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Review

Is prophylactic central neck dissection necessary for cN0 differentiated thyroid cancer patients at initial treatment? A meta-analysis of the literature

La dissezione linfonodale profilattica del compartimento centrale del collo è necessaria come trattamento iniziale nei pazienti affetti da carcinoma differenziato della tiroide cN0? Meta-analisi della letteratura

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SUMMARY

Central lymph node metastases are common in patients with differentiated thyroid cancer (DTC). The management of preoperatively node-negative (cN0) DTC is still under debate. The aim of this study was to analyse the difference in recurrence and surgical complications between thyroidectomy (TT) alone and TT combined with prophylactic central neck dissection (pCND) as initial treatments to DTC patients with cN0 and evaluate the clinic significance of pCND for these patients. PubMed, Ovid, Cochrane Library, and Web of Science databases were systematically searched using multiple search terms. Twenty-three articles with 6,823 patients were identified. The quality of evidence was assessed by Jadad quality scores and the Newcastle-Ottawa Quality assessment scale. The results showed that compared with patients who underwent TT alone, patients who underwent TT plus pCND had a significant higher rate of transient recurrent laryngeal nerve injury ($p = 0.023$), transient hypocalcaemia ($p < 0.01$) and permanent hypocalcaemia ($p<0.01$). There was a trend towards lower central neck recurrence rate in TT plus pCND ($p < 0.01$). Combined TT and pCND as initial treatment for DTC patients with cN0 may reduce the risk of recurrence, but increases the incidence of some complications. Methodologically high-quality comparative studies are needed for further evaluation.

KEY WORDS: Differentiated thyroid cancer • Prophylactic central neck dissection • cN0 • Meta-analysis

RIASSUNTO

Le metastasi ai linfonodi del compartimento centrale del collo sono comuni nei pazienti affetti da carcinoma differenziato della tiroide (DTC). La gestione dei pazienti con stadiazione preoperatoria cN0 è ancora dibattuta. L'obiettivo di questo lavoro è stato quello di analizzare le differenze in merito a ricorrenza e complicanze chirurgiche tra tiroidectomia (TT) isolata e TT associata a svuotamento linfonodale profilattico del compartimento centrale del collo (pCND) come trattamenti iniziali di pazienti con DTC cN0, e di valutare l'importanza clinica del pCND per questi pazienti. I database PubMed, Ovid, Cochrane Library e Web of Science sono stati analizzati scrupolosamente, e sono stati identificati ventitré articoli per un totale di 6823 pazienti. La qualità di evidenza è stata valutata tramite lo score di Jadad e tramite la Newcastle-Ottawa Quality assessment scale. I risultati hanno mostrato che i pazienti sottoposti a TT e pCND, se paragonati ai pazienti sottoposti a TT isolata, hanno avuto un tasso significativamente più alto di lesioni transitorie del nervo laringeo inferiore ($p = 0,023$), di ipocalcemia transitoria ($p < 0,01$) e di ipocalcemia permanente ($p < 0,01$). Inoltre è stato rilevato un trend in diminuzione per quel che riguarda il tasso di ricorrenza nei pazienti sottoposti a TT e pCND ($p < 0,01$). La tiroidectomia totale associata allo svuotamento del compartimento centrale del collo come trattamento iniziale per quei pazienti con cN0 potrebbe ridurre il rischio di ricorrenza di malattia, ma aumenta l'incidenza di alcune complicanze. Si rendono necessari ulteriori studi di maggior qualità metodologica.

PAROLE CHIAVE: *Carcinoma differenziato della tiroide • Svuotamento profilattico del compartimento centrale del collo • cN0 • Meta-analisi*

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Introduction

Well differentiated thyroid cancer (DTC) represents most cases of thyroid cancer, the incidence of which continues to increase. Papillary thyroid carcinoma (PTC) is the most common type of DTC and its incidence is increasing in developed countries, particularly in the United States and Western Europe [1 2]. Despite its relatively good prognosis with a 10-year cancer-specific survival above 90%, locoregional recurrence is a major cause of disease morbidity [3]. There are some regional lymph node compartments to which thyroid cancer is likely to metastasise, such as the central, lateral and mediastinal. Some studies have reported that the central compartment is the most common because it is located adjacent to the lower pole of

the thyroid. At present, a growing number of surgeons are performing routine central neck dissection at the time of total thyroidectomy (TT) for PTC [4-6].

Although LNM has no major impact on survival, it has been suggested that the presence of nodal involvement is commonly associated with extrathyroidal invasion and an increased rate of recurrence and distant metastasis. A therapeutic central neck dissection is recommended in patients with LNM in the central neck identified on cervical ultrasonography or at the time of surgery. Nevertheless, the role of prophylactic central neck dissection (pCND) in the absence of suspected cervical metastases on preoperative ultrasound (cN0) is still uncertain [5 7]. Many physicians consider pCND to have the potential benefits that can improve the accuracy of staging, enable better selection of patients for radioactive iodine (RAI) treatment and decrease postoperative recurrence in patients with cN0 PTC. Current American Thyroid Association (ATA) guidelines state that pCND may be performed in PTC patients with cN0, especially for T3 or T4 tumours [8]. European consensus does not recommend central dissection because there is no evidence that it improves recurrence or mortality rates [9]. However, these recommendations are grade C (expert opinion), and the controversy is still sustained due to the lack of prospective comparative studies with high levels of evidence.

Currently, the value of pCND for DTC patients with cN0 has been investigated in a number of trials worldwide. A definitive conclusion has not been reached. Thus, the aim of this study was to evaluate the clinical significance of pCND and provide surgeons additional information for clinical decision making.

Materials and methods

Search strategy

A systematic search was developed for all English language literature published from January 2000 to July 2015. The comprehensive search was performed using the electronic databases PubMed, Ovid, Cochrane Library and Web of Science. We used the following terms: differentiated thyroid cancer OR papillary thyroid cancer AND prophylactic central neck dissection AND (cN0 OR negative lymph node) OR complication OR recurrence OR hypocalcaemia OR hypoparathyroid OR (recurrent laryngeal nerve palsy OR recurrent laryngeal nerve injury). The search strategy was slightly adjusted according to the requirement of different databases. Review articles and bibliographies of other relevant identified investigations were hand-searched to identify additional studies.

Inclusion and exclusion criteria

The goal of pCND is to remove all lymphatic tissue en bloc. The area of dissection is bound superiorly by the hyoid bone, inferiorly by the suprasternal notch, laterally by the medial borders of the carotid sheath and dorsally by the prevertebral fascia. The lymph nodes that are removed include the prelaryngeal, pretracheal and paratracheal nodes. Also removed are nodes found along the RLN. Particular attention is given to identifying and preserving the RLN and parathyroid gland.

All clinical studies were required to meet the following criteria for this study: (1) proven diagnosis of DTC; (2) patients who performed pCND had no clinically apparent central LNM at presentation by neck ultrasonography as well as clinical exam; (3) clinical comparative trials, comparison of adverse outcomes of TT plus pCND to TT alone; (4) the studies had to report on at least one of the clinical outcomes mentioned below: hypocalcaemia, recurrent laryngeal nerve (RLN) injury and recurrence; (4) either one of the higher quality or the most recent study was included when two studies were published by the same institution or authors. The following articles were excluded: (1) studies exploring the results of lateral cervical node dissection or modified neck dissection instead of pCND; (2) TT with pCND was not performed as the initial treatment; (3) letters, comments, expert opinions, reviews, or case reports; (4) measured outcomes were not clearly presented in the literatures or it was impossible to extract the appropriate data from the articles.

Data extraction and quality assessment

Two reviewers reviewed each article independently. Discrepancies between the two reviewers were resolved through discussion, and when this did not resolve the differences, a third person made a final decision. The authors, publication years, country of investigators, sample size, follow-up period, clinical complications and postoperative recurrence were extracted. The quality of randomised controlled trials (RCTs) was evaluated using Jadad quality scores [10], and included secure methods for randomisation, allocation concealment, patient and observer blinding, and loss to follow-up. The studies were divided into a low quality group (score < 4) and a high quality group (score ≥ 4). The quality of observational studies was performed using the Newcastle-Ottawa Quality assessment scale [11 12]. Briefly, the overall star assessed three main categories on the following: selection of cohort, comparability of cohort and ascertainment of outcome. A trial can be awarded a maximum of 1 star for each numbered item within the selection and outcome categories. A maximum of 2 stars can be given for comparability. The total number of star was accumulated, with more stars reflecting a better methodological quality.

Statistical analysis

For dichotomous outcomes, we expressed the results using risk ratios (RRs) with 95% CIs. Heterogeneity between studies was tested qualitative by Q-test statistics with significance set at $p < 0.10$ and quantitatively tested by I^2 statistics, with I^2 more than 50% indicating large in-

consistency. A random effect model was used to calculate pooled RRs in the case of significant heterogeneity ($p < 0.10$ or $I^2 > 50\%$), otherwise, a fixed effect model was used. A $p < 0.05$ was considered statistically significant. Publication bias was estimated by visually assessing the asymmetry of Begg's funnel plot. Furthermore, Egger's test was also performed to provide quantitative evidence of publication bias [13,14]. Sensitivity analysis was performed by sequentially omitting individual study to check the stability of the result. Statistical analyses were performed using STATA version 12.0 software (Stata Corporation, College Station, Texas, USA) and Microsoft Excel 2010.

Results

Description of studies

The initial search strategy yielded 327 potentially relevant studies, 273 of which were excluded after the initial review of their titles and abstracts. After further consideration of the 54 remaining studies, 23 articles [4-7,15-33] were included in our study. The total number of patients included was 6,823, ranging from 83 to 1,087 patients per study. The rate of central LNM in TT combined with pCND ranged from 16.7% to 82.3%. Seventeen studies reported that patients received postoperative RAI [5-7,15-18,20-22,24-27,30,32,33]. Among the 23 articles, 7 were conducted in Italy [4,17,18,27,28,32,33], 5 in the United States [5,6,19,21,26], 4 in Korea [16,20,25,29], 2 in Australia [15,22], 1 in Columbia [7], 1 in UK [23], 1 in China [24], 1 in Poland [30] and 1 in France [31]. The characteristics and methodological quality assessment are shown in Table I.

Methodological quality of the studies

The quality of one RCT included was evaluated according to the Jadad scale, and one study was low quality according to the scores. Each of the remaining 22 eligible studies included in our meta-analysis was assessed for quality according to the Newcastle-Ottawa Quality scale. For quality, scores ranged from 0 to 9, and studies with scores of 6 or more were rated as high quality. The quality of all studies included varied from 4 to 8, with a mean of 6; 17 studies obtained scores of 6 or more in methodological assessment, indicating that they were of high quality (Table I).

Meta-analysis findings

A meta-analysis of combinable data was carried out to analyse the postoperative complications and recurrence, and the main results are shown in Table II. Eight studies reported data on haemorrhage, 15 studies reported data on transient RLN injury, 14 studies reported data on permanent RLN injury, 19 studies reported data on transient hypocalcaemia, 19 studies reported data on permanent hypocalcaemia, 13 studies reported data on postoperative recurrence,

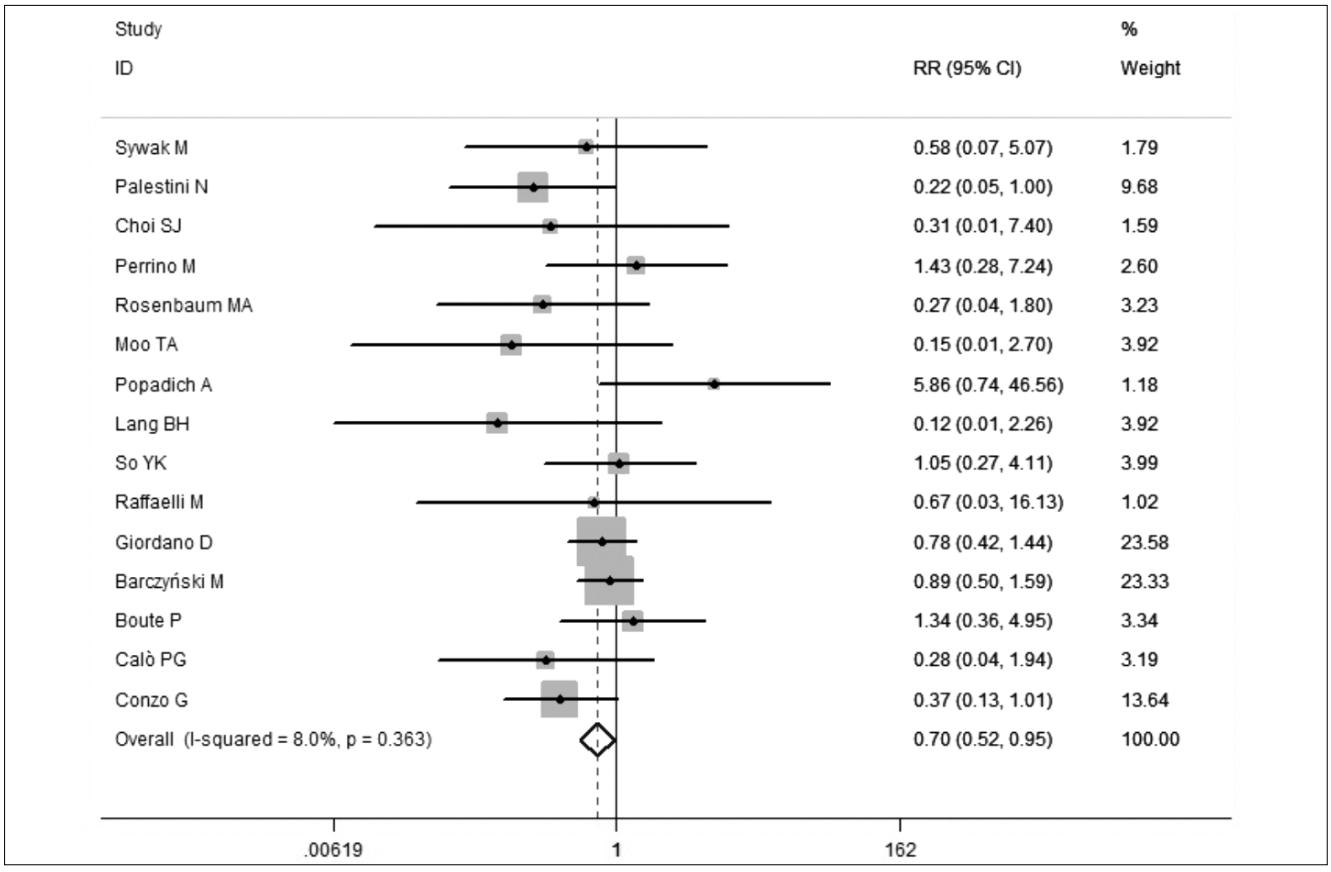

Fig. 1. Forest plot of risk ratio for transient RLN injury.

nine studies reported data on central neck recurrence and 10 studies reported data on lateral neck recurrence. When the data were pooled, transient RLN injury ($p = 0.023$) (Fig. 1), transient hypocalcaemia ($p < 0.01$), permanent hypocalcaemia ($p < 0.01$), postoperative recurrence ($p < 0.01$) and central neck recurrence ($p < 0.01$) were identified as statistically significant. Specifically, the pooled RRs (95% CIs) were as follows: 0.71 (0.521, 0.953) for transient RLN injury, 0.59 (0.531, 0.663) for transient hypocalcaemia, 0.61 (0.463, 0.801) for permanent hypocalcaemia, 1.78 (1.372, 2.302) for postoperative recurrence ($p < 0.01$) and 3.37 (2.028, 5.588) for central neck recurrence.

Sensitivity analysis and publication bias

A single study involved in this meta-analysis was deleted to reflect the influence of the individual data set to the pooled RRs, and the corresponding pooled RRs were not substantially altered. Begg's funnel plot and Egger's test were performed to access the publication bias of literatures. The shape of the funnel plot did not reveal any evidence of obvious asymmetry (Fig. 2). Next, the Egger's test was used to provide statistical evidence of funnel plot symmetry (Fig. 3). Similarly, the results did not suggest any evidence of publication bias.

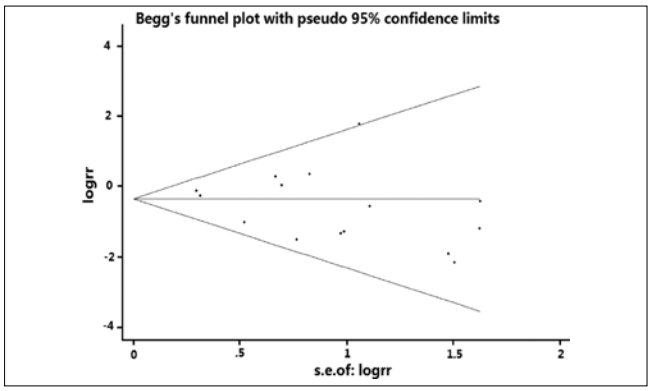

Fig. 2 Begg's funnel plot for visual assessment of overt publication bias for transient RLN injury.

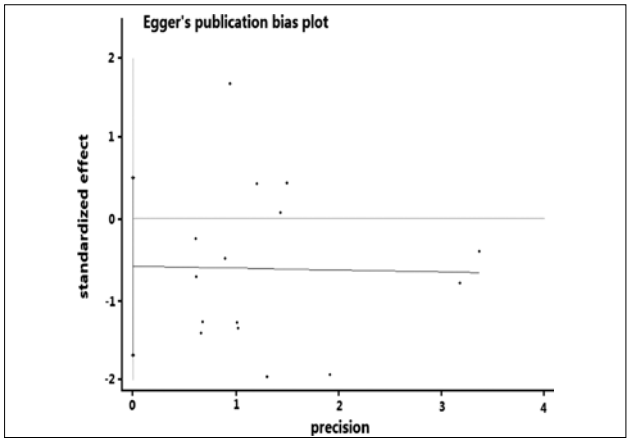

Fig. 3 Egger's publication bias plot showed no publication bias for transient RLN injury.

Discussion

Patients with DTC generally have excellent prognosis; however, it frequently tends to metastatise and often early to regional lymph nodes. About 80% of PTC patients have micrometastases in the central lymph nodes at diagnosis [4 34]. It remains difficult to identify which patients have central LNM before surgery due to the reduced sensitivity of ultrasound (especially in the central compartment) for nodal disease before TT [35 36]. Thus, the value of pCND in patients with cN0 is a matter of debate. In previous studies, some physicians described their experience of treating DTC in patients with pCND, and demonstrated that it had a tendency to upstage approximately 35% of patients, as detecting metastatic nodal disease moves N stage from N0 to N1a [21]. Improved staging might be useful for patients with tumours ≤ 1 cm and is the main advantage of pCND, which nevertheless remains a debated topic, because pCND can cause upstaging and potential overtreatment with the risk of treatment morbidity. In this study, our results showed that cN0 patients in TT plus pCND group had a relatively high incidence of central LNM, but the rate varied widely from 16.7% to 82.3%. We assume the reason may be the heterogeneity in surgical technique, especially in the setting of pCND in which the extent of surgery may vary by location and perceived risk factors. Simultaneously, it might also be a reflection of the quality of preoperative ultrasound evaluation and probably be a result of the quality of histological examination in different countries.

In contrast to other tumours, most DTC patients survive for more than 10 years, and might not have detectable LNM or recurrence until many years after initial surgery. Evaluating survivals as endpoints is difficult in clinical practice. The natural history of DTC would be for some patients to perform multiple neck operations that may result in a negative impact on quality of life. Thus, assessing the number of postoperative recurrences might be more realistic for patients with cN0. The rationale for pCND is based on the assumption that patients have high rates of metastases and regional recurrence in the central neck and that reoperation for central neck recurrence is difficult and carries an increased risk of hypocalcaemia and unintentional RLN injury [6 7 15 27]. However, improvements in survival and locoregional recurrence rates have not been consistently demonstrated with pCND. Although some studies have reported a decrease in neck recurrence after pCND, others do not demonstrate any effect [37-40]. In this study, pCND did show some advantage related to locoregional recurrence in cN0 disease. We found that TT plus pCND as initial treatment to DTC may reduce the risk of recurrence. Although there was variability in the rate of

Table I. Main characteristics of studies included.

Author	Country	Year	Design	Number of patients	Adverse events	Central LNM rate in TT+pCND	Follow-up	Number of postoperative RAI treatment	Quality of score
Sywak M [15]	Australia	2006	RT	TT: 391 TT+CND: 56	Hypocalcaemia, haemorrhage, RLN injury, recurrence	38%	TT: 70 months TT+CND:24.5 months	TT: 391 TT+CND: 56	8/9
Palestini N [4]	Italy	2008	RT	TT: 148 TT+CND: 157	Hypocalcaemia, haemorrhage, RLN injury	42%	NA	None	6/9
Choi SJ [16]	Korea	2008	RT	TT: 53 TT+CND: 48	RLN injury, recurrence	37.5%	24.4 months	TT: 53 TT+CND: 48	8/9
Costa S [17]	Italy	2009	RT	TT: 118 TT+CND: 126	Recurrence	47%	TT: 64 months TT+CND: 47 months	TT: 62 TT+CND: 87	6/9
Zuniga S [7]	Columbia	2009	RT	TT: 130 TT+CND: 136	Recurrence	82.3%	6.9 years	TT: 55 TT+CND: 79	7/9
Perrino M [18]	Italy	2009	RT	TT: 159 TT+CND: 92	Hypocalcaemia, RLN injury, recurrence	75.8%	69.2 months	140	6/9
Sadowski BM [19]	United States	2009	RT	TT: 130 TT+CND: 180	RLN injury, recurrence	46.7%	38.8 months	None	5/9
Roh JL [20]	Korea	2009	RCT	TT: 49 TT+CND: 148	Hypocalcaemia, recurrence	52.7%	36 months	TT: 49 TT+CND: 148	2
Rosenbaum MA [5]	United States	2009	RT	TT: 88 TT+CND: 22	Hypocalcaemia, RLN injury, recurrence	77%	TT:3.8 years TT+CND:2.8 years	TT: 56 TT+CND: 18	7/9
Hughes DT [21]	United States	2010	RT	TT: 65 TT+CND: 78	Hypocalcaemia, haemorrhage, RLN injury, recurrence	62%	TT: 27.5 months TT+CND: 19.1 months	TT: 56 TT+CND: 72	6/9
Moo TA [6]	United States	2010	RT	TT: 36 TT+CND: 45	Hypocalcaemia, RLN injury, recurrence	33%	3.1 years	TT: 36 TT+CND: 45	5/9
Popadich A [22]	Australia	2011	RT	TT: 347 TT+CND: 259	Hypocalcaemia, haemorrhage, RLN injury, recurrence	49%	TT: 50 months TT+CND: 32 months	TT: 347 TT+CND: 259	6/9
Mitra I [23]	UK	2011	RT	TT: 78 TT+CND: 49	Hypocalcaemia	59.2%	NA	None	5/9
Lang BH [24]	China	2012	RT	TT: 103 TT+CND: 82	Hypocalcaemia, haemorrhage, RLN injury, recurrence	54.9%	TT: 27.1 months TT+CND: 25.5 months	TT: 63 TT+CND: 62	6/9
So YK [25]	Korea	2012	RT	TT: 113 TT+CND: 119	Hypocalcaemia, haemorrhage, RLN injury, recurrence	37%	TT: 45.4 months TT+CND: 44.7 months	TT: 92 TT+CND: 101	6/9
Wang TS [26]	United States	2012	RT	TT: 37 TT+CND: 66	Hypocalcaemia, RLN injury	40.8%	21 months	TT: 12 TT+CND: 29	6/9
Raffaelli M [27]	Italy	2012	PT	TT: 62 TT+CND: 124	Hypocalcaemia, RLN injury, recurrence	35.5%	25.1 months	TT: 37 TT+CND: 90	8/9
Giordano D [28]	Italy	2012	RT	TT: 394 TT+CND: 693	Hypocalcaemia, RLN injury	NA	9 months	None	7/9
Hyun SM [29]	Korea	2012	RT	TT: 87 TT+CND: 65	Recurrence	44.6%	51.31 months	None	4/9
Barczyński M [30]	Poland	2013	RT	TT: 282 TT+CND: 358	Hypocalcaemia, RLN injury, recurrence	30.2%	TT: 128.8 months TT+CND: 126.4 months	TT: 79 TT+CND: 231	7/9
Boute P [31]	France	2013	RT	TT: 22 TT+CND: 61	Hypocalcaemia, RLN injury	16.7%	NA	None	5/9
Calò PG [32]	Italy	2013	RT	TT: 169 TT+CND: 46	Hypocalcaemia, haemorrhage, RLN injury, recurrence	30.4%	93 months	197	7/9
Conzo G [33]	Italy	2014	RT	TT: 390 TT+CND: 362	Hypocalcaemia, haemorrhage, RLN injury, recurrence	41.8%	9.5 years	652	8/9

RT: retrospective trial
PT: prospective trial
RCT: randomised controlled trial
TT: thyroidectomy
TT+pCND: thyroidectomy plus prophylactic central neck dissection
RLN: recurrent laryngeal nerve
LNM: lymph node metastasis
NA: not available
RAI: radioactive iodine

Table II. Statistical results of postoperative complications and recurrence between TT and TT+pCND groups.

	N	Cases	RR (95% CI)	Analytical model	P value
Haemorrhage	26	2885	0.66 (0.322, 1.353)	FEM	0.256
Transient RLN injury	175	5281	0.71 (0.521, 0.953)	FEM	0.023
Permanent RLN injury	75	5337	1.19 (0.748, 1.883)	FEM	0.467
Transient hypocalcaemia	1287	5851	0.59 (0.531, 0.663)	FEM	< 0.01
Permanent hypocalcaemia	204	5850	0.61 (0.463, 0.801)	FEM	< 0.01
Postoperative recurrence	261	4205	1.78 (1.372, 2.302)	FEM	< 0.01
Central neck	85	3422	3.37 (2.028, 5.588)	FEM	< 0.01
Lateral neck	88	3381	1.28 (0.840, 1.952)	FEM	0.250

FEM: fixed effects model
REM: random effects model
RLN: recurrent laryngeal nerve

recurrence among the individual series, there was a significant trend toward less recurrence in the central compartment in patients who underwent TT plus pCND compared to those who had TT only (1.0% *vs* 3.6%, $p < 0.01$). However, there was no significant difference in the rate of lateral recurrence whether or not a pCND was performed ($p = 0.25$). The biological behaviour of LNM may not always be predictable. While central LNM is often high, the recurrence rate remains low (0-15%), even in patients who underwent TT [7 41]. It has not been possible to explain this difference, but it might be related to the extreme aggressiveness of cancers, in which recurrence would not only depend on local procedures such as pCND.

The indication for pCND in PTC patients with cN0 is less well defined and remains controversial. The revised ATA guidelines were published with a modification in the recommendation for central neck dissection, and recommendation 27B was modified to state that "pCND may be performed with PTC with cN0, especially for T3 or T4 tumours" [8]. However, the strength was lowered to C, meaning that this was based on expert opinion. Although current guidelines offer varying recommendations for central neck dissection in the prophylactic setting, the decision for pCND is mostly at the discretion of the surgeon [7 21 24]. In some studies, patients who underwent pCND were generally more likely to have more risk factors, such as larger tumour size, extrathyroidal extension and multifocality which might have been easily detectable during surgery [7 24]. Ma et al. in their study identified several predictive factors for central LNM in cN0 patients, and proposed that certain risk factors, such as tumour size and extrathyroidal extension, could be considered in preoperative clinical decisions regarding the necessity of pCND in cN0 PTC patients [42]. We look forward to obtaining more information from larger samples for a better comprehension of treatment and authentication accuracy in a population-based collective of patients in the near future.

A benefit of pCND is accurate pathological assessment of nodal status, and these data may make it possible to take a selective approach in the use of postoperative RAI. A comprehensive literature review by Sawka et al. reported that there was no benefit from RAI in reducing cause-specific mortality or recurrence in DTC with early stage [43]. Sywak et al. also showed that there was no significant difference in recurrence in cN0 patients [15]. In accordance with previous results, we found that there was no significant difference between the two groups in terms of postoperative recurrence for patients who had adjuvant RAI. On the other hand, because of upstaging, it is expected that more patients would require RAI ablation and inevitably might be subjected to potential drawbacks from radiation, such as recurrent sialoadenitis, salivary gland swelling and increased risk of second primary malignancies in the long term [44-46]. Although RAI ablation is not recommended for patients with PTC in the absence of high-risk features according to the revised ATA guidelines [8], studies have yet to clarify whether these upstaged patients are overtreated. Whether current changes in decisions regarding RAI administration based on pCND have a long-term effect on outcomes for these patients remain to be seen.

Even with the benefits from pCND, central lymph node dissection inevitably results in a higher rate of RLN injury and hypocalcaemia than no dissection. Recent studies of lymph node dissection in DTC reported the development of transient hypocalcaemia in 14% to 60%, and permanent hypocalcaemia in 3% to 11%. This study showed that the rate of temporary hypocalcemia was indeed significantly higher when a pCND was performed (25.1% *vs* 14.3%, $p < 0.01$). This result is not unexpected, because more extensive dissection in the central neck may interfere with the blood supply to the parathyroid glands, particularly the inferior parathyroid glands. Simultaneously, a pCND did result in a significant increase in the incidence of permanent hypocalcaemia in our study (4.7% *vs* 1.7%, $p = 0.03$). Although the symptoms related to permanent hypocalcaemia may appear to be more acceptable in terms of quality of life than the voice disorders related to RLN injury, permanent hypocalcaemia can be difficult to treat and may require life-long calcium and vitamin D supplements. In terms of RLN injury, analysis of our data

suggests that pCND at the time of initial surgery was associated with a significantly higher rate of transient RLN injury. However, there was no significant difference in permanent RLN injury between the two groups.

The results of this study should be interpreted with caution because of some limitations. First, the studies included are not the highest-quality evidence, and such data might lead to less powerful results. Second, the number of patients in several studies is small, and most of the studies included were conducted in European and North American countries, which may not reflect the real situation. Third, the follow-up period in several studies included was relatively short. Because DTC has the characteristics associated with slow-growth, some patients might not have detectable LNM until many years after initial surgery. This might be a bias for clearly evaluating postoperative recurrence. Publication is a major concern for all forms of meta-analysis, and positive results tend to be accepted by journals, while negative results are often rejected or not even submitted. Although this study does not support publication bias, it should be noted that it could not completely exclude such biases. For example, our meta-analysis was restricted to papers published in English, which probably led to bias and might not allow a reliable conclusion.

Conclusions

In summary, pCND may result in the excision of occult central LNM for patients with cN0 DTC. Compared with TT alone, combined pCND by experienced surgeons may decrease the postoperative recurrence rate in this population. Nevertheless, pCND carries a greater risk of some complications, such as transient RLN injury, and transient and permanent hypocalcaemia. Balancing surgical morbidity and long-term benefits as well as better patient selection to undergo pCND are key. This study should be further updated whenever new and strong evidence is available.

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HEAD AND NECK

Modelling tumour volume variations in head and neck cancer: contribution of magnetic resonance imaging for patients undergoing induction chemotherapy

Modificazioni del volume nei tumori della testa e del collo: il contributo delle immagini di risonanza magnetica nei pazienti sottoposti a chemioterapia di induzione

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SUMMARY

Primary tumour volume evaluation has predictive value for estimating survival outcomes. Using volumetric data acquired by MRI in patients undergoing induction chemotherapy (IC) these outcomes were estimated before the radiotherapy course in head and neck cancer (HNC) patients. MRI performed before and after IC in 36 locally advanced HNC patients were analysed to measure primary tumour volume. The two volumes were correlated using the linear-log ratio (LLR) between the volume in the first MRI and the volume in the second. Cox's proportional hazards models (CPHM) were defined for loco-regional control (LRC), disease-free survival (DFS) and overall survival (OS). Strict evaluation of the influence of volume delineation uncertainties on prediction of final outcomes has been defined. LLR showed good predictive value for all survival outcomes in CPHM. Predictive models for LRC and DFS at 24 months showed optimal discrimination and prediction capability. Evaluation of primary tumour volume variations in HNC after IC provides an example of modelling that can be easily used even for other adaptive treatment approaches. A complete assessment of uncertainties in covariates required for running models is a prerequisite to create reliable clinically models.

KEY WORDS: Magnetic resonance imaging • Head and neck cancer • Induction chemotherapy • Survival modelling

RIASSUNTO

La valutazione del volume del tumore primitivo ha mostrato un valore predittivo per la stima dei risultati della sopravvivenza. Usando i dati volumetrici acquisiti con la Risonanza Magnetica (RM) nei pazienti sottoposti a chemioterapia di induzione (CI), tali risultati sono stati stimati nei pazienti con tumore del testa e collo, prima del trattamento radiante. Le immagini RM acquisite prima e dopo CI in 36 pazienti con tumore avanzato della testa e del collo sono state analizzate per valutarne il volume del tumore primitivo. I due volumi sono stati correlati utilizzando la regressione lineare locale tra i volume valutati nelle immagini della prima e quelli della seconda RM. Sono stati definiti i modelli di rischio proporzionale di COX per la valutazione del controllo locoregionale, la sopravvivenza libera da malattia e la sopravvivenza globale. La regressione lineare locale ha mostrato un buon valore predittivo per tutti i risultati di sopravvivenza nei modelli di rischio proporzionale di COX. I modelli predittivi per il controllo locoregionale di malattia e la sopravvivenza libera da malattia a 24 mesi ha mostrato una ottima discriminazione e capacità di previsione. La valutazione delle variazioni dei volumi dei tumori primitivi della testa e del collo dopo CI fornisce un esempio di modello che può essere facilmente utilizzato per altri approcci terapeutici. Una valutazione completa delle variabili nelle covariate è un prerequisito necessario per la creazione di modelli clinicamente attendibili.

PAROLE CHIAVE: *Risonanza magnetica • Tumori della testa e del collo • Chemioterapia di induzione • Modello di sopravvivenza*

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Introduction

Treatment of head-and-neck cancer (HNC) now requires complex multi-specialistic management and HNC types are typically characterised by wide heterogeneity of anatomical sites, primary treatment approaches and diagnostic pitfalls, the latter increasingly connected with procedures of optimisation of radiotherapy treatment planning [1-4]. Treatment of HNC is mainly based on integrating a chemoradiation approach [5-7], and in this context the use of induction chemotherapy (IC) has shown a consistent number of clinical responses before the start of radiotherapy [8]. The role of this approach is still under debate because of unclear advantages in final survival outcomes after definitive radiochemotherapy (RTCT) compared to

treatment based only on concurrent RTCT [9]. The individualisation of cancer treatment is an increasingly important topic in the medical literature [10]. Starting from this background, the use of IC provides the chance to analyse changes in tumour appearance, where imaging techniques can be evaluated moving towards adaptive radiotherapy strategies [11]. Traditional tumour evaluation approaches, such as tumour volume delineation on simulation computed tomography, have already proven to be consistent in terms of outcome correlations [12]. The aim of this study was to evaluate the feasibility and potential outcome classification for HNC patients undergoing IC, by using MRI based primary tumour volume shrinking evaluation after IC. After modelling, survival outcomes on imaging data a nomogram followed by strict model evaluation are assessed. This evaluation procedure is the main goal of this paper, being the base of further evaluations that can be extended to other adaptive treatment procedures.

Materials and methods

Patient characteristics

Thirty-six locally-advanced HNC patients were retrospectively analysed in our institution by selecting cases that underwent IC before the administration of definitive RTCT (34 patients) or before undergoing surgery (2 patients with oral cavity cancer, these latter underwent adjuvant RTCT after surgery). All patients gave informed consent for inclusion in the study. Main selection criteria were availability of complete on-site MRI diagnostics in patients with locally advanced HNC (stage III or IV, without distant metastases). Patient staging, chemotherapy and radiotherapy details are summarised in Table I. All patients were evaluated using MRI before and after IC. After RTCT patients were evaluated by MRI at 1st follow-up time to assess response to treatment. Subsequently, follow-up was routinely performed using CT, MRI and PET-CT to detect loco-regional or distant failures.

Treatment details

IC was administered using a TPF chemotherapy regimen: taxotere 75 mg/m^2 (day 1), cisplatin 75 mg/m^2 (day 1) and 5-flurouracil 750 mg/m^2 in continuous infusion (days 1-4) every three weeks for 3 cycles. Radiotherapy was delivered using a linear accelerator, and the dose and technique are summarised in Table 1. Lymphatic target volumes for radiation treatment were delineated according to Gregoire's indications for neck-positive and post-operative necks [13]. Concurrent cisplatin based chemotherapy (2 cycles at 100 mg/m^2 at the beginning of radiotherapy and after 3 weeks) was administered in 35 patients, while 1 patient received concurrent cetuximab at 250 mg/m^2. The outcomes evaluated were loco-regional control (LRC), disease-free survival (DFS) and overall survival (OS).

Table I. Summary of patient characteristics and RTCT treatment.

Patient characteristics		Number	(%)
Primary tumour site	Oropharynx	15	(41.7)
	Nasopharynx	14	(38.9)
	Larynx	3	(8.3)
	Oral cavity	2	(5.5)
	Hypopharynx	1	(2.8)
	Nasal cavity	1	(2.8)
Stage	IV*	34	(94.4)
	III	2	(5.6)
	Dose [Gy]		
PTV1 (primary + margin)	70.2	33	(91.6)
	68.4	1	(2.8)
	64.8	1	(2.8)
	50.4†	1	(2.8)
PTV2 (positive lymphatic compartment)	64.8	15	(41.6)
	61.2	2	(5.6)
	59.4	16	(44.4)
	50.4†	2	(5.6)
	36	1	(2.8)
PTV3 (elective lymph nodes)	64.8	2	(5.6)
	59.4	23	(63.8)
	50.4	9	(25)
	36	1	(2.8)
	30.6	1	(2.8)
RT technique	IMRT	35	(97.2)
	3D CRT	1	(2.8)
Concomitant chemotherapy	CDDP	35	(97.2)
	Cetuximab	1	(2.8)

** No distant metastases at diagnosis.*
† One of the two post-operative patients was treated using a two volumes approach in CTV delineation, with maximum delivered dose 50.4 Gy. All treatments were delivered at 1.8 Gy per fraction.

Magnetic resonance evaluation

Volumetric evaluation of primary tumours was performed using OsiriX Imaging Software (http://www.osirix-viewer.com): contours of primary lesions were manually outlined by two different radiologists who are expert in HNC imaging, in order to evaluate the impact of inter-observer differences in delineation. Volumes were delineated on axial T2w images, also using other sequences, mostly T1w images, before and after intravenous contrast injection, to better refine the delineation (Fig. 1). When both radiologists were satisfied with the outlines, tumour volumes were finally calculated.

