		25.6%(12)	9.5% (4)	45.2% (19)	14.3% (6)	2.3% (1)	100% (42)

threshold class (i.e. they moved from the profound to the severe or moderate hearing-loss group).

2. 33.3% of those with severe ISSNHL at onset (11 of 33), moved to a moderate or a slight hearing-loss class.
3. 59.3% of those with moderate ISSNHL at onset (48 of 81), improved their hearing and moved to a lower threshold class. The only outlier-value from this group originates from one subject classified in risk-class 3, who presented a threshold deterioration at the 30-day control.

These observations suggest that "better recovery" is a function of the initial residual hearing capacity. In this context, it can be hypothesised that better-hearing ears recover more threshold, than the worse-hearing ears (assuming no-age effects). These findings are summarised in Figure 4 (distributional data) and Table I. The latter shows the data expressed in a cross-tabulation format, which facilitates the observation of distribution changes within each hearing loss category, at 30 days. Additional details on the cross-tabulation are provided in the legend of Table I.

The analysis of variance did not suggest any significant effects between the variables "threshold recovery" and "risk-class". Nevertheless, the behaviour of each risk-class in terms of threshold recovery was different. Analytical data are presented only for risk-classes 2 and 3, since the patients from risk-class 1 did not present any cardiovascular risk factors.

The data from risk-class 2 are summarised in Table II. The percentage of profound cases at t = 0 was 7.1% which decreased to 2.3% at t = 30d, due to reclassification of two subjects in the slight and moderate HL groups. The percentage of moderate and severe cases at t = 0, was 61.90% and 23.80%, respectively. At t = 30d, the percentages changed to 45.2% and 14.3% due to re-classification of subjects to the normal and slight hearing-loss categories. No cases with threshold deterioration were seen.

The data from risk-class 3 are summarised in Table III. The percentage of profound cases at t = 0 was 14.4%, which decreased to 7.2% at t = 30d due to reclassification of four subjects in the moderate and 2 subjects in the severe HL groups. The percentage of moderate and severe cases at t = 0 was 57.9% and 22.9%, respectively. At t = 30d, these percentages changed to 28.9% and 20.5%. Interestingly, the only subject from the studied population who showed threshold deterioration at the 30-day control (from moderate to severe) belongs to this group.

Discussion

The pathogenesis of ISSNHL is still unknown, even if viral infections and vascular occlusions are among the most common mechanisms advocated. Concerning the viral hypothesis, although some studies have been able to demonstrate viral infection in patients with ISSNHL, the specific and direct pathogenic role of viral infections in inner ear structural damage has not yet been definitively demonstrated [1 13]. In support of the vascular occlusion thesis, impaired cochlear perfusion is the widely reported hypothesis although the location of the cochlea in the temporal bone makes the identification of a thrombotic occlusion difficult [1 14]. Previous studies have evaluated the possible role of different cardiovascular and thrombophilic factors in the pathogenesis of ISSNHL [15-20]. In a previous publication [7], we have studied the relation between the occurrence of ISSNHL and cardiovascular risk factors within this same group of subjects affected by ISSNHL; we found significant correlation between

Table III. Cross-tabulation of ISSNHL grade considering only subjects in risk class 3.

		ISSNHL grade at 30 days: Class 3 only					
		Normal	Slight	Moderate	Severe	Profound	Total
SSNHL grade at onset	Slight	100.0% (4)	0% (0)	–	–	–	4.8% (4)
	Moderate	45.8% (22)	16.7% (8)	35.4% (17)	2.1% (1)	–	57.9% (48)
	Severe	–	10.5% (2)	15.8% (3)	73.7% (14)	–	22.9% (19)
	Profound	–	–	33.3% (4)	16.7% (2)	50.0% (6)	14.4% (12)
Total ISSNHL at 30 days		31.3% (26)	10% (10)	28.9% (24)	20.5% (17)	7.2% (6)	100% (83)

the risk of ISSNHL and diabetes when matching ISSNHL subjects with a group from the general population. However, we did not study the relationship between the audiological features of the ISSNHL subjects and cardiovascular risk factors.

If little is known about the pathogenetic mechanisms that cause ISSNHL, even less information is available on the biological mechanism of threshold recovery. It has been reported that threshold recovery in ISSNHL depends on many factors, including: (i) HL duration; (ii) associated HL symptoms; and (iii) audiogram characteristics. Generally, patients with higher hearing thresholds at the onset of ISSNHL recover less in comparison to patients with initial mild losses [1 21]. The audiogram shape has also been shown to significantly impact hearing recovery. Data from the literature show that patients with low-frequency or mid-frequency hearing losses present higher rates of recovery in comparison to patients with flat or sloping-down audiometric profiles [1 22]. Additional factors that contribute to better recovery post-ISSNHL, are: (i) younger age; (ii) male gender; (iii) less time elapsed between the onset of hearing loss and beginning of treatment; and (iv) upward-sloping audiogram shape [22].

Comorbid symptoms and signs have also been investigated as prognostic indicators for ISSNHL. In some studies, complaints of imbalance or vertigo have been associated with a poorer prognosis for hearing recovery following ISSNHL [1 23]. This association was found to be non-significant in other studies [1 4-6]. Tinnitus has been investigated as an ISSNHL indicator, but the available data are not conclusive [1]. So far, no experimental data are available on the mechanisms underlying the physiopathological process of ISSNHL, its onset, the induced damage and possible recovery from ISSNHL. Thus, there are no means to predict the degree of hearing recovery following ISSNHL.

Conclusions

In conclusion, the data of the present study show that:

1. Subjects in risk class 3 have a higher average age than the other two groups;
2. Across all risk classes, 75% of patients affected by ISSNHL show recovery of hearing threshold;
3. The hearing threshold recovery has the same average value (18 dB HL) across the three risk classes and is independent of age, gender and cardiovascular risk class of origin. In this context, a subject belonging to risk-group 3 (two or more risk factors) and to risk-group 2 (a single risk factor) has the same possibility to have hearing threshold recovery compared with those belonging to risk class 1 (subjects with no cardiovascular risk factors). This conclusion was also confirmed by the application of the logistic regression model.

The data from the two studies we have conducted (one on the occurrence of ISSNHL and cardiovascular risk factors [7] and this study on hearing recovery after ISSNHL and cardiovascular risk factors), suggest that cardiovascular risk factors are involved in the occurrence/pathogenesis of ISSNHL, but that these factors do not seem to have any significant influence on the biological mechanism responsible for threshold recovery in ISSNHL.

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Audiology

Validity of the Italian version of Khalfa's Questionnaire on hyperacusis

Validazione della versione italiana del questionario sull'iperacusia di Khalfa

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SUMMARY

The present study aims to evaluate and validate the Italian version of Khalfa's questionnaire on hyperacusis (HQ). We recruited 117 patients (64 men, 53 women, mean age 53 years, range 14-88) with tinnitus for at least 3 months as a primary disorder. All patients completed the THI and the Italian version of the HQ and underwent audiometry, pitch and loudness tinnitus matching, otoacoustic emissions with distortion products (DPOAE) and uncomfortable loudness level (ULL). The overall performance of the tests was evaluated and compared using the area under the ROC curve (AUC) relative to the tests. The cut-off of the HQ was calculated. We also assessed the Cronbach's alpha (αC) for the HQ and its three major dimensions (attentional - αC1, emotional - αC2 and social - αC3). Statistical analysis showed no correlation between DPOAE, audiometry, ULL and gender. We observed a high correlation ($p < 0.05$) between hyperacusis and ULL described by the Spearman's ρ index ($rs = 0.72$). We found a cut-off of 16 indicative of hyperacusis comparing the area under the ROC curve (AUC) of HQ and audiometry, taken as a diagnostic reference, (sensitivity = 67.9% and specificity = 72.2%). The reliability of HQ was confirmed by a high αC = 0.89. The αC for the single dimensional scales were, respectively, αC1 = 0.73, αC2 = 0.72 and αC3 = 0.81. The Italian version of the HQ is recommended for proper and complete classification of patients with tinnitus and hyperacusis. From our study, we found a cut-off of 16 instead of the cut-off of 28 described as very high by other authors. Moreover, ULL was an important variable and can be discriminating in the evaluation of hyperacusis.

KEY WORDS: Khalfa • Hyperacusis questionnaire • Tinnitus • Uncomfortable loudness level (ULL)

RIASSUNTO

Il presente studio ha come scopo quello di valutare e dimostrare la validità della versione italiana del questionario sull'iperacusia di Khalfa (HQ). Sono stati reclutati 117 pazienti (64 uomini, 53 donne; età: 14-88 anni, media 53 anni) con acufene da almeno 3 mesi come disturbo primario. Tutti i pazienti hanno compilato il THI e la versione italiana del HQ e sono stati sottoposti ad esame audiometrico, acufenometria, otoemissioni acustiche con prodotti di distorsione (DPOAE) e soglia del fastidio (ULL). La performance complessiva dei test è stata valutata e confrontata usando l'area sotto le curve ROC (AUC) relative ai test. Il cut-off del HQ è stato calcolato. Inoltre abbiamo valutato l'alfa di Cronbach's (αC) per il HQ e per le sue tre scale dimensionali: attenzionale (αC1), emotiva (αC2) e sociale (αC3). La nostra analisi statistica non ha evidenziato alcuna correlazione tra DPOAE, esame audiometrico, ULL e sesso. Inoltre è stata osservata una elevata correlazione tra l'iperacusia e l'ULL, descritto dall'indice ρ di Spearman (rs = 0,72). È stato calcolato un cut-off di 16 indicativo di iperacusia, comparando l'area sotto la curva ROC (AUC) del HQ e l'audiometria, presa come riferimento diagnostico, (sensibilità = 67.89% e specificità = 72.22%). L'affidabilità del HQ è stata confermata da un alto αC = 0,89. L'αC per le singole scale dimensionali sono risultate rispettivamente αC1 = 0.73, αC2 = 0.72 e αC3 = 0.81. La versione italiana del HQ è consigliata per una classificazione appropriata e completa dei pazienti con iperacusia. Dal nostro studio abbiamo trovato un cut-off di 16 invece del cut-off di 28, descritto come molto alto da differenti autori. Inoltre, l'ULL risulta una importante variabile e può essere discriminante nella valutazione dell'iperacusia.

PAROLE CHIAVE: *Khalfa • Questionario iperacusia • Acufene • ULL*

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Introduction

Noise exposure is considered one of the most common causes of hearing loss that may lead to various disorders, such as loudness recruitment, tinnitus and hyperacusis [1]. Hyperacusis can be part of the clinical spectrum of auditory and vestibular disorders, such as acoustic shock injury, Meniere's disease, otosclerosis, perilymphatic fistula, Bell's palsy and superior semicircular canal dehiscence (SSCD) [2 3]. However, hyperacusis can also be due to several pathological conditions, some of which affect the neurological pathway (head injury, migraine, Lyme disease, Williams syndrome) or the psychological/psychiatric apparatus (acoustic spectrum disorders, chronic fatigue syndrome, fibromyalgia) [4 5]. Tinnitus is defined as an exaggerated perception of sound in absence of an external source. Hyperacusis is a disorder of loudness

perception, in which sound intensities that are considered comfortable by most people are perceived unbearably loud [5]. Hyperacusis is described as a reduced tolerance to ordinary environmental sounds and is characterised by consistently exaggerated or inappropriate responses to sounds [6 7]. Reported prevalence for sound intolerance in tinnitus patients ranges widely, from 30 [8] up to 79% [9 10]. This variability is probably due to the various techniques used in the literature to objectify hyperacusis complaints and results from the absence of a uniform standardised diagnostic procedure. Hyperacusis can occur without a loss of hearing thresholds [11]. For both tinnitus and hyperacusis, however, hearing loss is a major risk factor. As the incidence of hearing loss will increase with the aging of the population, the incidence of tinnitus and hyperacusis may also increase [12].

Hyperacusis does not imply a higher than normal threshold sensitivity to sound, nor loudness recruitment (the rapid growth in perceived loudness with increasing sound intensity that occurs with sensorineural hearing loss) [13]. Instead, in hyperacusis, sounds are not simply a bit loud, but truly unbearable [12].