Statistical analysis

Statistical analysis was performed using R Statistical Software (R Core Team 2013. http://www.R-project.org/). All statistical tests considered an alpha level of 5% to indicate statistical significance. The series of volumes delineated by each radiologist (labelled 'a' and 'b') be-

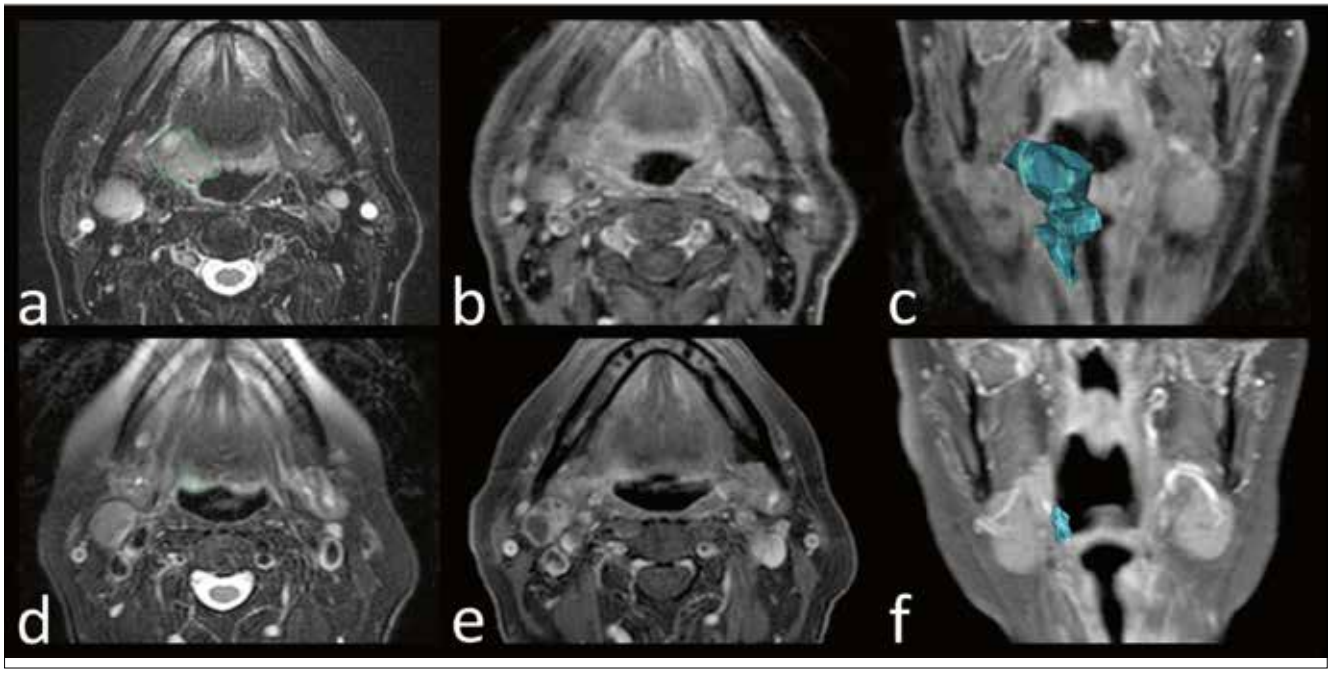

Fig. 1. Volumetric assessment of primary tumour volume. Squamous cell carcinoma of the oropharynx in a 73-year-old man. (a,d) Axial fat-saturated T2-weighted MR images. (b,e) Axial post-contrast T1-weighted fat-saturated MR images. (c,f) Coronal post-contrast T1-weighted fat-saturated MR images with 3D volumetric tumour reconstructions. Before induction chemotherapy (a,b,c): MR images show expansive/infiltrative tissue centred on the right glossopharyngeal fold, hyper-intense on T2-weighted image (a) with slight and faint enhancement on post-contrast T1-weighted fat-saturated MR image (b). The lesion was manually outlined (green closed line in a) to obtain a volumetric reconstruction of the tumour (blue volume in c). After induction chemotherapy (d,e,f) MR images show significant volumetric reduction of the lesion.

fore (V_1a, V_1b) and after (V_2a, V_2b) IC were analysed to detect normal distribution of values. Correlation between volumes measured at each step by each radiologist (V_1a vs. V_1b, V_2a vs V_2b) was calculated to assess homogeneity in delineation between the operators. Afterwards, two series of mean values of volumes for each diagnostic step

were calculated ($mV_1 = \frac{V_1a + V_1b}{2}$, $mV_2 = \frac{V_2a + V_2b}{2}$),

and used to divide patients in subsets to calculate stratified log-rank tests for survival outcomes. To define the relationship between volumes in the two diagnostic steps, two regression models were calculated, the first using linear regression between mV_2 over mV_1, and the second between mV_2 over $\log(mV_1)$. Considering the higher predictive power of the log-linear regression in describing the relationship between mV_2 and mV_1 (see results) a new score was calculated (Linear-Log-Ratio, *LLR*):

$$LLR = \frac{mV_2}{\log(mV_1)}$$

and used in Cox's proportional hazards model (CPHM) regression together with the values of mV_1 and mV_2 to find the best significant predictor for survival outcomes. Backward elimination process was used for determining the entry and removal of variables from the models with significance levels, respectively, of 0.05 and 0.15. After calculation of CPHMs, we tested the discrimination ability of the models using Harrell's c-index [14] calculated by bootstrap analysis on 1000 of a randomly created dataset based on the first. The prediction power of the models was defined using calibration plots drawn by resampling 200 cases for each model and calculating the mean prediction error as difference between observed - predicted outcome in the series [14]. Considering the problem of inter-subject (but also intra-subject) variations in volume delineation that arises from the literature in many anatomical sites and using different delineation procedures or imaging modalities [15-24], a procedure to evaluate the susceptibility of the model to variations in the delineation was defined: we created a function of mV_1 and mV_2 that gives the value of punctual uncertainty using the concept of '*gradient*' of a function in two variables. The chosen function for the analysis was DFS at 24 months, achieved by CPHM. Considering the appearance of the survival function at 24 months in a three-dimensional coordinates space (Fig. 2, DFS at 24 months) as a function of two variables (mV_1 and mV_2) linked by LLR, we can easily find portions of the surface where the slope of the function is higher, corresponding to values of survival prediction with high susceptibility to variability in volume delineation. In order to provide a quantification of the value of this susceptibility the *gradient* of survival function was

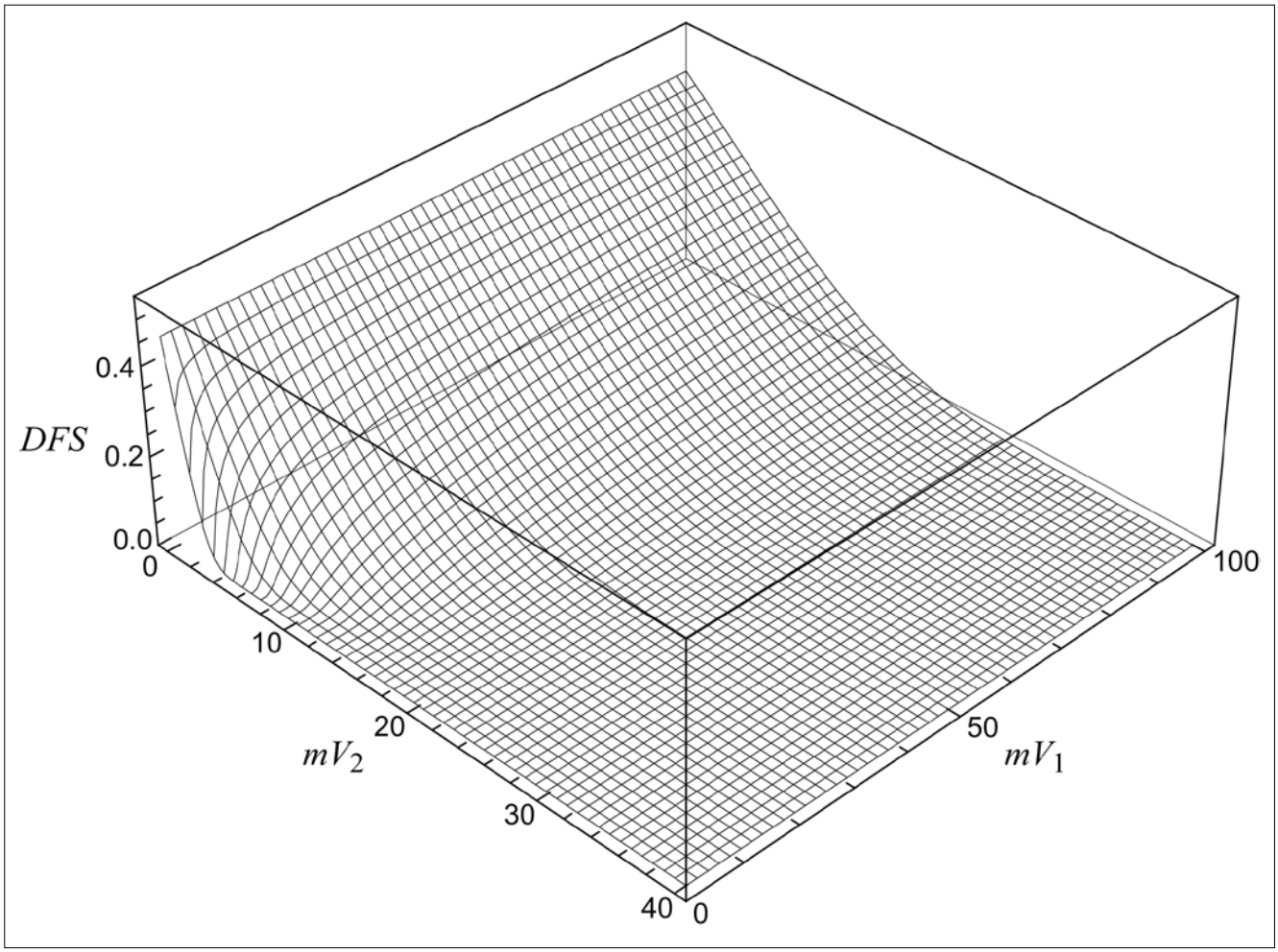

Fig. 2. Plot of 3D surface showing DFS at 24 months as a function of mean tumour volume before (mV_1) and after (mV_2) induction chemotherapy: the slope of the surface can vary according to the values of the two volumes, being steeper in the left corner of the plot, where the values of mV_1 and mV_2 are close to zero.

calculated using mV_1 and mV_2 as parameters of the function. Being:

$$S(mV_1, mV_2) = \exp\left\{-H_0 t \cdot \exp\left[\beta \cdot mV_2 / \log(mV_1)\right]\right\} \quad (1)$$

the value of survival function at time t (it can be used either for LRC or DFS) with $H_0 t$ the baseline hazard at chosen time (in our case 24 months), _ the coefficient of the covariate calculated with CPHM (see Table II), we first calculated the partial derivatives of S with respect to mV_1 and mV_2 and gave a first order estimate of the variation of S as a function of mV_1 and mV_2:

$$\Delta S(mV_1, mV_2) \cong \left|\left(\frac{\partial S}{\partial mV_1}, \frac{\partial S}{\partial mV_2}\right)\right| = \sqrt{\left(\frac{\partial S}{\partial mV_1}\right)^2 + \left(\frac{\partial S}{\partial mV_2}\right)^2} \quad (2)$$

The result of this equation is function of mV_1 and mV_2 only, and can be used to assess the sensitivity to error in volume delineation: it corresponds to the punctual maximum variation that survival function can show by changing the values of mV_1 and mV_2; looking at the 3D graph of survival function it is the module of the vector in one point, tangent to the direction of the highest steepness of the surface in such point. Using this method it is possible to determine both the predicted survival result and the level of uncertainty that matches the prediction given by the model. This last analysis was performed using Wolfram Mathematica® 9.0.1.0 (©1988-2013 Wolfram-Research Inc. Champaign, IL). Finally, a nomogram for DFS to directly calculate the predicted outcome using the two measures of MRI tumour volume, before and after IC (mV_1 and mV_2), was created. It was delineated using Pynomo, a program to create nomograms using Python interpreter (http://www.pynomo.org).

Results

The two volume series delineated by each radiologist (labelled 'a' and 'b') before (V_1a, V_1b) and after (V_2a, V_2b) IC analysed using D'Agostino-Pearson test showed non-

Table II. Summary of Cox's proportional hazards models for the LLR (Linear-Log-Ratio) covariate. All models and single LLR covariates in each model are largely significant (P-Values < 0.05 in all cases). The bootstrap over 1000 resampled series for each model allowed to calculate Harrell's c-index decreased by the 'optimism' for preventing model overfitting in starting case series. The performance of models is very close to the original c-index in all cases, meaning high discriminating power. The evaluation of the mean error of prediction of survival outcomes at 24 months, through calibration on 200 resampled cases, is also provided.

Summary of Cox's Proportional Hazards Regression models with unique significant covariate								
Outcome	Model P-value (Likelihood ratio test)	Standard error	P-value Pr(> |z|)	Standard error	Harrell's c-index (c)	Optimism (Op)	Corrected Harrell's c-index (c – Op/2)	Mean calibration error (24 months prediction)
LRC	0.0013060	0.4271	0.000788	0.1272	0.7668	0.0105	0.7615	0.073
DFS	0.0006376	0.3427	0.000234	0.0931	0.7546	0.0034	0.7529	0.056
OS	0.0008928	0.4905	0.000771	0.1458	0.8000	0.0155	0.7923	0.062

normal distribution (in all four series P < 0.0001). Thus, non-parametric tests were used to assess correlation between V_1a vs V_1b and V_2a vs V_2b (Spearman's coefficient of rank correlation and Kendall's Tau P < 0.0001 in both cases). These tests showed an optimal consistence in contouring procedure, and were not biased by different operators. All subsequent statistical analyses were carried out using the mean value of volume measures before (mV_1,) and after (mV_2) IC. Each series of mean values was used to determine factors for performing stratified Kaplan Meier (KM) analysis for LRC, DFS and OS: using the median value of mV_1 and mV_2 as cut-off (thus dividing the population into equal subsets, each one with 18 patients).

The KM Logrank test for mV_1 (cut-off: median = 12.9744 cc, range: 2.209 to 96.7385 cc) was not significant in all tested outcomes, while the same test for mV_2 (cut-off: median = 4.2181 cc, range: 0 to 39.3675 cc) was always significant (LRC: P-value = 0.0003, HR = 8.9437, 95% CI = 2.9196 to 27.3972; DFS: P = 0.0011, HR = 5.9193, 95% CI = 2.1952 to 15.9613; OS: P = 0.0097, HR = 5.7386, 95% CI = 1.8458 to 17.8412). In order to detect a correlation between mV_1 and mV_2 two regression functions were calculated and compared: the first, using a straight linear regression ($mV_2 = \beta \cdot mV_1 + \varepsilon$), showed a significant overall P < 0.0001, *b* P < 0.0001, *e* P = 0.402 (n.s.), adjusted R^2 = 0.3796; the second, using a regression with log-transformation of variable mV_1, ($mV_2 = \beta \cdot \log(mV_1) + \varepsilon$), showed a significant overall P < 0.0001, *b* P < 0.0001 and also an *e* P = 0.00525 (meaning that there is a better regression fit using log transformation rather than the simple correlation shown with lacking significance in the *e* term for the linear model) and finally a higher predictive power shown by higher adjusted R^2 = 0.4329.

Starting from this evidence, which links the concept of tumour regression with the biological assumption of fractional killing due to chemotherapy administration [25,26], an assumption proven for fractionated radiotherapy [27], we considered the possibility to create three different CPHM using LRC, DFS and OS as outcome, and both values of the volumes (mV_1 and mV_2) together with the value of linear-log ratio (*LLR*) as covariates, being such covariates dependent each other from the regression. Using the process of backward elimination of covariates we found a consistent significance for *LLR* that measures volume shrinkage against all checked survival outcomes, while mV_1 and mV_2 were no longer significant.

The results of CPHM are summarised in Table II. In order to assess the reliability of the models Harrel's c-index [14] was calculated for each model. Using a bootstrapping procedure, over 1000 randomly resampled datasets, we prevented the effect of overfitting by decreasing the value of the c-index: the value of *optimism* as defined by Harrel, performance of fitted model compared to that expected by chance [14], was always very low (Table II), showing an overall good discrimination performance of initially fitted models. The calibration plots [28] of the models showed a small underestimation of the predicted outcomes with respect to the observed outcomes (Table II).

Considering as sufficient the number of patients at risk [29] at 24 months of survival (16 patients), with a median follow-up time of 27 months (range 6-46), we developed a nomogram for DFS, calculating predicted survival as a direct function of mV_1 and mV_2 at 24 months. For OS the median follow-up time was considered too short to ensure a clinically reliable model, despite the significance in the modelling procedure, while LRC was considered redundant respect to the DSF evaluation in our case series. No stratification of patients according tumour primary site was performed, because of lack of significance in Kaplan-Meier LRC, DFS and OS according to this factor.

In Figure 3 the nomogram showing predicted DFS at 24months is shown. The use of this nomogram does not require calculating sums, but only placing a ruler connecting the value of the mV_1 on the right with the value of the mV_2 on the left. The predicted value of DFS at 24 months can be read on the oblique line showing the outcome prediction where the connection intersects this line. An aspect usually not analysed using current predictive models evaluation and nomogram drawing procedures [30] is the need to assess the level of uncertainty (that is *error*) in

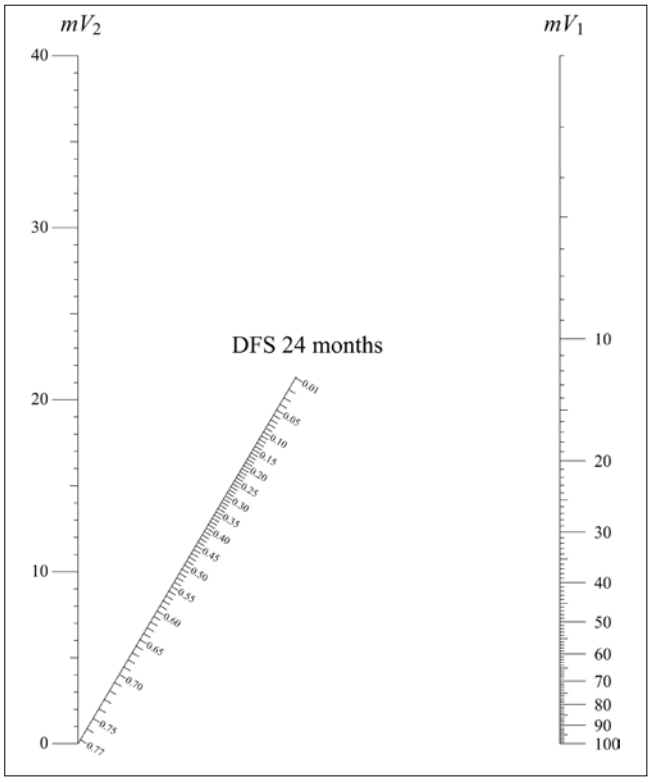

Fig. 3. Nomogram for calculating disease-free survival (DFS) at 24 months. Two vertical lines show the values of mean tumour volume before (mV_1) and after (mV_2) induction chemotherapy. Using a ruler to draw a straight line connecting the values of the two volumes on the oblique outcome line the predicted survival probability can be directly read.

measures of covariates put into the model. Covariates as sex, age and similar, used within predictive models, usually do not need to be evaluated for errors in detecting the value (especially when considering simple dummy variables). Indeed, when managing variables such as the value of delineated volume, as in our study, or similar measures subject to some kind of detection error, a verification of the variability in outcome prediction due to uncertainties in covariates should always be considered: as cited previously, there is much evidence for great variability in volume delineation procedures, that can vary to according anatomical site, imaging modality etc. [15-24 31].

This situation led us to use the procedure described in the previous section to assess the susceptibility of the model to variations in volume delineation using the gradient function derived from the survival functions calculated by CPHMs. The values of the *gradient* as a function of mV_1 and mV_2 for DFS are shown in Figure 4. This plot directly shows the maximum variation of survival prediction for small changes in covariates. In every point of the plot, the error in the determination can be always considered lower than or equal to the value shown by curved lines. As shown in Figure 4, only for very small values of mV_1 (< 12.5 cc) and mV_2 (< 6 cc) did the amount of delineation error become significant in affecting the predicted outcome, a prediction that can vary more than 5% in a very small portion of the parameter space (area shown by the continuous line in the down-left corner).

Discussion and conclusions

The evaluation of response in patients undergoing to treatment for different tumour types is one of the key-points in adaptive radiotherapy (ART), but the study of geometrical or volumetric changes in tumour volume and their subsequent impact on survival outcomes are still poor.

MR and CT are non-invasive imaging techniques that have an important role in assessing response to therapy, evaluating both morphological and "functional" parameters [32]. These parameters, as described in the literature, can be useful tools in oncological management. In fact, it is possible to characterise tissue cellularity by evaluating the motion of water molecules on MR diffusion-weighted imaging (DWI) and to provide information regarding tumour perfusion and permeability with MR perfusion-weighted imaging (PWI) or perfusion-CT (PCT) studies. DWI is described to be a predictor of response to therapy and a good tool to differentiate between recurrent tumours from post-radiation changes [3 33-34]. PWI may play a role in detecting residual disease and predict patient outcomes [35 36]. PCT, as well, may be helpful to differentiate between post-therapeutic changes and tumour recurrence, to monitor patients after radiotherapy and/or chemotherapy and predict response after induction chemotherapy [37 38]. In this study, we highlight the meaningful contribution of morphological MRI through the assessment of changes in tumour volume before and after CI to predict survival outcomes of HNC patients undergoing definitive RTCT.

This approach can be considered an assumption to be addressed by other types of strict radiotherapy 'adaptive' approaches. Indeed, despite the relative small number of cases, the relationship between survival outcomes and changes in tumour volumes shown by this analysis is consistent in HNC patients undergoing CI.

The modelling of CPHM using a non-linear relationship between two observed volumes allows to overcome the problem of co-linearity [39] when analysing parameters that are related each other, as the two volumes are. Furthermore, the use of LLR implies identification of the effect of treatment on tumour volume, as calculated by considering the possibility of tumour shrinkage rather than no change or enlargement in tumour volume. From a clinical point of view, this correlation has stronger predictive value than using the simple values of mV_1 and mV_2, because as a consequence of the assumptions of CPHM the two volumes should be independent in conditioning the outcome [40], but this assumption cannot be considered feasible in this model. The possibility to obtain a consistent 'benchmark' to refer the outcome prediction in a single predictor, being the definition of the LLR based on biological and clinical assumptions, confirms the result despite the small number

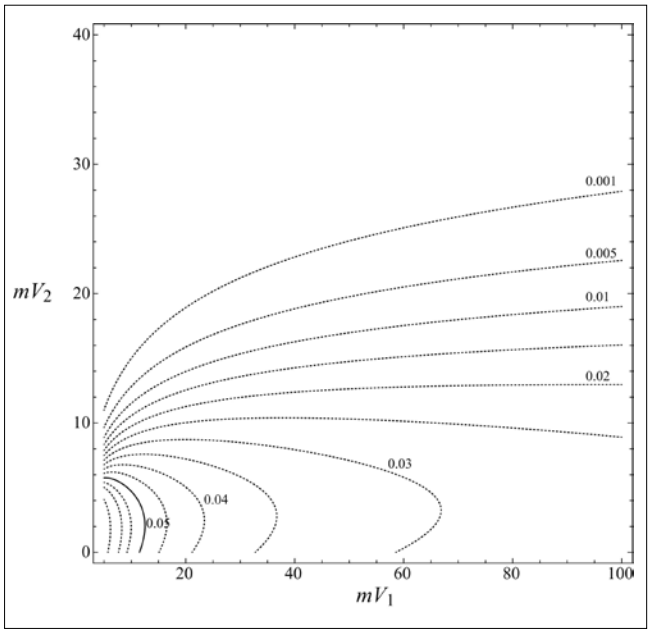

Fig. 4. Plot of gradient function. This plot shows the values of maximum variation in the outcome as a function of mV_1 and mV_2. It corresponds to maximum level of error for the survival function and can be used to assess the reliability of the prediction after survival calculation. In the down-left corner of the plot small variations of mV_1 and mV_2 can lead to outcome variations larger than 5% (continuous line). For most of the graph, the level of uncertainty can be considered reasonably low.

of cases and heterogeneity of the patient cohort. In the literature, there are already examples of correlations between survival outcomes and tumour volume assessment, but in HNC have been limited to diagnosis [12 41-43]. In cervical cancer, there is evidence of a semi-logarithmic relation between tumour volume at diagnosis and subsequent measures achieved by weekly MRI [44], and this relation was confirmed in our study for HNC after CI. As stated before, the semi-logarithmic volume reduction is the mathematical translation of the fractional killing phenomenon [25-27], due to constant proportion between the number of killed and surviving tumour cells for each therapeutic event that is the single radiotherapy fraction [44] or the single chemotherapy administration as in our study. The real value of the modelling procedure described in this work is not in the predictive value of the model itself, which gives only the evaluation of the results for this single case series and not yet validated by any external dataset, but rather it is in the *method* chosen for data analysis and evaluation of the pitfalls hidden in the volume assessment.

In this case, considering the small number of patients, better performance could be achieved by increasing the number of cases or refitting the models by introducing other clinical covariates that can improve the predictive power of the survival functions. The results of this study imply that measurement of the tumour volume can be considered a good predictor for patients undergoing CI and the strong correlation among outcomes and LLR can be an helpful parameter to be evaluated in perspective studies with 'adaptive' approaches to treatment. Hopefully, a similar approach to evaluate measures achieved by different diagnostic procedures could be used to better refine the actual impact of survival models in describing outcomes based on imaging studies.

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Head and neck

Overexpression of chromatin assembly factor-1/p60 predicts biological behaviour of laryngeal carcinomas

L'espressività della proteina CAF-1 p60 come fattore prognostico nei carcinomi laringei

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SUMMARY

This study analysed the immunohistochemical expression of the CAF-1/p60 protein in laryngeal cancers. CAF-1/p60 assumes an independent discriminative and prognostic value in laryngeal neoplasms; the presence of this protein in carcinoma in situ compared with laryngeal precancerous and larynx infiltrating tumours. We assessed the immunohistochemical expression of CAF-1/p60 in 30 cases of moderate and/or severe dysplasia, 30 cases of carcinoma in situ and 30 cases of laryngeal squamous cell carcinoma (LSCCs). CAF-1/p60 expression increased significantly according to the high index of neoplastic cellular replication; therefore, CAF-1/p60 was overexpressed in neoplastic cells and its moderate-severe expression is correlated with poorer prognosis compared to less expression. In conclusion, overexpression of the CAF-1/p60 protein is related to a risk of higher morbidity and mortality and is a reliable independent prognostic index of laryngeal carcinoma. CAF1-p60 protein overexpression can be used in cancer management as an indicator of malignant evolution, especially in carcinoma in situ.

KEY WORDS: CAF-1/p60 • Prognostic factor • Laryngeal cancer • Carcinoma in situ • Dysplasia • Tumoural marker

RIASSUNTO

CAF-1 è una proteina istonica trimerica implicata sia nella replicazione che nella riparazione del DNA, con il compito essenziale di stabilizzare la cromatina durante la replicazione; presenta un' azione di assemblaggio del tutto tipica, poiché unisce solo DNA che è andato incontro a replicazione. Di recente la proteina CAF-1p60 è stata proposta come marker della proliferazione cellulare nei tumori solidi, in particolare nel distretto testa- collo in virtù della sua iper-espressione nelle cellule in stato di proliferazione rispetto a quelle quiescenti in cui è down- regolata. Questa relazione con l'attività mitotica ha inoltre permesso di considerarla come possibile indice prognostico di aggressività neoplastica. In precedenti lavori, effettuati presso il nostro dipartimento, abbiamo documentato che tale proteina risulta essere iper-espressa nei tumori del cavo orale, delle ghiandole salivari e della tiroide. In questo studio abbiamo analizzato e confrontato l'espressività immunoistochimica della proteina CAF-1/p60 nelle neoformazioni laringee precancerose, nei carcinomi in situ e nei tumori maligni, in particolare: in 30 casi di displasia moderata e/o severa, 30 casi di carcinoma in situ e 30 casi di SCCs. CAF-1/p60 è iperespressa nelle cellule neoplastiche; l'espressione di CAF-1/p60 aumenta significativamente in correlazione all'alto indice di replicazione cellulare; inoltre la sua iperespressione moderata-severa è correlata con una prognosi peggiore comparata con la lieve e l'iperespressione della proteina CAF-1/p60 è correlata con un più alto rischio di morbilità e mortalità rappresentando un reale indice prognostico delle neoplasie laringee. CAF-1/p60 assume, pertanto, un valore prognostico indipendente nelle neoplasie laringe. Riteniamo, dunque, che l'iperespressione della proteina CAF-1/p60 che possa essere usata come indicatore di aggressività nell'evoluzione maligna specie dei carcinomi in situ ed impiegata nel follow-up per identificare le forme a più alto rischio prognostico che necessitano quindi controlli più ravvicinati nel tempo.

PAROLE CHIAVE: *Proteina CAF-1/p60 • Fattori prognostici • Carcinoma della laringe • Carcinoma in situ • Displasie laringee • Lesioni precancerose • Marker tumorali*

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Introduction

Laryngeal carcinoma (LC) represents one of the most common head and neck cancers and accounts for approximately 5.1% of all tumours in more developed areas and 3.5% in less developed areas, worldwide [1 2]. The estimated incidence of laryngeal cancer in 2012 is about 1.1% of all cancers, with nearly 157, 000 new cases in 2012 [3].

Currently, contrary to other anatomic districts (breast, colon etc.), for LC there are no specific and sensitive markers that can be used for early diagnosis and follow-up, beyond the available prognostic parameters [4 5]. Cell proliferative activity has been extensively investigated in head and neck tumours, including LC**,** as useful diagnostic and prognostic marker; however, its specific role has yet to be definitively established.

Over the last decade, it has been demonstrated that in head and neck carcinogenesis, the transition from normal epithelium to pre-malignancy, and finally to carcinoma is related to the accumulation of genetic and epigenetic alterations, is a multistep process. The most important epigenetic alterations include DNA methylation, histone modifications and RNA-mediated silencing. Chromatin assembly and remodelling is strictly regulated by histone chaperones. Chromatin assembly factor-1 (CAF-1), a histone chaperone, is an heterotrimeric protein complex formed of three subunits (p48, p60 and p150) [6 7]. CAF-1/p60 has recently been proposed as a new sensible proliferation marker in malignant tumours [8 9]. In particular, CAF-1/p60 is down regulated in quiescent cells, whereas it is overexpressed in hyper-proliferating and neoplastic cells [10].

CAF-1 plays a crucial role in the assembly and repositioning of nucleosomes on newly synthesised DNA, regulating replication processes and DNA repair [10-14]. The p150 subunit appears to be more active in repair processes, while CAF-1/p60 is more specifically connected to controlling cell replication. The p48 subunit intervenes on acetylation/deacetylation of histones by specific protein complexes [15-17].

In detail, CAF-1 mediates the epigenetic regulation of the state of chromatin aggregation, contributing to maintenance of chromosome structure before and after the formation of hairpin replication and appears to be involved in the transient destabilisation of nucleosomes required for the progression of hairpin replication.

CAF-1 has a typical assembly action, since it assembles only replication DNA. This is due to the fact that its activity necessarily requires interaction with the proliferating cell nuclear antigen (PCNA), which, as is known, specifically marks the newly synthesised DNA and is involved in the processes of replication, repair, recombination, repair of single strand breaks (SSB) and sister chromatid cohesion [18-22]. Ultimately, CAF-1 plays a critical role in maintaining the stability of chromatin during DNA replication and deregulation of this control mechanism can cause an uncontrolled proliferation, resulting in cancer [10 17 18].

In this study, we investigated the expression of CAF-1/p60 in laryngeal carcinomas to determine whether this protein could represent a reliable biological marker in evaluating tumour behaviour. Finally, we explored the possibility that this protein may represent a promising novel chance to plan for more concise follow-up, type of re-operation, or alternative treatment.

Materials and methods

Patients

The study population was selected from patients treated at the Otolaryngology, Head and Neck Surgery Department of the Federico II University, Naples, Italy, from 1 January 2000 to 31 December 2014. Inclusion criteria were:

- patients with laryngeal dysplastic precancerous lesions and laryngeal carcinoma in situ, treated surgically by excision biopsy or cordectomy (Type I, II, III, IV and V according to the classification of the European Laryngological Society, 2000) with the aid of CO_2 laser in accordance with the World Health Organization classification system (WHO) 2005 [23];
- patients with early laryngeal infiltrating neoplasm (squamous cell carcinoma) treated surgically with supraglottic, subtotal or total laryngectomy;
- post-operative patients during oncological follow-up in a specialised laboratory at our clinic, with ENT specialist examinations by direct fibre optic laryngoscopy through video recording and in accordance with the timetable guidelines for each dysplastic lesion or tumour stage;
- the same selected series of patients had been evaluated for CAF-1/ p60 by immunohistochemistry (Pathology section of the Department of Advanced Biomedical Sciences, Federico II University of Naples).

According to these inclusion criteria, the study population consists of 90 cases of laryngeal lesions. We divided this population into three subgroups:

a. moderate and/ or severe dysplasia: 30 cases;
b. carcinoma in situ: 30 cases;
c. infiltrating cancer: 30 cases.

The population of subgroup B is composed of all consecutive cases with definitive diagnosis of carcinoma in situ (diagnosis relatively less frequent) from 1 January 2000 to 31 December 2014. To highlight the features of CAF-1/p60 expression in carcinoma in situ, this subgroup was compared to the same number of consecutive cases of dysplasia (subgroup A) and infiltrating carcinomas (subgroup C) of the larynx, from a larger series, and that respected the following inclusion criteria:

- smoking more than 20 cigarettes per day for over 20 years and who continue to smoke upon diagnosis;
- negative anamnesis for alcohol abuse;
- negative anamnesis for exposure to environmental risk factors in the work place;
- absence of any clinical and anamnestic indirect signs of gastroesophageal reflux.

Formalin-fixed paraffin-embedded blocks were selected from the archive of the Department of Advanced Biomedical Sciences, Pathology Section, Federico II University II of Naples.

For each case, paraffin blocks containing tumour areas representative of the lesion were stained for CAF-1/p60 immunohistochemical expression by comparing A, B and C subgroups.

The study was performed in accordance with the guidelines of the Institutional Ethics Committee, Italian law, and the Declaration of Helsinki, as required for studies based on retrospective analyses on routine archival formalin-fixed, paraffin-embedded tissue. All patients provided written informed consent regarding use of data.

Immunohistochemistry

For each case, 4-µm-thick serial sections were cut and mounted on poly-L-lysine coated glass slides. Deparaffinised sections of all cases were boiled three times for 3 min in 1 mM sodium citrate buffer (pH 6.0) for antigen retrieval. In order to prevent the non-specific binding of the antibody, sections were pre-incubated with non-immune mouse serum (1:20, Dakopatts, Hamburg, Germany) diluted in PBS/BSA, 1%, for 25 min, at room temperature. After quenching of endogenous peroxidase activity was blocked by the incubation in 3% hydrogen peroxide for 30 min., followed by two rinses with Tris-HCl buffer, sections were incubated overnight at 4°C with the anti-CAF-1/p60 antibody (SS53 - ab8133, Abcam, Cambridge, MA, USA), diluted 1:300.

The standard streptavidin-biotin-peroxidase complex technique was performed, using sequential 30-min incubation with biotin-labelled secondary antibody and with peroxidase-labelled streptavidin for 30 min (DAKO LSAB kit HRP, Carpinteria, CA). For development of peroxidase activity, 3,3'-diaminobenzidine (DAB, Vector Laboratories, Burlingame, USA) was used as a substrate chromogen solution. Haematoxylin was used for nuclear counterstaining; sections were then mounted and cover-slipped with a synthetic mounting medium (Entellan, Merck, Germany). For each staining, sections from breast cancer were used as positive controls and for negative controls the sections were incubated with pre-immune serum instead of primary antibody. Only cells with definite brown nuclear staining were judged positive.

The expression of CAF-1/p60 was then rated semi-quantitatively according to an arbitrary scale, as follows: 0 (< 10% of positive cells); + (10% - < 20%); ++ (20% - < 30%); +++ (> 30% of positive cells) [24].

Statistical analysis

Statistical analysis was performed using Med-Calc (version 9.3.7.0), comparing the expressiveness of CAF-1/p60 between the A-B-C groups using the Wilcoxon/Mann-Whitney test for independent and non-parametric variables. According to literature, we assigned numeric values between 0 and 3 to different degrees of CAF-1/p60 expression (negative: 0; mild expression +: 1; moderate expressivity + + : 2 ; severe expressivity + + +: 3). We applied the Kaplan-Meier method, normalising the different categories by the log-rank Mantel-Haenszel test to compare overall and specific disease survival. A Cox proportional hazard model was used to assess the simultaneous contribution of multiple factors to the risk mortality. We performed multivariate Cox regression analysis for significant variables found in univariate analyses (overexpression, staging, grading, treatment strategy, progress and distant metastasis) to underline HR of patients with moderate-severe and severe CAF-1/p60 expression. In each test, a p value < *0.05* was considered statistically significant.

Results

The study population consists of 90 patients, 85 men and 5 women. The median age was 71 years (range, 37-86 years). Follow-up was performed in all patients (median: 33 months; range: 9-104 months).

Dysplasia

Patients with moderate and/or severe dysplasia showed a mild (+) expression of CAF-1/p60 in all cases evaluated (100%) (Fig. 1).

All patients underwent regular oncological follow-up at our clinic. During follow-up, direct laryngoscopy in 2 patients highlighted an area of suspected recurrence. These patients underwent a surgical procedure for enlargement of the previous excision (cordectomy type II vs. cordectomy type I). The histological examination relating to this procedure indicated mild dysplasia. Survival with organ and function preservation in such cases is 100% (Table I).

Carcinoma in situ

All malignant tumours showed a CAF-1/p60 overexpression (19 cases +, one case ++, 4 cases ++/ +++, 6 cases +++) (Fig. 1). During follow-up, 2 patients (6.6 %) died. The first of laryngeal carcinoma in situ (mild CAF-1/p60 expression) showed a metachronous oesophageal cancer and died of disease. In the second case, characterised by strong expression (+++) of CAF-1/p60, the death was due to recurrence and progression of disease. Twenty-eight patients (93.4%) continued oncological follow-up;

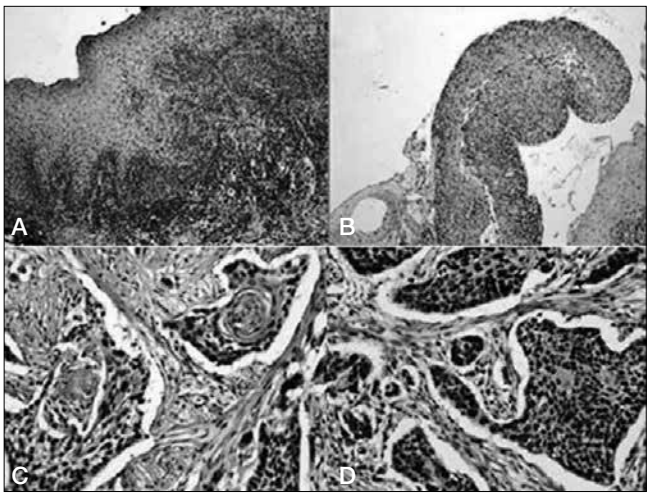

Fig. 1. A) Laryngeal mucosa with moderat-severe dysplasia and with mild (+) expression of CAF-1/p60; B) Laryngeal mucosa with cancer in situ and with severe (+++) expression of CAF-1/p60; C- D) High expression (+++) of CAF-1/ p60 in moderately differentiated cancer (G2) and in undifferentiated cancer (G3).

Table I. Dysplasia; correlation of CAF-1/p60 expression with surgical treatment and follow-up.

Case	Histological diagnosis	CAF1/P60	First treatment	F-UP	Second treatment	F-UP
18	Dysplasia	+	Cordectomy type I	Normal	-	-
10	Dysplasia	+	Cordectomy type II	Normal	-	-
2	Dysplasia	+	Cordectomy type I	Relapse	Cordectomy type II	Normal

in 19 patients, the follow-up was regular without the need for further treatment (CAF-1/p60 score was + in 17 of 19 cases and ++ and ++/+++ in 2 cases). Of the remaining 9 patients whose direct laryngoscopy revealed an area of suspected relapse, 2 (CAF-1/p60 score +++) underwent excisional biopsy, with histological examination revealing varying degrees of dysplasia, while the remaining 5 (CAF-1/p60 score, respectively, one case +, one case ++/+++, 3 cases +++) underwent a second surgical procedure to enlarge the previous excision because of the location of the lesion (anterior) and positive involvement of the resection margins. Histology revealed a recurrent carcinoma in situ. Only 2 cases (CAF-1/p60 score ++/+++) underwent laryngectomy (total and supraglottic) for laryngeal disease progression. Specific survival with organ and function preservation in such cases was 96.6% considering that one patient did not die of laryngeal disease (Table II).

Infiltrating carcinoma

Patients with invasive squamous cell carcinoma were treated surgically by laryngectomy. In 18 patients (60%) the tumour was glottic, in 9 patients (30%) supraglottic and 3 patients (10%) had subglottic extension. The grading of the neoplasm was moderately differentiated (G2) in 10 patients (33.3%), from moderately to poorly differentiated (G2-G3) in 9 patients (30 %) and undifferentiated (G3) in 11 patients (36.6%).

Among the cancers with an intermediate degree of differentiation (G2), 6 of 10 cases showed a moderate expression of CAF-1/p60 (++) and continued with regular follow-up; one patient died of disease (++), one case (++/+++) died of disease. The remaining 2 cases who had metastases at follow-up and died of the disease, showed a score of +++. All moderately to poorly differentiated (G2/G3) squamous cell carcinomas showed CAF-1/p60 moderate (++) staining in 3 cases, moderate-high (++/+++) in

Table II. Cancer in situ; correlation of CAF-1/p60 expression with surgical treatment and follow-up.

Cases	Histological diagnosis	CAF-1/P60	First treatment	Follow-up	Second treatment	Follow-up
3	Ca in situ	+	Cordectomy type I	Normal	-	-
5	Ca in situ	+	Cordectomy type II	Normal	-	-
4	Ca in situ	+	Cordectomy type III	Normal	-	-
4	Ca in situ	+	Cordectomy Type IV	Normal	-	-
1	Ca in situ	+	Cordectomy type Va	Normal	-	-
1	Ca in situ	+	Cordectomy type II	Relapse ca in situ	Cordectomy type IV	Normal
1	Ca in situ	+	Cordectomy type IV	† for esophagus metachronous cancer	-	-
1	Ca in situ	++	Cordectomy type III	Normal	-	-
1	Ca in situ	++/+++	Cordectomy type III	Normal	-	-
1	Ca in situ	++/+++	Cordectomy type III	Progression of disease	TL	Normal
1	Ca in situ	++/+++	Cordectomy type IV	Relapse ca in situ	Cordectomy type Va	Normal
1	Ca in situ	++/+++	Cordectomy type Vd	Progression of disease	SGPL	Normal
1	Ca in situ	+++	Cordectomy type III	Suspect of relapse	Excisional biopsy (grave dysplasia)	4 biopsy (light dysplasia)
1	Ca in situ	+++	Cordectomy type IV	Relapse ca in situ	Cordectomy type Va	Normal
2	Ca in situ	+++	Cordectomy type Va	Relapse ca in situ	Cordectomy type Vd	Normal
1	Ca in situ	+++	Cordectomy type Va	Suspect of relapse	Excisional biopsy (light dysplasia)	Normal
1	Ca in situ	+++	Cordectomy type Vd	† for progression of disease	-	-

† death
TL = total laryngectomy
SGPL = Supraglottic partial laryngectomy

3 cases and high (+++) in the remaining 3 cases. Three cases developed metastases during follow-up (2+++ and 1 ++/+++). In poorly-differentiated squamous cell carcinomas (G3), the CAF-1/p60 was expressed at moderate levels (score ++) in 5 cases, and high levels (+++) in 6 cases; 3 patients (2+++ and 1 ++) presented metastases during follow-up (*Figure 1*).

Post-operative patients received oncology counselling and radiotherapy and 22 patients (73.3%) received adjuvant chemotherapy and/or radiotherapy. During follow-up at our dedicated clinic, 10 patients (33.3 %) died of disease (CAF-1/p60 score ++ in 3 cases, ++/+++ in one case and +++ in 6 cases), 8 of whom presented distant metastasis (CAF-1/p60 score +++ in 6 cases, , ++/+++ in one case and ++ in one case). Five patients died of other diseases (CAF-1/p60 score ++ in 2 cases, ++/+++ in 2 cases and +++ in one case) and the remaining 15 patients had follow-up free of disease. Survival in such cases was 66.6% considering that five patients did not die of laryngeal disease (Table III).

Statistical analysis

Statistical analysis for non-parametric variables showed a $p = 0.0015$ in comparing group A, dysplasia, with group B, carcinomas in situ of the larynx (statistically significant). Comparison between group A, dysplasia, and group C, infiltrating tumours, showed a $p < 0.0001$ (highly statistically significant). Comparison between group B, carcinoma in situ, and group C, infiltrating tumours, revealed a $p = 0.0008$ (statistically significant). Analysis of the survival rate in the study population showed a statistically significant *p value* ($p = 0.0178$), which became more significant in specific disease survival ($p = 0.0070$) with a cumulative survival probability of 100% within 9 months of diagnosis in patients with severe expressiveness of the CAF-1/p60 protein (+++); 100% within 16 months of diagnosis in patients with moderate-severe expressiveness of CAF-1/p60 (++/+++); 100% within 32 months of diagnosis in patients with moderate expressiveness (++) of the p60 protein. No patient with mild expressiveness died of laryngeal cancer. We deduce that the probability of an adverse event (metastasis-death) is greater in cases with severe expression (score +++) of the p60 protein (Fig. 2). A Cox proportional hazards model was used to verify whether the overexpression of the p60 protein and other variables are independent prognostic factors for LSCC patients. Univariate analysis showed that overexpression, staging, grading, treatment strategy, progress and distant metastasis were associated with survival in patients with LSCC. Multivariate analysis of the same variables showed that those variables were independent prognostic factors for patients with LSCC, and overexpression of the p60 protein was significantly associated with poor prognosis.

Table III. Infiltrating cancer; correlation of CAF-1/p60 expression with surgical treatment and follow-up.

Case	Histological diagnosis	Grading/ staging	CAF-1 /P60	Surgical treatment	F-UP
1	LSCC	II/ G2	+++	SCPL	† - M+
2	LSCC	II/ G2	++	TL	Normal
3	LSCC	III/ G2-G3	++	SCPL	Normal
4	LSCC	III/ G2- G3	++	SGPL	†
5	LSCC	IVa/ G2	+++	SCPL	† - M+
6	LSCC	IVa/ G2	++	SCPL	Normal
7	LSCC	IVa/ G2- G3	++	SCPL	Normal
8	LSCC	IVa/ G3	++	SGPL	† for other disease
9	LSCC	IVa/ G3	++	SGPL	Normal
10	LSCC	IVa/ G3	++	SCPL	† - M+
11	LSCC	IVa/ G3	++	TL	† for other disease
12	LSCC	II/G2	++/+++	SCPL	† for other disease
13	LSCC	IVa/G2- G3	++/+++	SCPL	† for other disease
14	LSCC	II/ G2	++	SGPL	Normal
15	LSCC	III/ G3	++	SCPL	Normal
16	LSCC	IVa/ G2- G3	+++	SGPL	† - M+
17	LSCC	III/ G2	++	SGPL	Normal
18	LSCC	IVa/ G3	+++	SCPL	† - M+
19	LSCC	IVa/ G3	+++	TL	† - M+
20	LSCC	IVa/ G2	++	SGPL	Normal
21	LSCC	IVa/ G3	+++	SCPL	Normal
22	LSCC	III/ G2- G3	+++	SGPL	† for other disease
23	LSCC	IVa/ G2	++	SCPL	†
24	LSCC	IVa/ G3	+++	SCPL	Normal
25	LSCC	IVa/ G3	+++	TL	Normal
26	LSCC	IVa/ G2- G3	++/ +++	SCPL	† - M+
27	LSCC	II/ G2	++	SCPL	Normal
28	LSCC	IVa/ G3	+++	SCPL	Normal
29	LSCC	IVa/ G2- G3	++/+++	SCPL	Normal
30	LSCC	IVa/ G2- G3	+++	TL	† - M+

Abbreviations
LSCC: squamous cell carcinomas of the larynx; SCPL: supracricoid partial laryngectomy; SGPL: supraglottic partial laryngectomy; TL: total laryngectomy; † death; M+ metastasis

Discussion

Knowledge of the multistep laryngeal carcinogenesis mechanism is a prerequisite for the development of cancer prevention and treatment strategies. CAF1/p60 has been proposed as a new proliferation and prognostic marker in a series of different malignant tumours. The expression of the p60 subunit is particularly high in neoplasms with increased cellular proliferation: while CAF-1/p60 is down-regulated in resting cells, it is greatly overexpressed in neoplastic cells [8]. Moreover, the dissociation of CAF-1/p60 from hair-

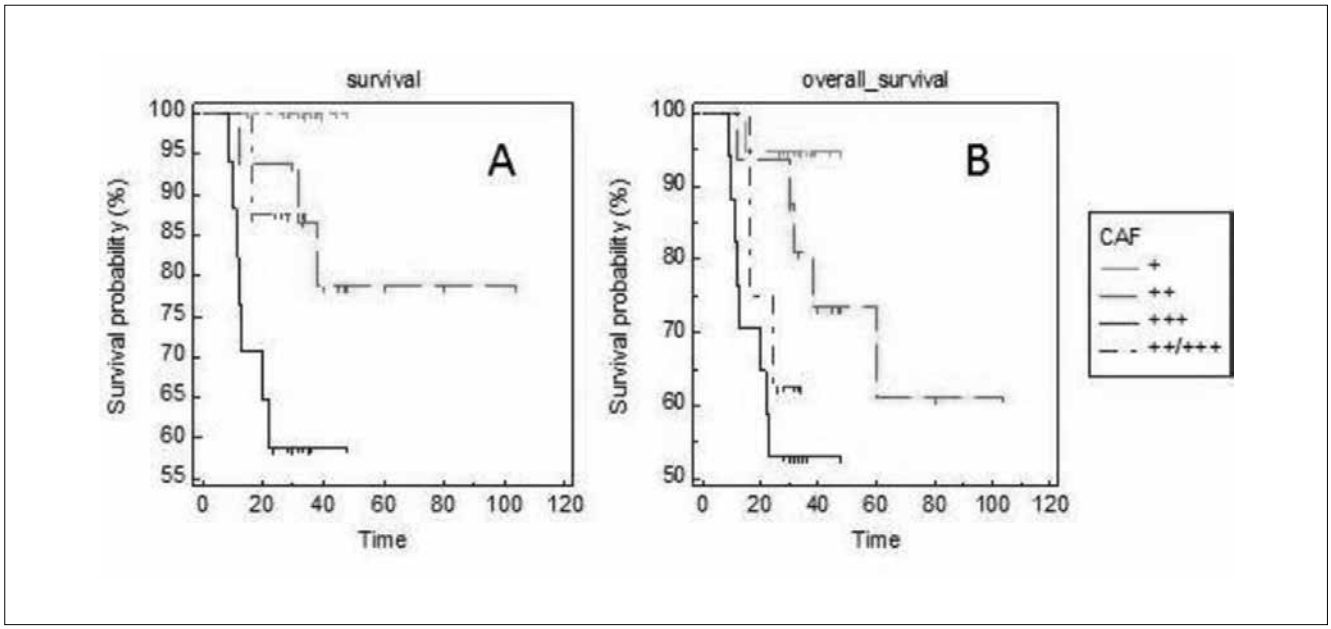

Fig. 2. Analysis of survival; A) disease-specific survival and B) overall survival.

pin replication causes the suspension of its activity in the nucleosomes assembly, with the arrest of hairpin replication, mitosis blocking during the cellular cycle and global alterations of chromatin in the S phase [25]. In addition to being a reliable marker of cellular proliferation, CAF-1/p60 also assumes a relevant value as reliable marker of tumour progression and as a predictor of biological behaviour [26 27].

In fact, several recent reports have highlighted the strict relationship between CAF-1/p60 over-expression and adverse behaviour of different malignant tumours, including prostate, tongue, salivary glands carcinomas and cutaneous melanoma [28-31]. These studies have confirmed the hypothesis that CAF-1/p60 is heavily involved in tumoural and metastatic processes, constituting an independent prognostic biomarker of tumour evolution [8 25 32-34].

Our results indicate that the levels of expression of the CAF-1/p60 assume a significant ($p < 0.05$) value for moderate/severe dysplastic lesions of the larynx compared with carcinomas in situ and when comparing both dysplasia and carcinomas in situ with infiltrating carcinomas of the larynx. Analysis of the surgical procedure allows us to confirm that excisional biopsy is a valid tool in treating the dysplasia, decisive in 100% of cases, while for carcinomas in situ excisional biopsy was the sole treatment performed in 19 patients, while a second surgical treatment was required in 9 patients. CAF-1/p60 expression was mild in all evaluated epithelial laryngeal dysplasia (100%). With reference to the clinical behaviour of carcinoma in situ, we can assume that mild overexpression of CAF-1/p60 (mild +) is connected to a regular progress; in patients with a moderate to severe expression on the other hand there is an increase in repeat surgery and relapses. It is possible to speculate that in cases of carcinoma in situ in which p60 is overexpressed, close follow-up is required to assess possible recurrence as promptly as possible and plan appropriate treatment. In infiltrating tumours of the larynx, p60 protein expression is moderate to high. In these patients, there is an increase in mortality and recurrence in accordance with the standard parameters of neoplasia (staging, grading); however, overexpression of CAF-1/p60 appears to be related to an independent risk of mortality associated with distant metastases, which are not common in tumours of the larynx. In fact, in 8 patients with moderate to high expression, the course of the disease was ominous, with distant metastases leading

Covariates	b	SE	P	HR Exp(b)	95% CI of Exp(b)	p
Overexpression	0,9484	0,3264	0,003669	2,5815	1,3659 to 4,8788	P = 0,0009
Staging	0,8045	0,2979	0,006912	2,2357	1,2507 to 3,9963	P = 0,0018
Grading	0,5950	0,2496	0,01711	1,8130	1,1145 to 2,9495	P = 0,0155
Treatment	1,0729	0,3301	0,001153	2,9239	1,5361 to 5,5655	P = 0,0001
Progress	2,8641	0,8813	0,001155	17,5332	3,1441 to 97,7750	P < 0,0001
Distant metastasis	2,6816	0,8430	0,001468	14,6078	2,8228 to 75,5952	P < 0,0001

Fig. 3. Univariate analyses of overexpression, staging, grading, treatment strategy, progression and distant metastases.

Covariates	b	SE	P	HR Exp(b)	95% CI of Exp(b)
Overexpression	1,1920	0,6607	0,07123	3,2936	0,9080 to 11,9462
Staging	-0,4976	0,5706	0,3832	0,6080	0,1998 to 1,8499
Grading	2,3724	0,9982	0,01747	10,7233	1,5311 to 75,1051
Treatment	-2,1509	0,9578	0,02472	0,1164	0,0180 to 0,7533
Progress	-8,0267	213,0050	0,9699	0,0003	0,0000 to 799E+174
Distant metastasis	12,0239	212,9991	0,9550	166695,1628	0,0000 to 403E+183

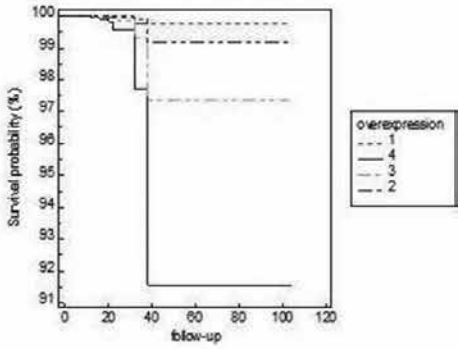

Fig. 4. Multivariate Cox regression analysis.

to death of both patients. This suggests that the overexpression of CAF can identify the subset of tumours with a more aggressive biological from among those having equivalent staging and grading.

In 36.6% of patients (11 cases) with carcinoma in situ, immunopositivity was moderate to high. In 9 patients, further surgery was required (5 cases of recurrence of carcinoma in situ, one case of laryngectomy and one patient death due to recurrence and progression of laryngeal cancer). The highest expression of protein characterised cases of metastatic SCC and the majority of carcinoma in situ (all except one) with a history of progressive disease (recurrence and/ or death due to disease). This clinical behaviour (need for further surgery, relapse and progression) highlights the need for a tighter follow-up timetable as a marker for such carcinoma in situ patients.

Analyses of survival indicate an increased risk of recurrence and mortality concurrent with the parameters assessed by univariate analyses that assume a predictive and statistical significance in each case (Fig. 3). Multivariate analysis of these variables showed that staging, grading, treatment strategy, progress, distant metastasis are all independent prognostic factors for patients with LSCCs, and overexpression of CAF-1/p60 protein is significantly associated with poor prognosis.

Multivariate analysis correlating risk of overexpression, considering the prognostic factors examined in the univariate analyse shows an increase in HR (2.58 versus 3.29) compared to univariate analysis with a $p < 0.001$ (Fig. 4). Also worthy of note is the HR of the distant metastases, an expression of the aggressiveness of the disease.

Conclusions

Our results show that CAF-1/p60 in an independent prognostic factor that may better predict the biological behaviour of LSCC associated with traditional prognostic features, but this exciting hypothesis needs to be validated by a larger and more representative series of cases. If confirmed, we may adopt a more pronounced oncological follow-up protocol for the subset of patients with carcinoma in situ overexpressing CAF-1/p60 and adjuvant therapy programmes in infiltrating ones.

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Dysphagia

Dysphagia screening in subacute care settings using the Italian version of the Royal Brisbane and Women's Hospital (I-RBWH) dysphagia screening tool

Screening della disfagia nelle unità di cure sub-acute utilizzando la versione italiana del Royal Brisbane and Women's Hospital (I-RBWH) dysphagia screening tool

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SUMMARY

The large majority of the available dysphagia screening tools has been developed for the stroke population. Only few screening tools are suitable for heterogeneous groups of patients admitted to a subacute care unit. The Royal Brisbane and Women's Hospital (RBWH) dysphagia screening tool is a nurse-administered, evidence-based swallow screening tool for generic acute hospital use that demonstrates excellent sensitivity and specificity. No Italian version of this tool is available to date. The aim of this study was to determine the reliability and screening accuracy of the Italian version of the RBWH (I-RBWH) dysphagia screening tool. A total of 105 patients consecutively admitted to a subacute care unit were enrolled. Using the I-RBWH tool, each patient was evaluated twice by trained nurses and once by a speech and language pathologist (SLP) blind to nurses' scores. The SLP also performed standardised clinical assessment of swallowing using the Mann assessment of swallowing ability (MASA). During the first and the second administration of the I-RBWH by nurses, 28 and 27 patients, respectively, were considered at risk of dysphagia, and 27 were considered at risk after SLP assessment. Intra- and inter-rater reliability was satisfactory. Comparison between nurse I-RBWH scores and MASA examination demonstrated a sensitivity and specificity of the I-RBWH dysphagia screening tool up to 93% and 96%, respectively; the positive and negative predictive values were 90% and 97%, respectively. Thus, the current findings support the reliability and accuracy of the I-RBWH tool for dysphagia screening of patients in subacute settings. Its application in clinical practice is recommended.

KEY WORDS: RBWH, Dysphagia • Screening • Subacute care unit

RIASSUNTO

La maggior parte dei test di screening per la disfagia è stato sviluppato per essere utilizzato in pazienti con stroke. Solo pochi strumenti risultano applicabili a popolazioni più eterogenee di pazienti, come quelli ricoverati nelle unità di cure per Sub-acuti. Tra questi, il Royal Brisbane and Women's Hospital (RBWH) dysphagia screening tool è stato concepito per essere utilizzato da personale infermieristico e possiede un'eccellente sensibilità e specificità. Al momento non è disponibile una versione italiana di questo strumento. Scopo del lavoro è di analizzare l'affidabilità e l'accuratezza nello screening della versione italiana del RBWH (I-RBWH) dysphagia screening tool. A tal fine sono stati arruolati 105 pazienti, tutti ricoverati presso l'unità di cure Sub-acute. Ogni paziente è stato valutato con il I-RBWH dysphagia screening tool da personale infermieristico (per due volte) e da una logopedista. Quest'ultima, non solo era all'oscuro dei risultati ottenuti durante la valutazione infermieristica, ma ha anche eseguito una valutazione standardizzata delle abilità deglutitorie utilizzando il Mann Assessment of Swallowing ability (MASA). L'affidabilità intra- e inter-rater si sono rilevate soddisfacenti. Il confronto tra i risultati ottenuti dal personale infermieristico durante la somministrazione del I-RBWH e i punteggi del MASA hanno dimostrato un'eccellente sensibilità (93%), specificità (96%), valore predittivo positivo (90%) e valore predittivo negativo (97%). Questi dati supportano l'affidabilità e l'accuratezza del I-RBWH dysphagia screening tool nello screening della disfagia nei pazienti ricoverati nelle unità di cure per Sub-acuti. Il suo utilizzo in clinica è pertanto raccomandabile.

PAROLE CHIAVE: *RBWH • Disfagia • Screening • Unità di cure per sub acuti*

Introduction

The estimated incidence of dysphagia in hospitalised patients ranges between 15% and 30% [1 2]. These findings are not surprising since the prevalence of dysphagia is high in common diseases – it exceeds 50% in stroke patients, is present in 10-30% of individuals older than 65 years [3] and may be as high as 84% in patients with Parkinson's disease [4]. Dysphagia limits the safe ingestion of adequate amounts of food and liquids thus placing the patient at increased risk for poor nutrition, dehydration, aspiration pneumonia and morbidity in general [5]. In addition, patients affected by dysphagia experience an increased risk of hospital admission, delayed discharge and dependence on health services (e.g. increased nursing time and physician consultations) thereby increasing health care costs [6]. For these reasons, early identification of dysphagia is mandatory since it can reduce the incidence of clinical complications and may improve outcomes in these patients [7-9]. Even if clinical and instrumental assessment using either videofluoroscopic swallowing study (VFSS) or fibre optic endoscopic evaluation of swallowing (FEES) is considered the "gold standard" for identification of swallowing dysfunctions [10-12], the approach has some limitations. Instrumental assessment is usually not available on a 24-hour basis and not all patients can be carried to the radiology department and correctly positioned, even with specially adapted chairs [13 14]. As a consequence, it appears difficult to assure a well-timed screening of dysphagia, especially outside standard working hours on weekdays. Moreover, instrumental assessment is usually not available at patient admission. In order to take decisions on feeding for newly admitted patients and to reduce the number of patients requiring VFSS or FEES and prevent dysphagia related complications, several dysphagia screening tools have been proposed [2 15-23].

The large majority of dysphagia screening tools have been developed for the stroke population and consequently cannot be readily used for a more heterogeneous population, such as that usually found in acute care settings, where more than half of the population presents a swallowing disorder due to a disease different from stroke [24]. Only few screening tools for patients with different diagnoses are available [17 20 21 23 25]. Unfortunately, these latter are too time-consuming to be considered acceptable for a screening assessment where the cost of the test and the time taken to administer it is fundamental [2 26]. The screening tool developed by Logemann et al. [17], in fact, is rather long as it includes 28 items in 5 categories: (1) 4 items on medical history variables; (2) 6 items on behavioural variables; (3) 2 items on gross motor variables; (4) 9 items on observations from oromotor testing; and (5) 7 items on observations during trial swallows. On the other hand, the screening tool developed by Stewart [20] focuses only on patients with intellectual disabilities. The volume-viscosity swallow test for clinical screening of dysphagia and aspiration developed by Rofes et al. [23] appears to be too time-consuming and complex; finally, in the Yale Swallow protocol developed by Suiter et al. [25] no exclusion criteria, with the exception of the absence of tracheostomy tube, were established. Therefore, while it is applicable to large population, a triaging approach is needed to reduce the number of patients who are screened by a water swallow test.

To overcome these difficulties, the Royal Brisbane and Women Hospital (RBWH) dysphagia screening tool has recently been developed [2]. This latter is a nurse-administered, evidence-based swallow screening tool for generic acute hospital use based on the triaging concept. It consists of three steps: 1. a two-phase question screen; 2. a water swallow test, as appropriate; 3. a swallowing management plan. The two-phase question screen reflects the perception that identification of 'at-risk' patients should come from a combination of (1) previous medical history/records and (2) specific clinical indicators. Phase 1 of the screening tool uses evidence-based medical diagnoses known to have a high association with dysphagia and risk for aspiration, such as stroke, neurological involvement, head injury, or head and neck surgery [18 27]. A negative indicator for any of the medical diagnoses allows the patient to start a general diet with liquids, while a positive indicator prompts the nurse to complete phase 2. Phase 2 gathers from the patient's (or their family/carer's) information specifically related to feeding and swallowing. If any of the phase 2 dysphagia indicators are present, the patient is placed nil by mouth (NBM) and referred for formal dysphagia assessment. Individuals without phase 2 dysphagia indicators proceed to step 2 and are administered a water swallow test with 90 ml of water. The nurse is prompted to observe for (1) coughing during or between swallows or up to one minute after swallowing, (2) wet or "gurgly" voice quality post-swallow and (3) increased respiratory rate post-swallow [2 16]. Depending on the results of the screening evaluation, patients with a positive result in the dysphagia screening examination are referred to a SLP.

In the original study by Cichero et al. [2], the RBHW dysphagia screening tool demonstrated a sensitivity of 95%, a specificity of 97%, a positive predictive value of 92% and a negative predictive value of 98% compared to formal clinical assessment. Nurse compliance with administering the tool on each new admission was 83%. In addition, the authors speculated that the use of the RBHW dysphagia screening tool may improve the quality of care and save healthcare costs. However, one of the major limitations of the original study lies in the lack of blinding, and consequently the interpretation of the screening results needs caution.

The aim of this study was to determine the reliability and screening accuracy of the Italian version of the Royal Brisbane and Women's Hospital (I-RBWH) dysphagia screen-

ing tool using a blinded format between nurse screening and clinician assessment. The underlying hypothesis is that the I-RBWH is both reliable and accurate for screening dysphagia in subacute care settings. The importance of the study lies in the need for a simple, quick and accurate dysphagia screening tool to be applied in everyday clinical practice for early recognition and management of manage dysphagia in a heterogeneous population.

Materials and methods

The study consisted of 4 phases: item generation (phase 1), nurse training (phase 2), reliability analysis (phase 3) and screening accuracy analysis (phase 4). All data were collected prospectively and each subject enrolled in the study provided written informed consent. Only patients admitted to the Subacute Care Unit of our hospital in the period between 1 May 2015 and 30 September 2015 were included. The Subacute Care Unit is a comprehensive inpatient care unit, designed for patients who have an acute illness, injury, or exacerbation of a disease process; it is a goal oriented treatment rendered immediately after, or instead of, acute hospitalisation to treat one or more specific active complex medical conditions or to administer one or more technically complex treatments, in the context of a person's underlying long-term condition and overall situation.

The study was carried out according to the Declaration of Helsinki. The study design was approved by the Institutional Review Boards of our hospital.

I-RBWH item generation (phase 1)

Cross-cultural adaptation of the RBWH dysphagia screening tool was performed using standard techniques [28 29]; the authors of the RBWH were contacted and permission was obtained to start the project. Items of the original RBWH dysphagia screening tool were translated into Italian by one professional translator and two bilingual investigators. Two independent phoneticians familiar with the process of instrument validation examined semantic, idiomatic and conceptual issues, and further refined these versions. A final consensus version was obtained and given to two professional translators to produce a literal translation into English. The two translators and an expert committee synthesised the results of the translations in an English back translated version that was compared with the original one to check that they had the same semantic value in order to obtain the final version of the I-RBWH dysphagia screening tool.

Nurse training (phase 2)

Similar to the study of Cichero et al. [2] the training package consisted of a 30-minute presentation prepared by the hospital speech pathology department and carried out by one SLP. During training, information regarding anatomy and physiology of the normal swallowing process, dysphagia, safe swallowing strategies, swallowing assessment, importance of dysphagia screening, I-RBWH dysphagia screening tool structure and aims were provided. In order to assess the effectiveness of the training, a short 20 true/false questionnaire was provided before and after training. A pass rate of 80% in the total score of the questionnaire was set. Nurses who failed to meet these criteria underwent further training. All the eight nurses employed in the subacute care Unit of our hospital were enrolled.

Table I. Aetiological factors leading to subacute care unit admission in the cohort of patients (n = 105).

	Number	Percentage	Sex	
			M	F
Clinical consequences of infectious diseases	32	30%	16	16
Complications of surgical or medical treatments	26	25%	11	15
Cardiac failure	21	20%	11	10
Diabetes mellitus	9	8%	3	6
Other	17	16%	8	9

Reliability analysis (phase 3)

Clinical data were obtained from 105 consecutive patients (49 men and 56 women). The mean age of participants was 76.7 ± 7.5 years (range 34-94). The mean age of female patients was 77.3 ± 10.3 years (range 34-94), while male patients were aged 76.1 ± 10.9 years (range 43-93). Clinical conditions leading to admission to the subacute care Unit are reported in Table I; the large majority of enrolled patients presented with comorbidities.

To assess intra-rater reliability of the I-RBWH dysphagia screening tool each patient was assessed twice, at admission to the Unit and within 24 hours, by the same nurse. This interval period was selected because no substantial change was expected to take place in subjects' deglutition abilities within this period. While completing the second I-RBWH dysphagia screening tool, nurses did not have any chance to check the results obtained during the first evaluation. To assess inter-rater reliability, each patient was also evaluated by a SLP specialised in swallowing disorders within 24 hours from admission. The SLP administered the I-RBWH dysphagia screening tool, but differently from the study of Cichero et al. [2], the SLP was blind to the results obtained during nurses' administration.

Screening accuracy (phase 4)

As in the original study, for analysis of screening accuracy of the I-RBWH dysphagia screening tool, each patient was evaluated by a SLP using standardised clinical assessment of swallowing with the Mann assessment of swallowing ability (MASA), a dysphagia clinical assessment tool validated for stroke patients [30]. This latter scores

oral motor/sensory features of swallowing, patient cooperation and comprehension, dietary recommendations and predictive risk rating of swallowing integrity. The SLP administered both the I-RBWH and the MASA at the same time. The results of MASA assessment were dichotomised to distinguish between patients with and without dysphagia (≤ 178 and > 178 points respectively). The MASA scores and the results of the I-RBWH dysphagia screening tool obtained during the nurse administration were compared to evaluate the sensitivity and specificity ratings and positive and negative predictive values of the I-RBWH dysphagia screening tool.

Statistical analysis

Statistical tests were performed using SPSS 21.0 statistical software (SPSS, Inc., Chicago, IL). The effect of nurse training was evaluated using Wilcoxon signed-ranks test. Differences in the age of male and female patients were evaluated using the Mann-Whitney test. Kappa coefficient was used to evaluate intra- and inter-rater reliability of I-RBWH dysphagia screening tool. The screening accuracy was evaluated by assessing the sensitivity, specificity, positive predictive value and negative predictive value of the I-RBWH dysphagia screening tool by comparing its results with the MASA scores. For all comparisons, a p value < 0.05 was considered significant.

Results

As far as the feeding recommendation is concerned, of the 105 consecutive patients enrolled, at admission 98 were following a regular diet, 6 were fed through a nasogastric tube (NGT) and 1 had PEG in situ. No differences were found in the distribution of age between male and female patients on Mann-Whitney test ($p = 0.39$).

Nurse training (phase 2)

A total of 8 nurses, with a bachelor university degree in nursing, were trained. The mean pre-training score of the 20 items questionnaire was 14.4 ± 2.7 (range 12-17) of a total possible score of 20. The mean post-training score was 18.7 ± 2.5 (range 18-20). The improvement in the score obtained by nurses after the training was significant by Wilcoxon signed rank test ($Z = 4.102$; $p < 0.001$).

After the training session, none of the nurses failed the pass rate of 80% in the total score of the questionnaire.

Reliability analysis (phase 3)

All 105 patients were involved in reliability analysis. The first screening was administered by a nurse immediately at admission to the subacute care Unit. The same nurse managed to repeat the screening within 24 hours. The time between the first administration of the I-RBWH dysphagia screening tool and the SLP assessment was almost always within one to six hours. The compliance rate was 100% for both nurses and the SLP. As far as the nurse's first I-RBWH dysphagia screening tool administration is concerned, 28 individuals (27%) failed the screening and were consequently identified as "at risk" for dysphagia and aspiration. During the nurse's second administration of the I-RBWH screening tool, 27 patients failed the screening, while 78 passed it. The kappa coefficient demonstrated a significant agreement between test and re-test condition (kappa = 0.92; $p < 0.001$) thus demonstrating a strong intra-rater reliability.

As far as inter-rater reliability is concerned, the I-RBWH results obtained during SLP's and nurse's administration of the tool on the same patient were compared. The kappa coefficient demonstrated a significant agreement between nurse and SLP results of I-RBWH (kappa = 0.88; $p < 0.001$), thus demonstrating a strong inter-rater reliability.

Screening accuracy (phase 4)

All 105 enrolled patients were screened by a total of 8 trained nurses at admission. The first phase of the I-RBWH dysphagia screening tool took approximately two minutes to complete, while 5-7 minutes were necessary to complete the second phase (which included questions specifically related to feeding and swallowing) and the water swallow test.

In Figure 1 the number of patients who passed or failed each phase of the I-RBWH dysphagia screening tool at first assessment is reported. Seventy-eight patients failed

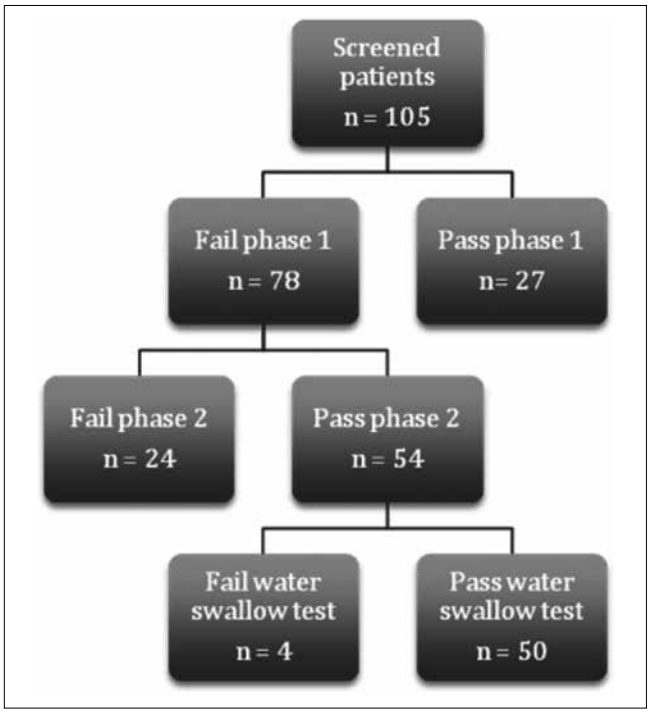

Fig. 1. Results of the first nurse administration of the I-RBWH dysphagia screening tool on 105 consecutive enrolled patients.

phase 1 of the I-RBWH dysphagia screening tool; the underlying causes of failure are reported in Table II. Twenty-four of the 78 patients who failed phase 1 also failed phase 2 and were placed on NBM. The remaining54 patients who failed phase 1 underwent a water swallow test; four failed the water swallow test and were placed on NBM.

Results of MASA evaluation performed by SLP showed that a total of 78 patients scored above 178 points (mean 194.7 ± 5.9; range 179-205) and were consequently considered not dysphagic. Twenty-seven patients scored equal or lower than 178 points (mean 154.2 ± 29.1; range 77-178) and were considered dysphagic. In order to evaluate the sensitivity, specificity, positive and negative predictive values for the dysphagia screening tool, nurse screening results were compared to MASA scores. Sensitivity rating for the I-RBWH dysphagia screening tool was 93%, while specificity was 96%. The positive predictive value was 90%, while the negative predictive value was 97% (Table III).

At the completion of the screen, nursing staff allocated 77 patients, who were found negative at I-RBWH screening, to an oral diet. Following SLP assessment, none of them was considered unsuitable for oral liquids. Fifty-six patients were confirmed on a general diet, while the remaining 21 required a modified texture diet because partially or fully edentulous. Twenty-eight patients failed the screening, were placed on NBM and referred to SLP: 21 required a modified texture diet, 6 were placed on NBM, while 1 was placed on regular diet (Table IV). Three of the 28 patients who failed the I-RBWH were not considered dysphagic at MASA: in one case the patient did not adequately manage oral secretions at nurse assessment, and were considered an appropriate over-referral; the remaining two cases were judging errors by nursing staff. Two of the 27 patients considered dysphagic at MASA passed the I-RBWH: in one case the nurse did not recognise the gurgly voice, and in the other case the nurse did not recognise the cough that occurred immediately after the swallow. In both cases, compensatory strategies by the SLP were sufficient to allow safe oral liquid administration.

Table II. Number of patients considered at risk of dysphagia during first phase of the I-RBWH dysphagia screening tool administered by nurses.