Hyperacusis is due to an alteration in the central processing of sound in the auditory pathways where there is an abnormally strong reaction from exposure to moderate sound levels. The cochlea is often completely normal, although patients frequently wrongly believe it is irreversibly damaged. Hyperacusis is often associated with tinnitus, but the mechanisms are largely unknown [14].

Many authors consider that hyperacusis seems to increase in extent at times of anxiety, tiredness or stress [15 16]. The pathophysiological mechanism that may explain this abnormal response during stress involves the release of endogenous dynorphins into the synaptic region beneath the inner hair cells. Subsequently, this mechanism leads to an enhanced glutamate neurotransmitter activity, causing the excessive loudness of the sound perceived [17]. Nowadays, based on the limited evidence related to hyperacusis, it has been pointed out as a concomitant occurrence with tinnitus [18]. Patients with hearing loss are exposed to a high risk to develop tinnitus and hyperacusis. Several studies showed a correlation but not causal relationships among hyperacusis, tinnitus and hearing loss [19].

Khalfa validated a questionnaire in order to screen several aspects of auditory symptoms and to quantify and evaluate the characteristics of hyperacusis [20]. The questionnaire is divided into 2 parts. The first includes 3 binary questions giving general information on auditory disorders and noise exposure. The second part comprises 14 self-rating items that will be scored over three major dimensions: attentional (questions 1-4), social (questions 5-10) and emotional (questions 11-14). Answers to each question/item are given on a 4-point scale, ranging from 'no' (scoring 0 points), 'yes a little' (scoring 1 point), 'yes, a lot' (scoring 2 points) to 'yes, quite a lot' (scoring 3 points). The hyperacusis questionnaire is highly sensitive to discriminate subjects with hyperacusis in the general population. A mean score greater than 28.4 is considered as indicative of hyperacusis. The questionnaire is useful in the quantification and characterisation of the clinical aspects of hyperacusis and is a valid instrument for follow-up. Meeus suggested that the cut-off value of 28 is too high and can underestimate some patients with hyperacusis [21]. Overall, no correlations were found between scores on questionnaires and audiometric values. Other questionnaires to assess subjective distress, related to hypersensitivity to sound, are available such as the Multiple-Activity Scale for hyperacusis (MASH) [22] and the self-rating questionnaire on hypersensitivity to sound (GUF) [23]. The MASH is designed for interview-based questioning and classifies hyperacusis into 4 grades from mild to very severe. The GUF is based on 15 questions and evaluates hyperacusis according to cognitive reactions, behavioural changes and emotional responses. The original GUF is in German, and a Spanish version is available [24]. The Khalfa questionnaire (HQ) is also translated in other languages, most recently in Japanese [25].

The present study aims to evaluate and validate the Italian version of the HQ in view of its use as an essential tool in the evaluation of hyperacusis. Our assessment considers all the possible factors that can lead to higher degrees of failure secondary to this symptom.

Materials and methods

The Italian validation of the HQ consisted of three different phases.

In the first, three native speakers of Italian, bilingual in English, independently translated the original questionnaire into Italian with the permission of the author. Subsequently, we formed the pooled version that was then reviewed for the linguistic quality. This version was back translated into English and compared with the original questionnaire. The initial Italian version of the questionnaire was formulated and administered to patients (Appendix 1).

Participants

From November 2011 to December 2012, the final version of HQ was administered to 117 consecutive outpatients [64 male (54.7%) and 53 female (45.3%), age range 14-88 years, (mean 53)], with a primary complaint of tinnitus to improve population homogeneity. All patients had tinnitus for at least 3 months. All questionnaires were filled in by patients in a self-administered way. Exclusion criteria were the presence of recruitment and Ménière's disease evaluated with anamnesis and audiological data. Patients with a previous diagnosis of psychiatric disease were also excluded. Informed consent was obtained from each participant before examination.

Patients were also asked to complete the Italian version of Tinnitus Handicap Inventory (THI).

All patients underwent ENT clinical examination with anamnesis, otoscopy and audiometric evaluation. Pure-tone audiometry was performed at 0.125, 0.25, 0.5, 1, 2, 3, 4, 6 and 8 kHz. Pitch and loudness tinnitus matching was carried out for each patient using the method of adjustment by Newman et al. [26]. Hyperacusis measurement includes uncomfortable loudness levels (ULL) which were measured at 0.25, 0.5, 1, 2, 4 and 8 kHz. Mild hyperacusis was considered in presence of ULL at 80-90 dB in 2 or more frequencies, moderate hyperacusis in presence of ULL at 65-75 dB in 2 or more frequencies and severe hyperacusis in presence of ULL at 60 dB or lower in 2 or more frequencies [27].

Otoacoustic emissions with distortion product (DPOAE) determined the hypothetical influence of hyperacusis on DPOAE parameters in tinnitus patients. DPOAE were recorded with f1/f2 = 1.22 and intensities of 65 dB (f1) and 55 dB (f2) SPL.

Statistical analysis

Cronbach's coefficient alpha (αC) assessed the scale reliability for the total score of HQ and for the three dimensions of the HQ (αC1, αC2 and αC3). Under the assumption that subjects were selected among those with some degree of acoustic impairment, a ROC analysis was carried out to estimate the performance of the HQ in discriminating medium and high levels of impairment. The diagnostic index variable used was the audiometric examination.

The αC was used to assess the validity of the Italian version of the HQ on the basis of internal consistency. Coefficients greater or equal to 0.70 were defined as acceptable, and those greater than or equal to 0.80 were defined as good. We compared the results to those obtained by Khalfa et al. (2002) on the original version of the HQ.

The statistical analysis preliminary studied the association among DPOAE, gender, ULL and audiometric examination, calculating pairwise Spearman correlations.

A proportional odds ratio (OR) model was worked out to account for the ordinal variable ULL with more than two categories. This model treated the variables gender (OR_{gender}) and HQ (OR_{HQ}) as regressors. The Brant test of parallel regression assumption was statistically significant ($p < 0.05$).

The analysis was carried out using STATA software version 12.

Results

We found that a cut-off of 16 was indicative of hyperacusis by comparing the ROC curves of HQ and audiometric examination (Fig. 1). The area under the ROC curve (AUC) of hyperacusis, evaluated by HQ, using audiometric examination as diagnostic reference variable (AUC = 0.67 ± 0.05), was statistically significant ($p < 0.05$). However, the AUC suggests a poorer performance of the HQ with respect to low and high ULL levels (optimal cut-off = 16, sensibility = 67.89%, specificity = 72.22%). Based on the cut off of 16 on HQ, 40.4% of patients with tinnitus also presented hyperacusis.

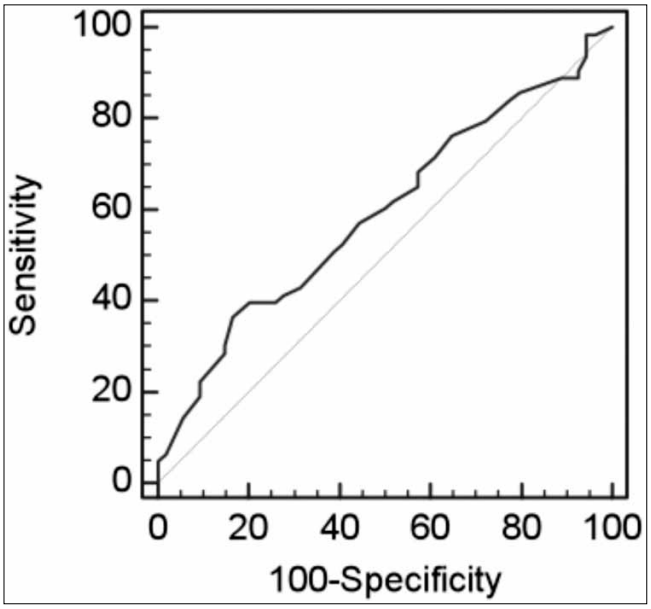

Fig. 1. AUC of hyperacusis calculated using HQ score and audiometric exam.

The scale reliability of the Khalfa questionnaire was statistically significant (αC = 0.89). We evaluated the three dimensions in which the questionnaire is divided and found that the αC indexes were, respectively, αC1 = 0.73, αC2 = 0.72 and αC3 = 0.81.

From statistical analysis, there was no association between DPOAE and audiometric examination.

We also observed statistically significant ($p < 0.05$) Spearman's ρ index ($r_s = 0.72$) between HQ and ULL.

We carried out an ordered logistic regression modelling having ULL (low, middle and high) as response variable with gender and HQ as regressors. The interpretation is that for a one unit increase in gender, i.e. going from 0 (male) to 1 (female), the odds of high ULL versus the combined middle and low categories are OR = 0.39 lower, given that all of the other variables in the model are held constant. The odds of the combined middle and high categories versus low ULL is OR = 0.39 times lower, given that all of the other variables are held constant. For each one unit increase in HQ, the odds of the high category of ULL versus the low and middle categories of ULL are OR = 1.07 times greater, given that the other variables in the model are held constant. Because of the proportional odds assumption, the same increase of OR = 1.07 times was found between low ULL and the combined categories of middle and high ULL (Table I). The likelihood ratio χ^2 of 14.16 with a $p < 0.05$ confirms that our model as a whole is statistically significant, compared to the null model with no predictors. Therefore, we can affirm that gender and HQ are significant and determinant of ULL scores.

Table I. Ordered logistic regression with ULL, gender and HQ. (OR: odds ratio. SE: standard error. P significant level, Cut 1: cut-off mild ULL. Cut2: cut-off moderate ULL. Cut3: cut-off severe ULL).

ULL	OR	SE	P
gender	0.39	0.18	0.039
HQ	1.07	0.028	0.007
Cut1	-3.86	0.84	
Cut2	1.83	0.55	
Cut3	3.57	0.66	

Table I reports the cut-off points of the latent structure model that can be used in a clinical context.

Discussion

As demonstrated for both the original version and the Dutch version [21] of the HQ, the Italian adaptation does not seem to be affected by age and gender and this result contributes to its general, cross-cultural validity as a self-report measure of perceived severity of hyperacusis.

In our study, we found a cut-off of 16 instead of the previous cut-off of 28 identified by Khalfa to represent strong auditory hypersensitivity, described as very high by different authors. This difference in cut-off could be due to the selection criteria of the subject sample. Khalfa selected the sample among the general population without a specific criterion since the main objective was solely to evaluate the sensitivity of the questionnaire among the general population [20]. Otherwise, Meeus et al. studied hyperacusis with the HQ and the MASH in 46 patients with a primary complaint of tinnitus. This study provided a Dutch validated version of the HQ and suggested that the cut-off value of 28 is too high and can underestimate some patients with hyperacusis [21].

The Italian version of HQ has an high and good internal consistency reliability for the total scale ($\alpha C = 0.89$, sensibility = 67.89%, specificity = 72.22%) and for the three dimensions: attentional dimension ($\alpha C\ 1 = 0.73$), social dimension ($\alpha C2 = 0.72$) and emotional dimension ($\alpha C3 = 0.81$). It is worth noting that the high specificity of the HQ is important in detecting the absence of the affection.

The original version demonstrated three dimensions with satisfactory internal consistency reliability according to αC values: respectively, 0.66 for attentional dimension, 0.68 for social dimension and 0.67 for emotional dimension [20]. The Dutch version of the HQ had a good internal consistency ($\alpha C = 0.85$). In contrast with other authors [21], we report good correlation between HQ and ULL scores.

Compared with hearing loss and tinnitus, little attention is given to hyperacusis.

In our study, we found hyperacusis in 40.4% of patients with tinnitus, as reported in the literature (40%) [8]. Schecklmann et al. indicated a rate of hyperacusis of 55% calculated among 1713 patients with tinnitus. In the study by Schecklmann et al., hyperacusis was investigated with the question "Do sounds cause you pain or physical discomfort?" of the Tinnitus Sample Case History Questionnaire (TSCHQ). This higher rate of hyperacusis could be explained by the use of a less specific screening tool for hyperacusis [28].

Hyperacusis may be very invalidating with consequent social isolation, anxiety and depression. Appropriate treatment involves a multidisciplinary approach with the general practitioner, neurologist, ENT and psychologist.