Aetiology identified as a risk factor for dysphagia	Number of patients
Dysphagia or aspiration on previous admission(s)	14
Chronic obstructive pulmonary disease ± upper gastrointestinal disorder	9
Cerebrovascular accident (stroke)	5
Neurological involvement	21
Head injury	1
Head and neck surgery	1
Chemotherapy/radiation to head and neck	1
Acutely unwell, frail aged with co-morbidities	18
Suspected aspiration pneumonia/recurrent chest infections	6
Severe disability (e.g. physical disability)	2
Total	78

Table III. Analysis of the I-RBWH dysphagia screening tool accuracy by comparing its results with MASA scores.

	MASA positive	MASA negative	Total
RBWH fail	25	3	28
RBWH pass	2	75	77
Total	27	78	105

Table IV. Feeding recommendations after SLP blind evaluation.

	Pass I-RBWH		Fail I-RBWH	
	N = 77	Percentage	N = 28	Percentage
Regular diet	56	74%	1	3%
Modified texture	21	26%	21	75%
NBM	0	0%	6	22%

Discussion

The RBWH dysphagia screening tool is unique since it uses diagnostic categories, patient self-report, nursing observation and – if applicable – the water swallow test. In the original study, high scores for sensitivity, specificity, positive and negative predictive values were found and the authors speculated that these results were related to the inclusion of all the above mentioned parameters in a single screening tool [2]. However, the lack of blinding between RBWH dysphagia screening tool scores and speech pathology clinical assessment of swallowing suggested caution in the interpretation of these results.

In the present study, the psychometric properties of the I-RBWH dysphagia screening tool were studied. The results showed strong intra- and inter-rater reliability and good screening accuracy. These results are similar to those of Cichero et al. [2] and further support the use of the I-RBWH screening tool in patients admitted to a subacute care unit. In addition, different from the original report, in the present study a blinding protocol for SLP evaluation was established, increasing confidence in the interpretation of the results.

Specific findings related to the I-RBWH dysphagia screening tool are noteworthy. In particular, the compliance rate was 100%. This high compliance rate may suggest that the nurses fully understood the importance of screening for dysphagia and consequently were more motivated in administration of the screening tool. It is possible that the nurses training program played an important role since information regarding swallowing assessment and importance of dysphagia screening were provided. In addition, it is possible to speculate that the I-RBWH dysphagia

screening tool is not a burdensome instrument and that it can be easily administered since the time required to complete the screening procedure never exceeded 9 minutes. Cichero et al. [2] reported a compliance rate of 84%. It is possible that the higher rate reported in the current study was related to the smaller number of nurses (n = 8) and hospital wards (n = 1) participating in the trial. In the original study, in fact, 2 general medical wards participated in the study, and a total of 38 nurses were trained.

As far as reliability of the I-RBWH dysphagia screening tool is concerned, both intra- and inter-rater reliability were analysed: the scores obtained support the idea that the I-RBWH has a high stability and reproducibility over time. In fact, the kappa coefficient for intra-rater reliability was 0.92, while that for inter-rater reliability was 0.88. No data regarding the reliability of the RBWH screening tool were provided in the original study [2].

In the current study, the sensitivity and specificity ratings for the I-RBWH were 93% and 96% respectively, while the positive predictive and negative predictive values were 90% and 97%, respectively, when the I-RBWH dysphagia screening tool scores were compared with MASA examination. These results appear slightly lower than those reported in the study of Cichero et al. [2]. However, it is possible that these differences are related to the lack of blinding of the original study. The SLPs who performed the MASA examination in the original study, in fact, knew the results of RBWH dysphagia screening tool.

Twenty-eight of the screened patients (26.67%) presented with dysphagia. This data is in agreement with previous reports [2 31-33]. In particular, Cichero et al. [2] reported that 25% of patients were positive at RBWH screening, while the estimated incidence of dysphagia in hospitalised patients ranges between 15% and 30% [1 31 32]. The high rate of dysphagia in the present study supports the need for a valid dysphagia screening tool in subacute care settings. A formal dysphagia screening tool, in fact, may reduce the risk of a patient starting oral intake inappropriately or unsafely. For this purpose, the I-RBWH dysphagia screening tool appears optimal since it demonstrated strong reliability and good accuracy. In addition, it is designed to be administered by nursing staff (present 24 hours a day, 7 days a week) and allows rapid patient identification and timely referral for ongoing care. The prevention of clinical complications related to dysphagia, such as malnutrition, dehydration and aspiration pneumonia [5 32], may reduce mortality and recovery time in hospital [2] and may also reduce healthcare costs. The findings of the present study support the application of the triaging concept; only 54 patients of the 105 (51.42%) required a water swallow test, while the remaining 51 patients (48.58%) were screened on the basis of phase 1 (information from clinical records) and 2 data (information from clinical records or from patients or caregivers). All the patients, except 1, who were referred to the SLP required a modified diet.

There are several limitations in the study. First, the study population included only 105 patients; therefore, although the data herein encourage the use of the I-RBWH, they should be considered preliminary. Second, the study included patients from a single subacute care unit; thus, it is unknown how these findings could be generalised to subacute care unit in general. Third, the accuracy of the I-RBWH screening tool was not tested against an instrumental assessment (such as FEES or VFSS); future studies are needed to further analyse the sensitivity and specificity of the I-RBWH.

Conclusions

In conclusion, our current findings support the reliability and screening accuracy of the I-RBWH dysphagia screening tool for the screening of patients in subacute care settings. The application of the I-RBWH dysphagia screening tool in daily clinical practice as well as in epidemiological, efficacy and outcome studies is recommended.

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Rhinology

The value of *Nigella sativa* in the treatment of experimentally induced rhinosinusitis

Potenziale della Nigella sativa nel trattamento della rinosinusite indotta in setting sperimentale

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SUMMARY

The aim of this study was to investigate the effect of *Nigella sativa* and cephalexin in the therapy of experimental bacterial rhinosinusitis. Bacterial rhinosinusitis was induced with *Staphylococcus aureus*. Rabbits were divided into five groups; control (n = 6), N. sativa 50 mg/kg/d (n = 6), *N. sativa* 100 mg/kg/d (n = 6), N. sativa 200 mg/kg/d (n = 6), and cephalexin 20 mg/kg/d (n = 6) groups. *N. sativa* was given orally for 7 days. The same volume of normal saline (0.9% NaCl) was given as a vehicle to the control group for the same period. After treatment period, sinus mucosa samples were evaluated using stereological and histopathological methods. Half of the maxillary sinus mucosa samples were frozen at -80^{0}C for further analysis of NO levels. Pathology revealed a severe acute inflammatory process in rabbits treated with saline. Only mild inflammation was determined in cephalexin group, *N. sativa* 100 mg/kg/d and *N. sativa* 200 mg/kg/d groups. The level of NO increased in the saline group was significantly reduced in all treatment groups. *N. sativa* may prevent histopathological changes of rhinosinusitis via decreased NO levels in a dose dependent manner and can be used in the treatment of rhinosinusitis diseases.

KEY WORDS: Nigella sativa • Rhinosinusitis • Nitric Oxide • Neutrophil • Rabbit

RIASSUNTO

Obiettivo del presente studio è stato quello di investigare l'effetto della Nigella sativa e della cefalexina nel trattamento della rinosinusite batterica indotta in setting sperimentale. La rinosinusite batterica è stata indotta mediante stafilococco aureo. I conigli sono stati suddivisi in 5 gruppi; uno di controllo (n = 6), N. sativa 50 mg/kg/d (n = 6), N. sativa 100 mg/kg/d (n = 6), N. sativa 200 mg/kg/d (n = 6), e cefalexina 20 mg/kg/d (n = 6). La N. sativa è stata somministrata per via orale per 7 giorni. Lo stesso volume di soluzione salina (% 0,9 NaCl) è stato quindi somministrato al gruppo di controllo per lo stesso periodo di tempo. Dopo il periodo di trattamento i campioni di mucosa dei seni mascellari sono stati valutati utilizzando metodologie istopatologiche e stereologiche. La metà dei campioni di mucosa del seno mascellare sono stati congelati a -80°C per una successiva analisi dei livelli di ossido nitrico. L'analisi patologica ha rivelato un intenso processo infiammatorio in atto nei conigli trattati con sola soluzione salina. Solo un lieve grado di infiammazione è stato invece rilevato nei conigli nei gruppi trattati con cefalexina, N. sativa 100 mg/kg/d, e N. sativa 200 mg/kg/d. Il livello di ossido nitrico, elevato nel gruppo placebo, è risultato invece essere ridotto negli altri gruppi. La N. sativa potrebbe prevenire i ambiamenti istopatologici indotti dalla rinosinusite mediante una riduzione dei livelli di ossido nitrico con andamento dose dipendente, e potrebbe essere usata nel trattamento della rinosinusite.

PAROLE CHIAVE: *Nigella sativa • Rinosinusite • Ossido nitrico • Neutrofili • Coniglio*

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Introduction

Rhinosinusitis is one of the most frequently reported chronic diseases. In contrast to acute rhinosinusitis, where bacterial or viral aetiology is well established, chronic rhinosinusitis has been defined as an inflammation of the mucous membrane of the paranasal sinuses resulting from impaired transport mechanisms [1]. Local and systemic host immune responses interact under such conditions, leading to bacterial and respiratory virus effects in the pathophysiological events which is characterised by hyperaemia, haemorrhage and submucosal oedema with polymorphonuclear infiltration of rhinosinusitis [2]. Therefore, treatment of rhinosinusitis must break the vicious cycle of inflammation, oedema formation and mucous hypersecretion, before antibiotic treatment. Although treatment of rhinosinusitis is usually based upon use of antibiotics and/or surgery, it may result in both high medical costs and development of multiple drug resistance in sinusitis-causing pathogenic microorganisms in humans [3 4]. In this regard, new antimicrobial substances from various sources like medicinal plants are arousing interest.

Nitric oxide (NO) is one of the most abundant free radicals in the body and acts as a signalling or toxic agent between cells. Excess NO production causes mitochondrial respiratory enzyme inhibition [5]. NO has been described in many cell types in a variety of tissues, where it acts as a regulator of vascular tone, neurotransmitters, acute and chronic inflammation and host defence mechanisms [6 7]. It is involved in innate immunity, which is generated during immune and inflammatory actions as a toxic agent towards infectious organisms [8 9]. Neutrophils are capable of sustained release of high levels of NO, initiated by inflammatory cytokines and bacterial products [10]. As a result, many types of infections may cause an increase in levels of NO.

Nigella sativa (N. sativa), belonging to the Ranunculaceae family, is an annual herbaceous plant native to Asian and Mediterranean countries. The plant is known as "black cumin" or "black seed". There are numerous reports on the phytochemical, pharmacological and toxicological properties of black cumin [11 12]. Recent studies have shown that *N. sativa* seeds possess various pharmacological effects including analgesic, antipyretic, anti-inflammatory and anti-nociceptive, antioxidant, anti-tumour and cytotoxic, anti-diabetic and anti-ulcer properties [9 13-21]. Many of these properties have been attributed to its quinone constituents, and especially the thymoquinone of black cumin seeds [17]. Many reports have also been cited the actions of *N. sativa* seed extracts or its oil on different bacterial isolates [11]. The oil or its extracts have been found to have a broad spectrum of antibacterial activity [13]. It has also been demonstrated that both crude alkaloid extracts and water extracts of *N. sativa* seeds were effective against a variety of organisms, isolated from human patients suffering from septic arthritis, and even many organisms that were resistant to antibiotics [22].

The aim of this study was to document the effects of three different doses of oral administration of *Nigella sativa* in a well-characterised rabbit model of rhinosinusitis, compared with cephalexin treatment, by analysing the number of neutrophils, histopathology and level of NO of the nasal mucosa following treatment.

Materials and methods

Animals

Thirty adult male albino rabbits weighing an average of 3 kg were obtained from Ataturk University Experimental Animal Laboratory of Medicinal and Experimental Application and Research Centre. The rabbits were maintained in our laboratory under controlled environmental conditions and fed *ad libitum* consumption of pelleted feed mixture that was formulated to meet or exceed National Research Council recommendations (National Research Council, 1995). Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the local animal care committee of Ataturk University.

Drugs

The seeds of *N. sativa* were purchased from a local market. *N. sativa* seed samples (200 g) were separately extracted four times with 600 ml of chloroform ($CHCl_3$) or ethanol (EtOH) at room temperature. After the organic solvents were evaporated to dryness under vacuum at low temperature using a rotary evaporator, 59.6 g liquid $CHCl_3$ extract (29.8% yield) and 51.8 g liquid ethanol extract (51.8% yield) were obtained.

Experimentally induced rhinosinusitis and treatment

The animals were inoculated with *Staphylococcus aureus (S. aureus)* after the right nasal cavity was packed with Merocel^R (Medtronic Xomed, Jacksonville, FL, USA). Twenty-four hours after bacterial inoculation, the Merocel^R in the right nasal cavities was removed. Thirty animals were assigned randomly to receive saline (per-oral, group 1), 50 mg/kg/d *N. sativa* per-oral (group 2), 100 mg/kg/d *N. sativa* per-oral (group 3); 200 mg/kg/d *N. sativa* per-oral (group 4) and 50 mg/kg/d cephalexin (i.m.) (group 5) daily for 7 days. Twenty-four hours after the treatment period, the animals were sacrificed with injection of sodium pentobarbital via the auricular vein and then decapitated after ensuring death.

Preparation of bacterial suspension and induction of rhinosinusitis

S. aureus strain ATCC 25923 was suspended at a concentration of $900x10^6$ cells/mlusing a McFarland Nephelometer Standard III at the Department of Microbiology, School of Medicine, Ataturk University, Erzurum, Turkey. After administration of a sedative, the nasal cavities were packed with Merocel^R for indirect obstruction of the maxillary sinus ostium [23]. The nasal dorsum was aseptically prepared with povidone-iodine before administration of a local anaesthetic to the skin and adjacent soft tissue. Next, 0.5 ml of bacterial suspension was injected to the right maxillary cavity using a hypodermic syringe.

Histological and stereological procedures

For analyses, dissected tissue samples were fixed in 10% formalin. After fixation, the tissue samples were decalcified with EDTA. The decalcified tissue samples were then washed, dehydrated in ascending graded alcohols, cleared in xylene, and embedded in paraffin. For stereological examination, 20 micron sections were taken from the blocked tissues using a systematically randomised sampling method [24 25]. For histological examination, 5 micron sections were taken from tissue blocks. After deparaffinisation and rehydration, serial sections were stained with haematoxylin and eosin.

An unbiased stereological technique, the optical dissector/fractionator method, was used to estimate neutrophil

numerical density the serial sections of the sinus mucosa [26 27]. The neutrophils in 20 micron sections were counted using a system for image analysis consisting of a light microscope (Leica DM4000 B, Cambridge, UK), equipped with an X-Y-Z movement-sensitive stage (Bio-Precision MAC 5000 controller system; Ludl Electronic Products, Hawthorne, NY, USA) and a video camera (Optronics MicroFire, Goleta, CA, USA) coupled to a computer containing Stereo Investigator software (Micro-BrightField, Williston, VT, USA).

Extraction of maxillary sinus mucosa and measurement of nitric oxide level

Sinus mucosa tissue NO levels were measured using the Griess reagent as previously described [28 29]. Griess reagent, a mixture (1:1) of 0.2% N-(1-napthyl)ethylenediamine and 2% sulphanilamide in 5% phosphoric acid gives a red-violet diazo dye with nitrite, and the resultant colour was measured at 540 nm. First, nitrate was converted to nitrite using nitrate reductase. The second step was the addition of Griess reagent, which converts nitrite to a deep purple azo compound; photometric measurement of the absorbance at 540 nm then determines the nitrite concentration. Protein interference was eliminated by treatment of the reacted samples with zinc sulphate and centrifugation for 5 min 10,000xg. Biochemical measurements were carried out using a Cecil CE 3041 (Cambridge, UK) spectrophotometer.

Statistical analysis

For all parameters studied, means and standard deviations were calculated. Statistical analysis was performed using SPSS 13 for Windows (SPSS Inc., Chicago, IL, USA). The analysis of variance with the post-hoc LSD (least significant difference) test was used to compare results for all the groups. A $p < 0.05$ was considered statistically significant.

Results

Physical examination

There was nasal discharge in all animals at the time of the removal of Merocel. Macroscopically, there was also oedema at varying degrees in the right maxillary sinus of all animals when sinus cavities were opened after the treatment period.

Histopathological lesions

In our study, the numerical density of neutrophils was also estimated. For all groups (control, cephalexin, *N. sativa* 50 mg/kg/d, *N. sativa* 100 mg/kg/d and *N. sativa* 200 mg/kg/d) the numerical density of neutrophils was 0.000351/μm^3, 0.000020/μm^3, 0.000072/μm^3, 0.000058/μm^3, 0.000068/μm^3 respectively (Table I and Fig. 1).

Severe neutrophil infiltration was detected in control rabbits treated with saline. Pathology revealed a severe acute inflammatory process, with neutrophil and oedema formation that permeated the maxillary sinus and surrounded a vasodilatation mucosal capillary. Several instances of vascular dilatation and haemorrhage were determined in section profiles.

Although the general architecture of the maxillary sinus in cephalexin groups was partly protected, no important pathological findings at the structural level were observed, but only mild inflammation (Fig. 2A). Neutrophil infiltration of the maxillary sinus was lower than in the saline group (Fig. 2A). Similar histopathological findings were observed in the *N. sativa* 100 mg/kg/d and *N. sativa* 200 mg/kg/d groups (Fig. 2C and 2D). In the *N. sativa* 50 mg/kg/d group, neither maxillary sinus nor vascular

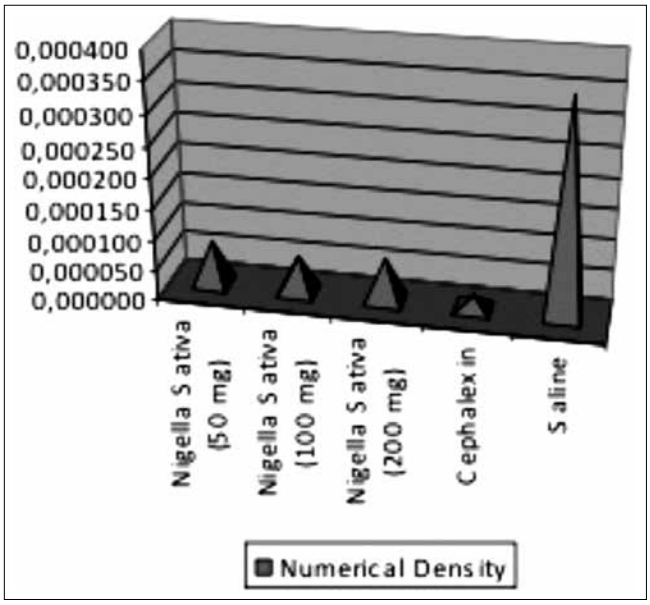

Fig. 1. Changes in mean neutrophil numerical density during treatment for 7 days in each group.

Table I. Neutrophil density in all the groups.

Groups	Numerical density of neutrophils (n/μm^3)	Standard deviation	Standard error mean	p
N. sativa (50 mg/kg/d, N = 6)	0.000072	0.00003950	0.00001613	< 0.001
N. sativa (100 mg/kg/d, N = 6)	0.000058	0.00004831	0.00002416	< 0.001
N. sativa (200 mg/kg/d, N = 6)	0.000068	0.00001058	0.00001067	< 0.001
Cephalexin (20 mg/kg/d, N = 6)	0.000020	0.00000496	0.00000202	< 0.001
Saline (N=6)	0.000351	0.00003726	0.00001862	< 0.001

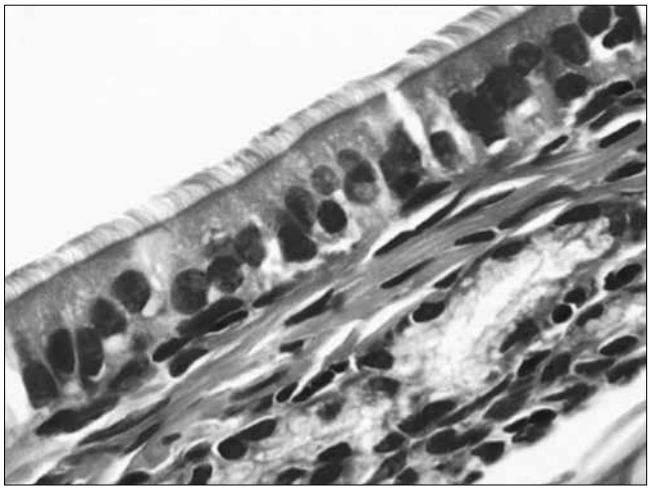

Fig. 2A. Histopathological section of rabbit maxillary sinus mucosa of treatment with cephalexin (H&E X200).

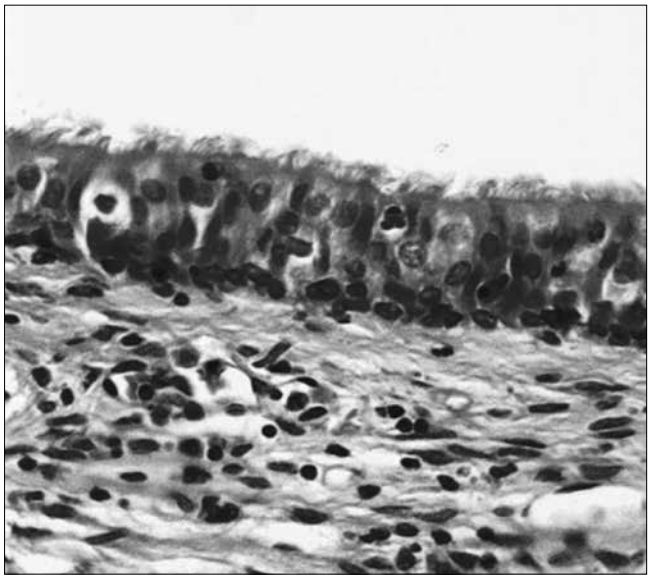

Fig. 2B. Histopathological section of rabbit maxillary sinus mucosa treated with *N. sativa* 50 mg/kg/d (H&E X100).

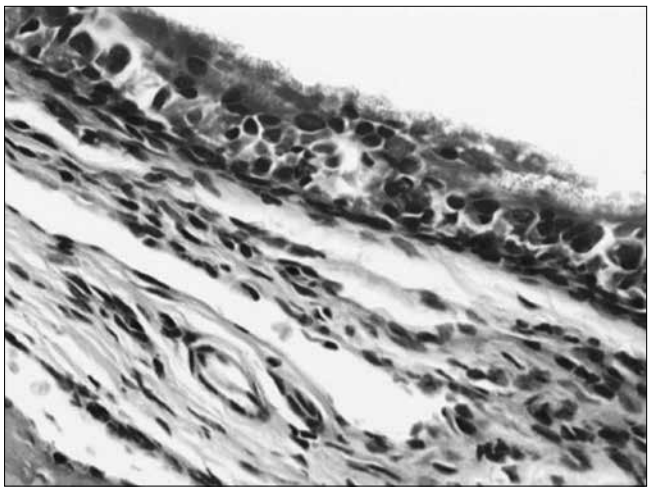

Fig. 2C. Histopathological section of rabbit maxillary sinus mucosa treated with *N. sativa* 100 mg/kg/d (H&E X100).

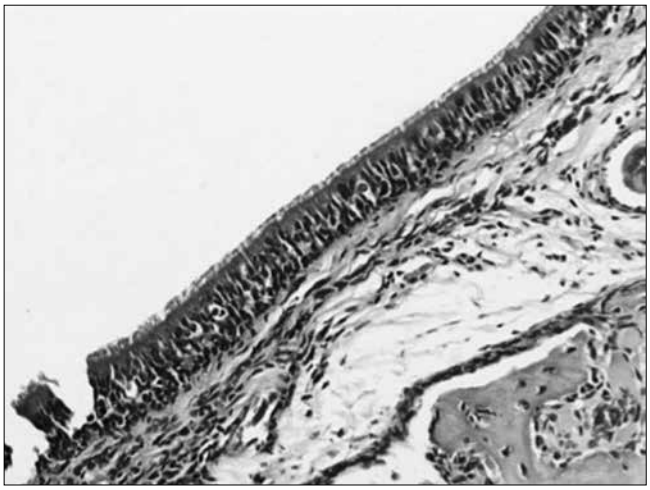

Fig. 2D. Histopathological section of rabbit maxillary sinus mucosa treated with *N. sativa* 200 mg/kg/d (H&E X50).

structures were normal in appearance compared with the cephalexin group (Fig. 2B).

Nitric oxide level

Table II shows that the level of NO increased in the saline treated group in comparison to the cephalexin 20 mg/kg/d, *N. sativa* 50 mg/kg/d, *N. sativa* 100 mg/kg/d and *N. sativa* 200 mg/kg/d groups. The mean tissue total NO levels were significantly ($p < 0.001$) higher in the saline group and correlated well with disease acuteness. Cephalexin 20 mg/kg/d, *N. sativa* 50 mg/kg/d, *N. sativa* 100 mg/kg/d and *N. sativa* 200 mg/kg/d had a similar effect on the levels of NO, all reducing its value, which were 163.31 µmol/ml, 166.40 µmol/ml, 165.20 µmol/ml and 166.43 µmol/ml in experimental rhinosinusitis, respectively. In the saline control group, the value of NO level was 184.17 µmol/ml.

Discussion

Nasal and paranasal sinus mucosa have a highly efficient system for the physiologic functions of olfaction, respiration, and protection [30]. The respiratory epithelial cell layer presents a physical barrier that prevents invasion by micro-

Table II. Effects of cefalexin and N. sativa on changes in the level of NO in sinus mucosa samples of rabbits in an experimental rhinosinusitis model. The groups treated with three different doses of N. sativa and cefalexin were compared with the saline group.

Groups	Number of Animals	NO (µmol/ml)	p
Control (Saline)	6	184.17 ± 3.09	-
Cefalexin 20 mg/kg/d	6	163.31 ± 2.98	< 0.001
N. sativa 50 mg/kg/d/	6	166.40 ± 0.22	< 0.001
N. sativa 100 mg/kg/d	6	165.20 ± 0.43	< 0.001
N. sativa 200 mg/kg/d	6	166.43 ± 0.43	< 0.001

*p < 0.001; ** p < 0.01.

organisms, and the mucociliary action prevents bacterial infection and protects the mucosa from injury and drying [31]. Sinusitis is one of the most frequently reported acute or chronic and heterogeneous diseases, which shows several types of aetiology. Where a bacterial or viral aetiology is well established, this has been defined as an inflammation of the mucous membrane of the paranasal sinuses resulting from impaired transport mechanisms [1]. Various systemic and local factors are known to be associated with nasal and sinus infections [32 33]. To maintain the physiologic condition of the nasal cavity and sinuses, it is known that nasal airflow, anatomical conditions, patency of the natural ostium, oxygen saturation in sinuses and mucociliary clearance all play important roles. When one of these physiologic conditions is changed, these abnormal conditions cause inflammatory reactions, due to an abnormal mucous membrane immunity, phagocytosis and bacteriologic action of the nasal secretion enzymes. The local and systemic host immune responses interact under such conditions, leading to bacterial and respiratory virus effects in the pathophysiological events, which are characterised by hyperaemia, haemorrhage and submucosal oedema with polymorphonuclear infiltration of rhinosinusitis [32].

The aim of this study was to compare the effectiveness of three different doses of *N. sativa*, which was of natural herbal origin, with that of cephalexin. With this therapy, we aimed to ameliorate neutrophil numbers and inhibit the inflammation that was a consequence of the NO level in the sinuses. Cingi et al. reported no significant difference histopathologically, among an antibiotic treated group, a thymoquinone treated group and a negative control group in their experimental study in which they investigated the role of thymoquinone, a phytochemical component of *N. sativa*, for sinusitis therapy [34]. They proposed that thymoquinone can be a bioactive agent used for treatment of rhinosinusitis and have the same histopathological effect with an antibiotic. We obtained similar results herein.

The reduction of the neutrophil numbers was histopathologically relevant and statistically significant in both treatment groups, compared with the saline group. It similar in the group of rabbits treated with *N. sativa* (100 mg/kg/d and 200 mg/kg/d) and in the group treated with cephalexin. The results of our study have shown that rhinosinusitis with cephalexin eradicate infection and reduce inflammation. The effectiveness of *N. sativa* is based on its known mechanisms of action, which are anti-inflammatory, antioxidant and antimicrobial [8 13 15-17].

NO may play a part in tissue damage, and its roles in the control of a variety of intracellular organisms have been described in some viral and fungal infections or protozoal infestations as a toxic agent towards infectious organisms [35-38]. However, the role of NO in bacterial infection has not been clearly defined; it may be cytostatic or cytotoxic, not only for invading microorganisms, but also for the cells that produce it and also for neighbouring cells. For example, NO is a mediator of NK cell killing of target cells and regulates NK cell function; it inhibits activation of mast cells and can enhance or inhibit neutrophil activation, depending on its concentration [39-41]. Another potential mechanism includes a direct microbiocidal effect, via the reaction of NO with iron or thiol groups on proteins that inactivate enzymes systems in mitochondria. It may also interact with oxygen-derived radicals to generate molecules that may enhance its cytotoxicity. In addition, NO has been found to react with superoxide to form reactive oxidants capable of damaging target cells [42].

Our results for tissue NO levels are in agreement with these data. Our results suggest that oxidative stress in tissue of rabbits with rhinosinusitis may cause an increase in NO, which arises because of tissue damage. *N. sativa* may be given to decrease the NO in patients with rhinosinusitis, with the hope of alleviating the observed toxicities. Since NO seems to have a dual role in bacterial rhinosinusitis, the high concentrations of NO could result in damage in sinus mucosa and *N. sativa* decreases the tissue level of NO via anti-inflammatory, microbiocidal or antioxidant functions.

Conclusions

Our findings underscore that acute bacterial rhinosinusitis can increase the level of NO and that high concentrations of NO may play an important role in the pathogenesis of rhinosinusitis of the impaired sinus tissue of rabbits. The results of our study suggest that *N. sativa* may prevent histopathological changes of rhinosinusitis via decreased NO levels in a dose dependent manner.

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Rhinology

Non-surgical management of chronic rhinosinusitis with nasal polyps based on clinical-cytological grading: a precision medicine-based approach

Trattamento medico della rinosinusite cronica con poliposi naso-sinusale sulla base del sistema di grading clinico-citologico per un approccio medico personalizzato

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SUMMARY

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common inflammatory disorder that strongly impacts patients' quality of life. CRSwNP is still a challenge for ENT specialists due to its unknown pathogenesis, difficult control and frequent relapse. We tested the hypothesis that a new standardised therapeutic approach based on individual clinical-cytological grading (CCG), may improve control of the disease and prevent the needing for surgery. We analysed 204 patients suffering from bilateral CRSwNP, 145 patients of whom regularly assumed therapy, respecting the planned check-up, and were considered cases; 59 patients were not assuming therapy as indicated and were considered as controls. After five years of standardised treatment, 15 of 145 (10.5%) improved endoscopic staging, 61 of 145 (42%) did not change their endoscopic staging, and 69 of 145 (47.5%) were worse. In the control group, 49 of 59 (83%) were worse by at least two stages ($p < 0.05$). Patients and controls were stratified basing on clinical and cytological grading as mild, moderate and severe. After patient stratification, in the mild group ($n = 27$) 92% patients had a constant trend, with no worsening and no need for surgery over a 5-year period, whereas in the mild CCG control group 1 of 59 (1.6%) required surgery ($p < 0.05$). In moderate GCC ($n = 83$), 44% of patients did not modify or improve endoscopic staging and 3.6% needed surgery, compared to 13.6% of controls with moderate GCC ($p < 0.05$). In severe CCG ($n = 35$), even though no patients achieved significant amelioration of endoscopic grading, 40% of patients were considered as "clinically controlled" and 5.7% of patients underwent surgery, but the percentage was significantly higher (49%) in the control group significant ($p = 0.0000$). Finally, statistical analyses revealed a clear trend that polyp size increased at a faster rate in the control group than in the treatment group and for each subgroup (low, moderate and severe). The present study suggests a new approach in the management of CRS according to clinical cytological grading that allows defining the grade of CRSwNP severity and to adapt the intensity of treatment. This approach limited the use of systemic corticosteroids to only moderate-severe CRSwNP with a low corticosteroid dosage in comparison with those previously suggested. Our protocol seems to improve the adherence by patients, control of disease and the need for surgery in the long-term.

KEY WORDS: Nasal polyps • Clinical grading • Cytological grading • Treatment

RIASSUNTO

La rinosinusite cronica con polipi nasali (CRSwNP) è una malattia cronica nasosinusale, a eziologia infiammatoria, con significativo impatto negativo sulla qualità di vita dei pazienti. La CRSwNP rappresenta ancora oggi una sfida terapeutica per lo specialista ORL, sia per la comprensione della sua eziopatogenesi, sia per il suo controllo clinico ed è questo è testimoniato dalla alta incidenza di recidiva dopo trattamento. Abbiamo voluto verificare l'ipotesi che un approccio terapeutico nuovo, standardizzato, e individualizzato sul grading clinico-citologico (clinical-cytological grading – CCG) consentisse un miglior controllo dei sintomi della malattia, e di ridurre la necessità di ricorrere alla chirurgia. Abbiamo pertanto reclutato 204 pazienti affetti da CRSwNP, di cui 145 hanno regolarmente assunto la terapia rispettando il protocollo proposto, e 59 pazienti, invece, che non hanno assunto la terapia in modo sistematico e sono stati quindi inclusi come controlli. Dopo 5 anni di trattamento standardizzato, abbiamo notato che 15 pazienti su 145 (10,3%) del gruppo con terapia standardizzata avevano avuto un miglioramento dello staging endoscopico, 61 su 145 (42%) si erano mantenuti costanti, mentre 69/145 (47,5%) erano andati incontro a un peggioramento. Nel gruppo di controllo, invece, i pazienti peggiorati erano ben 49 su 59 (83%), con un peggioramento significativo in termini di grading endoscopico di almeno due classi ($p < 0,05$). I pazienti e i controlli sono stati successivamente stratificati sulla base del CCG in 3 sottogruppi: pazienti con CCG lieve, moderata e grave. Dopo tale suddivisione in classi, è stato possibile evidenziare che nel gruppo con CCG lieve ($n = 27$), il 92% dei pazienti manteneva negli anni un trend costante, in assenza di peggioramenti e senza necessità di ricorrere alla chirurgia nei 5 anni di osservazione, mentre nel gruppo di controllo, 1 paziente su 59 (1,6%; $p = <0,05$) ricorreva a chirurgia. Nel gruppo con CCG moderato ($n = 83$), invece, il 44% dei pazienti "standardizzati" non aveva avuto un peggioramento di grading endoscopico, con un 3,6% di pazienti che aveva avuto necessità di ricorrere alla chirurgia, contro il 13,6% del gruppo controllo ($p < 0,05$). Nel gruppo dei pazienti con CCG grave ($n = 35$), anche se nessun paziente riusciva a ottenere un migliora-

mento del grading endoscopico, il 40% dei pazienti veniva comunque giudicato "controllato" da un punto di vista clinico. Nel gruppo dei pazienti con CCG grave, ben il 5,7% dei pazienti necessitava di trattamento chirurgico, ma anche in questo caso, la percentuale dei pazienti operati era significativamente maggiore (p = 0,0000) nel gruppo di controllo (49%). Infine, l'analisi statistica effettuata ha dimostrato chiaramente che, da un punto di vista obiettivo, le dimensioni dei polipi nasali tendevano ad aumentare a una velocità maggiore nel gruppo controllo che nel gruppo "standardizzato", con incrementi proporzionali nelle tre classi di CCG (lieve, moderato e grave). Lo studio attuale fornisce le basi per lo sviluppo e l'adozione di un nuovo approccio per la gestione della CRSwNP sulla base di uno score clinico e citologico (CCG) che permetta di stimare con accuratezza la gravità della CRSwNP e di adattarne il trattamento. Tale approccio limita l'uso degli steroidi sistemici alle sole classi CCG di entità moderata-grave con dosi di steroidi inferiori rispetto a quanto precedentemente suggerito in letteratura. Il nostro protocollo può migliorare pertanto l'aderenza terapeutica dei pazienti, il tasso di controllo della malattia e può ridurre il ricorso alla chirurgia nel corso degli anni.

PAROLE CHIAVE: *Polipi nasali • Grading clinico • Grading citologico • Trattamento*

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Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common inflammatory disorder, affecting about 4% of the population worldwide and strongly impacts the quality of life of affected patients [1]. CRSwNP remains a challenge for ENT specialists because of its unknown pathogenesis, difficult control and frequent relapse.

CRSwNP is characterised by different phenotypes depending on: comorbidity [2], endoscopic findings [3], radiological features [4] and cytology [5]. In this regard, a clinical-cytological grading (CCG) has been proposed for defining a prognostic index of relapse, as reported in Figure 1 [6].

Management of CRSwNP consists of a combination of medical therapy, careful follow-up, and appropriate surgery; this strategy should be individualised for each single patient. Corticosteroids (CS), both topical and systemic, and functional endoscopic sinus surgery (FESS) are the most common approaches [7 8]. Even though medical treatment usually allows satisfactory control in most patients [9],

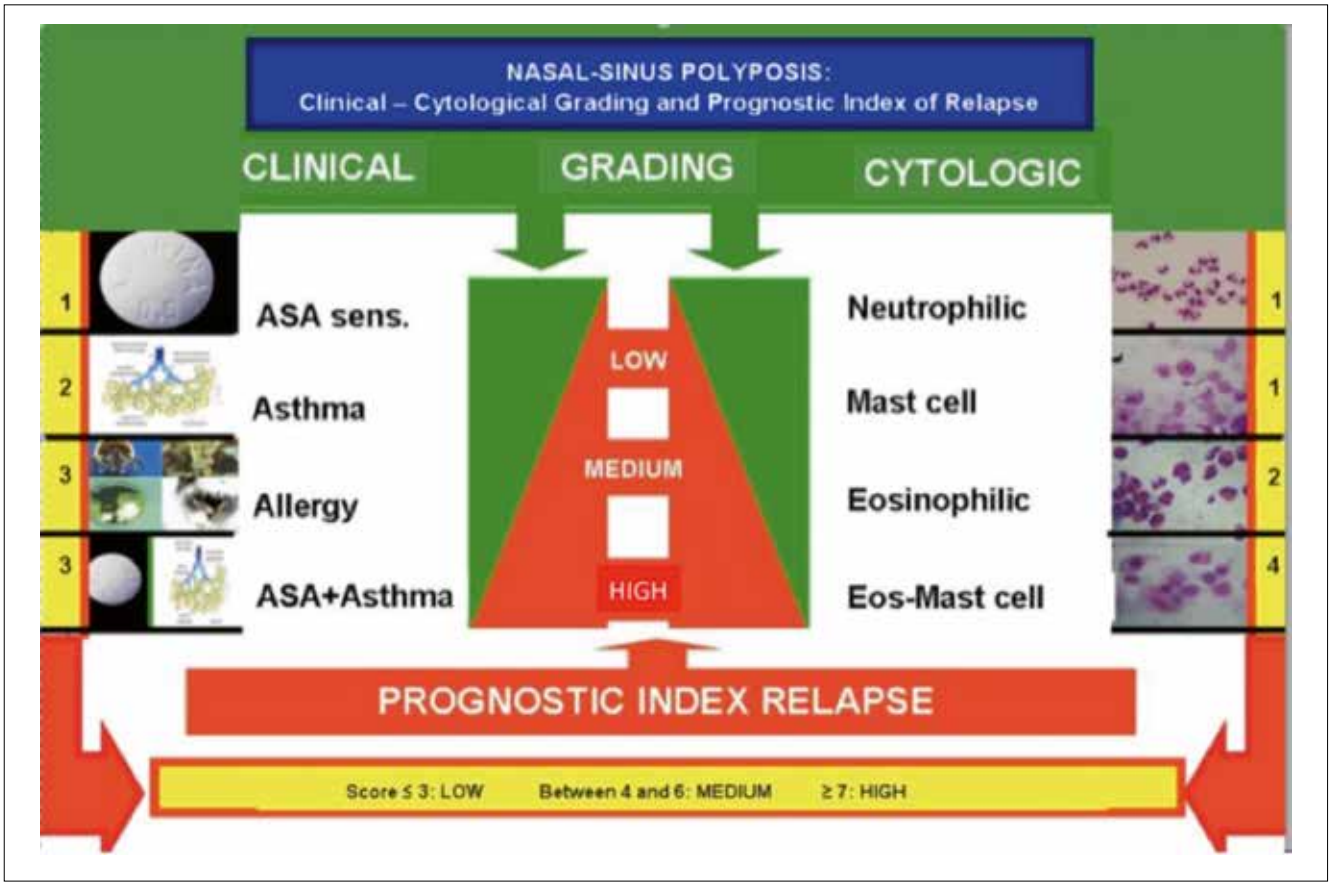

Fig. 1. Prognostic index of nasal polyp relapse based on clinical and cytologic grading.

some subjects need surgery [10]. Nevertheless, some patients, ranging between 15 to 87% of those undergoing surgery, may have relapse [11]. Therefore, there is the need for a therapeutic algorithm based on CRSwNP phenotyping. An appropriate strategy is mandatory to avoid under/overtreatment, prevent relapse and minimise the side effects of medication.

The present study examined the hypothesis that a new standardised therapeutic approach to CRSwNP based on clinical and cytological grading may improve control of disease and potentially prevent surgery and relapse.

Materials and methods

Two hundred four patients (117 males, 87 females, mean age 41 years, age range 28-71) suffering from bilateral CRSwNP were evaluated. CRSwNP diagnosis was performed by history (including ASA sensitivity), nasal endoscopy, cytology and allergy testing.

Nasal cytology was performed by scraping the middle part of the inferior turbinate with a Rhino-Probe® device (Arlington Scientific), according to validated criteria [12]. The inflammatory pattern of inflammation was in 91 of 204 (44.6%) patients with eosinophils; in 37 of 204 (18.1%) with mast cells; in 73 of 204 (35.7%) with mixed mast cells and eosinophils; finally, in only 3 of 204 (1.4%) patients with neutrophils. We never observed modification of inflammatory patterns over the years.

Skin prick test was performed according to validated criteria [13]. Allergy was detected in 93 of 204 (45%) patients, 71 of 204 (34.8%) were poly-sensitised, 32 of 204 (15.7%) patients were asthmatic; 23 of 204 (11.3%) patients had aspirin (ASA) intolerance and 59 of 204 (28.9%) had ASA sensitivity associated with asthma.

Clinical and cytologic grading and prognostic index of nasal polyps relapse are reported in Figure 1. Our diagnostic and therapeutic algorithm is shown in Figure 2.

The standardised therapeutic approach based on clinical and cytological grading (CCG) was followed by 145 of 204 patients who regularly assumed therapy and respected the planned check-up and were considered cases; 59 of 204 patients were not assuming the therapy as indicated and assumed only topical corticosteroids, and for this reason where considered as a control group.

CCG-treated patients and controls were stratified basing on clinical and cytological grading (15). Patients and controls were subdivided in three groups on the basis of CCG: group A: mild grade (CCG 1-3); group B: moderate (CCG 4-6); and group C: severe (CCG 7-10). Among cases, 27 of 145 (18%) were considered mild CCG; 83/ of 145 (57%)

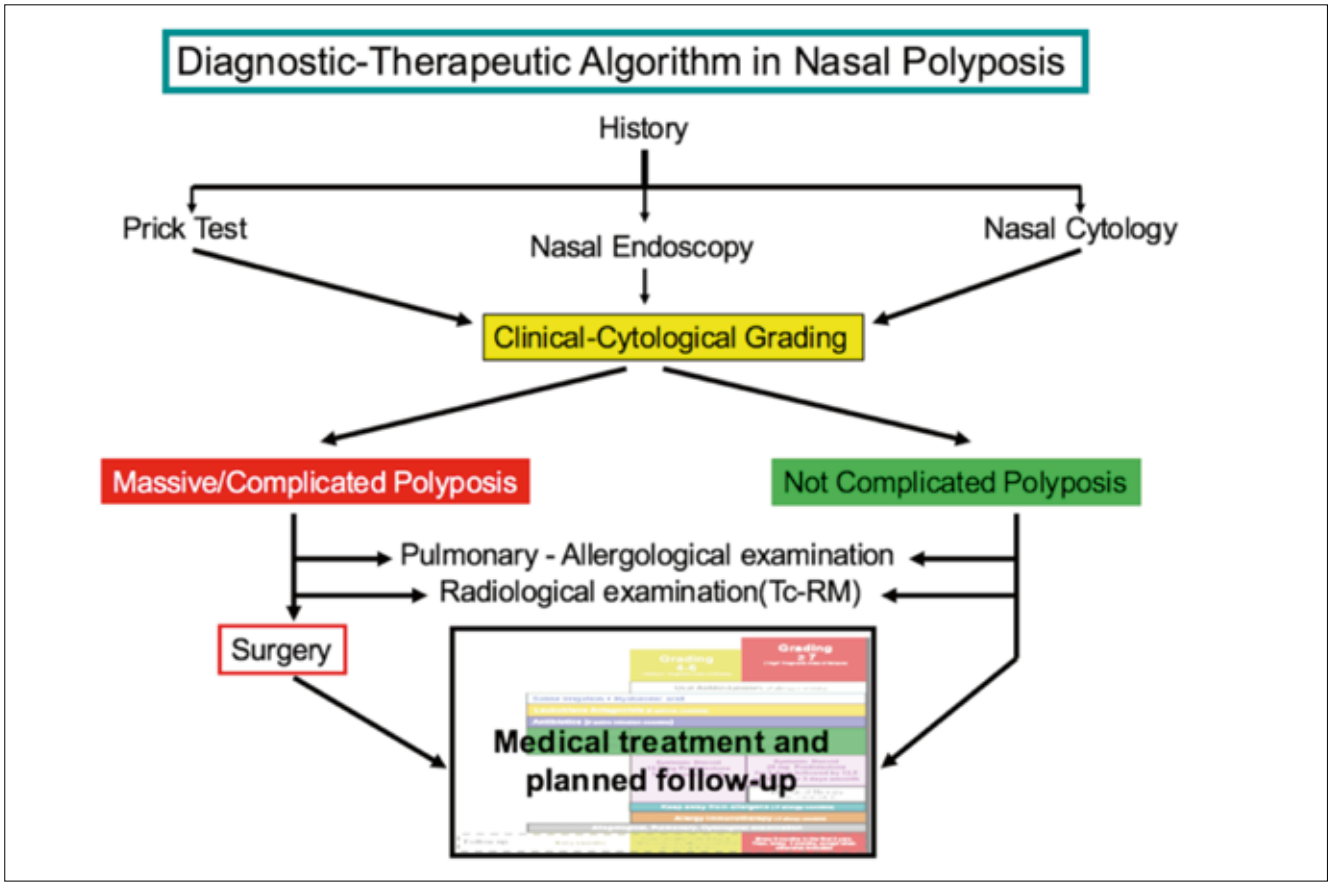

Fig. 2. Diagnostic and therapeutic algorithm for nasal polyposis.

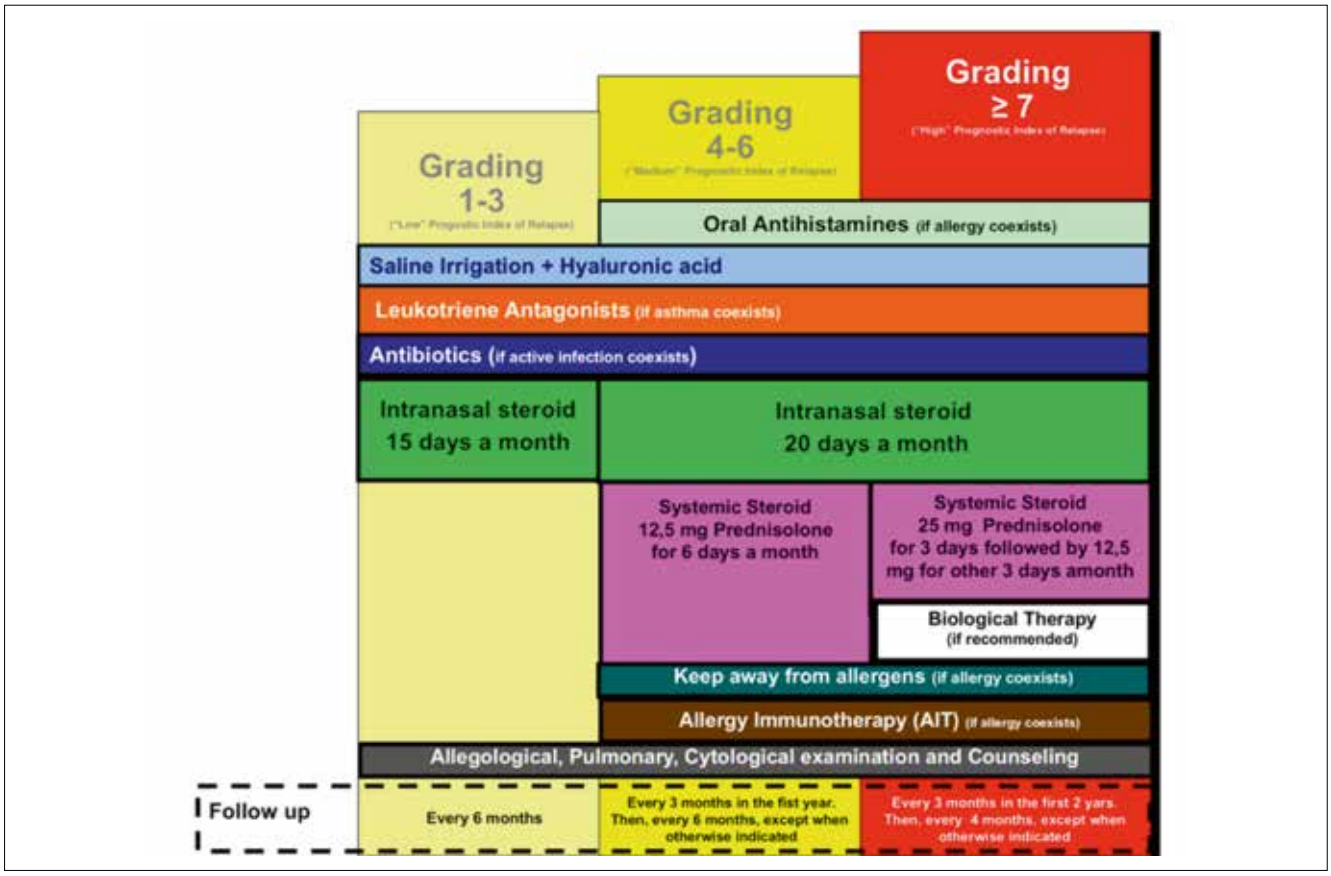

Fig. 3. Flow chart of nasal polyp treatment on the basis of proposed grading.

moderate CCG and 35 of 145 (24%) severe CCG. In the controls, 15 of 59 (25%) had mild CCG, 32/ of 59 (54%) had moderate and 12 of 59 (20%) had severe CCG.

The medical treatment prescribed based on clinical and cytological grading (CCG) is reported in Figure 3. Nasal irrigation using isotonic saline, at low pressure and large volume [14], was used by all patients. In low grade patients, furoate mometasone (200 mcg/day) was prescribed for 15 days a month, and montelukast 10 mg/day if asthma occurred. In patients with moderate grade, antihistamines and allergen immunotherapy (if indicated) were added; mometasone was prescribed for 20 days per month; and oral corticosteroid was added: prednisone 12.5 mg/day for 6 days per month. In patients with severe grade, 25 mg prednisone was prescribed per 3 days, then 12.5 mg for 3 days per month. Controls assumed only local corticosteroid furoate mometasone (200 mcg/day) which was prescribed for 15 days a month.

Nasal endoscopy was carried out by a 3.4 mm diameter flexible fiberscope (Vision-Sciences® ENT-2000). NP endoscopic classification proposed by Meltzer[3] was adopted (stage 0: no polyps visualised and open middle meatus; stage 1: small polyps noted in the middle meatus; Stage 2: middle meatus completely filled with polypoid disease; Stage 3: polyps extending out of the middle meatus but the above inferior turbinate; Stage 4: massive nasal polyposis filling the entire nasal cavity and spheno-ethmoid region).

The patients were followed for 5 years. Patient outcomes were evaluated at baseline (T0), after 1 year (T1), and after 5 years (T2). The Review Board of the Policlinico of Bari approved the procedure and all patients provided informed written consent.

Statistical analysis

Data came from an ordinal longitudinal clinical trial. The statistical analysis was mainly aimed to evaluate the effectiveness of a calibrated therapeutic protocol, based on the CCG versus topical corticosteroid therapy. The population sample was subdivided in three subgroups according to CCG score (A, B and C).

As a preliminary explorative data analysis, the discretised polyp size responses Y_{it} were plotted as a function of time τ conditioned on the treatment *tr* and on subsample A, B and C. A further summarised presentation of trend was generated in a conditioned boxplot.

The cumulative logit marginal model described in ([1], [3]) was fitted to data for each subsample A, B and C. The repeated response variable Y_{it} was the discretised polyp size of each subject ι in the study , classified on a five-level ordinal scale with possible values y = 0, 1, 2, 3, 4 (0 = low;4

Table I. 5-year follow-up after treatment by endoscopic staging, stratifying results according to the clinical cytological grading.

	Improved		Not modified		Worsening	
	Cases	Controls	Cases	Controls	Cases	Controls
GCC 1-3	9/27 (33.3%)	0/15 (0%)	16/27 (59.2%)	2/15 (13.3%)	2/27 (7.4%)	13/15 (86.6%)
GCC 4-6	6/83 (7.2%)	0/32 (0%)	31/83 (37.34%)	8/32 (25%)	46/83 (55%)	24/32 (75%)
GCC ≥7	0/35 (0%)	0/12 (0%)	14/35 (40%)	0/12 (0%)	21/35 (60%)	12/12 (100%)
Totale	15/145* (10.3%)	0 (0%)	61/145* (42%)	10/59 (16.9%)	69/145* (47.5%)	49/59 (83%)

*$p < 0.05$

= high), at time t = 0 (baseline classification) and at two follow-up times: one year (t = 1) and five years (t = 5).
Data were acquired and analysed using R 3.0.1 software (R Core Team 2015 https://www.R-project.org).

Results

After one year of standardised treatment (T1), 10 cases of 145 (6.8%) improved endoscopic staging by at least one stage, 75 of 145 (51.7%) did not change their stage and 50 of 145 (34.48%) were worse at endoscopic staging of no more than one stage. In the control group, 22 of 59 (37.2%) did not change their staging, whereas 37 of 59 (62.7%) were worse by at least one-two stages.
After five years of standardised treatment (T2), 15 of 145 (10.3%) improved endoscopic staging, 61 of 145 (42%) did not change endoscopic staging and 69 and 145 (47.5%) were worse. In the control group, 49 of 59 (83%) were worse by at least two stages.
The outcomes after treatment according to clinical cytological staging are reported in Table 1. In group A (mild CCG), 92% of patients did not modify or improve endoscopic staging; no patients in this group required surgery; in mild GCC of the control group 1 of 59 (1.6%) required surgery ($p < 0.05$). In group B (moderate CCG), 44% of patients did not modify or improve endoscopic staging and 3 of 83 patients (3.6%) required functional endoscopic sinus surgery versus 8/59 (13.55%) in controls ($p < 0.05$). In group C (severe GCC), no patients improved endoscopic staging, 40% of patients did not worsen; 2 of 35 patients (5.7%) required surgery *versus* 20 of 59 (33.8%) in the control group. During the five-year follow-up, 5 of 145 treated patients (3.4%) underwent functional endoscopic sinus surgery, and in particular 3 with moderate CCG and 2 with severe CCG; in the control group, 29 of 59 (49%) underwent surgery ($p = 0.0000$).
Figure 4 shows a plot of the discretised polyp size response Y_{it}, for each subject ι, as a function of time τ, conditioned on group (control, treatment) and on subsample (A,B,C). From the plot a clear trend is revealed; i.e., on average polyp size increased at a faster rate in the control group than in the treatment group, for each subsample. Figure 5 shows a summary of the same trend with boxplots.

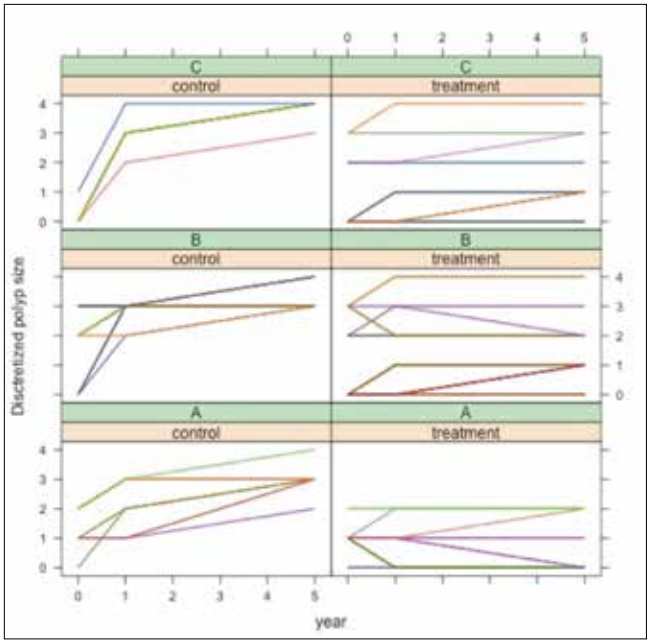

Fig. 4. Plot of the discretised polyp size for each subject, as a function of time, conditioned on group and subsample.

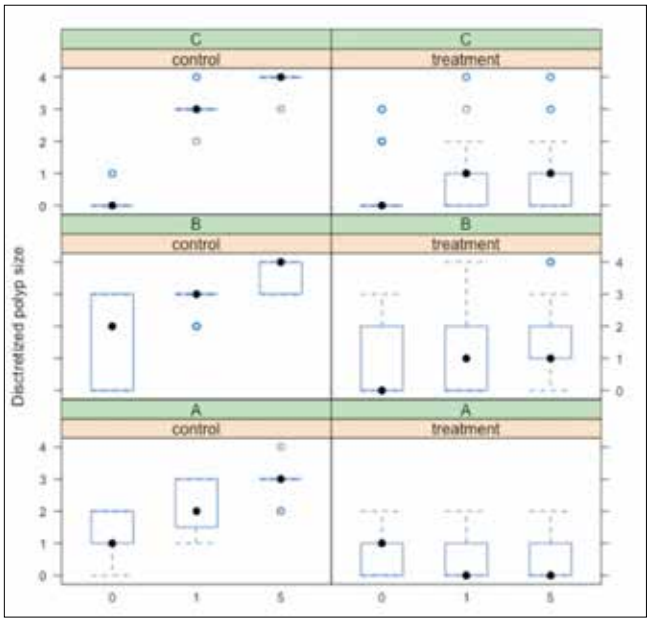

Fig. 5. Boxplot of the discretised polyp size for each subject, as a function of time, conditioned on group and subsample.

Table II. Parameter estimates, standard errors, z-statistics and p-values for each subsample.

	Parameter	Estimate	Std. Error	z-statistic	P value
A	β^{tr}	3.910	0.814	4.803	< 0.001
	$\beta^{t/tr}$	3.024	0.741	4.079	< 0.001
B	β^{tr}	4.574	0.787	5.810	< 0.001
	$\beta^{t/tr}$	2.184	0.545	4.009	< 0.001
C	β^{tr}	3.511	0.875	4.012	< 0.001
	$\beta^{t/tr}$	2.077	0.598	3.474	< 0.001

The GEE estimates of the relevant parameters β^{tr} and $\beta t^{t/tr}$, the related estimated standard errors based on the sandwich covariance matrix, Wald's z-statistics and p-values, for each subsample A, B and C, are reported in Table II.

In each subgroup, the effect of calibrated treatment at time t = 1 year was positive and statically significant at α= 0:05 (H0: β^{tr} = 0 rejected) as well as the interaction effect (H0: $\beta t^{t/tr}$ = 0 rejected).

For subsample A, the estimated cumulative odds of subjects in the treatment group was 49.88 (t = 1 year) and 1026.38 (t = 5 years) times those of subjects in the control group.

For subsample B, the estimated cumulative odds of subjects in the treatment group was 96.97 (t = 1 year) and 861.61 (t = 5 years) times those of subjects in the control group.

For sub sample C, the estimated cumulative odds of subjects in the treatment group was 45.20 (t = 1 year) and 267.24 (t = 5 years) times those of subjects in the control group.

Discussion

Many studies [15-21] have conducted on CRSwNP to attempt to clarify the aetiology of the disease, and even though several hypothesis have been postulated, none has been universally accepted by the international scientific community. Furthermore, despite improvements in medical and surgical therapy for NP, no significant efforts have been made in phenotyping the disease.

From a cytological point of view, recent studies have shown that CRSwNP is highly associated with an immune-inflammatory state, characterised by eosinophils and mast cells, sometimes associated, albeit more rarely, with neutrophils [22]. From a clinical point of view, it is well known that CRSwNP is commonly associated with other comorbidities (allergy, asthma, ASA-intolerance) that are able to influence the course of NP [23 24].

All these factors confirm the clinical impression that different phenotypes exist and that treatment modalities should be tailored to them. Recent evidence suggests the possibility of "precision medicine" [25], based on the specific phenotype of each patient, allowing tailored treatment, minimising collateral effects and reducing the risks of under/overtreatment. Moreover, in a chronic disease scenario, it is mandatory to have the most precise diagnosis possible: this may help to develop more accurate therapy, with more satisfactory results and more long-term adherence to treatment.

This is the first innovative longitudinal study based on "personalised" medical treatment accordingly to clinical cytological grading (CCG) and therefore tailored to each patient.

In our model, every patient with CRSwNP has a unique identity, based not only on clinical features, such as the size of the polyp and comorbidities, but also by the cellular infiltrate. The combination of all these elements determines the clinical course of CRSwNP. Therefore, the different groups were subdivided on the basis of CCG, a stratification system for CRSwNP that we first developed and applied in our clinical practice in 2009 [6], in order to associate different phenotypes to different treatment modalities.

Our approach has given us the advantage of treating patients with low CCG with exclusive topical steroids, whereas patients with higher CCG (> 4) were treated with systemic steroids, but with doses and treatment duration that are significantly lower in comparison to that reported in the literature.

In fact, no more than 75 mg and 112.5 mg of prednisone per month (in one week of therapy) were administered to the moderate and severe CCG groups, respectively, which is a much lower dose compared to that suggested by Hissaria [26] (50 mg per day for 2 weeks, with a total dose of 700 mg prednisone per month), Vaidayanathan [27] (25 mg per day for 2 weeks, with a total dose of 350 mg prednisone per month), and Alobid [28] (prednisone 170 mg in one week). We believe that a rational and weighted use of systemic steroids is one of the key elements to obtain high adherence to treatment by patients, especially in those at risk for steroid-associated comorbidities (e.g.: diabetes, hypertension, glaucoma, gastritis). Compliance to therapy and basic information on the disease features, coupled with instructions on the use of nasal steroids, were, in our opinion, one of the possible reasons for the low percentage of surgical procedures in our patients with CRSwNP.

Moreover, our study demonstrated that a personalised use of steroids, with our posology and time intervals, was able to control CRSwNP much better in CCG groups than in the control group (p = 0.0000). Steroids, both topical and systemic, are the main agents responsible for reducing the inflammatory infiltrate, especially when eosinophils and mast cells are present in association. It has been demonstrated that the presence of both mast cells and eosinophils is the main factor responsible for high CCG [6].

In the group with mild CCG, 92% of patients had a constant trend, with no worsening and no need for surgery over a 5-year period, whereas in mild CCG of the control group 1 of 59 patients (1.6%) required surgery (p < 0.05). In the group with moderate CCG (n = 83), 44% of patients did not modify or improve endoscopic staging and 3.6% needed surgery, *versus* 13.55% of controls with moderate CCG (p < 0.05). In the group with severe CCG, even

though no patients achieved significant amelioration of endoscopic grading, 40% of patients were considered as "clinically controlled", namely with sufficient respiratory function, absence of complications and a good quality of life. In patients with severe CCG, 5.7% of patients underwent surgery that was performed in case of obstructive CRSwNP, in presence of complications or a low quality of life, but this percentage was significantly higher (49%) in the control group and the difference was statistically significant (p = 0.0000). Finally, statistical analyses revealed a clear trend that polyp size increased at a faster rate in the control group than in the treatment group, for each subgroup (low, moderate and severe).

In a very recent study by Oscarsson et al. [29], patients with untreated CRSwNP were observed for 13 years with no specific therapeutic intervention: interestingly, they showed that CRSwNP is a chronic entity, with a variable course over the years, that does not necessarily evolve into a more serious condition. Even though not specifically indicated by the authors, it is clear that there is a clinical CRSwNP phenotype, with a lower "grading" and a more benign course.

One of the most challenging aspects of CRSwNP is their relapse after surgery, which is still a common event despite continuous refinements of surgical techniques and therapy modalities. Hopkins and colleagues [30] described a 4% revision rate at 12 months post-operatively, which increased to 11% at 36-month follow-up. Masterson et al. [31] reported a revision rate of 12.3%, while in 2012 EPOS (12) depicted a highly variable percentage of recurrence, varying from 4% to 60%, with a mean of 20%. In our opinion, this elevated heterogeneity in CRSwNP recurrence reflects the variability of CCG grading in patients, which could be only hypothesised in the prior studies, but which could be demonstrated in larger groups of patients. Our low percentage of "surgical" patients demonstrates that our tailored approach is successful and that this is the consequence of adequate phenotyping of CRSwNP. In addition, we believe that proper counseling, tailored information and useful advice to manage CRSwNP symptoms are the basic elements for efficacious management of such a complex disease. On the other hand, emerging data [32 33] are now available from studies about the possibility of targeting therapy to different endotype of CRS with NP, focusing on prominent cytokines, which are key features of eosinophilic CRSwNP. With the availability of various hmAbs to specifically target cytokines and their receptors, we cannot exclude a refinement of our approach integrating indication, and in particular to control severe disease.

Conclusions

Nowadays, personalised medicine represents a new frontier in medical progress. Several respiratory and head-neck disorders have been explored, but CRSwNP remains a unclear area. In this regard, CRSwNP phenotyping is a fruitful attempt to tailor the best treatment and to avoid under or overtreatment. The present experience is the first longitudinal study aimed to calibrate medical treatment according to CCG. Each phenotype represents a fingerprint of pathophysiological mechanisms involved in CRSwNP, specific for each patient. CCG allows to define the grade of CRSwNP severity and to adapt the intensity of treatment. This approach limits the use of systemic corticosteroids to only moderate-severe CRSwNP. In addition, the proposed schedules consist of low CS dosage in comparison with those previously reported. Our protocol seems to improve adherence by patients, frequently suffering from co-morbidities, the rate of control disease and need forsurgery in the long-term.

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Sleep apnoea

Transoral robotic surgery (TORS): a new tool for high risk tracheostomy decannulation

La chirurgia robotica transorale (TORS): una nuova applicazione nelle decannulazioni ad alto rischio

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SUMMARY

Tracheostomy decannulation has always been considered a procedure with an attendant risk, especially in patients with a reduced upper airway diameter as is commonly observed in the obstructive sleep apnoea (OSA) population. We report on 4 cases where transoral robotic surgery (TORS) helped in the management of long-term cannulated patients. The aims of our paper are: 1. To demonstrate how the otolaryngology team can help identify patients at high risk for decannulation failure; and 2. To demonstrate how TORS may aid in the decannulation process of patients at high risk for failure due to severe tongue base hypertrophy. From our experience, TORS appears to offer an effective option to aid in the decannulation of patients with a severe hypertrophy of the base of tongue and floppy epiglottis.

KEY WORDS: Transoral Robotic Surgery • Tongue base • Tracheostomy

RIASSUNTO

La decannulazione è sempre stata considerata una procedura con un certo grado di rischio, specie nei pazienti con ridotti diametri delle via aeree, come nel caso della sindrome delle apnee ostruttive (OSA). Presentiamo 4 casi nei quali la chirurgia robotica transorale (TORS) ha permesso un appropriato management di pazienti tracheotomizzati da divers mesi. Gli obiettivi del nostro lavoro sono: 1. Dimostrare come il team otorinolaringoiatrico possa favorire il riconoscimento di pazienti ad alto rischio di decannulazione inefficace e 2. Evidenziare il ruolo nella TORS nel trattamento dell'ipertrofia della base della lingua, responsabile dell'ostruzione delle vie aeree superiori. Dalla nostra esperienza la TORS appare una tecnica efficace nella decannulazione di pazienti affetti da ipertrofia della base della lingua e da epiglottide flottante.

PAROLE CHIAVE: *Chirurgia robotica transorale • Base della lingua • Tracheostomia*

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Introduction

Tracheostomy decannulation has always been considered a procedure with an attendant risk, especially in patients with a reduced upper airway diameter as is commonly observed in the obstructive sleep apnoea (OSA) population. From a recent survey, most clinicians would consider decannulation failure, if reinsertion of an artificial airway occurs within 48 to 96 hours following planned tracheostomy removal [1].

The cause for re-intubation or a new tracheostomy intervention after decannulation is multifactorial: level of consciousness, cough effectiveness, secretions and oxygenation seem to be the most important factors contributing to the success or failure. In addition, anaesthesiologists consider the difficulty of intubation to be another important contributing element [1]. Cormack and Lehane, in particular, described anatomic conditions associated with difficult intubation (grades 3 and 4) [2]. The main anatomic obstacles are a narrow incisor distance, micrognathia, and hypertrophy of the base of tongue (BOT) with or without floppy epiglottis. The latter, in particular, is very common in patients affected by OSA. In our Institutions (Forlì, Italy and Ann Arbor, Michigan, USA) more than 650 cases have been treated with trans-oral robotic surgery (TORS).

The Morgagni-Pierantoni Hospital is a tertiary care hospital located in Forli, Italy. A multidisciplinary airway board has been developed to assist in the management of

tracheostomy patients and others with long-term airway requirements. The board includes otolaryngologist and anaesthesiologists of our Institution and takes advantage of consulting from others Italian and international centres. As part of this effort, the board evaluates known difficult airway patients and patients for whom difficulty in tracheostomy decannulation is anticipated or who have failed prior attempts at decannulation. This team approach has led to the application of TORS and other techniques in providing patients with effective airway management leading to successful decannulation.

Recently, this technique has gained popularity as an effective therapeutic option both for OSA and head and neck cancer [3-5]. The aims of our paper are: 1. To demonstrate how the otolaryngology team can help identify patients at high risk for decannulation failure and 2. To demonstrate how TORS may aid in the decannulation process of patients at high risk for failure due to severe tongue base hypertrophy. We present the following case series.

Case series

We report on 4 cases where TORS helped in the management of long-term cannulated patients.

All patients gave their consent to the procedure. TORS was carried out by the same team (C.V. and F.M.) with an Intuitive da Vinci robot. The operative setting was the same as that described by Weinstein [5] for the tongue base neoplasms. The robot is set up on the right side of the patient. The eyes and teeth are protected by means of specific devices. After the insertion of a mouth gag, the da Vinci robotic arms are placed in the oral cavity. Visualisation is achieved with a 30x magnification, 3-dimensional endoscope. Surgery begins with the visualisation of the epiglottis to orientate the surgeon. Then a piecemeal resection of the BOT is performed using a step-by-step approach. First the medial and paramedial portions of the tongue base are addressed and then the lateral parts. In this way, it is possible to identify and preserve the noble structures.

Case #1: D.S.F., male, 69 years, came to our attention in October 2014 for evaluation of tracheostomy removal. The tracheostomy was performed in January 2014 for a severe respiratory impairment that occurred after a stroke. Several unsuccessful trials of decannulation were attempted before our evaluation.

The patient's past medical history was complicated type 2 diabetes, arterial hypertension, ischaemic cardiopathy (he underwent 7 coronary stents in 1995) and both alcohol and tobacco addiction.

The endoscopic examination highlighted hypertrophy of the BOT and oedema of the epiglottis causing near complete occlusion of the upper airway (Fig. 1). As part of his evaluation for failed decannulation, the patient also underwent both a CT scan and an MRI scan of the neck with contrast (Fig. 2).

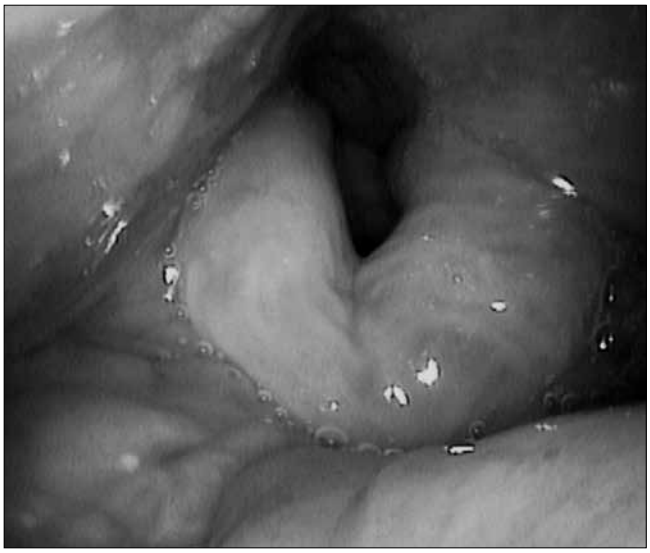

Fig. 1. Endoscopic examination before TORS highlighted hypertrophy of the BOT associated to an oedema of the epiglottis occluding almost completely the upper airway.

The case was discussed at the multidisciplinary airway conference where it was recommended that the patient undergo TORS BOT reduction and supraglottoplasty (SGP). The patient underwent TORS BOT resection and SGP in October 2014; a total of 12 mls of lingual tissue was resected. The patient was successfully decannulated in January 2015. At 6 months follow-up, the patient did not complain any further symptoms and did not require replacement of the tracheostomy (Fig. 3).

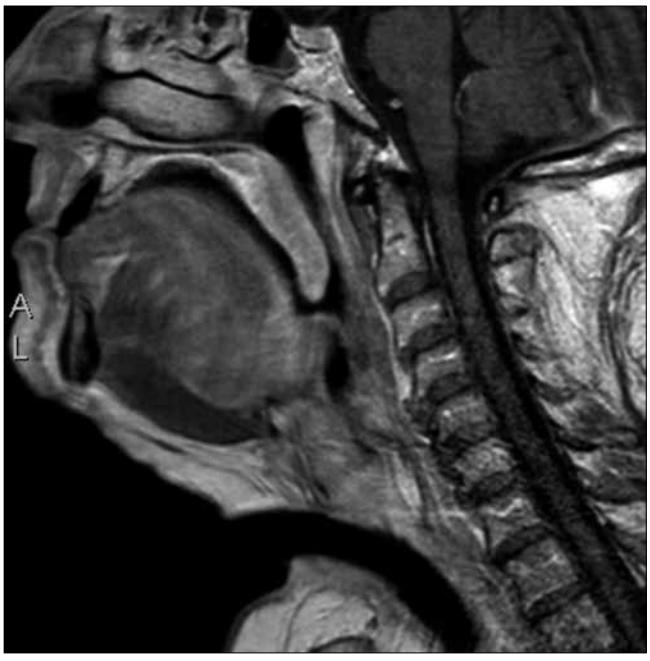

Fig. 2. MRI examination highlighted a muscular verticalised BOT with a significant narrowing of the upper airway.

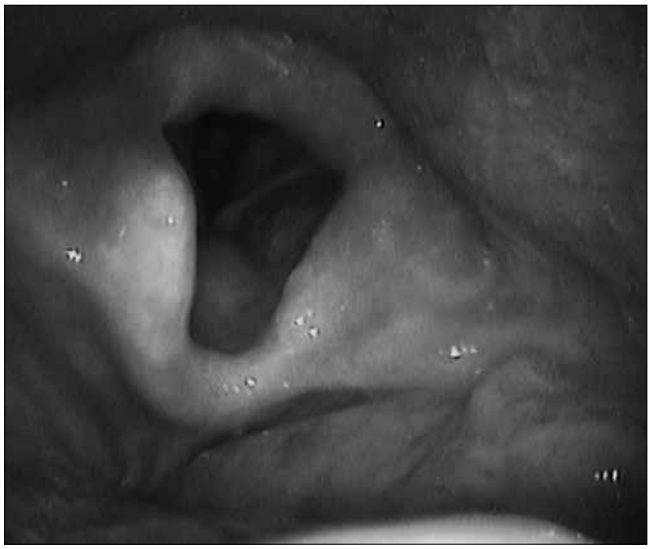

Fig. 3. Endoscopic view after 6 months of follow-up.

Case #2: M.MA. is a 61-year-old woman who developed a severe post-operative oedema of the BOT in January 2014 following microlaryngoscopy and biopsy of BOT lymphatic tissue. For this reason, the patient underwent an urgent tracheostomy performed by otolaryngologists at another Institution.

Several months later, the patient was referred for consultation. Interestingly, the patient had also undergone a previous cervical spine surgery secondary to severe trauma 10 years before the above-reported event. The patient's physical examination demonstrated hypertrophy of the BOT (Fig. 4).

Considering the patient's limited cervical mobility, reduced upper airway volumes due to BOT hypertrophy, several unsuccessful trials of tracheostomy removal, and the high risk of re-intubation failure, our team opted for TORS. In September 2014 the patient underwent TORS BOT resection and SGP; a total volume of 7 mls of lymphoid tissue was removed from the BOT. Three months later in December 2014 the patient was successfully decannulated, and six months later, upon routine follow-up, she continues to do well without a tracheostomy (Fig. 5).