Gu et al. [11] studied the sound-evoked fMRI activation in the subcortical centres such as the inferior colliculus (IC) and medial geniculate body (MGB) and in the primary auditory cortex (PAC) in people with and without tinnitus. In this study, the signal change in the fMRI in the subcortical centres was significantly correlated with ULL and a sound level tolerance questionnaire (SLTQ) score. There was little or no effect of tinnitus on the sound-evoked activation levels of these subcortical structures. In contrast to the subcortical centres, some of the cortical areas also showed an effect of tinnitus. Even in the cortical areas the correlation between the percentage signal change and STL measures was significant.

These results directly show a physiological correlate of abnormal SLT that is indicative of hyperacusis, ranging from mild to severe. This means that hyperacusis could be more directly related to tinnitus than generally appreciated.

There was no statistically significant association between DPOAE and ULL ($p > 0.05$), DPOAE and audiometric examination ($p > 0.05$), or DPOAE and gender ($p > 0.05$).

The Spearman correlation between ULL and HQ was statistically significant ($p < 0.05$), ($r_s = 0.72$). The AUC of HQ using audiometric examination as a diagnostic reference variable (AUC = 0.67 ± 0.05) was statistically significant ($p < 0.05$), suggesting a poor performance of the test compared to low and high ULL levels (optimal cut-off = 16, sensibility = 67.89%, specificity = 72.22%).

The OR model showed an adequate fitting ($\chi^2 = 14.16$, $p < 0.05$). $OR_{gender} = 0.39 \pm 0.18$ ($p < 0.05$). The odds interpretation can be that the high ULL level vs. the combined middle and low categories is less than 1, i.e. women have a lower odds of a high ULL level if hyperacusis is held constant.

$OR_{HQ} = 1.07 \pm 0.03$ ($p < 0.05$). Odds of the high category of ULL versus the low and middle categories of ULL are 1.07 times greater within each gender. Because of the proportional odds assumption the same increase, 1.07 times is found between low ULL and the combined categories of middle and high ULL.

Other factors, such as long-term stress, need to be taken in consideration when assessing hyperacusis with a questionnaire. As reported by Hasson et al., women with high levels of emotional exhaustion become more sensitive to sound after an acute stress task and have reduced thresh-

olds to loudness. Patients with normal ULL but seeking help for hyperacusis should be assessed for emotional exhaustion with control of plasma cortisol concentration and estradiol [29 30]. We also confirm, as Hasson et al. [29], that the strongest correlation was found for the social dimension of HQ. This result may suggest that social aspects revealed by the questionnaire HQ correspond best to ULL. Moreover, it was pointed out a sex-related biological difference between male and female subjects. In fact, in women with high emotional stress levels the prevalence increase of the ULL levels was more pronounced than in men [29].

In addition, many patients are unaware that their problem has a name or do not know the significance of "hyperacusis". The use of HQ represents a screening tool to evaluate the subjective distress related to hypersensitivity to sound and to guide the results of the therapy. However, the present study presents a limit in that we administered the HQ only to patients with tinnitus, while a control group of normal subjects is not present.

A future prospective will be to establish which index between audiometry, DPOAE and ULL is most predictive of hyperacusis in relation to the degree obtained with score on the HQ.

Conclusions

In our study, we found a cut-off of 16 instead of the previous cut-off of 28, described as very high by different authors. The αC in our study is also very significant and, consequently, the Italian version of the HQ should be introduced among questionnaires necessary for the classification of patients with tinnitus and intolerance to sounds, possibly using adjusted scores. Moreover, from our study, ULL was an important variable that can be discriminating in the evaluation of the hyperacusis. The HQ is a valid and easy instrument to evaluate hyperacusis, which is often undervalued in patients with hearing disorders.

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Appendix 1.
The Italian translation of the hyperacusis questionnaire by Khalfa S. et al., 2002.

Questionario sull'iperacusia

Cognome, Nome: _____ Data: _____

Sesso: [M] [F] Età: _____

Professione: _____

Città di residenza: _____

Telefono fisso / mobile: _____

È stato o è esposto al rumore? _____

Tollera il rumore meno bene di qualche anno fa? _____

Ha mai avuto problemi di udito? Se sì di che tipo? _____

	No	Raramente	Spesso	Sempre
1. Ha l'abitudine ad usare tappi o cuffie per ridurre la percezione del rumore (non consideri l'utilizzo di protezioni auricolari durante situazioni di anormali od elevati rumori)?				
2. Le riesce difficile ignorare i suoni circostanti in situazioni quotidiane?				
3. Ha difficoltà a leggere in ambienti rumorosi?				
4. Ha difficoltà a concentrarsi in situazioni rumorose?				
5. Ha difficoltà a seguire la conversazione in ambienti rumorosi?				
6. Qualcuno le ha detto che tollera poco il rumore o alcuni suoni?				
7. È particolarmente sensibile o disturbato dai rumori della strada?				
8. Trova il rumore sgradevole in alcune situazioni sociali (night club, pub, bar, concerti, rinfreschi, spettacoli pirotecnici)?				
9. Quando le propongono qualcosa (uscire, andare al cinema, andare ad un concerto) pensa immediatamente al rumore al quale potrà essere esposto?				
10. Rinuncia mai ad inviti o ad uscire a causa del rumore a cui potrebbe essere esposto?				
11. Il rumore o particolari suoni la disturbano maggiormente in un luogo silenzioso piuttosto che in presenza di un leggero rumore di fondo?				
12. Lo stress e la stanchezza riducono la sua capacità di concentrazione in presenza di rumore?				
13. La sua capacità di concentrazione in presenza di rumore diminuisce verso la fine della giornata?				
14. Il rumore o alcuni suoni le causano stress od irritabilità?				

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VESTIBOLOGY

Canal switch and re-entry phenomenon in benign paroxysmal positional vertigo: difference between immediate and delayed occurrence

Conversione canalare e fenomeno del rientro nella vertigine parossistica posizionale benigna: differenze tra forma immediata e ritardata

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SUMMARY

This prospective study was designed to evaluate the differences between immediate and delayed canal re-entry of otoliths after therapeutic manoeuvres in patients with benign paroxysmal positional vertigo (BPPV). A total of 196 patients with BPPV were visited and 127 matched our inclusion criteria. The mean age was 54.74 years. The horizontal semicircular canal (HSC) was involved in 30 cases and the posterior semicircular canal (PSC) in 97 patients. Patients with hearing loss in the ear affected by BPPV have a more recurrent form, compared to those with normal hearing. An immediate canal re-entry was recorded in 3 patients with HSC BPPV, all with geotropic nystagmus. In 7 patients with PSC BPPV, the immediate canal re-entry was detected and the delayed form was noted in 5 patients. The patients with the delayed canal re-entry underwent more than 2 previous manoeuvres. The canal re-entry was not related to the manoeuvre performed. The timing of the Dix-Hallpike test to verify the resolution of the BPPV had a significant role in immediate canal re-entry. A recurrence in the follow-up at least one month after treatment was recorded in 20 patients and was more frequent in patients that had canal re-entry. The canal re-entry or canal switch is a clinical entity that should be kept in mind of the neurotologist when approaching BPPV patients. It is important to distinguish it from recurrence when delayed and from manoeuvre failure when immediate. The timing of manoeuvre performing, in particular the final verification test after therapeutic sessions, is important to prevent the immediate reflux of particles into canals.

KEY WORDS: Benign paroxysmal positional vertigo • Canal conversion • canal switch • Vertigo • Dizziness

RIASSUNTO

Studio prospettico ideato per la valutazione delle differenze tra la conversione canalare o il rientro degli otoliti nei canali semicircolari successivo alle manovre terapeutiche nei pazienti affetti da VPPB. Sono stati valutati 196 pazienti affetti da VPPB, 127 dei quali corrispondevano ai criteri di inclusione. L'età media dei pazienti era di 54.74 anni. Il canale orizzontale è stato coinvolto in 30 casi e il canale posteriore in 97 pazienti. I pazienti con sordità neurosensoriale presentavano forme ricorrenti di VPPB, rispetto a quelli con udito normale. L'immediato rientro canalare è stato diagnosticato in 3 pazienti con VPPB del canale laterale, tutti con nistagmo geotropo. 7 pazienti con VPPB del canale posteriore hanno presentato un rientro canalare immediato e 5 la forma ritardata. I pazienti con rientro canalare ritardato avevano precedentemente subito più di 2 manovre di riposizionamento. Il rientro canalare non è risultato connesso al tipo di manovra eseguita. Il tempo di attesa tra l'esecuzione della manovra liberatoria e il test di verifica si è rivelato importante ai fini del rientro canalare immediato. La recidiva della BPPV dopo un mese dalle manovre liberatorie si è riscontrata in 20 pazienti ed è stata più frequente in quei pazienti che hanno avuto un fenomeno di rientro canalare. La conversione canalare ed il fenomeno del rientro canalare rappresentano delle entità cliniche che devono essere considerate dal medico che tratta le VPPB. Appare importante distinguere un rientro da un fallimento della manovra in caso di forme immediate, o da una recidiva di patologia in caso di forme ritardate. L'esecuzione del test di verifica del successo terapeutico dopo manovre di riposizionamento deve avere un distacco temporale sufficientemente ampio al fine di evitare il reflusso immediato di otoliti nei canali.

PAROLE CHIAVE: *Vertigine parossistica posizionale benigna • Rientro canalare • Conversione canalare • Vppb • Manovre • Vertigini • Disequilibrio • Otologia • Neurotologia*

Acta Otorhinolaryngol Ital 2015;35:116-120

Introduction

Benign paroxysmal positional vertigo (BPPV) accounts for about 20% of vestibular complaint [1]. Being a mechanical disorder of the posterior labyrinth, management consists of a "mechanical" repositioning of the otoconial debris detached from vestibular sensorineural epithelia. The posterior semicircular canal (PSC) is the most involved by BPPV with approximately 90% of cases, while horizontal semicircular canal (HSC) is the next most common [2]. Both

canalithiasis and cupulolithiasis theories are accepted in the pathophysiology of the BPPV, which are also confirmed by intraoperative findings of otoconial debris into canal [3]. The repositioning manoeuvres to treat canalithiasis are well established and widely used, with some variation recently reported in literature [4-6].

The recurrence of BPPV may be linked to some systemic diseases [7-9], but true recurrence should be differentiated from true persistence of canalithiasis, which is often due to a reflux of otoliths.

Although repositioning manoeuvres are free of major complications, a form of canalithiasis called "re-entry BPPV" may appear after therapeutic manoeuvres [10]. This type of positional vertigo can also be called "canal switch BPPV" if the canal involved is different from the firstly affected canal [11] before any repositioning session. These clinical entities arise when manoeuvres became common in clinical practice, hence the clinician should consider a quick differential diagnosis to distinguish a re-entry form from recurrent BPPV by an early verifying test.

In our practice, we have noted two forms of canal re-entry and/or canal switch. The first is immediate, occurring some minutes after the repositioning session. The second is delayed, occurring after one or two days after the manoeuvres were done.

In our present work, we report the differences between the two clinical forms of canal re-entry and provide some suggestions to avoid phenomenon supported by clinical evidence.

Materials and methods

The study was conducted prospectively in the period from January to July 2013. The setting of the study was in an ENT and audiology departments in a referrals centre for diagnosis and management of equilibrium diseases. Patients affected by BPPV were consecutively included in the study and exclusion criteria were: atypical nystagmus [12 13], associated Meniere's disease, bilateral BPPV, multicanalar BPPV, secondary BPPV, recent whiplash injury [14], clinical suspect for cupulolithiasis, unable to undergo repositioning manoeuvre for physical limitations.

Diagnosis of canalithiasis BPPV was done with and without Frenzel lens to allow the fixation of a specific point (nose of the examiner) during the examination to reduce the variability of nystagmus description among patients, which may be influenced by gaze. The nystagmus was then described observing the movement of iris-pupil complex and the ocular globe. A torsional nystagmus beating toward the more dependent side (geotropic) in Dix-Hallpike position was considered as canalithiasis of ampullary arm of ipsilateral PSC; if the torsional nystagmus in the Dix-Hallpike position was beating towards the unexamined side (apogeotropic) it was considered as canalithiasis of non-ampullary arm of examined PSC according to the description of Vannucchi et al. [15] The superior semicircular canal (SSC) BPPV was examined in the head-hanging position, although the side is difficult to determine [6]. The HSC was examined with the roll-test eliciting the horizontal nystagmus, and considering the affected ear the side with more intense nystagmus in case of geotropic form, while the side with less intense nystagmus in case of apogeotropic form.