Case #3: S.V. is a 43-year-old man affected by achondroplasia. As part of his anatomic phenotype he presented with a pronounced hypoplasia of the upper maxilla. He underwent a tracheostomy due to acute respiratory failure likely associated with his progressive, severe pattern of OSA. The Apnoea Hypopnoea Index (AHI) was 84, Oxygen Desaturation Index (ODI) 87, Lowest Oxygen Saturation (LOS) 60%, Epworth Scale Score (ESS) 12 and Body Mass Index (BMI) 25.

The patient came to our attention for the evaluation of his OSA syndrome and the possibility of tracheostomy removal. The endoscopic examination highlighted hypertrophy of the BOT completely obstructing the hypopharynx.

Our team recommended a comprehensive evaluation of the upper airway using drug induced sedation endoscopy (DISE) to precisely identify the sites of obstruction contributing to both his OSA and potential decannulation failure [6]. Using the NOHL classification system described by Vicini et al., the patient was found to have severe (75%) transverse (lateral) collapse at the level of the oropharynx (O) and complete (100%) circumferential collapse at the hypopharynx (H) [7].

The patient underwent TORS BOT resection in October 2015 to treat the OSA syndrome and to allow a safer decannulation procedure. A total of 5 mls of tissue was robotically removed from the BOT.

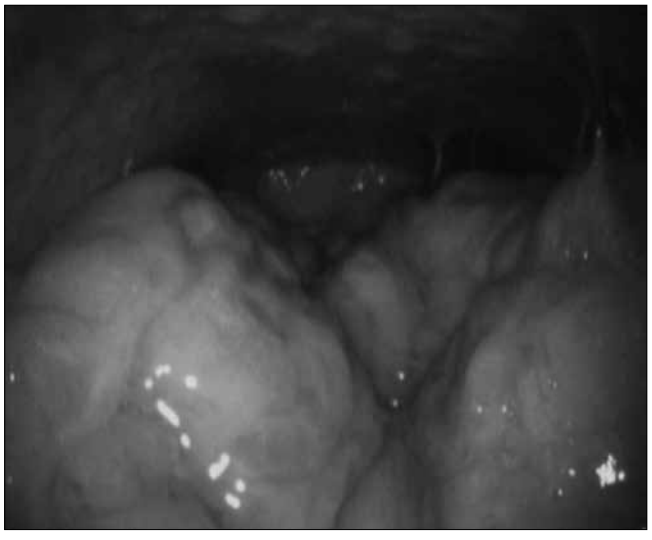

Fig. 4. Endoscopic view shows a lymphatic hypertrophy of the BOT before TORS.

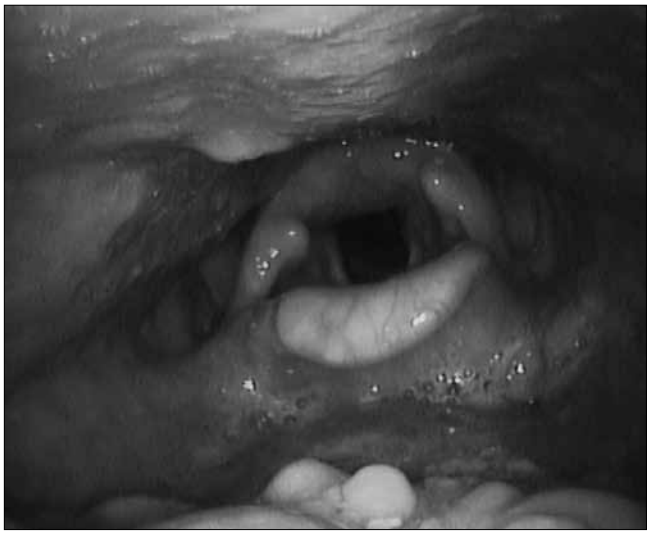

Fig. 5. Endoscopic view after 6 months of follow-up.

In December 2015, the tracheostomy was removed and the patient underwent repeat polysomnography; a significant improvement of the sleep parameters were registered: AHI 16.5, ODI 17.8, LOS 88%. At 6 months follow-up, the patient did not require replacement of the tracheostomy and reported subjective resolution of OSA symptoms.
Case #4: T.L. is a 49-year-old male who underwent a tracheostomy for prolonged intubation (1 week) due to respiratory failure following an epileptic crisis. As part of his evaluation for decannulation, endoscopic evaluation revealed a 40% subglottic stenosis, probably due to the tracheostomy, significant hypertrophy of the BOT and a floppy epiglottis.
In September 2015 the patient underwent a TORS BOT resection with the removal of 18 mls of lymphoid tissue and a SGP to stabilise the epiglottis. In November 2015, the patient underwent serial laser excisions of the suprastomal tissue, and was successfully decannulated. At 6 months follow-up, the patient did not complain of further symptoms and no relapses were highlighted.

Discussion

TORS has been shown to be an effective and safe procedure for the treatment of OSA in patients with hypertrophy of the BOT and floppy epiglottis [3 4]. A low rate of major complications, such as intra-operative or post-operative massive bleeding, has been reported [3 4]. On the other hand, 14.2% of patients do report transient hypogeusia [3].
Furthermore, much attention has focused on TORS as a surgical option for head and neck cancer, in particular, for oropharyngeal and laryngeal carcinomas, reporting promising outcomes [5].
In this paper, a new role for TORS in the management of long-term tracheostomy patients is reported. The indications for placement of a tracheostomy tube are several and include prolonged mechanical ventilation, impaired neurologic status, excessive secretions, and severe upper-airway obstructions [8]. Tracheostomy decannulation is generally accepted when there is no longer a need for airway protection or mechanical ventilation [9]. Specific decannulation strategies are frequently institution-dependent. Some authors consider it once the patient has had the tracheostomy tube plugged for 48 hours or more, whereas others consider it once a speaking valve is tolerated [10].
Endoscopic inspection of the airway prior to decannulation, although not essential, can be helpful. In one study, 67% of patients with tracheostomies were found to have airway abnormalities during bronchoscopy (i.e. tracheal granulomas, tracheomalacia, tracheal stenosis and vocal cord dysfunction) [11].
The epiglottis and tongue base may present additional challenges in successful decannulation. In some cases, anatomical variants preclude easy intubation. Cormack and Lehane, describe anatomic conditions where severe difficulty during intubation can be anticipated (grades 3 and 4). Several anaesthesiologists consider the difficulty of intubation an influencing element in the evaluation of decannulation.
Patients at risk for reintubation following decannulation should be carefully evaluated so that this risk is minimised. A multidisciplinary approach including otolaryngologists and anaesthesiologists must play a lead role. The role of bedside endoscopy, imaging and DISE should be considered on an individualised basis. In our series, three patients came to our attention several months after a prolonged tracheostomy due to difficult reintubation.
The most common airway abnormality in our series was significant hypertrophy of the BOT and a floppy epiglottis. In our opinion, this clinical pattern may be an obstacle for future intubations and revision tracheostomy for repositioning of the endotracheal tube, which carries a higher risk of complications (i.e. tracheomalacia), could be required.
In the second patient, hypertrophy of the BOT was accompanied by reduced cervical mobility, while significant hypoplasia of the upper maxilla characterised the third case, and subglottic stenosis the fourth patient.
All these anatomic patterns may represent an obstacle to effective decannulation and/or to any attempt of re-intubation. In fact, the first two patients experienced several unsuccessful trials of tracheostomy removal before undergoing TORS. The third patient was also affected by OSA and refused the use of positive airway pressure therapy before undergoing tracheostomy. In this case, TORS was strongly indicated and led to two satisfying results: an amelioration of the apnoea and a safer and effective decannulation.

Conclusions

In conclusion, from our experience TORS appears to offer an effective option to aid in the decannulation of patients with severe hypertrophy of the BOT and floppy epiglottis. In our opinion, physicians should be aware of the potential pathological role of these two anatomic conditions and the possible use of TORS as a safe and effective tool for the treatment of these patients.

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Otology

Quality of life measurements for patients with chronic suppurative otitis media: Italian adaptation of "Chronic Ear Survey"

La misura della qualità della vita in pazienti con otite media suppurativa cronica: adattamento in italiano del "Chronic Ear Survey"

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SUMMARY

The chronic ear survey (CES) is a sensitive and disease specific quality of life (QoL) measurement tool in patients with chronic suppurative otitis media (CSOM). It is a 13-item survey that evaluates the frequency, duration and severity of problems associated with this disease. It is composed of three subscales that describe activity restrictions, symptoms and medical resource utilisation. Based on patient's answers, it is possible to obtain a score resulting in a scale ranging from 0 to 100; the highest indicates the best health, while the lowest denotes poor health. The questionnaire was originally created in English. The aim of this study is to validate the CES questionnaire in Italian (CES-I). Translation was made following international guidelines. The application follows the stages of translation from English to Italian and linguistic adaptation, and grammatical and idiomatic equivalence review. The CES-I and the Short Form Health Survey 36 (SF-36) questionnaires were administered to 54 patients with CSOM. A cross-sectional design was used to examine the internal consistency (Cronbach's alpha) and concurrent validity (Pearson's product moment correlation). To confirm the external validity of CES-I, Pearson correlation coefficient, considering the total score and single subscales of CES and the 8 scales of the SF-36, was calculated. Cronbach's alpha coefficient for internal consistency was 0.737. The intraclass correlation coefficient, measured through mixed effects, was 0.737 (95% CI: 0.600–0.835, $p < 0.001$) for average measures and 0.412 (95%CI: 0.273–0.559, $p < 0.001$) for individual measures. According to our results, CES-I is a reliable tool for evaluation of QoL in patients with CSOM among the Italian-speaking population.

KEY WORDS: Quality of life • Chronic suppurative otitis media • Chronic ear survey • Validation • SF-36

RIASSUNTO

Il Chronic Ear Survey (CES) è una misura specifica della Qualità della Vita (QoL) nei pazienti affetti da Otite Media Suppurativa Cronica (CSOM). È un questionario composto da 13 domande che indagano frequenza, durata e severità dei sintomi associati a questa malattia. Il CES genera tre sottoscale con rispettivo punteggio che riguardano limitazioni nelle attività fisiche e sociali, sintomi e trattamento medico. Attraverso le risposte ottenute dai pazienti è possibile ricavare un punteggio che va da 0 a 100; il punteggio più alto indica una QoL migliore, mentre quello più basso indica una QoL peggiore. Il questionario è stato creato in lingua inglese. Lo scopo del lavoro è di validare in lingua italiana il CES. La traduzione è stata condotta seguendo le linee guida internazionali. La versione italiana del CES (CES-I) è stata proposta a 54 pazienti con CSOM. Nello stesso tempo, è stato somministrato a tutti i pazienti anche il questionario SF-36. Un modello trasversale è stato usato per esaminare la consistenza interna (Cronbach alpha) e la validità esterna (coefficiente di Pearson). Per confermare la validità esterna del CES-I è stato poi analizzato il test di correlazione di Pearson considerando il punteggio totale, le singole sottoscale del CES e le 8 scale dello Short Form Health Survey (SF-36). Il coefficiente di Cronbach è stato pari a 0.737. Il coefficiente di correlazione interno ha dato un risultato pari a 0.737 (95% CI: 0.600-0.835, $p < 0.001$) di media e 0.412 (95% CI: 0.237-0.559, $p < 0.001$) per le singole misure. Sulla base dei nostri risultati il questionario CES-I è risultato essere concorde con l'originale in lingua inglese e può essere considerato uno strumento adeguato per valutare la Qualità della Vita nei pazienti con CSOM di lingua italiana.

PAROLE CHIAVE: *Qualità della vita • Otite media suppurativa cronica • Chronic ear survey • Validazione • SF-36*

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Introduction

Chronic suppurative otitis media (CSOM) is characterised by an evident and definite perforation of the tympanic membrane and by constant or intermittent middle ear inflammation often associated to a chronic or intermittent otorrhoea [1]

From a clinical point of view, the CSOM presents significant functional limitations of hearing. Other unpleasant symptoms include malodorous ear, fullness, ear pain, headaches, and vertigo [2]. Tinnitus, although a common symptom also associated to a variety of other conditions [3]

may be present. Although during the recent decades the incidence of CSOM has significantly decreased in developed countries [4] thanks to improvements in housing, hygiene, and antibiotic use, it still represents a dangerous disease with severe complications such as cholesteatoma. Spontaneous healing is rare and the cure is possible only through a medical and surgical therapeutic strategy.

CSOM can severely impact quality of life (QoL) of patients. The concept of QoL emerged in the 1970s as an important new outcome for healthcare [5]. The World Health Organization (WHO) defines the QoL as the individual's perception of his/her position in life, in the context of the culture and value system in which he/she is inserted and in relation to his/her goals, expectations, patterns and worries [6].

The notion of health-related quality of life (HRQoL) has evolved since the 1980s as a subjective and multi-dimensional concept that includes domains related to physical, mental, emotional and social functioning. It goes beyond direct measures of population health, life expectancy, causes of death and focuses on the impact of health status on quality of life [7].

The measurement of general HRQoL is usually performed using a questionnaire called the Short Form 36 Health Survey (SF-36) [8]. The SF-36 includes one multi-item scale that assesses eight different health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions [9]. The eight scales are scored individually and then combined, resulting in a score ranging from 0 to 100; the highest score indicates the best health, while the lowest denotes poor health. An Italian validated version of SF-36 is available since 2000 [10].

It has been demonstrated that SF-36 is not very sensitive to assess the impact of a single disease on HRQoL; moreover, it does not focus on individual symptoms ot mirror the subjective experience of patients [11].

In 2000, Wang and Nadol [12] analysed results of the SF-36 questionnaire in patients with CSOM vs. a control group of healthy subjects: although scores for several of the SF-36 subsections were lower in the CSOM group, no statistically significant differences were found between the CSOM and control groups. Moreover, postoperative SF-36 score did not change significantly compared to the preoperative score.

To overcome these difficulties for patients with CSOM, Nadol proposed and developed the chronic ear survey (CES) questionnaire [13], a sensitive and disease-specific QoL measurement in patients with CSOM. CES is a 13-item survey that evaluates the frequency, duration and severity of problems associated with CSOM, by analysing the total score and 3 subscale scores (activity restrictions, symptoms and medical resource utilisation) that objectively evaluate overall CSOM patient discomfort and the effects of medical and surgical management. The final score ranges from 0 to 100; the highest score indicates the best health, while the lowest denotes poor health.

The CES score has the aim of calculating objective discomforts in patients and the effects of medical and surgical management of CSOM patients. To calculate the total score of the CES questionnaire, it is necessary to apply a definite value from 0 to 100 for every answer. The total values obtained from each section (Activity Restriction, Symptom and Medical Resource) are then divided by the number of the questions (i.e. in the Activity Restriction section the values obtained are added and divided by 3; in the Symptom section are divided by 7). Total values for the three sections (A+S+M) are summed and then divided by 3, resulting in the final value of the questionnaire.

The questionnaire was originally written in English and has been translated and validated in Chinese and Korean [14 15].

The term "validity" indicates the robustness and reliability of a survey, which is a real correspondence between the real world and research findings, and refers to how well a test measures what it is purported to measure [16]. It is essential, therefore, that the questionnaire actually measures what the researcher is intending to measure [17]. The validation process goes beyond simple translation, and a validated questionnaire gives the opportunity to collect and compare data from populations with different languages.

The CES questionnaire has been shown by Nadol et al. [13] to be a valid, disease-specific health measure that can be used to evaluate adult patients with CSOM; however, this tool is not available in the Italian language and therefore cannot be used in the Italian speaking population.

The aim of this paper is to propose a translated version of the CES questionnaire in the Italian language (CES-I) following international guidelines and to assess its validity in an Italian sample of patients affected by unilateral or bilateral CSOM.

Materials and methods

The study was approved by the Ethics Committee of the "Sapienza" University. All patients signed specific informed consent forms.

Translation and adaptation

In the first phase of this study, the CES questionnaire was translated from English into Italian (CES-I). The process of translating the CES questionnaire followed international guidelines through a process of reviews and modifications [18]. Two expert otorhinolaryngologists and two

Table I. Chronic Ear Survey (CES).

Activity Restriction-Based Subscale	
A1	Because of your ear problem, you don't swim or shower without protecting your ear.
	❑ definitely true ❑ true ❑ don't know ❑ false ❑ definitely false
A2	At the present time, how severe a limitation is the necessity to keep water out of your ears?
	❑ very severe ❑ severe ❑ moderate ❑ mild ❑ very mild ❑ none
A3	In the past 4 weeks, has your ear problem interfered with your social activities with friends, family, or groups?
	❑all of the time ❑ most of the time ❑ a good bit of the time ❑ some of the time ❑ a little of the time ❑ none
Symptom Subscale	
S1	Your hearing loss is:
	❑ very severe ❑ severe ❑ moderate ❑ mild ❑ very mild ❑ none
S2	Drainage from your ear is:
	❑ very severe ❑ severe ❑ moderate ❑ mild ❑ very mild ❑ none
S3	Pain from your ear is:
	❑ very severe ❑ severe ❑ moderate ❑ mild ❑ very mild ❑ none
S4	Odor from your ear is very bothersome to you and/or others:
	❑ definitely true ❑ true ❑ don't know ❑ false ❑ definitely false
S5	The hearing loss in your affected ear bothers you:
	❑ all of the time ❑ most of the time ❑ a good bit of the time ❑ some of the time ❑ a little of the time ❑ none
S6	In the past 6 months, please estimate the frequency that your affected ear has drained:
	❑ constantly ❑ >5 times, but not constantly ❑ 3-4 times ❑ 1-2 times ❑ not at all
S7	The odor from your affected ear bothers you and/or others:
	❑ all of the time ❑ most of the time ❑ a good bit of the time ❑ some of the time ❑ a little of the time ❑ none
Medical Resource Utilisation Subscale	
M1	In the past 6 months, how many separate times have you visited your physician, specifically about your ear problem?
	❑ >6 times ❑ >5 times, but not constantly ❑ 3-4 times ❑ 1-2 times ❑ not at all
M2	In the past 6 months, how many separate times have you used oral antibiotics to treat your ear infection?
	❑ >6 times ❑ >5 times, but not constantly ❑ 3-4 times ❑ 1-2 times ❑ not at all
M3	In the past 6 months, how many separate times have ear drops been necessary to treat your ear condition?
	❑ >6 times ❑ >5 times, but not constantly ❑ 3-4 times ❑ 1-2 times ❑ not at all

Table II. Chronic Ear Survey (CES) score calculation.

Activity Restriction: (A1 + A2 + A3) / 3 = A	
A1	0-25-50-75-100
A2	0-20-40-60-80-100
A3	0-20-40-60-80-100
Symptoms: (S1 + S2 + S3 + S4 + S5 + S6 + S7) / 7 = S	
S1	0-20-40-60-80-100
S2	0-20-40-60-80-100
S3	0-20-40-60-80-100
S4	0-25-50-75-100
S5	0-20-40-60-80-100
S6	0-25-50-75-100
S7	0-20-40-60-80-100
Medical Resource: (M1 + M2 + M3) / 3 = M	
M1	0-25-50-75-100
M2	0-25-50-75-100
M3	0-25-50-75-100

psychologists performed, separately, an initial translation from the English language. The translated versions were then discussed and adjusted to obtain consensus and close equivalence to the original version. The text was then back translated from Italian into English by a bilingual person with a professional academic level of Italian and English and by a native English speaker. The original and back-translated English versions were compared by the two translators and, if discrepancies were found, the new version was adjusted to optimise the conceptual overlap.

Study validation

In the second phase of this study, we enrolled 54 patients affected by CSOM presenting to our clinic between November 2014 and November 2015 to evaluate the validity of the CES-I questionnaire and compare the results to those obtained with the Italian validated SF-36 survey. Diagnosis of CSOM was performed with medical history,

Table III. Italian version of Chronic Ear survey (CES-I).

Limitazione delle attività	
A1	A causa della malattia dell'orecchio, non può nuotare o fare la doccia senza proteggerlo.
	❑ sicuramente vero ❑ vero ❑ non so ❑ falso ❑ sicuramente falso
A2	In questo momento quanto è grave dover tenere l'acqua lontano dall'orecchio?
	❑ molto grave ❑ grave ❑ moderato ❑ medio ❑ lieve ❑ nullo
A3	Nelle ultime 4 settimane la malattia dell'orecchio ha condizionato le sue attività in famiglia o con gli amici?
	❑ sempre ❑ molto spesso ❑ una buona parte del tempo ❑ talvolta ❑ per un breve periodo ❑ mai
Sintomi	
S1	Ora, la perdita di udito è:
	❑ molto grave ❑ grave ❑ moderata ❑ media ❑ lieve ❑ nulla
S2	Ora, la secrezione dell'orecchio è:
	❑ molto abbondante ❑ abbondante ❑ moderata ❑ media ❑ lieve ❑ nulla
S3	Ora, il dolore dell'orecchio è:
	❑ molto grave ❑ grave ❑ moderato ❑ medio ❑ lieve ❑ nullo
S4	L'odore dell'orecchio la preoccupa molto e/o preoccupa gli altri:
	❑ sicuramente vero ❑ vero ❑ non so ❑ falso ❑ sicuramente falso
S5	La perdita di udito la preoccupa:
	❑ sempre ❑ molto spesso ❑ una buona parte del tempo ❑ talvolta ❑ per un breve periodo ❑ mai
S6	Negli ultimi 6 mesi quante volte l'orecchio ha prodotto pus:
	❑ costantemente ❑ 5 o più volte ma non costantemente ❑ 3-4 volte ❑ 1-2 volte ❑ mai
S7	L'odore dell'orecchio la preoccupa e/o preoccupa gli altri:
	❑ sempre ❑ molto spesso ❑ una buona parte del tempo ❑ talvolta ❑ per un breve periodo ❑ mai
Interventi medici	
M1	Negli ultimi 6 mesi, quante volte è stato visitato dal suo medico per l'orecchio:
	❑ più di 6 volte ❑ 5-6 volte ❑ 3-4 volte ❑ 1-2 volte ❑ nessuna
M2	Negli ultimi 6 mesi, quante volte ha usato antibiotici orali per curare l'infezione dell'orecchio:
	❑ più di 6 volte ❑ 5-6 volte ❑ 3-4 volte ❑ 1-2 volte ❑ nessuna
M3	Negli ultimi 6 mesi quante volte sono stati necessari periodi di cura con gocce auricolari?
	❑ più di 6 volte ❑ 5-6 volte ❑ 3-4 volte ❑ 1-2 volte ❑ nessuna

general ENT examination (including micro-otoscopy), pure tone audiometry (PTA) and high resolution computerised tomography (CT) of temporal bone. Further data (age, gender, unilateral or bilateral disease) were collected. After obtaining written consent, the CES-I and SF-36 forms were administered to all patients.

Statistical analysis

Collected data were analysed statistically. Measures of central tendency (mean and median) as well as dispersion measures (standard deviation, SD; range: minimum – maximum) were calculated. Test-retest reliability of the CES-I was determined by the intraclass correlation coefficient (ICC). A cross-sectional design was used to examine the internal consistency (Cronbach's alpha) and concurrent validity (Pearson's product moment correlation). Pearson correlation coefficient between the total score and single subscales of CES and the 8 scales of the SF-36 was used to examine the correlation between the CES-I and SF36. Physical composite score (PCS) and mental composite score (MCS) were calculated as summary criteria for the HRQoL. Statistical significance was set at $p < 0.05$. Statistical analysis was carried out using SPSS 22.0.

Results

54 patients were enrolled in the study, 26 (48.1%) were females and 28 (51.9%) males, with a median age of 42 (range 24-61) years. Bilateral CSOM was diagnosed in 18% of subjects.

In our sample, the CES-I presented a median value for activity restriction, symptoms and medical resources of 8, 22.5 and 9, respectively. The total score had a median value of 38.5 (range 14-53). The median PCS and MCS scores of SF-36 were 50.3 and 47.5, respectively, which are close to the median values for the Italian population (PCS = 53.3 and MCS = 49.3)

The validity analysis of the CES-I questionnaire was strongly supported by our statistical analysis: Cronbach's

Table IV. Characteristics of the patients according to the CES-I and SF-36 questionnaires.

Variable	Mean	Median	SD, Range (min-max)
Chronic Ear Survey - I (CES-I)			
Activity restriction sum	8.04	8.00	3.17 (1-13)
Symptoms sum	22.31	22.50	4.75 (11-31)
Medical resources sum	8.70	9.00	2.52 (0-12)
Total sum	39.06	38.50	7.02 (14-53)
Short Form 36 (SF-36)			
Physical composite score (PCS)	49.28	50.35	8.55 (27.14-64.08)
Mental composite score (MCS)	45.54	47.57	10.16 (20.22-60.53)

alpha was 0.737, while the intraclass correlation coefficient (ICC), measured through mixed effects, was 0.737 (95% CI: 0.600 – 0.835, $p < 0.001$) for average measures and 0.412 (95% CI: 0.273–0.559, $p < 0.001$) for individual measures.

Moreover, correlation analysis between the CES-I and SF-36 scores (the 8 areas and the two composite scores, PCS and MCS) was performed: a significant correlation was found between AR sum and the physical function (PF) score ($r = 0.282$, $p = 0.039$), between MR sum and role emotional score ($r = - 0.303$, $p = 0.026$), and between the MR sum and the MCS score ($r = - 0.273$, $p = 0.045$)

Discussion

Over the last decade, there has been growing interest in developing instruments to define surgical and nonsurgical outcomes from a patient's perspective. Patient-reported outcome measures (PROMs) currently play a significant role in the assessment of outcome for reflective practice, audit and research. These PROMs consist of different methods of data collection such as tests, behavioural observations, content analysis, interviews, questionnaires, physiological and neuropsychological measures, inventories and personality scales measuring attitudes.

The questionnaire is a very effective tool for data collection in terms of reliability and validity of the data. The word validation is often used indiscriminately to define a process of survey evaluation, whereas certain tests, such as evaluating internal consistency, are not truly tests of validity. The evaluation of survey instruments comes under the branch of survey research known as psychometrics. Generally, this process can be split into the evaluation of reliability and validity. Reliability takes the form of features such as test-retest reliability, alternate-form reliability, internal consistency, interobserver reliability and intraobserver reliability. Validation, on the other hand, takes the form of content validity, criterion validity and construct validity [19].

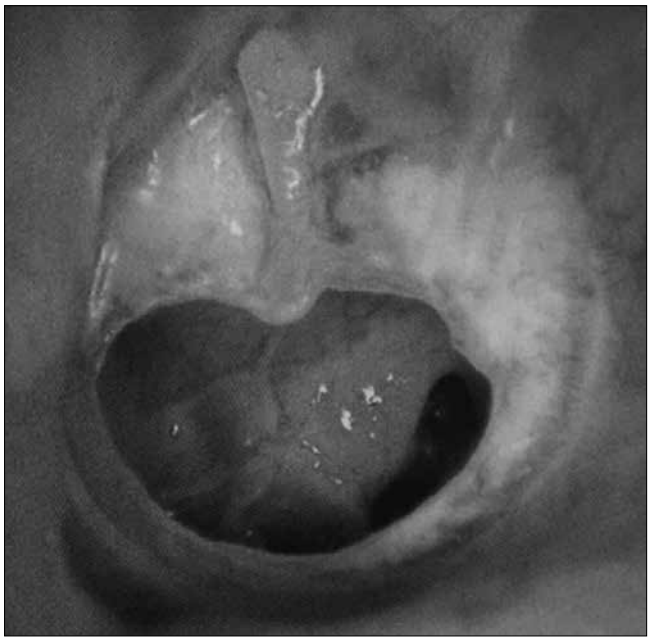

Fig. 1. Otoscopic image of CSOM with central perforation of the tympanic membrane.

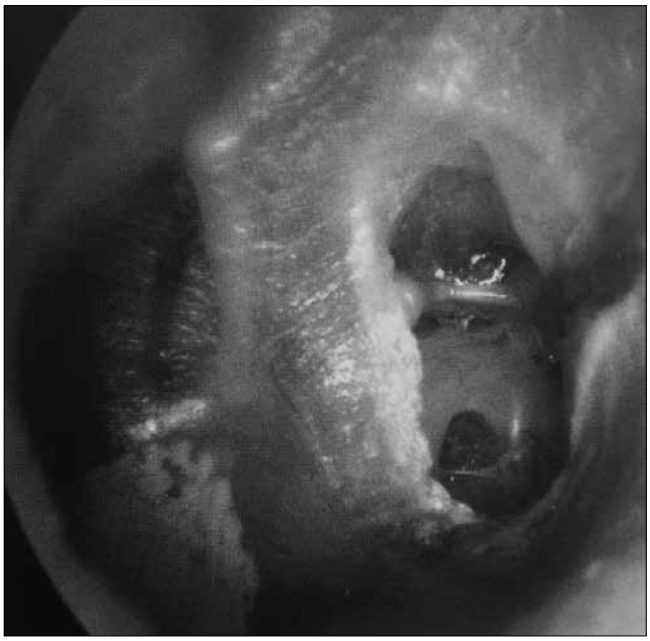

Fig. 2. Otoscopic image of CSOM with marginal perforation of the tympanic membrane.

Questionnaire validation is not a single exercise, and to achieve some forms of validation, such as construct validation, the process involves gathering a group of different types of data over a multitude of settings and populations over a number of years.

A number of studies have investigated association between social aspects and health. More information on social aspects among people with chronic illness could increase our understanding of the processes involved in the wide variety of situations [20]. Using questionnaires in different languages gives us the opportunity to study and compare different populations and cultures, gather information from various health systems and understand the importance of a disease and its treatment adequacy.

So far, there has been no Italian instrument available to assess QoL for patients with CSOM; although an Italian version of the SF-36 survey was proposed in 2000, it has proven not to be sensitive enough for this condition. In this study, we demonstrate the CES-I to be a valid, disease-specific health measure that can be used to evaluate adult patients with CSOM among the Italian speaking population.

In our validation process, we found a Cronbach's alpha of 0.737, demonstrating very good reliability of this tool in the Italian setting. Correlation of the CES-I with the validated Italian SF-36 general health measure was used as a test of convergent validity: when comparing our results to the findings of Nadol et al., the CES total survey score had significant correlation with several subscales of the SF-36: a significant correlation was found between AR sum and PF score, between MR sum and role emotional score, and between MR sum and MCS score.

In the AR subscale, there are questions about the restriction of social activity by hearing loss, and this correlation means that there may be an important influence of QoL in patients who experience improvements in hearing.

In our results, the MR and AR subscale scores are lower than those on other subscales. This may be attributable to the easy accessibility of medical resources and suggests that patients included in this study were adapted to their status, including their hearing loss, and were more cautious in their daily life. Neither the CES nor the CCES (as demonstrated in the Chinese validation study) were significantly correlated with PTA: this is a finding that should be further analysed in future studies. The CES questionnaire, and consequently the CEI-I in the Italian population, appeared to be a valid, reliable and sensitive disease-specific health measure that adds another dimension to our understanding of the impact of the disease on patients with CSOM.

Conclusions

CSOM is a common disease that has a significant health impact on general population and is far from being eradicated. The use of tools that are able to evaluate QoL in patients affected by this disease is useful for greater awareness of the results of surgical and medical treatments. The CES questionnaire was a valid tool to assess QoL in CSOM patients; however, the absence of an Italian version of this tool makes it difficult to use it among Italian speaking patients. Based on the results of our study, the CES-I questionnaire appears to be a reliable and valid instrument for the investigation of health status among Italian speaking patients with CSOM.

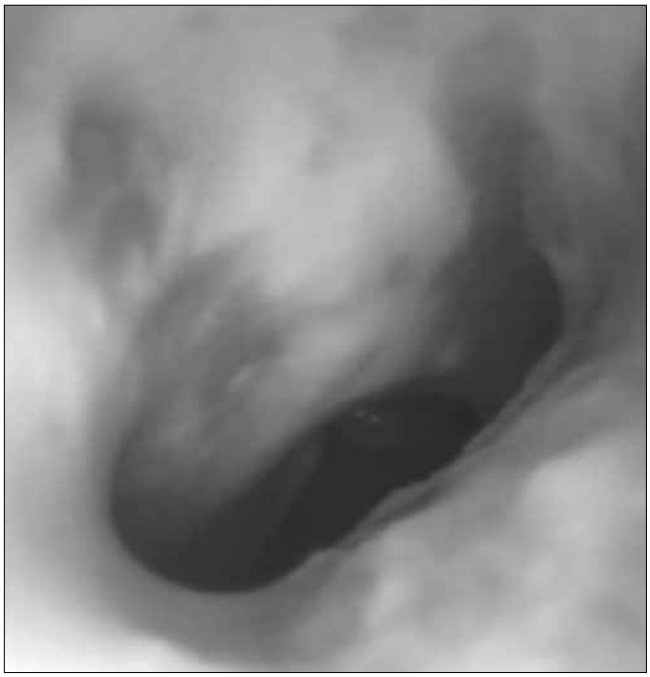

Fig. 3. Otoscopic image of "active" CSOM with anterior perforation of the tympanic membrane.

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Audiology

Influence of temporal resolution skills in speech discrimination abilities of older subjects

Influenza della risoluzione temporale nelle abilità di discriminazione verbale dei soggetti anziani

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SUMMARY

Compromised temporal resolving power of the auditory system can be one of the main factors contributing to poor speech perception skills in the elderly. Very few of the earlier studies have systematically examined this aspect. Hence, the current study was designed with the following objectives: 1) To establish normative database of Gaps in Noise (GIN) test in elderly population in an Indian context. 2) To determine the relationship between speech discrimination skills and temporal resolution abilities in elderly individuals with normal hearing sensitivity. Thirty normal hearing elderly individuals (age range: 55-75 years; mean age: 59.86 ± 4.11 years) participated in the study. The audiological evaluation comprised of tympanometry, puretone and speech audiometry (Speech Reception Threshold-SRT, Speech Discrimination Score-SDS) and GIN. The results of the present study revealed mean Gap Detection Threshold (GDT) of 8.7 msec (SD = 3.38) in the right ear and 8.83 msec (SD = 2.86) in the left ear for the older participants. The mean Total Percentage Score (TPS) in the right ear was 47% (SD = 11.92) and 45% (SD = 11.29) in the left ear. These results suggest that temporal resolution abilities are poor in the elderly compared to the young and middle-aged group. There was no significant ear based difference on either GDT or TPS. The GDT was inversely correlated with speech discrimination performance. The TPS was positively correlated with SDS. This study clearly demonstrated a positive relationship between temporal resolution abilities and speech discrimination. The current database might be useful when assessing temporal resolution abilities in hearing impaired elderly individuals. Furthermore, all elderly individuals should undergo temporal resolution evaluation, irrespective of their hearing status, during audiological assessment.

KEY WORDS: Temporal resolution• Elderly group • Speech discrimination • GIN

RIASSUNTO

Un alterata capacità di risoluzione temporale del sistema uditivo può essere uno dei fattori alla base della ridotta discriminazione verbale nei soggetti anziani. Ben pochi studi in passato hanno approfondito questo aspetto in modo sistematico. Il presente studio si è pertanto posto come obiettivi: 1) Stabilire una normativa in una popolazione di anziani in un contesto culturale Indiano per il test Gaps in Noise (GIN); 2) Stabilire la relazione fra la discriminazione verbale e le capacità di risoluzione temporale in una popolazione di individui anziani normo udenti. Sono stati arruolati complessivamente trenta pazienti anziani normo udenti (età: 55-75 anni; età media: 59,86 ± 4,11 anni). La valutazione audiologica si è composta di una timpanometria, audiometria tonale e vocale (Speech Reception Threshold-SRT, Speech Discrimination Score-SDS) e il GIN. I risultati del presente studio ci hanno permesso di determinare per la popolazione studiata un GDT (Gap Detection Threshold) medio di 8,7 millisecondi (SD : 3,38) per l'orecchio destro e di 8,83 millisecondi (SD : 2,86) per l'orecchio sinistro. Il TPS (Total Percentage Score) medio per l'orecchio destro è stato del 47% (SD : 11,92) e per l'orecchio sinistro del 45% (SD : 11,29). I nostri risultati suggeriscono che le abilità di discriminazione temporale siano peggiori nei soggetti anziani rispetto ai soggetti giovani e adulti. Né il TPS né il GDT hanno mostrato differenze interaurali significative. Il GDT ha presentato una correlazione inversa con le performance di discriminazione verbale. Il TPS ha mostrato una correlazione diretta con l'SDS. Il presente studio dimostra in modo chiaro la relazione che intercorre fra la discriminazione verbale e le abilità di risoluzione temporale. Il presente database di dati rappresenta una risorsa per la valutazione delle abilità di risoluzione temporale in soggetti anziani affetti da ipoacusia. Riteniamo che tutti i soggetti anziani vadano sottoposti a una valutazione delle abilità di risoluzione temporale nel corso di una valutazione dell'udito, indipendentemente dal fatto che siano o meno affetti da alterazioni uditive.

PAROLE CHIAVE: *Risoluzione temporale • Anziani • Discriminazione verbale • GIN*

Acta Otorhinolaryngol Ital 2017;37:58-62

Introduction

Hearing loss (presbycusis) affects approximately one in three people between the ages of 65 and 74 years. By 75 years of age, nearly one-half of all individuals suffer from presbycusis [1]. Earlier, presbycusis was thought to be primarily a deficit in the function of the ear; however, recent studies revealed that there may be involvement of the central auditory system as well [2].

Most elderly individuals, even without greater degree of hearing loss, have difficulty in understanding speech compared with younger adults in both noisy and quiet situations [3 4]. Elderly people also experience difficulty with all types of altered speech, i.e., fast speech [5 6], interrupted speech [5 7] and reverberated speech [5]. Smith and Prather [8] found a decrement in performance for elderly listeners in comparison to young listeners for speech discrimination of consonant-vowel (CV) nonsense syllables across a range of sensation levels (SLs) and signal-to-noise (S/N) ratios.

Temporal cues in auditory signals are the basis for speech discrimination [9] especially for detecting rise/fall time [10], voice onset time (VOT) [11-14] and other transient part of the stimuli (onset of syllables). Accurate speech discrimination requires precise temporal processing [15]. According to Shinn [16], temporal processing may be conceptualised as four sub-processes which include temporal resolution, temporal patterning, temporal integration and temporal masking. These skills are particularly crucial for phonemic distinction, lexical and prosodic distinctions and auditory closure [17].

Reduced temporal resolving power of the auditory system can be one of the main factors contributing to poor speech perception skills in elderly. It is reported that temporal resolution skills decline before the age of 60 years even in individuals with normal hearing sensitivity [18 19].