The manoeuvres to treat the canalithiasis BPPV were: Semont manoeuvre or Gans manoeuvre for PSC BPPV [4 16], Gufoni maneuverer for HSC [2]. The choice between the Semont and Gans manoeuvre was determined by randomising patients with PSC BPPV, undergoing one of the two manoeuvres. All treatments were performed at the same visit until the verifying Dix-Hallpike/Roll-test was without evidence of nystagmus or symptoms. During the manoeuvre, patients kept the position for 120 sec, and the following manoeuvre (when needed) was done after an additional 120 sec. To determine the influence of the time lapse between the repositioning manoeuvre and the final Dix-Hallpike/Roll-test, we randomly divided patients into 3 groups and the final test was done, respectively, after 5, 10 and 15 minutes after the repositioning manoeuvre. The minimum follow-up was 3 months after the manoeuvre.

Data were entered in a database created with Excel 5.0. Data analysis was performed using EpiInfo 3.5.1 software. Absolute and relative frequencies were calculated for qualitative variables, while quantitative variables were summarised as means (standard deviation). Differences by groups for categorical variables were analysed using the chi-square test. Differences in means were compared with a Student's t-test.

Univariate analysis between BBPV and possible factors associated was performed. The difference between the PSC and the HSC related to the different manoeuvres was analysed. A $p < 0.05$ (two-tailed) was considered statistically significant.

Institutional review board approval was obtained for this study without human experimental procedures. The choice of the manoeuvre used was done among manoeuvres with equal success rates, as often reported in the literature.

Table I. Hearing loss is statistically associated with recurrent BPPV.

	Previous BPPV			p value
n = 127	0	1	> 2	
		n (%)		
Tinnitus				
- Yes	9 (45.0)	3 (15.0)	8 (40.0)	0.22
- No	46 (43.0)	34 (21.8)	27 (25.2)	
Hearing Loss				
- Yes	6 (42.9)	0	8 (57.1)	< 0.01
- No	49 (43.4)	37 (32.7)	27 (23.9)	

Table II. Patients with delayed canal re-entry had more than two manoeuvres in the same session to treat BPPV.

PSC (n = 97)	Canal Re-entry				
	None	Immediate	Delayed	No	Yes
		n (%)		n (%)	
Number of manoeuvres in a single session					
- 1	62 (92.5)	5 (7.5)	0 (0.0)	62 (92.5)	5 (7.5)
- 2	18 (90.0)	2 (10.0)	0 (0.0)	18 (90.0)	2 (10.0)
- 3	5 (71.4)	0 (0.0)	2 (28.6)	5 (71.4)	2 (28.6)
- 4	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)
- 5	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2 (100.0)

Table III. Influence on re-entry phenomenon of the timing to repeat the diagnostic manoeuvre after treatment.

Minutes waited before last Dix-Hallpike test	Canal Re-entry			p-value	Canal Re-entry		p value
	None	Delayed	Immediate		No	Yes	
		n (%)			n (%)		
- 5	29 (70.7)	2 (4.9)	10 (24.4)	> 0.001	29 (70.7)	12 (29.3)	> 0.001
- 10	41 (95.3)	2 (4.7)	0 (0.0)		41 (95.3)	2 (4.7)	
- 15	42 (97.7)	1 (2.3)	0 (0.0)		42 (97.7)	1 (2.3)	

Results

In the period considered, we consecutively visited 196 patients with BPPV, of which 69 matched our exclusion criteria and were not included in the analysis. The sex distribution was 49 men and 78 women. The mean age was 54.74 years. In 55 patients, the episode of BPPV was the first, while 72 patients had had a previous BPPV episode more than 6 months before. The statistical analysis of relationship between recurrence and comorbidities or personal patients data did not demonstrate any significance.

The HSC was involved in 30 cases of which 5 presented an apogeotropic nystagmus. In 97 patients, PSC BPPV was diagnosed and 13 cases showed an apogeotropic nystagmus.

The patients with hearing loss in the ear affected by BPPV seemed to have a more recurrent form compared with those with normal hearing (Table I).

An immediate canal re-entry was recorded in 3 patients with HSC BPPV, which was promptly managed with a Gufoni manoeuvre. These patients, having geotropic nystagmus before the manoeuvre, had the classical freeing nystagmus during the Gufoni manoeuvre, but geotropic nystagmus persisted at the verifying test. In patients with HSC BPPV, we recorded canal switch only for those cases with geotropic nystagmus, probably because the otolith mass lied near the vestibular opening of the PSC.

In patients with PSC BPPV, a canal re-entry was noted in 12 patients; 7 patients had an immediate canal re-entry (with changed nystagmus direction to apogeotropic form), and 5 patients the delayed form. As shown in Table II, the patients with the delayed canal re-entry underwent more than 2 manoeuvres in the same session to achieve the disappearance of the positional nystagmus. Although the result is quite evident, statistical significance was not obtained probably due to the low number of cases.

The canal re-entry was not related to the manoeuvre performed.

As shown in Table III, the timing of the Dix-Hallpike test to verify the resolution of the BPPV had a significant role in immediate canal re-entry, which occurred only for those patients who had a Dix-Hallpike after only 5 min from the last repositioning manoeuvre.

A recurrence in the follow-up at least one month after treatment was recorded in 20 patients and was more frequent in patients who had a canal re-entry ($p > 0.001$) (Table IV).

Table IV. Relationship between re-entry phenomenon and recurrence of BPPV.

Recurrence	Canal Re-entry		p value
	Yes	No	
	n (%)		
- Yes	11 (55.0)	9 (45.0)	> 0.001
- No	4 (3.7)	103 (96.3)	

Discussion

The treatment of BPPV is often simple and immediate, providing a prompt resolution of symptoms. On occasion, neurotologists encounter patients with resistant BPPV requiring several manoeuvres to obtain results, or patients who after an initial resolution of symptoms show some delayed positional nystagmus due to a canal re-entry of otoliths.

The immediate reflux of otolith into the PSC after a repositioning manoeuvre could be mistaken for a contralateral SSC BPPV, as these two forms of nystagmus are similar. As reported by Foster et al. [10], such a form of nystagmus should be differentiated because in PSC reflux it is finest due to inhibition of endolymphatic flow. However, as that discrimination is very difficult to detect, the clinician should consider that it is highly unlikely that a contralateral disease, previously undiagnosed, appears after a repositioning procedure.

The apogeotropic nystagmus in PSC during head-hanging position is due to otolith into the non-ampullary arm of PSC [15 17]. It could be possible that the apogeotropic nystagmus observed after retest could be caused by otolith mass stopping in the ampullary arm, although we observed in all cases a liberatory nystagmus after the first repositioning procedure. For this reason, we believe that the hypothesis of the otolith stopping in the ampullary arm is a less probably cause of apogeotropic nystagmus in this series.

Our clinical experience in detecting some canal conversion after reposition procedure was also recently reported by Babic et al. who described the transitional BPPV and, as in our present series, the conversion occurred after the final check to assess the freeing of the semicircular canal [18]. The large number of conversions in HSC cupulolithiasis is in our opinion not real, but influenced by the fact that the authors considered all apogeotropic nystagmus in HSC as cupulolithiasis, rather than contemplate the possibility of otolith in the ampullary arm of HSC, which is manageable with appropriate manoeuvre [2].

BPPV patients may have a variable otoliths mass, ranging from fine particles unable to elicit clinically-evident nystagmus [19] to high mass particles visible with the operating microscope [3]. The mass of otoliths, in our opinion, supported by the results obtained in this study, have a role in determining not only the difficulty to obtain particle repositioning, but also in the type of canal re-entry if present.

We agree with the theory that patients cleared with a single manoeuvre are likely to have some high mass or aggregated particles [10]. In patients with an immediate reflux episode, particles after the treatment procedure were probably located near the openings of the common crus and HSC, so that the re-entry was more simple in case of high mass particles rather than dispersed otoliths. This event was compatible with our findings, as our patients with immediate reflux underwent less than 2 manoeuvres to free the canal involved.

The majority of patients with BPPV requires a single manoeuvre to clean the canal. Patients requiring more than 3 repositioning manoeuvres to achieve cleaning of the canal (negative Dix-Hallpike control test) probably have a large number of low-mass otoliths that are dispersed into the utricle during the numerous manoeuvres performed. Effectively, in our series, patients with delayed re-entry/canal switch had more than 2 manoeuvres in the same session and a negative final Dix-Hallpike/Roll test. The canal re-entry or switch was evident after an average of 2-3 days; it is possible that this time lapse was necessary to assemble particles into the utricle before casual re-entry.

Undoubtedly, to distinguish delayed canal re-entry from recurrence can be difficult. The only consideration that leads us to believe that after 2-3 days a recurrence is effectively a re-entry phenomenon is the direction change of the nystagmus (i.e. a geotropic before the treatment becomes apogeotropic for PSC). For canal switch from PSC to LSC BPPV, the observation is different because it is very likely that a new episode of BPPV after a few days following treatment affecting a different canal of the same side is due to re-entry of otoliths.

Similar to Foster et al., we noted a relationship between timing of final test with canal re-entry [10]. These findings lead to couple the cause of re-entry BPPV or canal switch with the repetition of Dix-Hallpike test to assess cleaning of the canal. In our series, there was evidence that the minimum time that we should wait before doing a verification test, to reduce the risk of immediate re-entry/canal switch, was 10 min. Delayed canal re-entry is not preventable by increasing the time before performing the verification test, but it likely depends on a casual position/movements done by patients.

Conclusions

Canal re-entry or canal switch is a clinical entity that should be kept in mind by the neurotologist when approaching BPPV patients. It is important to distinguish it from recurrence when delayed and from manoeuvre failure when immediate. It is likely that the mass of otoliths has a role in determining the type of canal re-entry. The timing of performing manoeuvres, in particular the final verification test after therapeutic sessions, is important to prevent the immediate reflux of particles into canals.

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CLINICAL TECHNIQUES AND TECHNOLOGY

Supraclavicular artery island flap reconstruction of a contralateral partial laryngopharyngeal defect

Lembo sovraclaveare ad isola nella ricostruzione di un difetto faringo-laringeo parziale controlaterale

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SUMMARY

Oncologic resection of head and neck cancers often results in complex reconstructive problems that can require local, regional, or free flaps. Herein, we present a case of a 67-year-old female with a history of floor of mouth cancer who had a second primary carcinoma in the left side of pharynx, 9 years after initial therapy. She underwent a wide oncologic resection requiring laryngopharyngeal reconstruction. Significant scar formation on the left side, due to previous surgery and radiotherapy, prevented us from harvesting an ipsilateral flap. Therefore, we used a right sided supraclavicular artery island flap for reconstruction of the contralateral defect. The patient healed completely with hyperbaric oxygen therapy and conservative local wound care. Supraclavicular artery island flap is a viable option for poor microvascular surgical candidates. It is easy and quick to harvest, and significantly decreases operative times. It is thus a versatile option for contralateral laryngopharyngeal reconstruction.

KEY WORDS: Head and neck reconstruction • Laryngopharyngeal defect • Supraclavicular artery island flap

RIASSUNTO

La resezione dei tumori della regione testa-collo spesso comporta complessi problemi ricostruttivi che possono necessitare di lembi locali, regionali o liberi. Presentiamo il caso di una donna di 67 anni con storia pregressa di tumore del pavimento buccale, che a 9 anni dalla prima neoplasia ha presentato un secondo carcinoma primitivo del faringe. La paziente è stata sottoposta ad un'ampia resezione che ha richiesto una ricostruzione faringo-laringea. La presenza di un ampia area cicatriziale omolaterale dovuta al precedente intervento chirurgico e alla successiva radioterapia ci ha impedito di prendere un lembo ipsilaterale. Pertanto, per la ricostruzione del difetto, abbiamo usato un lembo sovraclaveare ad isola controlaterale. La completa restitutio ad integrum è stata ottenuta con l'ausilio di ossigenoterapia iperbarica e terapia locale della ferita chirurgica. Il lembo sovraclaveare ad isola è un opzione interessante e percorribile in quei pazienti che per condizioni locali e generali siano candidati sub-ottimali al lembo microvascolare. Il lembo è inoltre di facile e rapida preparazione, e consente una significativa riduzione dei tempi operatori. Per tali motivi rappresenta un opzione versatile per la ricostruzione faringo-laringea controlaterale.

PAROLE CHIAVE: *Ricostruzione testa-collo • Difetto faringo-laringeo • Lembo sovraclaveare ad isola*

Acta Otorhinolaryngol Ital 2015;35:121-124

Introduction

Reconstruction of complex head and neck surgical defects remains one of the most challenging aspects of head and neck surgical oncology. The goal of reconstruction is not only limited to cover the defect, but also to restore anatomical function with similar skin colour and texture match at the recipient site [1]. The ideal surgical reconstruction should have the lowest morbidity with minimal fistula or stricture rates, and allow early swallowing and speech rehabilitation. Oncologic resection of head and neck cancers often results in complex reconstructive problems that require dependable local, regional, or free flaps. Free tissue transfer has revolutionised reconstructive surgery but remains a complex procedure requiring microvascular equipment and surgeon expertise, availability of recipient vessels and a patient who can tolerate major surgery [2]. Since advanced cancer patients are debilitated and malnourished, quick and simple reconstructions with local flaps are usually preferred [1]. Supraclavicular artery island flap (SCAIF) is a safe, reliable, versatile, regional fasciocutaneous flap for the reconstruction of head and neck defects. It can be harvested easily and quickly with minimal donor-site morbidity.

The aim of this study is to evaluate the feasibility and efficacy of SCAIF for the reconstruction of a contralateral laryngopharyngeal defect. To the best of our knowledge, this is the first case demonstrating the use of SCAIF to reconstruct the opposite side.

Materials and methods

Preoperative evaluation

A 67-year-old woman with a history of floor of mouth cancer presented for evaluation of a pharyngeal mass. She had been treated with partial glossectomy and partial mandibulectomy with bilateral selective neck dissection as well as radiation therapy 10 years ago. One year after the operation, during regular follow-up a neck recurrence was detected and she underwent left radical neck dissection with intraoperative radiation therapy. During long term follow-up, she presented with a second primary carcinoma in the left side of pharynx 9-year after initial therapy. On endoscopic examination, the inferior oropharynx demonstrated a left posterior oropharyngeal lesion extending to the left pyriform sinus, approximately 3 × 3 cm in size. The larynx was intact with normal true vocal cord mobility bilaterally. The patient agreed to proceed with surgery. Total tumour resection with partial laryngopharyngectomy was planned. Since the patient had previously undergone left radical neck dissections as well as radiation therapy, the significant scarring on her left side limited to harvest a left sided flap. The decision was to use a right sided supraclavicular island flap for reconstruction of the left sided laryngopharyngeal defect.

Surgical technique

Under operative conditions, with the use of previous neck incision scar, sub-platysmal flaps were elevated and midline anterior pharyngotomy was performed at the level of the vallecula. This gave us good visualisation of the tumour. Resection included left pyriform sinus, left upper cervical oesophagus, up to the parapharyngeal tissues and prevertebral fascia together with the tonsillar fossa as well as lateral and posterior pharyngeal wall. After total tumour excision and confirmation of negative surgical margins with frozen sections, reconstruction was started.

A Doppler probe was used to identify the right supraclavicular vessels coursing from the transverse cervical pedicle in the triangle demarcated by the clavicle, sternocleidomastoid and external jugular vein. (Fig. 1) An ellipse shaped flap was planned out according to the pharyngeal defect.

The skin, subcutaneous tissue, and the fascia down to the deltoid and pectoralis major muscles were incised. Further dissection was continued in the subfascial plane over the deltoid muscle and the flap was raised in the distal to proximal direction. A 3-4 cm pre-vertebral space tunnel was created at the level of larynx to transpose the right SCAIF to the left sided surgical defect. (Fig. 2) A diameter of approximately 3 cm of soft tissue was maintained around the origin of supraclavicular artery. A proximal portion of the skin paddle which would be lying under the tunnel was de-epithelialised. The flap was advanced through the tunnel, behind the larynx into the left laryngopharyngeal defect. (Fig. 3) A tension free reconstruction of the pharynx was achieved all the way to base of tongue. (Figs. 4, 5) The donor site was closed primarily by extensive subcutaneous undermining and approximation of wound edges.

The patient recovered well postoperatively. She was initially monitored in the intensive care unit, but had no perioperative complications and was transferred to the floor on postoperative first day. She developed pharyngocutaneous fistula that healed completely in a couple of weeks with hyperbaric oxygen treatment and conservative local wound care.

Discussion

Extensive cancer resections of the face and neck often result in complex reconstructive problems that affect functional and aesthetic outcomes. Oro- and hypopharyngeal reconstructions can be a challenge due to their functional complexity and exposure to saliva and digestive enzymes [3].

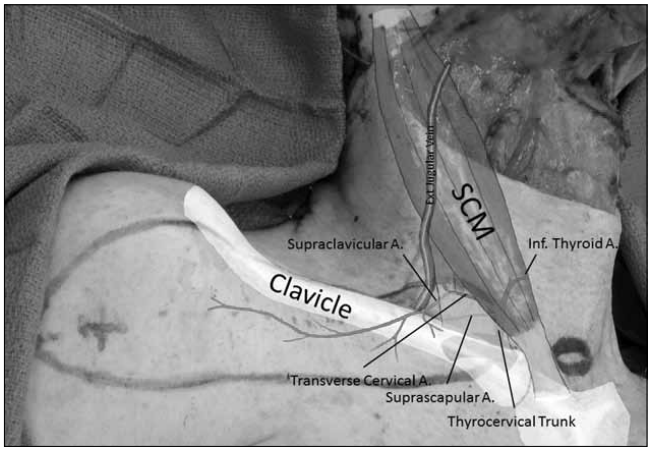

Fig. 1. The origin of supraclavicular artery is demonstrated in the triangle formed by clavicle, SCM and external jugular vein. SCM: sternocleidomastoid muscle; Ext: external; inf.: inferior; A.: artery.

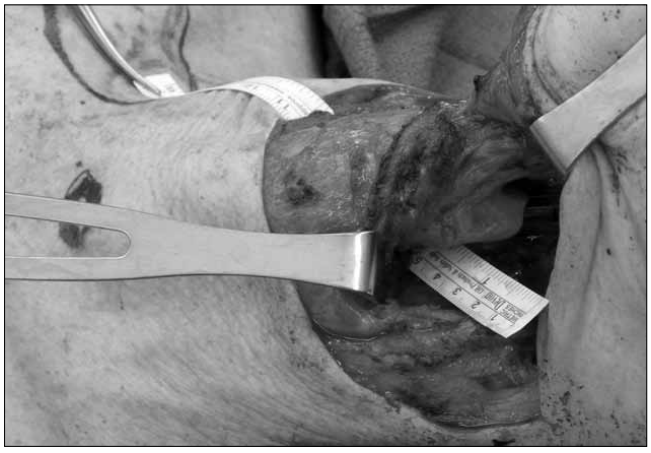

Fig. 2. View of the pre-vertebral space tunnel at the level of larynx to transpose the right SCAIF to the left sided surgical defect. SCAIF: supraclavicular artery island flap.

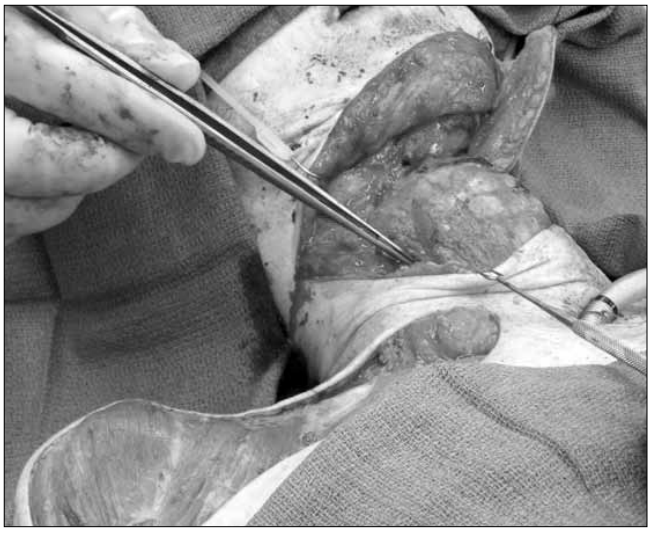

Fig. 3. Transposition of the flap through the tunnel.

Fig. 4. View of laryngopharyngeal defect after flap transposition was completed.

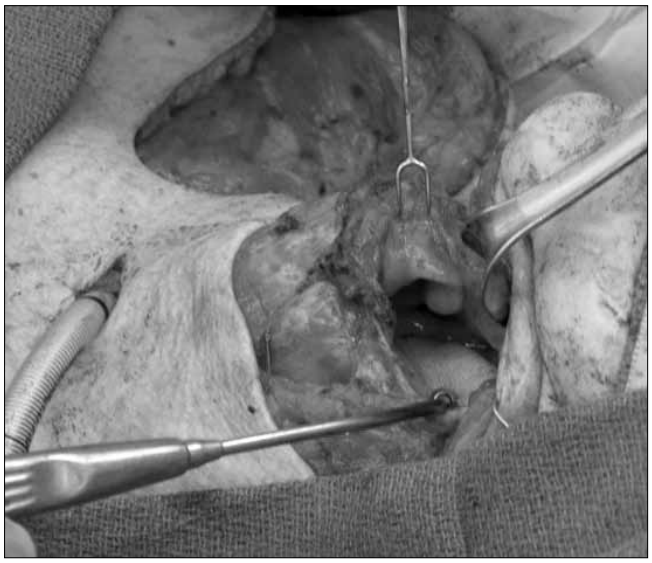

Fig. 5. The final position of the flap before suturing.

For surgical reconstruction of pharyngeal defects, the ideal flap should allow early speech and swallowing function rehabilitation with minimal fistula and stricture rates [4].

Microsurgical transplantation of free flaps (radial forearm, anterolateral thigh, and fibula flap) provides pliable thin vascularised tissue to fill large defects, though they lack colour match to the face and neck. In addition, these flaps need trained surgeons for microsurgical techniques and special postoperative monitoring. High-risk patients (those with advanced age, advanced tumours, poor nutrition, or multiple medical issues) are not always good candidates for potentially prolonged microsurgery; therefore, regional flaps remain the preferred technique in these complicated cases [5].

Microsurgical reconstruction may be severely hampered in patients with a history of prior neck dissection and/ or irradiation, as in our patient, or contraindicated in patients with poor clinical conditions. In this context, The SCAIF is an ideal flap with a thin pliable tissue with superior texture and colour match for face and neck reconstruction [5].

Although fasciocutaneous flaps based on the supraclavicular artery have been described for several decades, the use of has it recently popularised among head and neck surgeons. In 1997, Pallua et al. found that the supraclavicular artery island flap was both reliable and safe for releasing post-burn neck contractures after resecting cervical scars [3]. In 2000, they demonstrated the use of the flap to reconstruct skin defects of the cheek, chin, and neck [1]. Di Benedetto et al. in 2005, reported SCAIF as reliable for covering oral cavity defects after oncologic resections [4]. Liu et al. described the use of this flap for functional pharyngeal reconstructions [5].

The SCAIF is a viable option for poor microvascular surgical candidates, or for those who do not wish to have a free tissue transfer. It can also serve an excellent first option in many other types of lower face and neck reconstructions, leaving major free flap surgeries and their higher associated costs as a backup option. SCAIF is easy and quick to harvest, and significantly decreases operative times. Flap donor site is in the same operative field and which can be closed primarily with minimal morbidity. The thin pedicle allows easy transfer of the island flap, which can be tunneled into a defect in the head and neck. The pedicle helps to fill the defect created by the neck dissection and covers the vessels of the neck [3]. It is a versatile flap that should be kept as a valid option for head and neck reconstruction.

There are some limitations on the use of SCAIF that it cannot be used in patients undergoing radical neck dissection in whom the supraclavicular artery has been damaged. In addition, because of the variability in the vascular pattern, this flap cannot be used in patients with vascular anomalies or those with damage to the transverse vessels resulting from previous neck dissection [3 5]. For successful use of the extensive pedicled SCAIF, consideration of

vascular anatomy is extremely important, and preoperative assessment is essential.