As per the technical report by the American Speech-Language-Hearing Association [20], temporal resolution is considered as an important component of central auditory processing. Gap detection (GD) is one of the most common measures to assess temporal resolution. Numerous studies have reported elevated GD thresholds in elderly individuals with hearing loss [21-23]. A few studies have also reported elevated GD thresholds in elderly individuals with normal hearing sensitivity [24 25].

Very few of the earlier studies have systematically examined changes in auditory temporal resolution and speech perception performance in elderly individuals with normal hearing sensitivity. Hence, the current study was designed to focus on the influence of temporal resolution skills (measured by GD) on speech discrimination abilities in elderly individuals with normal hearing sensitivity (as investigated by puretone audiometry). GD measures were obtained using Gaps-In-Noise (GIN) test. The present study had two objectives: 1) To establish normative database of GIN test in the elderly population in an Indian context, 2) To determine the relationship between speech discrimination skills and temporal resolution abilities in elderly individuals with normal hearing sensitivity.

Materials and methods

Thirty normal hearing (puretone thresholds of ≤ 25 dB hearing level-HL from 0.25 kHz to 8.0 kHz) elderly individuals (age range: 55-75 years; mean age: 59.86 ± 4.11 years) participated in the study. All had normal middle ear function as shown by 'A' type tympanogram. None had history of noise exposure, ear infections, or any other neurological/psychiatric conditions. Informed consent was obtained from all participants.

The audiological evaluation comprised of tympanometry, puretone and speech audiometry (Speech Reception Threshold-SRT, Speech Discrimination Score-SDS) and GIN. For obtaining SRT, the presentation level was set at intensity 20 dB higher than the puretone average. The subject was presented with spondee words one at a time. The lowest intensity level at which 50% of correct responses were obtained was considered as the SRT. For obtaining SDS, the presentation level was set at intensity 40 dB higher than the SRT. The subject was presented with 20 phonetically balanced monosyllables. Each correct repetition of the syllable carried 5% and the SDS was calculated using the formula:

$$\text{SDS} = \frac{\text{Number of correct responses}}{\text{Total number of stimuli}} \times 100.$$

The GIN test was presented through dual channel audiometer (GSI 61 clinical-diagnostic audiometer with TDH-50 P earphones). The test CD was played using a CD player connected to the audiometer. The GIN test was administered bilaterally and scoring was done as per the standard criteria [26]. The stimulus was presented at 35-50 decibel Sensation Level (dB SL) with reference to puretone average. The indices used were Gap Detection Threshold (GDT) and Total Percentage Score (TPS). Practice list provided in the test was used to train the participants for comprehension of the task. The test was comprised of four different lists containing up to 36 signal segments of 6 seconds white noise in each list. The number of gaps of silence in each signal varied from 0-3. The duration of each gap were either 2, 3, 4, 5, 6, 8, 10, 12, 15 or 20 msec with each silence gap duration occurring six times in each GIN list. Thus, each GIN list consisted of a total of 60 gaps and the order of gap durations were randomised. A five sec gap of silence separated each six sec noise segment. One list was administered in each ear. While administering the test, subjects were instructed to listen for any silence gap that may or may not occur within each noise burst. As soon as the gap was detected, the subject had to respond by pressing a button.

The GDT was calculated by considering: (1) minimum gap duration correctly identified 4 out of 6 times and (2) similar or better performance for longer gap durations. The TPS was calculated by dividing the total number of gap durations correctly identified by the total number of gap durations presented (n = 60) multiplied by 100. False positives were noted separately. More than two false positives per ear were counted as errors and subtracted from the number of gap durations correctly identified.

The entire test procedure was carried out in a sound-treated room. The ambient noise levels were maintained within the permissible levels according to American National Standards Institute [27].

Results

Demographic data

Among participants, there were 20 males (66%) and 10 (34%) females. All participants presented with normal hearing sensitivity whose mean and standard deviation (SD) of Puretone Average (PTA) (average of air conduction-AC thresholds at 500 Hz, 1 kHz and 2 kHz) and All Frequency Average -AFA (average of AC thresholds at 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz, 6 kHz & 8 kHz) of each ear were computed separately (Table I).

Speech Discrimination Score (SDS)

Descriptive statistical analysis was done and calculated the mean (*M*) and standard deviation (*SD*) for SDS in the right ear (*M* = 91.83%, *SD* = 8.54) and in the left ear (*M* = 90.33%, *SD* = 8.89) for all participants. A parametric, two-tailed paired *t* test was used to analyse the ear- based performance difference on SDS. The results revealed that there was no significant difference between the performance of the right and left ears on SDS (t (29) = 1.964, $p = 0.059$).

Performance on Gaps-In- Noise (GIN) test

The two measures derived were GDT and TPS. Mean and standard deviation for both measures were obtained. The ear based performance was analysed for both measures using two-tailed paired *t* test. There was no significant ear based difference on either GDT (Table II) or TPS (Table III). Both ears performed equally for the GIN test.

The other objective of the current study was to determine whether there is any relationship between performance on the GIN test and speech discrimination. To address this issue, the Pearson *r* correlation analysis was computed using the following variables: GDT, TPS and SDS (Table IV).

The correlation analysis revealed two significant correlations of interest. The GDT was inversely correlated with speech discrimination performance as shown below: For GDT right and SDS right (r = -0.566, $p < 0.05$) and for GDT left and SDS left (r =-0.644, $p < 0.05$). These results indicated that as the GDT improved, speech discrimination abilities were better.

The TPS was positively correlated with SDS. It should be noted that the correlation between these variables was assessed for both ears (i.e., for TPS and SDS in right ear, $r = 0.389$, $p < 0.05$ and for TPS and SDS in left ear, $r = 0.506$, $p < 0.05$).

Table I. Mean (M) and standard deviation (SD) of PTA and AFA in the elderly population.

n= 30	MEAN (M)	SD
PTA RT (dB)	17.60	3.75
PTA LT (dB)	19.06	6.69
AFA RT (dB)	20.40	4.48
AFA LT (dB)	2l. 42	5.65

PTA, pure tone average; AFA, all frequency average; RT for right ear and LT for left ear

Table II. Results of t-test and descriptive statistics for Gap Detection Threshold (GDT) in the right and left ears.

	Right ear (n = 30)	Left ear (n = 30)	t	t	Df	95% CI of the difference
	Mean (SD)	Mean (SD)				
GDT (msec)	8.7 (3.28)	8.83 (2.86)	-0.394		29	-0.824, 0.558

p = 0.696
Statistical significance at p < 0.05.

Table III. Results of t-test and descriptive statistics for Total Percentage Score (TPS) in the right and left ears.

	Right ear (n = 30)	Left ear (n = 30)	t	t	Df	95% CI of the difference
	Mean (SD)	Mean (SD)				
TPS	47.96 (11.92)	45.03 (11.29)	0.769		29	-1.771, 3.920

p = 0.448
Statistical significance at p < 0.05.

Discussion

Temporal resolution is, most likely, one of the basic components for speech perception and its assessment may provide information about the neural integrity of the central auditory nervous system [2]. The results of the present study documented a mean GDT of 8.7 msec (SD = 3.38) in the right ear and 8.83 msec (SD = 2.86) in the left ear for the older participants. The mean TPS in the right ear was 47% (SD = 11.92) and 45% (SD = 11.29) in the left ear. These findings are different from those observed for the adult population previously. Museik et al. [26] found relatively lower mean GDT (right ear = 4.9 msec and left ear = 4.8 msec) and comparatively higher TPS score (70% bilaterally) for the adult population of the age range of 13 to 46 years. When the GIN test was employed in an Indian

Table IV. Result of Pearson r Correlation among three variables of the elderly participants.

		SDS (RT)	SDS(LT)	GDT(RT)	GDT LT	TPS (RT)	TPS (LT)
SDS (RT)	Pearson Correlation	1	0.88**	-0.566**	-0.445*	0.389*	0.459*
	Sig. (2-tailed)		0.000		0.014	0.034	0.011
	N	30	30	30	30	30	30
SDS (LT)	Pearson Correlation	0.885**	1	-0.599**	-0.644**	0.493**	0.506**
	Sig. (2-tailed)	0.000		0.000	0.001	0.006	0.004
	N	30	30	30	30	30	30
GDT (RT)	Pearson Correlation	-0.566**	-0.599**	1	0.827**	-0.846**	-0.742**
	Sig. (2-tailed)	0.001	0.000		0.000	0.000	0.000
	N	30	30	30	30	30	30
GDT (LT)	Pearson Correlation	-0.445*	-.0644**	0.827**	1	-0.740**	-0.698**
	Sig. (2-tailed)	0.014	0.000	0.000		0.000	0.000
	N	30	30	30	30	30	30
TPS (RT)	Pearson Correlation	0.389*	0.493**	-0.846**	-0.740**	1	0.787**
	Sig. (2-tailed)	0.034	0.006	0.000	0.000		0.000
	N	30	30	30	30	30	30
TPS (LT)	Pearson Correlation	0.459*	0.506**	-0.742**	-0.698**	0.787**	1
	Sig. (2-tailed)	0.011	0.004	0.000	0.000	0.000	
	N	30	30	30	30	30	30

*** Correlation is significant at the 0.01 level (2-tailed).*
** Correlation is significant at the 0.05 level (2-tailed).*

context for the normative data (age range = 17-60 years), the mean GDT obtained was 5.82 msec in the right ear and 5.84 in the left ear. Mean TPS in the right ear was 62.99% and 63.25% in the left ear [28]. However, results similar to the present findings were observed in a study on older women (n = 33, age range = early 60s) with overall average GDT of 8.3 msec. TPS was not considered in that study [29]. Hence, it is clear that older individuals demonstrate poor temporal resolution skills in comparison to younger and middle-aged individuals. The study done by Snell & Frisina [30] also supported this finding. These results suggest that age-related changes in the central auditory system can contribute to deterioration of temporal resolution abilities in the elderly group in spite of normal hearing sensitivity.

The performance between ears of older individuals did not show any significant difference for either GDT or TPS. This suggests equal performance of both ears on the GIN test. Similar findings were observed by Shinn, Chermak & Musiek [31] in a very young population (n = 72) and in an adult population [28].

To establish the relationship between temporal resolution skills and speech discrimination abilities in elderly individuals, a correlation analysis was done. Speech discrimination abilities demonstrated positive correlation with TPS and negative correlation with GDT. Similar findings in middle-aged women were reported by Helfer & Vargo [19], using the GIN test and considering speech understanding ability in competing speech situations.

From the present study, we obtained a normative database of the GIN test for the elderly population. This may be useful when assessing temporal resolution abilities in hearing impaired elderly individuals.

It should also be noted that the proportion of elderly individuals have tremendously increased in modern society because of advancements in medical care. Hence, the current study has great social relevance. The subtle mechanisms involved in speech understanding difficulty in elders needs to be clearly understood. Such information may be crucial in planning rehabilitation strategies. This study also has relevance in the Indian context as the relationship between temporal resolution and speech understanding has not been thoroughly investigated.

The present study could have considered a larger database of elderly population for better validation. Furthermore, gender-based differences in terms of temporal resolution in elderly were not assessed. These aspects can be considered as the limitations of the study.

The elderly should be counseled to take effective steps to avoid further deterioration in auditory function by getting exposed to noisy environments, overuse of mobile phones or persisting with harmful habits like smoking. Additionally, in our opinion all elderly individuals should undergo temporal resolution evaluation irrespective of their hearing status during audiological assessment.

Conclusions

The present study clearly indicated that temporal resolution skills are impaired even in normal hearing elderly individuals. The observed decline in temporal and speech processing abilities in normal hearing older population might be due to compromised function of central auditory system. Furthermore, the normative database that was obtained for GIN test in elderly population can be useful

in clinical assessment of hearing impaired elderly individuals. Future research should focus on comparing temporal resolution skills in the normal hearing elderly group with age-matched subjects with varying types of pathologies (conductive, sensory and neural) to understand the effects of each on temporal resolution.

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Letter to the Editor

Beware of the dangers along the path towards the diagnosis of HPV-driven oropharyngeal squamous cell carcinoma

Attenzione alle insidie riservate dalla diagnosi del carcinoma squamocellulare orofaringeo HPV-relato

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Dear Editor,

A growing incidence of oropharyngeal squamous cell carcinoma (OPSCC) has been observed in many areas in high income countries. This so-called cancer epidemic has been attributed to an increasing causal role of high-risk alpha human papillomavirus (HR α-HPV) in this malignancy. HR α-HPV types, mainly HPV type 16, are now ascertained to be aetiologically associated with a subset of OPSCC arising from the crypt epithelium of the palatine and lingual tonsils [1]. In addition, HR α-HPVs are responsable for a substantial fraction of SCCs from unknown primary metastatic to the neck nodes (CUP) [2].

Identifying those HR α-HPV infections that are the real driving force behind the oropharyngeal carcinogenesis process is of paramount importance. Unlike cervical and other anogenital cancers, HR α-HPVs play a causal role only in a subset of OPSCCs to which they confer a significantly better prognosis [1]. Furthermore, several ongoing clinical trials are exploring the feasability of treatment de-escalation in this subset of OPSCC in the attempt to reduce toxicity, while maintaining the same efficacy of traditional treatment regimens [3].

Detection of HPV E6/E7 mRNA by polymerase chain reaction (PCR) in frozen samples is considered the gold standard for diagnosis of oncologically-relevant HPV infections. A spliced version of the E6 transcript, the ultra-short E6*I mRNA, can be successfully analysed even in formalin-fixed, paraffin-embedded (FFPE) specimens [4]. In a clinical setting, HPV-status is mainly assessed using FFPE-based diagnostic tests such as HR α-HPV DNA detection (by PCR or *in situ* hybridisation) or immunostaining for cyclin-dependent kinase inhibitor $p16^{INK4a}$, considered a surrogate marker for active HPV involvement in OPSCC tumourigenesis. Unfortunately, both markers lack adequate sensitivity and/or specificity. In particular, HR α-HPV DNA testing positive to PCR sequencing may be linked to transient, non-transforming HPV infection or to specimen processing/laboratory contamination [5]. In addition, $p16^{INK4a}$ over-expression could be triggered by HPV-independent deregulation of $p16^{INK4a}$/RB signalling pathway [6]. Finally, fluorescence in situ hybridisation, which is characterised by acceptable specificity, lacks sufficient sensitivity [7].

Consistently, prognostic stratification based on HR α-HPV-DNA or $p16^{INK4a}$ immunostaining as standalone tests is unsatisfactory compared to those based on more accurate markers of transforming infection such as E6/E7 mRNA, high viral load, or E6 seropositivity [8 9]. On the other hand, double positivity to HR α-HPV-DNA and $p16^{INK4a}$ immunostaining is largely accepted to be a good compromise when oncologically-relevant infections are being determined in a clinical context [10].

Surprisingly, most of the ongoing clinical trials exploring treatment de-escalation in patients with supposed HR α-HPV-related OPSCC are recruiting patients on the basis of $p16^{INK4a}$ immunostaining positivity alone [3], a modality that raises ethical concerns because it would expose a fraction of the patients whose $p16^{INK4a}$ over-expression is triggered by a HPV-independent mechanism to sub-optimal treatment. In addition, translating the results of those studies into clinical practice/decision-making in areas where there is a low prevalence of HR α-HPV-driven OPSCC could prove to be even riskier given the modest predictive value of $p16^{INK4a}$ positivity.

Given these considerations, we strongly recommend aiming for at least positivity to *both* HR α-HPV-DNA and $p16^{INK4a}$ immunostaining to estimate the burden of HR α-HPV-driven OPSCC and to obtain more accurate predictive and prognostic information. $p16^{INK4a}$ immunostaining can be used as screening test and followed by HR α-HPV-DNA testing on $p16^{INK4a}$-positive cases. We also

recommend to routinely assess HPV-status in patients with CUP, as it may guide successful identification of the primary tumour in the oropharynx, provide important prognostic information and help to prevent comprehensive prophylactic mucosal irradiation of the entire upper aero-digestive tract.

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Medical legal problems

A proposal for limited criminal liability in high-accuracy endoscopic sinus surgery

Una proposta di ridotta responsabilità penale nella chirurgia endoscopica sinusale

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SUMMARY

The aim of the present study is to propose legal reform limiting surgeons' criminal liability in high-accuracy and high-risk surgery such as endoscopic sinus surgery (ESS). The study includes a review of the medical literature, focusing on identifying and examining reasons why ESS carries a very high risk of serious complications related to inaccurate surgical manoeuvers and reviewing British and Italian legal theory and case-law on medical negligence, especially with regard to Italian Law 189/2012 (so called "Balduzzi" Law). It was found that serious complications due to inaccurate surgical manoeuvers may occur in ESS regardless of the skill, experience and prudence/diligence of the surgeon. Subjectivity should be essential to medical negligence, especially regarding high-accuracy surgery. Italian Law 189/2012 represents a good basis for the limitation of criminal liability resulting from inaccurate manoeuvres in high-accuracy surgery such as ESS. It is concluded that ESS surgeons should be relieved of criminal liability in cases of simple/ordinary negligence where guidelines have been observed.

KEY WORDS: Endoscopic sinus surgery • Malpractice • Medical liability • Gross negligence • Italian Law 189/2012

RIASSUNTO

Lo studio ha lo scopo di sollecitare una riforma della responsabilità penale che preveda una riduzione di responsabilità legale per la chirurgia ad alta precisione, per quella ad alto rischio, come per esempio la chirurgia endoscopica sinusale (ESS). Il contributo comprende una revisione della letteratura medica, concentrandosi sull'identificazione e sull'esame dei motivi per cui la tecnica di ESS corre un rischio molto elevato di produrre gravi complicazioni dovute a manovre chirurgiche inesatte. Tale contributo, prevede anche una revisione della teoria del diritto e della giurisprudenza britannica e italiana in merito alla negligenza medica, soprattutto con riferimento alla L. italiana n. 189 del 2012 ("Decreto Balduzzi"). Si è constatato che gravi complicanze dovute a manovre chirurgiche non corrette di ESS possono verificarsi, indipendentemente dalla prudenza/diligenza del chirurgo. La soggettività in termini giuridici risulta essenziale per la negligenza medica, soprattutto con riferimento alla chirurgia ad alta precisione. La legge italiana 189/2012 rappresenta una buona base per la limitazione della responsabilità penale derivante da manovre imprecise in chirurgia ad alta precisione, come appunto l'ESS. In conclusione, si considera che i chirurghi che eseguono ESS dovrebbero essere esonerati da responsabilità penale in caso di negligenza lieve sopravvenuta nonostante il rispetto delle line guida emanate.

PAROLE CHIAVE: *Chirurgia sinusale endoscopica • Negligenza • Responsabilità medica • Lieve negligenza/grave negligenza • Legge Italiana N. 189/2012 ("Decreto Balduzzi")*

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Introduction

Functional endoscopic sinus surgery (ESS) has been developed over the last 20 years [1] and is being adopted more commonly. A US study for the period 1985-2005 reports that 70% of malpractice claims in ENT referred to rhinology, and most involved ESS [2 3]. This is due to the fact that modern techniques (i.e. FESS) involve penetration into inaccessible areas and deal with conditions including chronic or recurrent rhinosinusitis (CRS) [3] and polyposis (in 73% and 12% of cases, respectively) [4]. The aim of ESS is to restore the physiology of the nasal mucosa [5]. FESS has high success rates (~ 90%) [6], but widespread use of such methods results in an increase in potential complications. While refinement of these methods reduces complications, it cannot eliminate them [3].

Given that during ESS a marginally negligent manoeuvre may cause serious injury and complications, it is one of the techniques most vulnerable to malpractice litigation. The 76% of ESS-related malpractice litigation refers to negligence [4]. Common serious injuries caused by FESS include: CSF leak and diplopia (24% and 17%, respectively, according to one study), blindness, intracranial brain injury, life-threatening haemorrhage and nerve injuries [4 7].

Endoscopic sinus surgery: very high-accuracy (high-risk) surgery

ESS is a high-accuracy (high-risk) form of surgery. The risk of error depends largely on the patient's characteristics, i.e. on the "specific bio-individual reactions" or the so-called 'endogenous risks' (anatomical variations, type of disease, individual reactions, co-morbidities and medications), rather than on 'exogenous risks' (including type of method chosen, type of instruments, type of anaesthesia administered, positioning of the patient, skill and experience of the surgeon) [7]. The risk of error during a medical (surgical) procedure can be reduced (or minimised) with a thorough preoperative evaluation of individual anatomical variations using CT scan in axial, coronal and parasagittal planes [1 7 8], the use of image guidance (IG) surgery [7 9] and limiting the possibility of excessive intraoperative bleeding (using reverse Trendelenburg body positioning, maintaining low arterial blood pressure etc.) [10]. However, such risks cannot be completely eliminated [3 11]. Endoscopic sinus surgery is performed on anatomical regions featuring close proximity of anatomical structures, including nerves (e.g. optic nerve), skull-base, dura mater, blood vessels (e.g. internal carotid artery), orbit, lachrymal duct, etc. The position of anatomical structures, both individually and in relation to each other, may vary, the thickness of a bone covering vessels and nerves may be minimal, or there may be some dehiscence. Some vessels or nerves crossing a cavity (e.g. pneumatised sphenoid sinus, SS) may be in a mesentery position.

The extremely high-risk surgical field of ESS

The narrow, complex and inaccessible nature, and relative limited visibility, of the operative field of ESS, in addition to the probability of individual anatomical variations (congenital or subsequent), together with the close proximity of critical anatomical structures, often make serious complications inevitable, even for the most skilled and experienced surgeon familiar with endoscopic anatomy and the use of instrumentation, especially during FESS or ETTS (Endoscopic Transnasal Transsphenoidal Surgery) and in the case of revision surgery. The operative field may become more restricted and more obscure (poor) whenever anatomy is distorted in some regions (e.g. in revision surgery with potential scarring, syndromes including Kartagener syndrome, cystic fibrosis, connective tissue disorders, diffuse polyposis with connective tissue growth etc. [7]. The common causes of an obscure operative field include polyposis or intraoperative bleeding, especially during revision surgery [3 11], which predisposes intervention to major complications, although this has been disputed recently [12]. Thus, a surgeon may be denied the possibility of visualising some well-known anatomical (surgical) landmarks that generally facilitates surgery, including the maxillary sinus ostium [13], the intact middle turbinate for identifying the maxillary sinus ostium [13], the point of contact between the third nerve and the tentorial edge for protecting the fourth nerve from an erroneous surgical procedure during skull-base surgery [14], and the inferior turbinate and vertical middle turbinate attachment that guide the extent of cartilage incision during ESS [15].

A well-known complication of ESS, rare (≤ 1%) though serious, is skull-base injury [16]. The most common cause of iatrogenic skull-base injury is ESS, during which an injury to the ethmoid roof and lateral lamella of the cribriform plate occurs intraoperatively, resulting in an iatrogenic cerebrospinal fluid leak (CSF leak) [17]. CSF leak may occur even during surgery by the most skilled hands. The anatomical variations in skull-base anatomy, the complexity of the case (e.g. in revision surgery) and a surgeon's inadequate experience and familiarity with the method used, may function as risk factors for CSF leak [18]. Good preoperative testing and planning may reduce the possibility of CSF leak to a considerable degree. Anatomical elements predisposing a patient to a CSF leak due to FESS include a "steep skull-base angle at the sagittal plane, a greater slope of the skull base at the coronal plane and a low cribriform height relative to the ethmoid roof" [19]. A preoperative review of imaging in terms of these particular anatomical variations is recommended, along with adoption of the Keros classification, which is used to describe the ethmoid skull-base configuration/height and helps physicians avoid iatrogenic injury to the cribriform region and medial ethmoid roof [20]. The osseous lamina at the ethmoid roof is extremely thin (0.05 mm at the frontal bone), the lateral lamella of the cribriform plate is 0.2 mm thick on average and the ethmoidal sulcus is 0.05 mm thick on average [21]. Thus, they are vulnerable to the surgical manoeuvres of ESS (high risk of perforation or split). Evaluation of the ethmoid skull-base height is important to avoid any complications during surgery [22]. A low-lying skull base is risky: the ethmoid skull base (ESB) extends from the superior attachment of the cribriform plate's lateral lamella to the junction of the lamina papyracea, and varies between 3.7 mm and 15.4 mm in thickness [23].

The upper medial border of FESS is determined by lamina papyracea (LP) and lamina cribrosa and its lateral lamella (the deeper the former the thinner the latter), while the upper lateral border is the anterior ethmoidal artery (AEA). LP may be in an excessively medialised position relative to the lateral nasal wall, thus rendering orbital penetration more likely [24]. Attachment of the uncinate process to LP is a high-risk variation (similar to the lateral surface of the middle turbinate or the anterior skull base) [8]. LP is tested for dehiscence or orbital fat protrusion into the ethmoid sinus or maxillary sinus (at a rate of 0.5-10%) [25]. When the olfactory fossa is deep, depending on the angle

formed by the lateral and perpendicular walls, then the risk of skull-base injury increases [26].

In (F)ESS, any anatomical variations of the most variable cavity of the human body, i.e. the SS, must be evaluated before surgery [1]. Pneumatisation (of conchal, presellar, or sellar type) of the SS varies greatly among individuals, and also varies according to age, gender, and race [27]. It may extend to the (anterior) clinoid process (in 6-17% of cases) [1], which is dangerous. Pneumatisation may be present at the pterygoid process (25-57%), carotid, optic nerve, Vidian canal, foramen rotundum and greater wing [1]. Optic nerve dehiscence is reported in 6% and prominence in 40% of patients [28]. Given that asymmetry is also reported at the ethmoid (> 2 mm in 8% of patients) [29], it is possible that the surgeon may misinterpret the patient's anatomy and believe he/she is at the posterior ethmoid, while he/she is in fact at the SS.

In 3-42% of patients [7], a so-called (sphenoethmoid) "Onodi cell" is found; this is a posterior ethmoid cell pneumatising into the superolateral aspect of the SS [1 7]. It represents a risk for the ESS surgeon, since it is very close to the optic nerve, internal carotid artery and sellar floor, and associated with dehiscence of the optic nerve and internal carotid artery [30]. Dehiscence of the carotid canal, optic canal, foramen rotundum etc. leads to a high risk of serious complications. Such dehiscence is reported in 4-25% of patients [1].

The occurrence of accessory bony septa or crests, as well as the occurrence of bony intramaxillary sinus septa in the SS (or in the optic or Vidian canal), also represents a risk for the surgeon [1 8]. 91% of AEAs (Anterior Ethmoidal Arteries) "are located within the skull base or 1-2 mm below, while 9% are suspended in a mesentery hanging" [7]. The artery may be 5 mm from the skull base (which is a highly risky variation). A high rate of AEAs (up to 43% according to a study) move freely within the ethmoid cells [8]. Aggressive disease removal and removal of SS septa attached to the bone just forward of the carotid artery may result in its injury.

The carotid artery aims at the lateral wall of the SS at a rate of 71-98% [7]. In 88% of cases, the bone that covers the artery is < 5 mm thick [7]. In 4-22% of cases, it is dehiscent (up to grossly dehiscent) [7]. Accordingly, as the internal carotid artery and the optic nerve cross the Onodi cell, they may be dehiscent (4-8%), covered by a thin sinonasal layer, while in 78% of cases, they are covered by a thin layer of bone (< 5 mm thick) [31]. If the anterior clinoid of the SS is pneumatised, then the optic nerve or the AEA may be in mesentery hanging across the roof of the sphenoid [26] (which is highly dangerous). Basak et al. note that an extreme medial location of the carotid canal and the bulging of the optic canal into the sphenoid sinus are very dangerous [8].

Often the optic nerve or the carotid artery is very highly susceptible to injuries due to these anatomical variations, or even due to minimal surgical manipulation.

Measurement, identification and evaluation of anatomical structures, and accuracy of these techniques

Preoperatively, a high-definition multi-slice helical CT scan of sinuses must be performed (following maximal medical therapy), principally in the axial, coronal and parasagittal planes (slices between 0.5 mm and 1 mm) [1 7 8 30]. Even the most minute anatomical variations can be detected with utmost accuracy [32]. CT is mandatory before ESS, insofar as the safety of ESS "depends on a surgeon's knowledge and experience to a great extent" [30]. According to one study, however, a CT scan can hardly evaluate whether there is any dehiscence of the optic nerve or of the carotid artery (or both) as they cross the SS [1].

During surgery, the surgeon is often assisted by image guidance (IG) systems; however, such systems are unable to assess the sinus and skull-base anatomy accurately, due to the so-called "target recognition error" (TRE) in terms of the location of the corresponding targets on CT (2 mm, approximately) [33 34]. During the use of image guidance technology there may be some discrepancy between anatomic endoscopic visualisation and the computer image. It is reported that "the skull base or the orbit should be given at least 2-3 mm as a buffer safety zone". TRE is a statistical distribution, and for each point in the image, the TRE may be different. "It is rare to remove the disease right to the skull base or orbit using computer guidance" [35]. It has been argued that "not using IG does not necessarily make one more vulnerable to malpractice litigation" [9].

Instruments used and risks resulting from their use. Spatial orientation disorders among surgeons

The type of instruments used may lead to an increased risk of medical error, e.g. the use of angled endoscopes and instruments makes surgical error more likely for a right-handed surgeon when the lesion is on the right side (and for a left-handed surgeon when the lesion is on the left side) [3 7 35)]. When the instrument is at a relatively parallel position to the ethmoid roof during ESS surgery, a surgeon may have a distorted perception of orientation [7]. Stankiewicz notes that "during ESS, a right-handed surgeon has to deal with an anatomic illusion on the left side. The left ethmoid sinuses are actually more medial than appreciated by a right-handed surgeon" [35].

Power instruments increase the possibility of error, and more specifically of the severity of complications (since they exert a cutting and suction action), mostly due to penetration into the endocranium and the orbit. If the lamina papyracea is injured, it may be sucked to orbital fat resulting in injury of the rectus muscle and diplopia [7], especially if there is injury at the third posterior of the lamina papyracea, where there is less fat between the papyracea and the muscle. It is not clear whether the balloon catheter systems used to dilate the sinus ostia are at least equally safe as other ESS techniques [7 36 37].

Risks due to anaesthesia or to physicians (surgeons or anaesthesiologists)

A surgical procedure may cause injury because general anaesthesia reduces the sensitivity of anatomical structures (patient feedback) when the ESS surgeon approaches sensitive structures such as the lamina papyracea and the skull base or the orbit [3 35]. Thus, the surgeon may penetrate very risky anatomical regions unhindered. Furthermore, in case of minimal movement by the patient, the ESS surgeon may find him/herself involved in medical litigation without any negligence on his/her part [38].

Even the most skilled and experienced surgeons are "at risk"

In ESS it is very possible that a surgeon will follow the guidelines and still be found guilty, despite the fact that his/her negligence was ordinary and possibly inadvertent, or that there was no deviation from his/her standard of care. Complications are uncommon but very serious, and depend highly on the patient's characteristics. The physician's experience is fundamental in reducing them, but cannot eliminate them altogether. Complications are possible even for the most skilful and experienced surgeons [3 5 35 39]. A minimal surgical manipulation (e.g. a marginally negligent manoeuvre) may result in serious iatrogenic injury.

Legal action may easily lead to finding a physician guilty whenever there is a complication. "The occurrence of a complication puts a surgeon at risk, even if he or she was not at fault". "The severity of the patient's disability, rather than the occurrence of an adverse event due to negligence, was predictive of payment to the plaintiff". "The standard of medical litigation performs poorly in malpractice litigation" [40]. A surgical error may be committed entirely unwittingly instead of negligently, for example it is not easy for surgeons "to know for themselves how much force they are exerting as they handle surgical tools" [41].

Italian Law No. 189/2012 ("Balduzzi" Law): a reform to restrict medical malpractice litigation

In Italy, Article 3 of Law No. 189/8.11.2012, which amended Decree-Law No. 158/2012 and entered into force on 11.11.2012, provides for the decriminalisation of simple/ordinary negligence (*culpa levis*) of a physician provided that he/she followed the guidelines and "good medical practice" accredited by the scientific community while carrying out his/her activities [42]. It may be of some concern that the original version of Art. 3 contained in Decree 158/2012 was slightly different from the latest version contained in Law 189/2012. Indeed, the original version stated that: "*Withstanding the provisions of Article 2236 of the Civil Code, the Judge – pursuant to Article 1176 of the Civil Code – in the assessment of negligence in the health workers' activity, shall take into account in particular the observance, in the concrete case, of guidelines and good practices accredited by the national and international scientific community*".

Successively, Art. 3 of Law 189 was changed to the following: "*Health workers who in carrying out their activities adhere to guidelines and good practices accredited by the scientific community are not liable for criminal negligence. In such cases there is no prejudice of Article 2043 of the Civil Code. The judge, when determining damages, shall also take due account of conduct in accordance with the first paragraph*".

It is clear that in updated Art. 3 there is an explicit reduction of liability in the criminal field, to *culpa levis*. In other words, Italian legislation introduced a partial *abolition criminis*. Such a limitation of the physician's criminal liability is not extended to civil liability. However, in determining injuries to the patient, the judge has to consider that the physician followed the provisions of the guidelines and nevertheless caused injury to the patient (third sentence of Article 3, 1st Subsection of Italian Law 189/2012) [43].

The distinction between ordinary and gross negligence (*culpa lata*) determines not only the *quantum*, but also the *an respondeatur.* Therefore, a sharp *discrimen* is a requirement for not violating the important principle of criminal law *nullum crimen nulla poena sine lege*. Gross negligence implies an unjustifiable degree of negligence from an objective point of view [38 44]. Such legislation leads to a limitation of "defensive medical practice", favouring a good relationship between physician and patient, patient safety and quality healthcare.

Guidelines and constitutionality of the Law

There are several reasons, for example those involving financial issues and the protection of legal rights, for the establishment of guidelines which can play a role in limiting criminal cases [45]. Guidelines need to be "indisputable" if they are to attain such a decriminalising role. However, they have no clear legal force; as a result, the absolute value of the guidelines has been disputed by the Italian Court of Cassation [46].

Furthermore, adherence to guidelines would render medical liability objective [47]. The assessment of medical liability is by its very nature subjective. A physician is under an obligation to provide the means, and not to obtain a result. However, in recent years there has been a tendency to "objectify" medical liability (in countries such as Greece [47], Italy [48] and Portugal [49]).

It is possible that the patient's interest in a particular case is better served by a deviation from the guidelines, rather than adherence to them [50]. Non-adherence to these guidelines is not indicative of negligence, and on the contrary, adherence to them does not result in an "automatic de-

fence of negligence" [51]. It seems that the application of guidelines protects physicians in terms of criminal liability in cases of marginal negligence, which may be more easily characterised as ordinary. Italian legislation implies that the guidelines resemble a navigation chart: if a surgeon follows them, s/he can only be held responsible for hitting the visible shelf. If s/he does not follow them, s/he can also be held responsible for hitting the reef [52].

Law No. 189/2012 has been discussed because of different interpretations in both Italian courts [53] and the legal literature [54]. It has been said that "from an analysis of case law, it is possible to conclude that there is interpretative doubt about the field of application of this reform. At the moment, the Supreme Court has taken a clear position, but this does not exclude potential future action by legislators" [55]. Moreover, Law No. 189/2012 was interpreted by the Italian Supreme Court of Cassation [56] and also by the Italian Constitutional Court [57].

To conclude, we believe that the legislative change introduced by Article 3 of Italian Law 189/2012 is a fundamental step towards ensuring the smooth-running of both the provision of healthcare services and administration of justice in the courts.

Advertent and inadvertent medical negligence

Gross negligence is usually based on advertent negligence (recklessness), as being an expression of a highly antisocial attitude. Gross negligence involves extreme carelessness or incompetence, which is expressed, however, through some serious form of negligent conduct (unjustifiable risk taking) [58]. Liability depends on foresight, on the conscientious taking of an unjustified risk. Utilitarians put social needs above individual needs, and advocate punishment in order to protect the community, since subjectivists are unable to make a clear distinction between knowledge and awareness of a risk [58]. In this paper, we stand with subjectivists regarding liability for inaccurate manoeuvres in high-accuracy surgery. In our opinion, an external distinction between gross and simple negligence roughly corresponds to an internal distinction between advertent and inadvertent negligence. This position, although we refer to "roughly corresponding", is not in contrast with the principle *nullum crimen nulla poena sine lege*, because it is maintained (in British legal theory) that the distinction between recklessness and gross negligence is subtle (they are almost identical) [59], although recklessness is based on advertence, while gross negligence is based on the violation of a duty-of-care rule [60]. If the distinction between *culpa levis* and *culpa lata* (simple/ordinary and gross negligence) can hardly determine the *an respondeatur* (and not the *quantum respondeatur*), this is even more true for the distinction between inadvertent and advertent negligence (recklessness).

As medical liability tends to be "objectified", the possibility of determining *an respondeatur* through the distinction between advertent and inadvertent negligence is invalidated. However, this should not be the case as far as high-accuracy surgical manoeuvres, where subjectivity has a crucial role, are concerned. The risk of certain surgical errors is particularly high and, in some cases, it is independent of the skill, experience and even prudence/diligence of the surgeon. Therefore, in ESS-related malpractice litigation the "objectification" of medical liability seems to be completely unjustified. During this type of surgery, a serious complication (result) may occur independently of the skill, experience and even prudence/diligence of the surgeon.

Conclusion – Proposal

Given that the guidelines acquired a crucial role, we consider – from a legal point of view – with great emphasis on subjectivity, and propose that a surgeon shall only be held criminally liable for gross negligence. Subjectivity is essential to medical negligence, especially when dealing with high-accuracy surgical procedures such as ESS. A good "tool" to ascertain not only the an respondeatur, but also the quantum respondeatur, is the judgment as to whether this constitutes negligence, because reference is made to subjectivity. An ESS surgeon should have received special and well-established training before performing surgical procedures. In our view, the attachment of a great deal of importance to subjectivity counterbalances the "objectification" of medical liability resulting from adherence to guidelines.

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CASE SERIES AND REPORTS

Pneumo-thorax/mediastinum/(retro)peritoneum/scrotum – a full house of complications following JET ventilation

Pneumotorace/pneumomediastino/pneumoretroperitoneo/pneumoscroto – complicanze a seguito di ventilazione JET

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SUMMARY

We present a patient who presented to our clinic with airway obstruction secondary to oropharygeal cancer. He underwent emergent tracheostomy with JET ventilation, the latter resulting in a "full house" of barotraumatic complications including pneumothorax, pneumomediastinum, pneumoperitoneum, pneumoretroperitoneum and pneumo-scrotum. Free air, while sometimes dramatic as in our case, need not always be a cause for alarm and can often be managed expectantly. Our patient was treated with only a chest drain and otherwise made an uneventful recovery.