As patients with advanced tumour usually present with impaired clinical conditions, shorter hospital stay with minimal surgical morbidity is preferred in their treatment. The SCAIF provides adequate tissue to perform even quite large and complex reconstructions. In our patient, prior radical neck dissection and extensive neck radiation on the lesion side resulted in severe fibrosis, vascular injury and tissue adhesions. Excessive scarring and muscle fibrosis can lead to formation of oropharyngeal stricture. This prevented us from using an ipsilateral SCAIF or other locoregional flaps, such as pectoralis major flap and deltopectoral flap. Therefore, our reconstructive method of choice was contralateral SCAIF.

Conclusions

SCAIF is a feasible and reliable local fasciocutaneous flap option with exceptional skin colour and texture match. Easy and quick harvest, minimal donor site morbidity and shorter operative time make it an ideal flap alternative for laryngopharyngeal and cutaneous reconstructions. Although technically challenging, the use of SCAIF in reconstruction of contralateral laryngopharyngeal defects is a convenient technique and should be reserved for selected cases.

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CASE SERIES AND REPORTS

Two new cases of chronic tuberculous otomastoiditis in children

Due nuovi casi di otomastoidite cronica tubercolare in età pediatrica

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SUMMARY

This report focuses on tuberculous otomastoiditis treated at a third level Italian paediatric hospital. We reviewed the clinical charts of 4077 children who underwent middle ear surgery at the Audiology and Otology Unit of the Institution's ENT Department from January 1995 to December 2011. A tubercular aetiology was identified in 2 cases: a 4-year old boy who presented with primary ear involvement, i.e. with no other infected sites but the middle ear, and a 5-year old girl with secondary tuberculous otomastoidits, who was treated for pulmonary and mediastinal tuberculosis at the age of 7 months.

KEY WORDS: Tuberculous otomastoiditis • *Mycobacterium tuberculosis* • Middle ear • Children

RIASSUNTO

Il presente lavoro descrive l'esperienza di un ospedale pediatrico italiano di terzo livello sulle otomastoiditi tubercolari. Sono state revisionate le cartelle cliniche di 4077 bambini sottoposti dal gennaio 1995 al dicembre 2011 a chirurgia dell'orecchio medio presso la UO di Audiologia ed Otologia della divisione ORL dell'Ospedale. La diagnosi eziologica di tubercolosi è stata posta in 2 casi: in un bambino di 4 anni affetto da otomastoidite cronica tubercolare primaria, ossia senza segni di infezione in altri distretti corporei al di fuori dell'orecchio medio, e in una bambina di 5 anni affetta da otomastoidite cronica tubercolare secondaria, essendo stata trattata all'età di 7 mesi per tubercolosi polmonare e mediastinica.

PAROLE CHIAVE: *Otomastoidite tubercolare • Mycobacterium tuberculosis • Orecchio medio • Bambini*

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Introduction

Tuberculosis (TB) remains a major health concern in developed countries. In children, approximately 85% of reported cases are limited to the lung; the remaining 15% involve only extra-pulmonary or both pulmonary and extra-pulmonary sites [1 2]. Among the extra-pulmonary presentations, tuberculous otitis media (TOM) with otorrhoea is extremely rare, accounting for 0.05-0.9% of chronic infections of the middle ear [3].

The pathogenesis of TOM is controversial. The *Mycobacterium* can reach the middle ear via a haematogenous route, via mucus aspiration through the Eustachian tube or by direct implantation through the external auditory canal and tympanic membrane perforation [4].

Diagnosis of TOM may be difficult and delayed, mainly because of a low index of suspicion, its low prevalence and non-specific clinical signs mimicking chronic otomastoiditis (COM), such as painless otorrhoea refractory to standard antibiotics, tympanic membrane perforation and unilateral conductive hearing loss [1 5 6]. As in all COM cases, imaging is mandatory in order to study the extension of the disease and any possible complications, even if it is of little benefit in differential diagnosis, since radiological findings are not specific and signs of aggressiveness are common to other middle ear infections. Thus, identification of *Mycobacterium tuberculosis* remains the gold standard of diagnosis and therefore a necessary step in the presence of a high clinical suspicion. In fact, prompt diagnosis as well as early treatment are very important to avoid severe complications such as facial paralysis [1], sensorineural hearing loss [1] and abscess of the parotid [7] or brain [8].

The aim of this paper is to report on the experience of "Bambino Gesù" Paediatric Hospital with TOM in children. By reviewing the available clinical charts of 4077 cases of COM undergoing middle ear surgery at the Audiology and Otology Unit of the Institution's ENT Department from January 1995 to December 2011, the Authors retrieved three children. One patient had *Mycobacterium avium* isolated from the ear culture and, consequently,

was excluded from the study. This case was the object of a previously published case report [9]. The remaining two cases were caused by *Mycobacterium tuberculosis* and are described in the present report.

Description of clinical cases

Case 1. In September 2008, a 4-year old Moldovan boy was referred to our ENT Department for a 2-year history of recurrent, painless, purulent otorrhoea in his right ear, which was refractory to broad spectrum local and systemic antibiotics. Otoscopy showed a subtotal perforation of the tympanic membrane through which granulomatous and whitish epitympanic material could be seen. The boy's hearing was normal on the left side, and a moderate-to-severe conductive hearing loss (ACPTA = 61 dB HL, BCPTA = 10 dB HL) was found on the right side (Fig. 1). A CT scan of the temporal bones showed soft tissue-like material occupying the mastoid cells and the tympanic cavity, eroding the ossicles (Fig. 2). A cholesteatomatous otomastoiditis (CCOM) was suspected and in January 2009 a canal-wall up tympanomastoidectomy was performed with tympanic membrane reconstruction with temporalis fascia. Intraoperatively, the mastoid and the middle ear appeared entirely occupied by granulomatous material. Histological examination revealed granulomatous, non-necrotising tissue that was negative on Ziehl Nielsen staining.

After a healthy period, in October 2010 the patient was hospitalised for a recurrence of otomastoiditis with otorrhoea. On this occasion, the patient's mother reported a family history of TB (uncle). Therefore, an ear swab was performed, revealing acid fast bacilli. The purulent material, sent for culture, was positive for MT, and susceptible to routine anti-TB drugs. A tuberculin skin test and a QUANTIferon test were positive. The patient underwent a chest radiography, which was negative for pneumonia, suggesting a diagnosis of primary TOM. He was started on a three-drug antituberculous treatment (rifampin, isoniazid, pyrazinamide) and administered local therapy with boric acid and tobramycin/dexamethasone droplets. Over the following months, the otorrhoea subsided gradually, with no clinical recurrence after 3 years of follow-up. A CT scan of the temporal bones (Fig. 3) showed moderate opacification of the mastoid cavity and of the middle ear, with no signs of local recurrence. Finally, on the right side, the hearing threshold improved slightly (ACPTA = 45 dB HL; BCPTA = 15 dB HL).

Case 2. In January 2009, a 5-year old Romanian girl was referred to our hospital for a 2-year history of recurrent otorrhoea that was refractory to broad-spectrum antibiotic therapy. She had a history of bilateral pulmonary and mediastinal TB at 7 months of age that had been treated with anti-TB chemotherapy (isoniazid, rifampin and streptomycin).

Otoscopy revealed subtotal perforation of the tympanic membrane, through which whitish, simil-cholesteatomatous material could be seen occupying the tympanic cavity. Pure tone audiometry showed mild conductive hearing loss on the right side (ACPTA = 35 dB HL, BC PTA = 8 dB HL) and normal hearing on the left side. A CT scan of the temporal bones showed complete obliteration of the right mastoid cavity, with sclerotic remodelling of the mastoid and erosion of the mastoid cortical bone. The middle ear was also entirely filled with isodense material, without apparent erosion of the ossicular chain (Fig. 4).

The patient underwent canal-wall up tympanomastoidectomy in July 2009. Intraoperatively, abundant granulation tissue was found in the mastoid, in the antrum and in the tympanic cavity. The ossicular chain was intact. Histology did not confirm the suspected CCOM and bacterial culture of specimens was negative.

A few months later, otoscopy revealed successful eardrum reconstruction, but no effective ear ventilation. The neotympanic membrane was retracted, whitish and thick. Consequently, in December 2010 the patient underwent a myringocentesis with insertion of a ventilation tube in the right ear. Intraoperatively, after incision of the tympanic membrane, abundant whitish caseous material was found. Suspecting a local recurrent TB infection, the surgeon abstained from inserting a ventilation tube and sent the material for culture, which grew isoniazid and rifampin resistant MT. As MT was also cultured in a broncho-alveolar lavage, a secondary TOM diagnosis was made. CT of the temporal bones showed complete re-obliteration of the mastoid cavity by soft tissue-like material, which fully occupied the middle ear (Fig. 4). Due to growth of a drug-resistant TB strain upon culture, treatment with linezolid and moxifloxacin was prescribed for 12 months. In the following 18 months, the patient's ear appeared dry, the reconstructed tympanic membrane normal and the tympanic cavity well ventilated, with no signs of TOM recurrence. Audiometry showed unchanged conductive hearing loss on the right side (ACPTA = 35 dB HL, BC PTA = 8 dB HL) and normal hearing on the left side.

At the end of the therapy, CT of the temporal bones showed a remarkable improvement of middle ear ventilation with only mild signs of effusion in the epitympanic space (Fig. 5).

Discussion

Tuberculosis remains the leading infectious cause of death worldwide. Although the incidence of TB in Italy has decreased over the last century, the recent increase in immigration of people from areas with a high incidence of TB has contributed to reversing this downward trend and to raising again the debate on TB as a matter of public health concern [10]. Among the extra-pulmonary presentations, TOM with otorrhoea is extremely rare, accounting for 0.05-0.9% of chronic infections of the middle ear [3]. Consistent with the literature [1 3 11], in our hospital the inci-

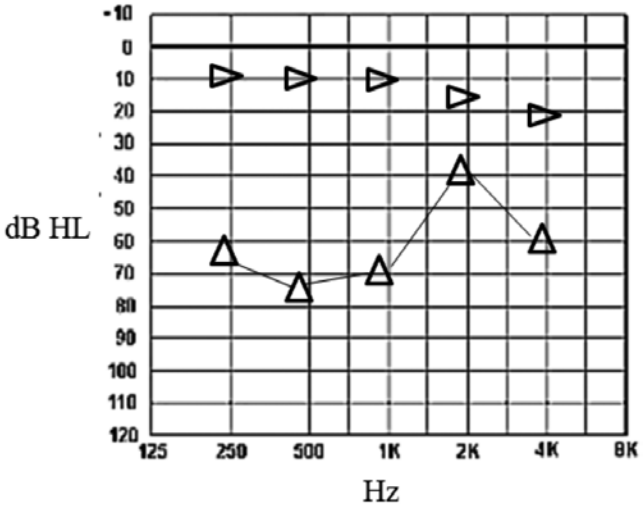

Fig. 1. Pre-operative pure tone audiometry of case 1.

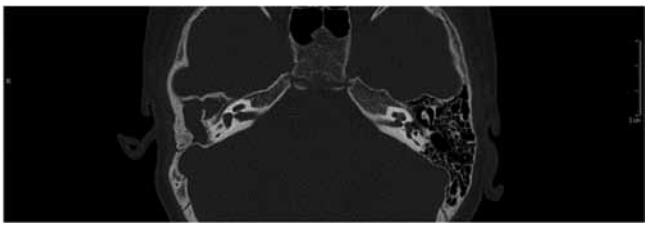

Fig. 2. Pre-operative temporal bone CT scan of case 1.

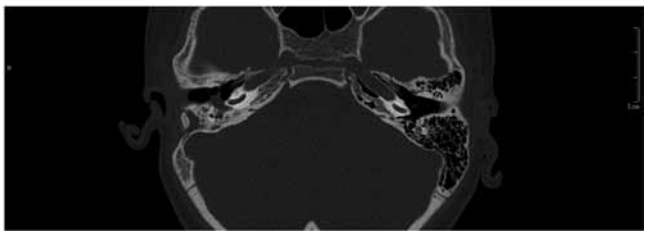

Fig. 3. Post-operative temporal bone CT scan of case 1.

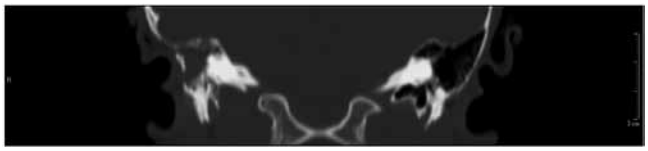

Fig. 4. Pre-anti-TB treatment temporal bone CT scan of case report 2.

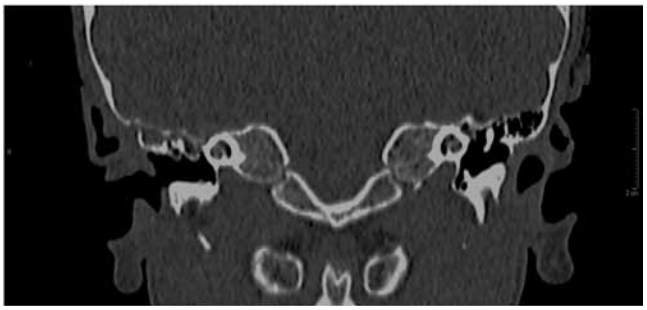

Fig. 5. Post-anti-TB treatment temporal bone CT scan of case 2.

dence of TOM in children undergoing middle ear surgery is 0.05% to date. It is by no means surprising that the 2 cases recorded at our institution are from 2008-2011 and were diagnosed in children originating from areas with a high incidence of TB. In the light of this, we expect the incidence of TOM to increase in the next years. In the literature, a considerable delay before the diagnosis is often reported because of its low prevalence and insidious clinical signs. Likewise, in our experience TOM presented with unspecific findings that did not allow early diagnosis. In fact, in both cases the clinical symptoms mimicked CCOM, and CT did not show any of the characteristic alterations such as those identified by Rho [12]. On the other hand, magnetic resonance imaging has no demonstrated role in the differential diagnosis of TOM and requires general anaesthesia in children. Therefore, clinicians did not think it would have provided useful information in the cases reported herein.

Because of the low specificity of imaging, the gold standard for TOM diagnosis remains microscopy, culture, drug sensitivity testing and histopathological examination of specimens obtained from the middle ear, as recommended by European guidelines [13]. It is with hindsight that the Authors acknowledge that an ear swab should have been obtained immediately and sent with a special request for culture and PCR analysis. However, this can be done if a high degree of clinical suspicion is present, which is rarely the case, since for most clinicians TOM is often far down in the list of possible differential diagnoses of chronic otorrhoea. At present, this conception of TOM as a rare disease entity should be revisited on account of the latest increase of migratory flows from Eastern Europe.

Nonetheless, MT identification through middle ear biopsy is not easy to obtain, in that it requires general anaesthesia in paediatric subjects.

The gold standard of TOM treatment is still a matter of debate: although the indication for chemotherapy is well defined, there is no consensus whether middle ear surgery should be attempted in these patients. The guidelines issued by the American Thoracic Society, CDC and Infectious Diseases Society of America in 2003 [14] recommend that extrapulmonary TB be treated with the same drug regimens as pulmonary disease, and do not mention surgery as a treatment option. The rationale of performing a tympanomastoidectomy in TOM is that anti-tuberculosis drugs alone do not completely penetrate the middle ear. Conversely, the effectiveness of surgery has not been yet demonstrated [6 15]. Some authors even recommend against it, unless complications are present [16]. Finally, recent studies have demonstrated that higher rates of dry ear are achieved when surgery precedes chemotherapy, as compared to chemotherapy alone [1 17]. In our experience, in both cases tympanomastoidectomy preceded anti-TB chemotherapy due to the delay of aetiological diagnosis. Nonetheless, surgery alone was effective only in the short term,

signs of local TB recurrence appearing 6-12 months after surgery, and specific anti-TB chemotherapy was essential in inducing a lasting remission of the disease. Therefore, when TOM diagnosis is not accidental, surgery should be considered optional as first-line treatment of TOM, whereas anti-TB chemotherapy with the drug regimen recommended by International Guidelines [14] should be the initial treatment. Surgery remains mandatory in two cases: for a diagnostic purpose when the clinical suspicion is high and bacteriological and PCR analysis of ear discharge fail to identify the MT, and to complete treatment when chemotherapy is not sufficient, in order to eradicate the infectious process from the mastoid and middle ear.

Once surgery is attempted, a "second look" should not be planned in the short term, since re-opening a potentially infected middle ear and mastoid cavities could facilitate disease dissemination. Finally, close follow-up is required to recognise early complications or recurrence of disease. In addition to otoscopic and audiometric examinations, radiological studies may be useful to monitor the disease and to modulate pharmacological therapy. In particular, CT of the temporal bones is the most appropriate exam for this purpose.

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CASE SEIRES AND REPORTS

Simultaneous nasopharyngeal and parotid gland Warthin's tumour: a case report

Un caso raro di tumore di Warthin sincrono della ghiandola parotide e del rinofaringe

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SUMMARY

Herein, a rare case of synchronous cystoadenolymphoma (Warthin's tumour) of the right parotid gland and the nasopharyngeal space is described. Although Warthin's tumour (WT) of the parotid gland is a common benign pathology, the occurrence of extra-parotid cystoadenolymphoma is rare. Extra-parotid WT have been mainly localised in the submandibular gland, periparotid region and occasionally in other sites, such as the oral cavity, hard palate and nasopharynx. The simultaneous occurrence of an intra-parotid and extra-parotid WT localisation, as in the case presented, is extremely uncommon.

KEY WORDS: Warthin's tumour • Parotid Gland • Nasopharynx

RIASSUNTO

Obiettivo del presente lavoro è descrivere un raro caso di cistoadenolinfoma (tumore di Warthin) sincrono della ghiandola parotide destra e del rinofaringe. Il tumore di Warthin della ghiandola parotide è una neoplasia benigna relativamente comune; più raro è il suo riscontro in ambito extra-parotideo (per lo più, a livello della ghiandola sottomandibolare, del cavo orale o del rinofaringe). Molto più raro è poi il riscontro di due localizzazioni simultanee, una intra-parotidea ed una extra-parotidea, come nel caso qui descritto.

PAROLE CHIAVE: *Tumore di Warthin • Ghiandola Parotide • Rinofaringe*

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Introduction

Warthin's tumour (WT) is a benign salivary gland tumour, almost exclusively located in the parotid gland and periparotid region [1]. From 12-19% of patients develop more than one WT, frequently with a bilateral parotid involvement (incidence: 5-14%) [1]. WT has been rarely reported to occur at multiple sites and at the same time; to the best of our knowledge, in the literature there is only another case report of a simultaneous intra-parotid and extra-parotid WT [2].

Case report

A 63-year-old man was referred to the ENT department of the University Hospital of Ferrara for the assessment of a right, non-painful, parotid mass. The lesion had been present for 4-6 months, but was increasing in size very slowly. He was also complaining the onset of snoring since 6 months with occasional nasal discharge.

Apart from a 40-year habit of smoking 20 cigarettes a day and the onset of a non-insulin dependent diabetes mellitus two years ago, his medical history was unremarkable.

ENT examination revealed a mobile 3.5 × 2.0 cm mass just behind the angle of the jaw (right side). Facial nerve function was normal. Nasopharyngeal endoscopy revealed an oval mass (1.0 cm) with a smooth surface in the left side of the nasopharynx. There were no other notable findings on physical examination.

Fine needle aspiration of the right parotid mass was performed. Histological examination revealed mixed lymphoid cells (lymphocytes and macrophages). The exact anatomical location of both lesions was confirmed by MRI (Fig. 1A-B).

Under general anaesthesia, the parotid mass was excised, via a superficial parotidectomy; at the same time, the nasopharyngeal mass was removed under FESS guidance. At histological examination, a synchronous parotid and nasopharyngeal WT was diagnosed (Figs. 2, 3). There have been no signs of tumour recurrence at 16 months postoperatively.

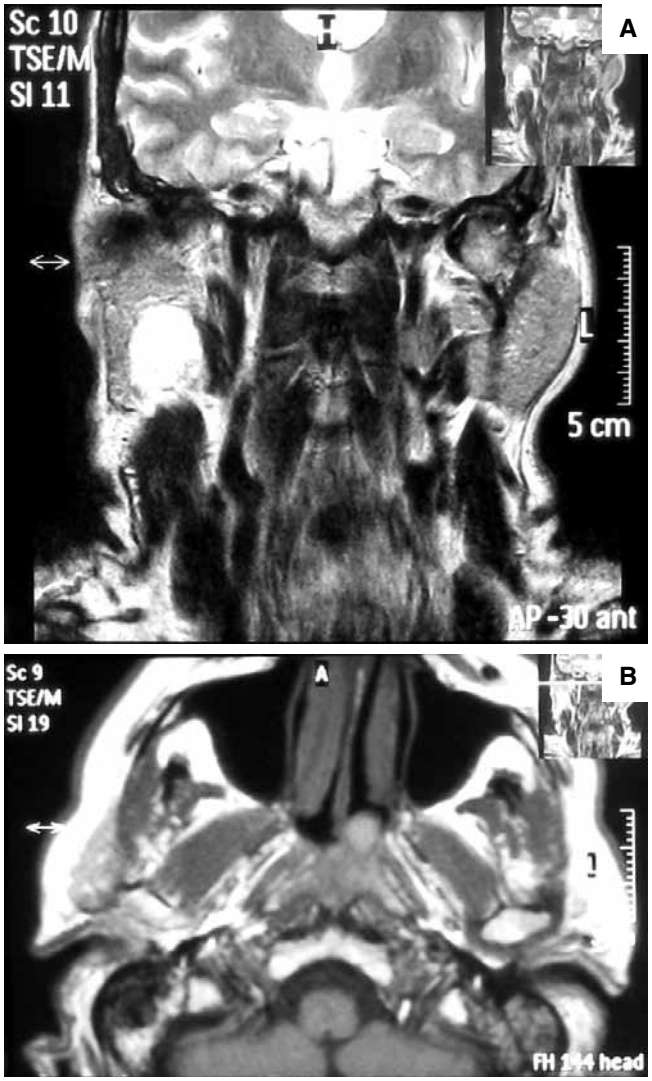

Fig. 1. A. T1 weighted MRI scan, coronal section: a 3.5 × 2.0 cm mass is located in the superficial lobe of the right parotid gland. B. T1 weighted MRI scan, axial section: an oval mass of about 1.0 cm in diameter is located in the left side of the nasopharynx.

Discussion

Warthin's tumour (adenolymphoma, papillary cystadenoma lymphomatosum, cystoadenolymphoma) is one of the most common benign salivary gland tumours, generally involving the parotid gland [1-3]. Even if benign tumours of the salivary glands are more common in women, WT, on the other hand, is found more frequently in men between the ages of 55 and 70 years. An association with cigarette smoking has been described [3-5].

Although some cases have been reported in extra-parotid locations such as the cervical lymph nodes, submandibular gland, lip, cheek, tongue and hard palate, WT of the nasopharynx are extremely rare [2-10]. Only few isolated cases of primitive nasopharyngeal involvement have been described so far, and we could find (through a PubMed database search performed in January 2011) only one published case of a nasopharyngeal WT with a simultaneous associated parotid tumour [2]. A synchronous intra-parotid and extra-parotid WT, as in our case, can therefore be considered exceptional.

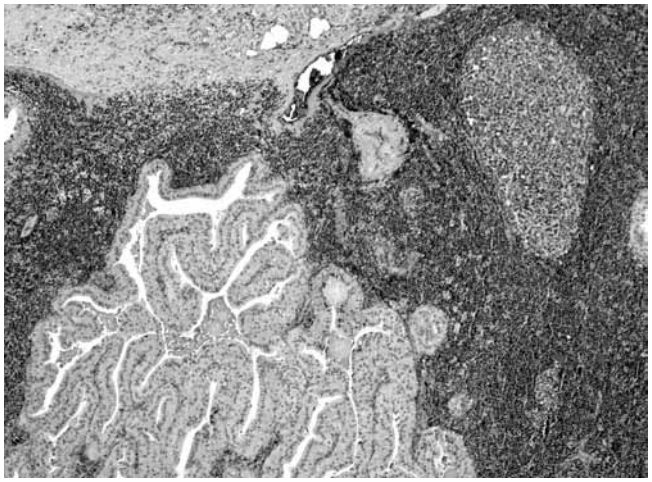

Fig. 2. Parotid lesion showing the classic histological pattern of Warthin's tumour: bilayered oncoctic epithelium and lymphoid stromal tissue. Haematoxylin-eosin, magnification 20×.