KEY WORDS: Jet ventilation • Chest drain • Pneumothorax • Pneumomediastinum • Pneumoperitoneum • Pneumoretroperitoneum • Pneumo-scrotum • Free air • Subcutaneous emphysema

RIASSUNTO

Presentiamo il caso di un paziente che è giunto alla nostra clinica per un'ostruzione respiratoria secondaria a un carcinoma orofaringeo. Il paziente è stato sottoposto a una tracheotomia in emergenza e a una ventilazione JET, che ha dato come conseguenza un insieme di complicanze barotraumatiche, fra le quali un pneumotorace, penumomediastino, pneumoperitoneo, pneumoretroperitoneo, e pneumoscroto. Tale tipologia di ventilazione, benché talvolta dalle conseguenze drammatiche, come nel nostro caso, non è per questo necessariamente da temere e può essere ben gestita. Il nostro paziente è stato trattato con un drenaggio toracico ed è andato incontro a un recupero completo.

PAROLE CHIAVE: *Ventilazione Jet • Drenaggio toracico • Pneumotorace • Penumomediastino • Pneumoperitoneo • Pneumoretroperitoneo • Pneumoscroto • Ventilazione • Enfisema sottocutaneo*

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Introduction

Free air is often the sign of a ruptured viscera and must be taken seriously. For example, in ENT practice, oesophageal rupture following rigid oesophagoscopy must be treated appropriately. Free air can, however, present in other settings where it does not always mandate intervention. Iatrogenic free air can often be managed conservatively or with only minimal intervention providing the patient is appropriately monitored.

Case report

The 55-year-old man had originally been treated for a right sided cT3N2b tonsillar cancer infiltrating the tongue base and vallecula with chemoradiotherapy followed by a staged neck dissection in 2002. After 5 year follow-up he had been discharged, only to present some 10 years after the initial therapy with a cT3N0M1 biopsy-proven recurrence. Despite the thoracic surgeons being confident of achieving a curative resection of the distant metastasis, from an ENT point of view, the primary was unresectable and he had already received his maximal radiotherapy dose. He was therefore treated palliatively with cetuximab, cisplatin and 5-fluorouracil.

At his regular oncology appointment he was noted to have increasing airway compromise and sent for ENT consultation. On examination the patient showed stable cardiopulmonary conditions but with inspiratory stridor. Oral examination was almost impossible due to extreme trismus. Transnasal fibreendoscopy showed the epiglottis to be grossly swollen and contacting the posterior pharynx. No view beyond could be obtained.

The situation was discussed with the patient and the decision made for emergent tracheostomy.

Before planned fibreoptic transnasal intubation, a Ravussin needle was inserted to improve oxygenation. The anaesthetic team however quickly spotted subcutaneous emphysema and the patient's breathing became worse. The decision was taken to perform an awake cricothyroidotomy under local anaesthesia. Once the cricothyroidotomy was completed, the patient was fully anaesthetised and the cricothyroidotomy converted to a surgical tracheotomy.
Post-operatively, the patient continued to have breathing difficulties and chest auscultation revealed reduced breath sounds on the right together with a hyper-expanded and hyper-resonant right chest. Chest X-ray (Fig. 1) confirmed clinical diagnosis of a pneumothorax and 20 ch chest drain was placed in standard fashion. The chest X-ray also showed bilateral sub-diaphragmatic free air and pneumo-mediastinum. Repeat chest X-ray showed appropriate placement of the chest drain and the lung to have re-expanded (not shown).
Clinically, the patient's cardiovascular status was stable with no signs of engorged neck veins. Furthermore, the abdomen was soft with normal bowel sounds. Scrotal examination showed an enlarged and tender scrotum and the clinical diagnosis of pneumo-scrotum was made. The chest drain was swinging normally without any bubbles. Blood tests revealed mild leukocytosis and C-reactive protein of 85. To better evaluate the extent of mediastinal/ abdominal/ scrotal free air, a triple contrast chest/abdominal CT. This showed copious bilateral subcutaneous emphysema, small pneumothoraces bilaterally, mediastinal, intra-abdominal, retroperitoneal and scrotal free air (Figs. 2, 3).
Regular general surgical and thorax surgical consults were made in the following days and the patient remained clinically stable. The chest drain was removed on post-operative day 5. Clinically the subcutaneous emphysema and scrotal air resolved over the following days such that the patient could be discharged back to oncological care.

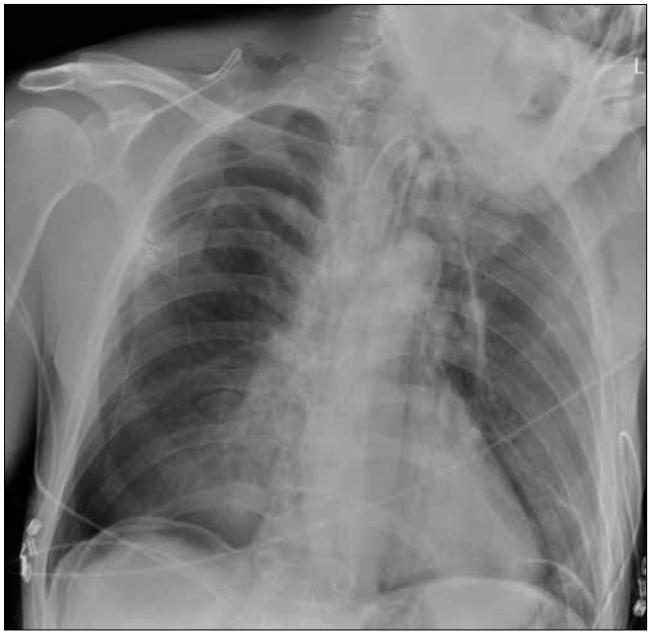

Fig. 1. Chest X-ray showing large right sided pneumothorax, pneumo-mediastinum and bilateral sub-diaphragmatic free air.

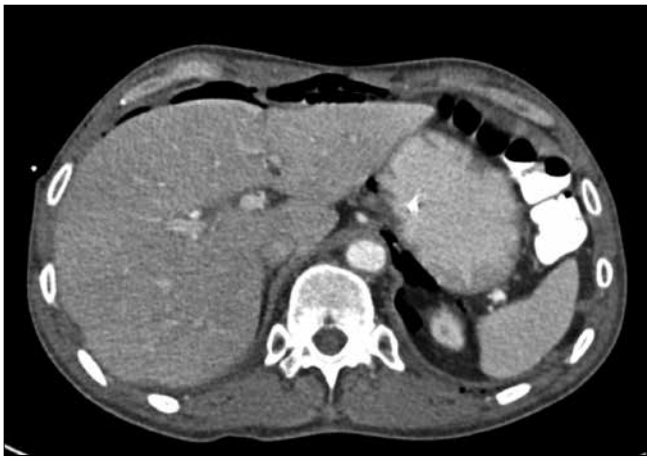

Fig. 2. Axial slice triple contrast chest/abdominal CT at the level of the right adrenal gland showing intra-abdominal and retroperitoneal free air.

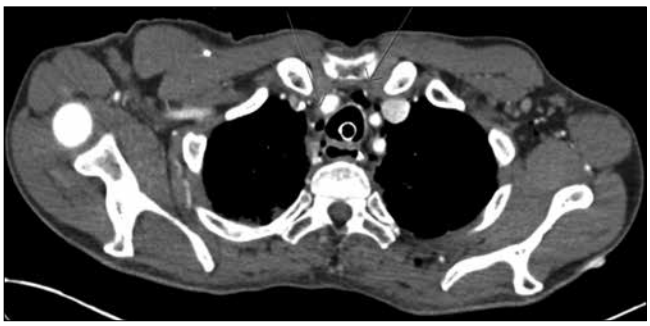

Fig. 3. Axial slice triple contrast chest/abdominal CT showing mediastinal free air (arrows) and subcutaneous emphysema on the left.

Discussion

Barotrauma

Pulmonary barotrauma is a recognised complication of mechanical ventilation, especially in neonates. Other patients thought to be particularly at risk include asthmatics and those with chronic interstitial lung disease or acute respiratory distress syndrome (ARDS).
Positive pressure ventilation results in an elevated transalveolar pressure and can lead to alveolar rupture and air escape [1]. Previously, it was estimated that roughly 10% of patients undergoing mechanical ventilation suffered barotrauma, but the increasing use of low tidal volume ventilation is thought to have reduced this.

The escaping then air dissects along tissue planes. Two theories explain how the air can exit the thoracic compartment: (a) direct passage through pleural and diaphragmatic defects and/or (b) via perivascular connective tissue to the retroperitoneum and finally to the peritoneum [2]. Serious complications including tension pneumothorax, tension pnemo-mediastinum and tension pneumo-peritoneum have been reported.

Several ventilation strategies have been suggested to reduce the risk of barotrauma. Reducing the peak airway pressure seems to have little effect, although reducing plateau airway pressures (a more accurate measure of alveolar pressure) to < 35 cm H_2O is effective.

Needle cricothyroidotomy ventilation

Needle cricothyroidotomy (NC) was first described in the 1950s [3] and can be used to temporalise a critical airway until more definitive measures are taken. This has the benefit of achieving oxygenation without requiring sedation or muscle relaxant. Indeed, a similar technique (SCOOP®) is often used by chronic obstructive pulmonary disease patients for long-term oxygen therapy. Oxygen can be delivered at continuous positive pressure or via high frequency, low tidal volume ventilation (JET) [4].

Needle cricothyroidotomy has proven itself invaluable in "can't intubate, can't ventilate scenarios" for example following trauma or gross swelling of the upper airway from infection/allergy/chemical or thermal burns. However, the technique is not without complications, which include possible barotrauma, hypercapnia and ongoing aspiration risk. Contraindications to NC include situations where the airway can safely be maintained with non-invasive measures. An assessment must also be made of an effective "chimney" for the air to escape through the mouth and nose. Indeed, in normal patients, up to a third of the insufflated air is thought to escape superiorly without ever passing through the lungs. An inadequate chimney, for example in the setting of upper airway obstruction, can result in massive distention of the lungs and barotrauma [5]. This complication can be mitigated by using prolonged expiratory times (I:E ratio 1:10 at a respiratory rate of 5-6/minute), a lower insufflation pressure (< 350kPa in adults and < 200kPa in children) and a larger internal diameter catheters.

The risk of hypercapnia due to inadequate ventilation despite adequate oxygenation is thought to result within 45 min [6]. In certain circumstance however, hypercapnia may be tolerated though particular attention should be paid to possible rising intracranial pressure.

It should finally be emphasised that NC does not protect the airway. Aspiration risk can be considerable, especially in the setting of trauma and bleeding. The position of the needle in the trachea should mean, however, a very low risk of insufflating air into the stomach.

Management of complications

Herien, we focus on the complications of barotrauma outside of the lungs. Our patient had bilateral pneumothoraces, pneumo-mediastinum, pneumo-peritoneum/ retroperitonuem/scrotum.

Pneumothorax

Close observation of small pneumothoraces is acceptable, but larger pneumothoraces, or those progressing to airway compromise or tension will require needle decompression and definitive chest drain insertion. Smaller drains are generally easier to insert and are more comfortable for the patient and are as efficacious as larger drains [7]. Chest tubes should be connected to an underwater seal, normally without suction (to avoid re-expansion pulmonary oedema [8]). Failure of re-expansion or persistent leak can be managed with video-assisted thorascopic surgery with/without pleurodesis or open thoracostomy. Once no longer bubbling, the drain may be clamped for a period of observation (often including check chest X-ray) before being removed.

Pneumo-mediastinum

Pneumo-mediastinum can also be managed expectantly, though rarely patients will develop tension pneumo-mediastinum which can be similarly be managed acutely with needle decompression followed by mediastinotomy and tube placement. Loss of cardiac output due to reduced venous return with bulging neck veins are worrying signs which should prompt immediate cardio-thoracic consultation.

Pneumoperitoneum

Pneumoperitoneum is a recognised complication of a perforated abdominal viscera, but can occur in settings without obvious perforation. Clearly, post-abdominal procedure free air is a normal finding, but free air can sometimes also be seen post-colonoscopy or secondary to transvaginal procedures or intestinal *Pneumatosis cystoides* infection. The management of these latter causes of pneumo-peritoneum is patient specific and depends on clinical findings, vital parameters and blood results. Often a conservative management can be tried before exploratory laparoscopy/laparotomy.

Following barotrauma, pneumo-peritoneum is usually self-limiting with 97% of cases resolving within 5 days [9]. Occasionally patients can suffer abdominal compartment syndrome and require intervention.

Conclusions

Needle cricothyroidotomy is an invaluable weapon in the anaesthetist's armamentarium, but is not risk-free and can only temporalise an airway. Pulmonary baro-

trauma is a real possibility and appears to increase intensive care length of stay and mortality [10]. Anaesthesiologists, intensive care personnel and other staff need to be aware of what method of airway management was used intra-operatively in order to be appropriately vigilant post-operatively and recognise findings in the appropriate context. Free air, while sometimes dramatic as in our case, need not always be a cause for alarm and can often be managed expectantly.

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Case series and reports

Idiopathic SIADH in young patients: don't forget the nose

SIADH idiopatica in pazienti di giovane età: non dimenticare il naso

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SUMMARY

Olfactory neuroblastoma (ONB) is an uncommon neuroendocrine sinonasal cancer associated by many authors to ectopic production of several biologically active substances. We report a case of a 31-year-old male patient who presented with idiopathic syndrome of inappropriate secretion of antidiuretic hormone (SIADH). During diagnostic work-up, a CT scan of the head was performed and an ethmoidal ONB was detected. Endoscopical surgery followed by radiotherapy was carried out. Immediately after surgery natraemia levels normalised. Five years later the patient is disease-free. To our knowledge, 17 cases of SIADH associated to ONB have been published. In nine reports, idiopathic SIADH promptly led to the diagnosis of the sinonasal mass as in our clinical case, however, in many reports, correct diagnosis was accomplished months to years later. In young patients with idiopathic inappropriate antidiuretic hormone secretion, a neuroendocrine malignancy of the sinonasal area must be excluded.

KEY WORDS: Olfactory neuroblastoma • SIADH • Paraneoplastic syndrome • Arginine vasopressin • Sinonasal

RIASSUNTO

Il neuroblastoma olfattivo (ONB) è un raro tumore neuroendocrino dei seni paranasali associato, secondo molti autori, alla produzione di molteplici sostanze biologicamente attive. In questo lavoro descriviamo il caso di un paziente di sesso maschile di 31 anni giunto alla nostra osservazione presentando la sindrome idiopatica da inappropriata secrezione di ormone antidiuretico (SIADH). Durante il work-up diagnostico il paziente è stato sottoposto a TAC del massiccio facciale, che documentava la presenza di un estesioneuroblastoma etmoidale. è stato eseguito un trattamento di chirurgia endoscopica e successiva radioterapia. Subito dopo l'intervento chirurgico i livelli di natremia si sono normalizzati. Cinque anni più tardi il paziente risulta essere libero da malattia. Attualmente in letteratura sono stati pubblicati 17 casi di SIADH associata a ONB. In nove lavori la SIADH idiopatica ha tempestivamente portato alla diagnosi di masse sinusali come nel nostro caso, tuttavia, in molti casi, si è giunti alla diagnosi corretta mesi o anni dopo. Nei giovani pazienti con sindrome idiopatica da inappropriata secrezione di ormone antidiuretico è necessario escludere un tumore maligno neuroendocrino dei seni paranasali.

PAROLE CHIAVE: *Neuroblastoma olfattivo • SIADH • Sindrome paraneoplastica • Arginina vasopressina • Seni paranasali*

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Introduction

Syndrome of inappropriate secretion of antidiuretic hormone (SIADH) or Schwartz-Bartter syndrome is characterised by excessive levels or activity of arginine vasopressine (AVP) with hyponatraemia and hyposmolality. High urinary sodium concentration without salt and water intake alterations is present. Usual causes of SIADH are drugs, intracranial pathology, trauma and predominantly paraneoplastic syndromes. Most commonly paraneoplastic syndrome is related to small-cell carcinoma of the lung. Many studies have also been conducted about incidence of SIADH in head and neck cancer [1 2] reporting 3% of patients affected by apparently idiopathic hyponatraemia. Most tumours are squamous cell carcinomas, but a small number of neuroendocrine sinonasal carcinomas have been reported. The prevailing phenotype is esthesioneuroblastoma (ENB) or olfactory neuroblastoma, first described by Berger in 1924 [3], which is known to be capable of producing biologically active substances such as somatostatin, calcitonin and vasoactive polypeptides. Olfactory neuroblastoma has been reported to be responsible for development of SIADH, especially in young patients. To our knowledge, 17 reports [4-18] of SIADH induced by olfactory neuroblastoma have been published.

Case report

A 31-year-old man was admitted to a North American hospital with complaints of nausea, dizziness and weak-

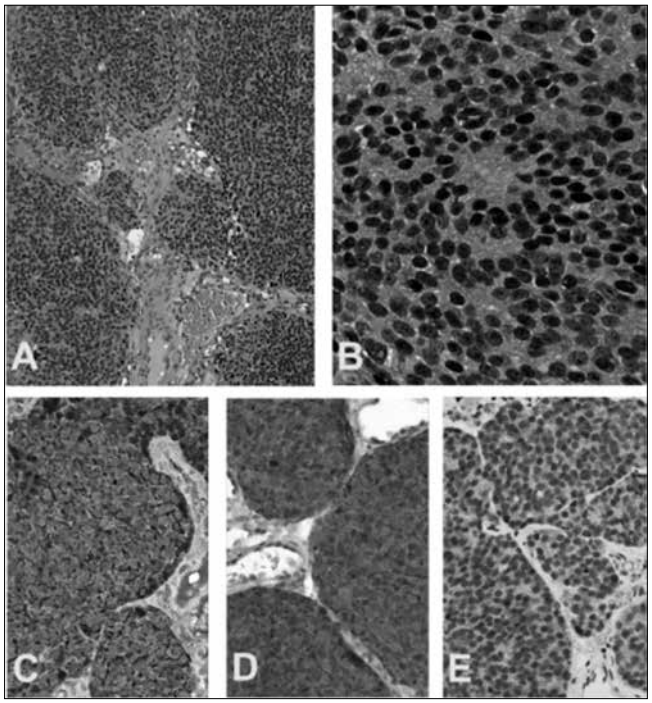

Fig. 1A. The tumour shows a nodular grow pattern composed by monomorphic round to oval neoplastic cells. (Haematoxylin and eosin, original magnification ×100). B. Tumour cells with finely granular nuclei and scant cytoplasm with neurofibrillary matrix forming Homer-Wright rosettes. (Haematoxylin and eosin, original magnification ×400). C-D-E. Tumour cells exhibiting diffuse cytoplasmatic immunoreactivity for chromogranin A (C), synaptophysin (D) and NSE (E). (Haematoxylin counterstaining, original magnification ×200).

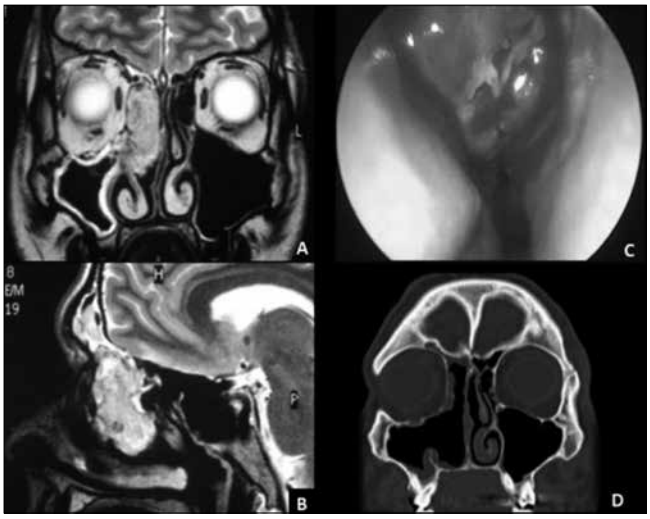

Fig. 2A-B. Coronal and sagittal T2-weighted images. Pre-operative MRI shows a large, well-defined mass expanding the right ethmoid. The mass lesion exhibits intermediate signal on T2-weighted images, due to its high cellularity; there is no evidence of infiltration of the orbital fat and of the fovea ethmoidalis. Inflammatory/obstructive changes are seen in both right frontal and maxillary sinuses due to mass effect on the ostio-meatal unit. Right lamina papyracea was compressed by the mass, but not interrupted; the orbit, anterior cranial fossa and brain were disease-free. C. Endoscopic view of the right nasal fossa: a fairly well circumscribed, multi-lobulated smooth rounded mass, involving the right ethmoid sinus and the region of middle turbinate is showed. Bony destruction was not recognisable, but the mass effect was prominent. D. Post-operative coronal CT image demonstrates the ethmoido-maxillectomy, without evidence of residual/recurrent disease.

ness for about a week. The patient was seen in an Urgent Care Centre and at physical examination temperature, heart rate, blood pressure and other vital criteria were normal: no cervical lymphadenopathy, no oedema was noted. The laboratory data reported a hypotonic hyponatraemia (serum sodium 111 mmol/L, serum osmolality 237 mmol/kg) in a euvolemic, non-oedematous patient with normal levels of potassium, chloride, bicarbonate and creatinine. Urinary sodium was 58 mmol/L, urinary osmolality was 266 mmol/kg. The patient's hyponatraemia was interpreted as a chronic disease given the fact that even with a sodium of 111 mmol/L on admission he had no change in mental status. He was treated with fluid restriction and furosemide for 2 days but his sodium increased to only 116. As patient continued to remain hyponatraemic despite resolution of nausea and pain, a CT scan of head, neck, chest and abdomen was performed, demonstrating a homogeneous mass lesion that expanded the right ethmoid. No evidence of lung masses or adenopathy were found. ENT consult was obtained and differential diagnosis was reported as a malignant lesion versus aggressive fungal infection. The patient was started on demeclocycline to inhibit ADH action with an increase in sodium to 129 on the day of discharge. A few days later an endoscopic biopsy of the right sinonasal mass under local anaesthesia was performed. At microscopic examination, the tumour was highly cellular and composed of round to oval neoplastic cells with slight nuclear pleomorphism and scant cytoplasm, organised in solid nests with sparse Homer-Wright rosettes. Scattered mitotic figures (up to 8 x 10 high power fields (HPF)) but no necrosis were found (Fig. 1). The proliferative index was 20% (MIB1/Ki-67). The neoplastic cells were stained positively with synaptophysin, chromogranin, NSE and CD56-NCAM. No immunostaining was observed for AE1/AE3, Cam 5.2 and Bcl-2. These findings, together with the morphology, were consistent with the diagnosis of olfactory neuroblastoma.

The patient at this point made return to his home country and was admitted to our hospital for therapeutic planning. MR scan of the head and neck was performed (Fig. 2 A-B), results were consistent with the CT data, additional information were precise dimensions (38x16 mm), slight nasal septum and nasolacrimal duct deformation. Endoscopic nasal evaluation was performed (Fig. 2C). According to Kadish grading[19], the lesion was classified as stage B. After tumour board discussion, an endoscopic resection of the lesion without lamina cribra resection and duroplasty was performed (Fig. 2D). Serum natraemia levels normalised the day after surgery. Postoperative radiation therapy was administered. The patient underwent regular endoscopic and radiological follow-up and 5 years later is disease-free.

Table I. Reported cases of Siadh secondary to olfactory neuroblastoma.

Author	Age/Sex	Presentation	Tissue demonstration of AVP secretion
Bouche 1967	34/M	Concomitant	No
Singh 1980	17/F	Concomitant	Yes
Pope 1980	56/F	Concomitant	No
Sringley 1983	33/F	SIADH (4 years earlier)	No
Wade 1984	59/F	Concomitant	No
Osterman 1986	28/M	SIADH (6 years earlier)	Yes
Cullen 1986	26/F	SIADH (10 years earlier)	Yes
Myers 1994	79/F	Concomitant	No
Al Ahwal 1994	27/M	Concomitant	No
Bernard 2000	22/M	Concomitant	Yes
Muller 2000	47/M	SIADH (15 months earlier)	No
Miura 2001	56/M	Concomitant	Yes
Plasencia 2006	34/F	SIADH unmasked ONB relapse after 16 years	No
Renneboog 2008	28/F	SIADH (8 years earlier)	No
Gray patient 1 2012	29/M	SIADH (5 months earlier)	Yes
Gray patient 2 2012	25/F	SIADH (3 years earlier)	Yes
Gray patient 3 2012	32/F	SIADH (8months earlier)	Yes

Discussion

Over the past 45 years 17 cases of SIADH associated with olfactory neuroblastoma were reported [4-18]. In all cases, the secretion of the neurohypophysial hormone manifested itself prior to the diagnosis of ONB. In most cases, the time between first determination of hyponatraemia and detection of the sinonasal mass was short, thus allowing to consider the two aspect concomitants. Limited reports demonstrate preexisting SIADH, in a patient otherwise asymptomatic, which was accordingly considered idiopathic for months to years (Table I).

Only in a few of the above-mentioned studies was AVP secretion directly demonstrated on frozen section sections, most commonly the relationship between high blood levels of AVP and aberrant neuroendocrine tumour secretion was considered consequential as natraemia rapidly increased to standard levels after successful treatment of ONB. In the totality of the reports, in fact, natraemia levels normalised immediately after ONB treatment was accomplished, independently from the oncologic outcome of the patient.

An interesting aspect, already mentioned by Gray and colleagues [18] is the relatively young age of the small group of patients affected by this peculiar clinical lesion: average age at diagnosis was 37 years. Olfactory neuroblastoma mainly arises in two decades, the second and the fifth, although it is most commonly reported around the fiftieth year [20].

SIADH as a paraneoplastic syndrome most commonly is related to small cell carcinoma of the lung and it reveals itself usually as a mild clinical picture. The accidental detection of a slight natraemia alteration, in fact, often leads to pulmonary investigation and allows to connect the two clinical aspects. On the contrary, as previously noticed [16], most of the diagnoses of idiopathic hyponatraemia which revealed the neuroendocrine nasal disease were accomplished for severe nervous or systemic diseases. Nonetheless, in our opinion, even in the presence of mild clinical case it is advisable to suspect early a sinonasal involvement after pulmonary lesions are excluded, especially in young patients.

In a previous report [16], alterations in serum sodium metabolism preannounced an olfactory neuroblastoma relapse, 16 years after first diagnosis, with no history of SIADH at the time of the first diagnosis. Pathological hyponatraemia and late lymph nodal relapse [8] were reported as well. This aspects lead to consider that natraemia is a valuable follow-up tool in patients affected by olfactory neuroblastoma, with or without history of SIADH. Serum sodium evaluation is also an inexpensive, practical exam that can be easily performed during follow-up.

Conclusions

In patients affected by idiopathic SIADH, after a pulmonary primitive lesion has been excluded, the study of the sinonasal area must be included in diagnostic work-up. In patients with history of ONB, it is advisable to continue follow-up lifelong to detect late recurrences as early as possible, which unfortunately are very common in neuroendocrine tumours. Along with clinical and radiological inspection, we found that periodic natraemia evaluation is useful as an inexpensive, smart and safe means to detect ectopic production of AVP. SIADH, in fact, can arise as an indirect sign for ONB recurrence even if not assessed at first diagnosis.

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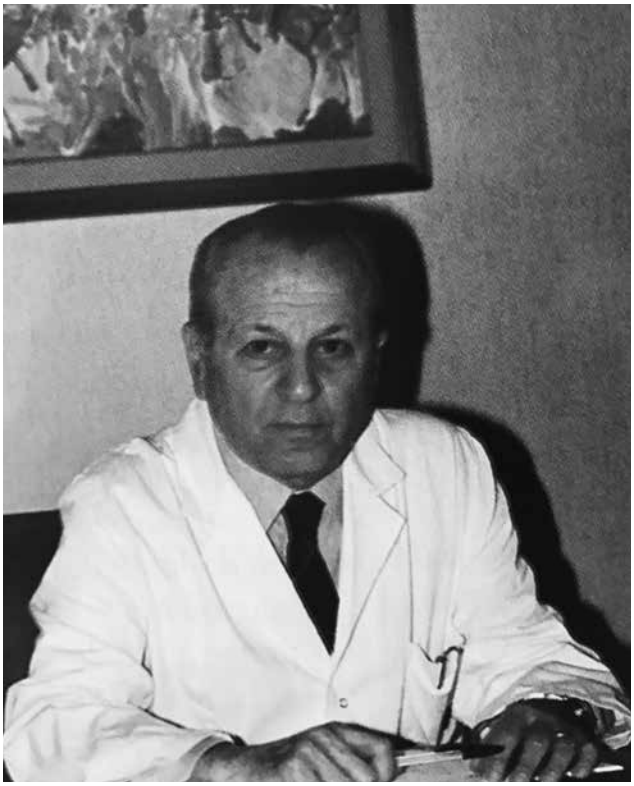
In Memoriam Prof. Italo de Vincentiis
(1926-2016)



Professor Italo de Vincentiis passed away on the morning of Sunday, December 11, 2016, a few days before his 90th birthday. His death marks the passing of one of the most charismatic figures in ENT in Italy in recent decades, and a master of science and life who greatly enriched those who had the good fortune to be close to him, even for a short time, during his long academic career. As an original and precise researcher, he always perfectly combined scientific activity, broad and diversified in all subspecialties, with excellent teaching capabilities and outstanding clinical and surgical skills, and certainly personified the ideal university professor.

Born in Campo di Giove in Aquila on 15 December, 1926, he received his medical degree from the University of Rome in 1951, and finished his specialisation in ENT in 1954. From 1953 to 1962, he was at the ENT clinic at the University of Perugia under the direction of Prof. Domenico Filipo, and during that time distinguished his scientific, educational and surgical abilities and earned the profound esteem of both colleagues and faculty. In 1962, when Prof. Filipo moved to Rome, the university wanted to promote him to department head. However, this never happened since Prof. Filipo wanted him by his side in Rome. In 1969, following the division of the Chair of Otolaryngology at the University "La Sapienza", Prof. de Vincentiis became the co-head of ENT and in 1970 was appointed full professor.

Italo de Vincentiis was author of a large number of presentations at national congresses and published many studies in national and international journals. One of his main areas of interest was artificial larynxes, which was preceded by studies on vocal organ reconstruction in animals and humans. He also worked intensely on the pathogenesis and diagnosis of Meniere's disease, which in 1964 led to the discovery of osmotic therapy and the role that glycerol has in this labyrinthopathy. He also contributed many studies on cochlear and retrocochlear hearing loss and cranio-facial pain.

Professor Italo de Vincentiis stood out by advancing the ENT field in Italy, by opposing indiscriminate tonsillectomy and by promoting more 'major' ENT surgery. In 1971, he was one of the founders of the AUORL, and became its president soon thereafter; in 1980, he established a department for maxillofacial surgery; in 1986, he was president of the SIO, and in the same year contributed to the foundation of the EUFOS in Paris; for about two decades he was director of the journal 'Il Valsalva'; he was coordinator of a national working group on labyrinthopathies for the Italian National Research Council; he was a longstanding member of the Italian National Health Council and a staunch supporter of the need for the SIO to acquire a permanent seat in Rome (currently in via Pigorini).

His human qualities are no less important: a pragmatic man, with a broad intellectual culture, and exceptional in many ways, which was perfectly described in two autobiographical texts, "Il ragazzo della valle" and "I paralipomeni al ragazzo della valle" ["The Valley Boy" and "Chronicles of the Valley Boy"], recollecting his adolescence and youth as part of the social world at his birthplace, Campo di Giove, and recalling a simple pastoral life of rare beauty. Being in love with his birthplace and the nearby mountains, Italo de Vincentiis was also a protagonist, during the German occupation of the Second World War, of acts of heroism by rescuing, near Campo di Giove, many young Italians, Jews and military allies. In an open letter dated 22 February, 2012 to the Mayor of Campo di Giove in which he requested official recognition for the town considering what his fellowmen did in 1943-44, he concluded by writing that "I have reached my last mile, the finish is still far away, but it will come".

The finish for the "Valley Boy" came on Sunday, December 11, 2016, but we students, and many of those who knew him, will never forget.

Adelchi Croce

Calendar of events – Italian and International Meetings and Courses

Acta Otorhinolaryngol Ital 2017;37:81-82

Information, following the style of the present list, should be submitted to the Editorial Secretariat of Acta Otorhinolaryngologica Italica (actaitalicaorl@rm.unicatt.it).
In accordance with the Regulations of S.I.O. and Ch.C.-F. (Art. 8) Members of the Society organising Courses, Congresses or other scientific events should inform the Secretary of the Association (A.U.O.R.L., A.O.O.I.) within the deadlines set down in the respective Statutes and Regulations.

MARCH-DECEMBER 2017

12th SURGICAL ANATOMY IN HEAD & NECK CANCERS PROCEDURES • ***March 1-3, 2017 • Arezzo – Italy***

Course Directors: M. Benazzo, F.G. Chiesa – Website: www.iclo.eu

CURSO DE MICROCIRUGÍA DEL OÍDO Y DISECCIÓN DEL HUESO TEMPORAL
TEMPORAL BONE SURGICAL DISSECTION COURSE
March 1-3, 2017 • Barcelona – Spain

Sra. Conchi Castilla – Tel. 93 205 02 04 – Fax 93 205 43 67 – E-mail: entsecretaria@hotmail.es – info@iogi.org

CORSO DI ANATOMIA CHIRURGICA ENDOSCOPICA DEI SENI PARANASALI
March 5-7, 2017 • Arezzo – Italy

Responsabili Scientifici: Enzo Emanuelli, Fabio Pagella, Stefano Pelucchi – Website: www.iclo.eu

PREMIMA HANDS-ON COURSE, ADVANCED – Extended endoscopic transnasal surgical approaches to the skull base • ***March 21-23, 2017 • Varese – Italy***

Course Directors: Paolo Castelnuovo, Davide Locatelli, Manfred Tschabitscher – Website: www.milanomasterclass.it

9th MILANO MASTERCLASS • ***March 24-28, 2017 • Milan – Italy***

Charimen: Paolo Castelnuovo and Pietro Palma – Website: www.milanomasterclass.it

1° CORSO DI RINOALLERGOLOGIA E RINOCHIRURGIA • ***April 4-8, 2017 • Baronissi – Italy***

Chairman: Francesco Antonio Salzano – Website: www.iclo.eu

THE 13th INTERNATIONAL NETHERLANDS CANCER INSTITUTE HEAD AND NECK SYMPOSIUM – Diagnosis and treatment of sinonasal cancer and nasopharyngeal cancer
April 6-7, 2017 • Amsterdam – The Netherlands

E-mail: kno@nki.nl – Website: http://www.hoofdhalskanker.info/symposium-head-and-neck-cancer/

2nd WORLD CONGRESS ON ENDOSCOPIC EAR SURGERY • ***April 27-29, 2017 • Bologna – Italy***

Chairmen: Livio Presutti, Muaaz Tarabichi, Daniele Marchioni – http://www.eesworldcongress2017.com/

XXV INTERNATIONAL EVOKED RESPONSE AUDIOMETRY STUDY GROUP (IERASG) BIENNIAL SYMPOSIUM • ***May 21-25, 2017 • Warsaw – Poland***

Website: ierasg2017.com/

104° CONGRESSO NAZIONALE SIO – SOCIETA ITALIANA DI OTORINOLARINGOLOGIA E CHIRURGIA CERVICO-FACCIALE • ***May 24-27, 2017 • Sorrento – Italy***

President: Carlo Antonio Leone – Website: www.sioechcf.it

HANDS-ON COURSE, BASIC - SINUS & SKULL BASE SURGERY: ANATOMICAL DISSECTION, DIAGNOSTICS AND OPERATIVE TECHNIQUES • ***June 12-14, 2017 • Varese – Italy***

Website: www.attingo-edu.it

IFOS PARIS 2017 - ENT WORLD CONGRESS • *June 24-28, 2017 • Paris – France*

Website: www.ifosparis2017.org

53rd GRAZ COURSE ON RHINOSURGERY • *July 12-15, 2017 • Graz – Austria*

Website: www.ent-graz.com – E-mail: claire.zwerina@klinikum-graz.at

SWISS ENDOSCOPIC EAR SURGERY COURSE SEES – HANDS-ON ENDOSCOPIC EAR AND LATERAL SKULL BASE SURGERY • *September 4-5, 2017 • Bern – Switzerland*

Website: http://sees.swiss-meeting.org

ENDOSCOPIC PARANASAL SINUS & SKULL BASE HANDS ON COURSE PSSB
September 7-8, 2017 • Bern – Switzerland

Website: http://paranasal.swiss-meeting.org

VII CORSO TEORICO PRATICO DI AUDIOLOGIA E VESTIBOLOGIA "GIANNI MODUGNO"
September 25-27, 2017 • Benevento – Italy

Direttore: Luigi Califano – E-mail: vertigobn@hotmail.com

XXXVI CONGRESSO NAZIONALE DELLA SOCIETÀ ITALIANA DI AUDIOLOGIA E FONIATRIA
September 27-30, 2017 • Siena – Italy

Website: www.congresso-siaf2017.it/

XVI CONGRESSO NAZIONALE AIOLP • *October 6-7, 2017 • Venice – Italy*

Website: www.aiolp.it/

4th CONGRESS OF THE EUROPEAN ORL-HNS • *October 7-11, 2017 • Barcelona – Spain*

Website: www.ceorlhns2017.com/ – E-mail: orl-hns2017@topkon.com – E-mail: scientific_orl-hns2017@top-kon.com

RHINOPLASTY & FACIAL PLASTIC SURGERY • *October 20-21, 2017 • Porto – Portugal*

Website: www.portofacialplastic.com

AUDIOVESTIBOLOGIA... IN CORSO D'OPERA - III EDIZIONE
October 23-25, 2017 • *Benevento – Italy*

Direttore: Luigi Califano – E-mail: vertigobn@hotmail.com

CORSI PRATICI DI VIDEOCHIRURGIA ENDOSCOPICA NASO-SINUALE E DEL BASICRANIO
November 13-17, 2017 • Milan – Italy

Direttore: Alberto Dragonetti – Website: http://www.dragonettialberto.it/corsi.html

17th ASEAN ORL HNS CONGRESS • *November 16-18, 2017 • Myanmar*

Website: www.entnet.org/content/17th-asean-orl-hns-congress

JANUARY-DECEMBER 2018

15th INTERNATIONAL CONFERENCE ON COCHLEAR IMPLANTS AND OTHER IMPLANTABLE AUDITORY TECHNOLOGIES • *June 13-16, 2018 • Antwerp – Belgium*

Chairman: Paul Van de Heyning – E-mail: vincent.van.rompaey@uza.be – Website: www.ci2018.org

6th WORLD CONGRESS OF THE INTERNATIONAL FEDERATION OF HEAD AND NECK ONCOLOGIC SOCIETIES • *September 1-5, 2018 • Buenos Aires – Argentina*

Website: http://ifhnos2018.org/

JANUARY-DECEMBER 2019

14th ASIA-OCEANIA ORL-HNS CONGRESS 2019 • *January 9-13, 2019 • Hyderabad – India*

Email: info@14asiaoceania.com – Website: http://14asiaoceania.com/