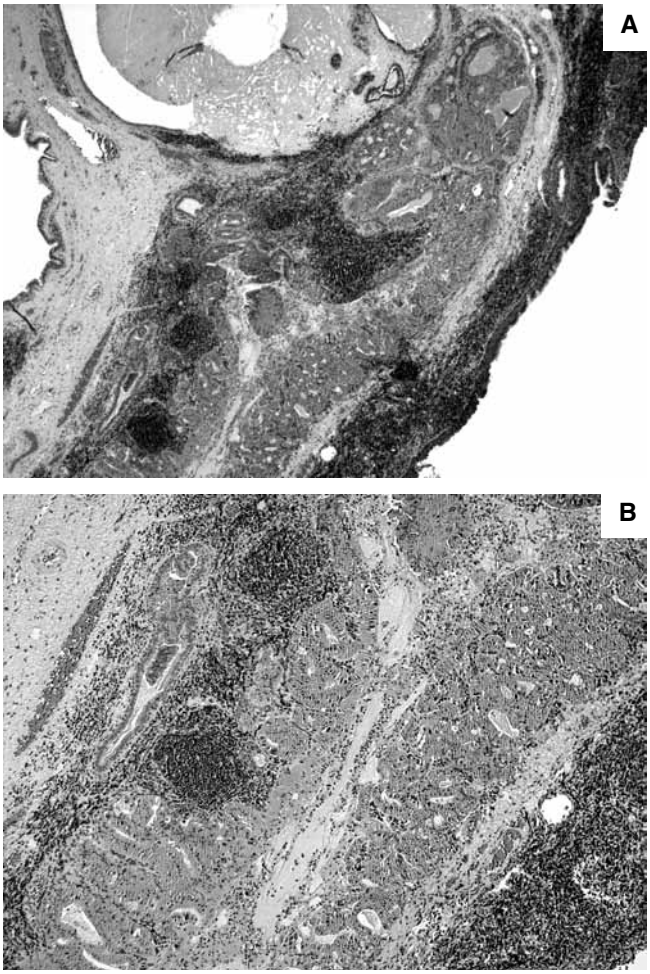

Fig. 3. The nasopharyngeal lesion shows the same histological pattern of Warthin's tumour. A. Haematoxylin-eosin, magnification 10×. B. Haematoxylin-eosin, magnification 20×.

Histologically, WT is an adenoma in which bilayered columnar and basaloid oncocytic epithelium forms multiple cysts with multiple papillae and accompanied by a proliferation of lymphoid tissue [1]. Sometimes, oncocytic cells can also form in nodal tissue. Our case was judged to meet these criteria and the lesion was diagnosed as WT.

It is difficult to explain the occurrence of a synchronous intra-parotid and extra-parotid WT. To date, there is no evidence in either the previously reported case [2] or our patient that one or more systemic or local factors might have influenced or induced the development of WT in two different sites: the hypothesis that we can make is of a multiple and simultaneous origin.

The origin of WT is still controversial and has been much debated. The most accepted hypothesis suggests that parotid WT could arise from salivary duct epithelium inclusions in the parotid gland lymph nodes, during ontogeny [2 11]. Nonetheless, other authors propose that the lymphocytic component is the result of an immunological reaction to the epithelial component, or could arise as an inflammatory response [2 3].

At the same time, the pathogenesis of nasopharyngeal WT also remains unclear. By using monoclonal antibodies, Fantozzi et al. found the ratio of T to B cells in extraparotideal WT to be similar to that of a normal lymph node, thus strengthening the theory of salivary organogenesis rather than that of reactive proliferation or hypersensitivity [12]. Extraparotideal WT may then arise from components of the minor salivary glands that are engaged in a preexisting lymphoid stroma, and chronic inflammation in the nasopharynx could induce the formation of oncocytic metaplasia of glandular tissues in the stroma [2 9].

The question also arises as to whether cigarette smoking, a chronic inflammatory stimulus, as well as a reported risk factor for the onset of WT [3-5], could have acted as a trigger for the simultaneous occurrence of WT in this case.

In conclusion, WT is a predominantly benign lesion almost exclusively found in the parotid, which can exceptionally appear simultaneously in other areas, such as the nasopharynx. Head and neck surgeons should always be aware of extra parotideal WT and consider performing MRI in highly suspicious cases during initial workup. Available data [2 6] support a surgical, conservative, approach to the management of WT, even in case of synchronous lesions, as in the patient presented.

It is likely that once the details of the pathogenesis of WT are better clarified, it will be possible to understand the occurrence of synchronous and/or multifocal lesions.

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Calendar of events – Italian and International Meetings and Courses

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Information, following the style of the present list, should be submitted to the Editorial Secretariat of Acta Otorhinolaryngologica Italica (actaitalicaorl@rm.unicatt.it).
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APRIL-DECEMBER 2015

CENACOLO ITALIANO DI AUDIOVESTIBOLOGIA • ***March 21 - May 16, 2015 • Chieti – Italy***

Director: Giampiero Neri – E-mail: info@nsmcongressi.it – Website: www.nsmcongressi.it

3° MASTER DI LARINGOLOGIA OTOLOGICA • ***March 30 - April 2, 2015 • Vittorio Veneto (TV) – Italy***

Director: M. Lucioni – Chairman: G. Rizzotto – E-mail: mail@nordestcongressi.it – Website: www.nordestcongressi.it

INTERNATIONAL CONGRESS OF KOREAN SOCIETY OF OTORHINOLARYNGOLOGY-HEAD & NECK SURGERY
April 24-26, 2015 • Seoul – Korea

President: Sang Hag Lee

5th INTERNATIONAL HANDS-ON COURSE "TRANSNASAL CORRIDORS TO SKULL BASE AND ORBIT"
April 28-30, 2015 • Wien – Austria

Course Directors: P. Castelnuovo, P. Nicolai, M. Tschabitscher – Organizing Secretariat: informazioni@attingo-edu.it – E-mail: www.attingo-edu.it

16th WORLD CONGRESS OF RHINOLOGY • ***April 30 - May 2, 2015 • São Paulo – Brazil***

President: Aldo Stamm – E-mail: secretaria@malulosso.com.br – Website: http://www.rhinology2015.com/Scientific-program.htm

VI CONGRESO IBEROAMERICANO DE IMPLANTES COCLEARES Y CIENCIAS AFINES
May 20-23, 2015 • Sao Paulo – Brasil

Tel. 55 11 5081 7028

VERTIGO ACADEMY INTERNATIONAL • ***May 22-23, 2015 • Moscow – Russia***

Chairman: O. Nuri Ozgirgin (Turkey) – Website: www.vainternational.org

102nd NATIONAL CONGRESS SIO, ITALIAN SOCIETY OF OTORHINOLARYNGOLOGY HEAD AND NECK SURGERY • ***May 27-30, 2015 • Rome – Italy***

President: Giuseppe Spriano – Organizing Secretariat: NordEst Congressi – Tel. +39 06 68807925 – Fax +39 06 68212211 – E-mail: nec@nordestcongressi.it – Website: www.sio2015.com

3rd CONGRESS OF EUR OPEAN ORL-HNS • ***June 7-11, 2015 • Prague – Czech Republic***

President: Jan Betka – E mail: orl-hns2015@guarant.cz – Website: http://www.europeanorl-hnsprague2015.com

27° CONGRESSO NAZIONALE SPIGC • ***June 11-13, 2015 • Brescia – Italy***

President: Gian Luca Pariscenti – Website: www.spigc.it

5th HANDS ON DISSECTION ADVANCED COURSE: "FROM REMOVAL TO RECONSTRUCTION IN HEAD & NECK CANCERS" • *June 16-19, 2015 • Paris – France*

Directors: Marco Benazzo, Department of Otolaryngology HN Surgery, University of Pavia; Fausto Giuseppe Chiesa, Department of Otolaryngology HN Surgery, IEO Milan. Organizing Secretariat: Bquadro Congressi srl, via S. Giovanni in Borgo 4, 27100 Pavia. Tel. +39 0382 302859 – Fax +39 0382 27697 – E-mail: bolla@bquadro-congressi.it – Werbsite: www.bquadro-congressi.it

41° CONGRESSO CONVENTUS SOCIETAS ORL LATINA • *July 6-8, 2015 • Torino – Italy*

President: Roberto Albera, Francesco Pia

22nd INTERNATIONAL CONGRESS ON THE EDUCATION OF THE DEAF • *July 6-9, 2015 • Athens – Greece*

Website: www.iced2015.com

WORLD CONGRESS ON LARYNX CANCER 2015 • *July 26-30, 2015 • Queensland – Australia*

Website: www.wclc2015.org

27th INTERNATIONAL COURSE ON ENDOSCOPIC SURGERY OF THE PARANASAL SINUSES & SCULL BASE *August 26-29, 2015 • Ghent – Belgium*

Course Director: Claus Bachert – Tel. +32(0)92338597 – Email: Fess@semico.be

SYMPOSIUM & 52nd INNER EAR BIOLOGY WORKSHOP • *September 12-15, 2015 • Rome – Italy*

Directors: Gaetano Paludetti and Diana Troiani – Website: www.ieb2015.it

2nd SIR (SOCIETÀ ITALIANA DI RINOLOGIA) NATIONAL CONGRESS • *September 17-19, 2015 • Udine – Italy*

President: Marco Piemonte – E-mail: nec@nordestcongressi.it – Website: www.nordestcongressi.it

V CORSO TEORICO-PRATICO DI AUDIOLOGIA E VESTIBOLOGIA
September 28-30, 2015 • Benevento – Italy

Segreteria Scientifica: Luigi Califano, Maria Grazia Melillo – E-mail: luigi.califano@tin.it, vertigobn@hotmail.com

INTERNATIONAL WORKSHOP "OPEN PARTIAL HORIZONTAL LARYNGECTOMIES (OPHL) VERSUS TRANSORAL LASER MICROSURGERY (TLM) IN LARYNX CANCER"
October 8-10, 2015 • Castel Brando (TV) – Italy

Chairman: G. Rizzotto – Email: mail@nordestcongressi.it – Websites: www.nordestcongressi.it, www.oncolarynx.it

XXXIX CONVEGNO NAZIONALE AOOI (ASSOCIAZIONE OTORINOLARINGOLOGI OSPEDALIERI ITALIANI) *October 16-17, 2015 • Genova – Italy*

President: Felice Scasso – E-mail: nec@nordestcongressi.it – Website: www.nordestcongressi.it

7th INTERNATIONAL SYMPOSIUM ON MENIERE'S DISEASE AND INNER EAR DISORDERS
October 17-20, 2015 • Rome – Italy

Website: meniere2015.eu

VII INTERNATIONAL SYMPOSIUM ON RECENT ADVANCES IN RHINOSINUSITIS AND NASAL POLYPOSIS
October 22-25, 2015 • Panama

Information: congresors2015@gmail.com

3rd VIS (SOCIETÀ ITALIANA DI VESTIBOLOGIA) CONGRESS • *October 30-31, 2015 • Modena – Italy*

Website: www.vestibologyitaliansociety.com

SIOP (SOCIETÀ ITALIANA DI OTORINOLARINGOIATRIA PEDIATRICA) NATIONAL CONGRESS
November 5-7, 2015 • Rome – Italy

E-mail: info@formazionedeventisrl.it – Website: www.formazionedeventisrl.it

XXXV CONGRESSO NAZIONALE SIAF – AGGIORNAMENTI IN AUDIOLOGIA INFANTILE
December 16, 2015 • Milan – Italy

Chairman: Antonio Cesarani – Website: www.sia-f.it

JANUARY-DECEMBER 2016

6° CONGRESSO NAZIONALE CO.R.TE. • ***March 10-12, 2016 • Rome – Italy***

President: Nicolò Scuderi – Tel. +39 06 35497114 – Email: corte@jaka.it

15th INTERNATIONAL MEETING OF THE MEDITERRANEAN SOCIETY OF OTOLOGY AND AUDIOLOGY
April 28-30, 2016 • Cappadocia – Turkey

President: S. Armagan Incesulu – Website: www.msoa2016.org

INSTRUCTIONAL WORKSHOP EUROPEAN ACADEMY OF OTOLOGY AND NEURO-OTOLOGY
September 28 - October 1, 2016 • İzmir, Turkey

President: O. Nuri Ozgirgin – Website: www.eaono.org