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Contents

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Editorial

Surgical management of head and neck tumours during the SARS-CoV (COVID-19) pandemic

Gestione chirurgica dei tumori testa e collo durante la pandemia da SARS-CoV (COVID-19)

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The health emergency caused by the SARS-CoV (COVID-19) pandemic calls for the utmost commitment on the part of every member of the healthcare team and involves a reorganisation of the health system to meet exceptional care needs efficiently and effectively.

In Lombardy – one of the Italian regions most affected by this pandemic – many public and private hospitals have been wholly reconverted to ensure the treatment of COVID-19 patients. At the same time, other "COVID-free" hospitals have been identified and designated to treat all other diseases and conditions safely. As a consequence of this reorganisation, specific cancer centres, including the European Institute of Oncology, have become regional reference "hubs" for the treatment of cancer patients. A series of organisational and clinical problems has therefore to be addressed as the number of patients to be evaluated and treated increases, while at the same time, it is necessary to ensure that the "hub" remains as "COVID-19-free" as possible over time.

These dedicated centres are employed in the treatment of patients referring from different areas, with a significant increase in workload, where, alongside the complexity of oncological disease, there is the overlapping problem of coronavirus infection.

The "urgency of treatment" of an oncology patient in this COVID-19 era becomes increasingly pressing when we add the distinctive degree of fragility of patients suffering from head and neck cancer – generally patients are debilitated by dysphagia-related malnutrition or by the disease itself, and on average require urgent treatments (less than 30 days) both due to the stage of the disease itself and due to the possible complications typical of this type of cancer: risk of suffocation arising from obstruction of the upper respiratory tract (larynx/pharynx) or the risk of haemorrhage which is often fatal.

The sudden increase in the number of patients entering the reference center is determined by the available resources, such as: the number of beds, availability of the operating room, availability of any intensive care units, and availability of diagnostic slots for staging examinations.

This discrepancy does not allow the requirements of the healthcare region to be fully met, and has led to an urgent remodelling of management and of certain concepts and recommendations that were considered the "gold standard" in times of "normality". Measures had to be undertaken in order to be able to respond to the national health emergency during a pandemic.

Ethically, it is challenging to define the right compromise between the necessary cancer treatments and the risk of infection. The biology of the tumour, the health of the patients, and the "viral" integrity (COVID-19-free nature) of the

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hospital must be taken into account in treatment decision-making. This integrity must be tested over time to rule out the presence of the COVID-19 virus, to prevent the infectious disease from causing management difficulties (ad hoc measures should be provided for COVID-19 + patients), and major postoperative complications that are difficult to resolve, with consequent prolongations of hospital stay, increased bed occupancy, delays in starting any adjuvant therapies, as well as an increase in deaths.
It is also imperative to reduce the risk of in-hospital transmission of the virus among other patients and healthcare staff [1].
In this perspective, measures are needed which provide support for a decision-making process that is as streamlined as possible and must be agreed and in harmony with the decisions of the crisis unit led by the hospital's medical directorate.
The following measures are the result of the experience gained day by day in an oncological hub addressing the emerging needs of head and neck cancer management, and of what has recently been published [2-4].

Action plan of an ENT regional cancer hub during the COVID-19 pandemic

The individual patient performs all the procedures below. No accompanying person has the right of entry inside the centre in the absence of the patient's proven and demonstrable needs. All patients who are allowed entry must wear a surgical mask (provided at the entrance) that is removed only during the execution of the diagnostic investigations, in order not to transmit possible contagion as asymptomatic subject.

Assessment of outpatients

Before entering the facility, all patients undergo an initial triage where temperature is measured. After passing the first check, they are assessed oncologically. All clinical assessments such as laryngoscopy, nasal endoscopy, digital palpation of the oral cavity and oropharynx are considered as aerosol- and droplet-generating procedures (AGP), which require the adoption of medical devices by the examining doctor in the form of personal protective equipment (PPE): FFP2 mask, associated with a surgical mask, water-repellent disposable gown or apron, gloves, and protective goggles or visor.

Staging

One-day staging and pre-hospitalisation should be scheduled to minimise patient access to the hospital. Staging tests must be kept to the minimum necessary to accurately determine the correct extension of the tumour and the general condition of the patient. During the pre-hospitalization even in apyretic patients, pharyngeal swab and ad hoc blood tests are performed for the diagnosis of COVID-19: PCR, complete blood count with formula and LDH are conducted, and if found to be altered, a Chest CT scan should be carried out.

Therapeutic planning (multidisciplinary team)

It is essential to maintain the weekly frequency of multidisciplinary meetings. If necessary, these can be conducted in live streaming, by limiting their participation to those specialists who are indispensable, such as the surgeon, radiotherapist, medical oncologist, radiologist, and pathologist. Before hospitalisation, the multidisciplinary team must discuss all complex cases, such as patients with advanced malignancies, to decide and share the most appropriate treatment plan. On the other hand, cases in which therapy is well standardised and consolidated (e.g., early laryngeal or oral tumours, nasopharyngeal cancers) may not need to be discussed in the multidisciplinary meeting.

Treatments

It is advisable to delay the treatment of low-risk tumours, for example, differentiated thyroid cancers; reduce, where possible, the duration of surgery by choosing a minimally-invasive rather than an open approach (e.g., in borderline laryngeal or paranasal sinus tumours); choose transoral surgery vs. major surgery (e.g. in borderline cancers of the oral cavity); reconstruct surgical defects with local flaps rather than revascularised flaps; minimise the presence in the operating room of people not involved in surgical and anaesthesiological procedures (fellows, attendees, and clinical observers).

Hospital stay

All patients are to be considered as possible healthy carriers and are therefore obliged to wear a surgical mask. Patients with a tracheotomy have a high droplet production and the healthcare professional must pay scrupulous attention when carrying out any actions, manoeuvres, positioning, procedures etc. on the patient. So, the patient on the ward, with a tracheotomy, must be managed with suitable PPE: FFP2 mask, associated with a surgical mask, water-repellent disposable gown or apron, gloves, and protective goggles or visor [3].

Follow-up

All patients with scheduled periodic checks should be contacted by telephone by a staff doctor; if the patient is asymptomatic, the clinical check must be postponed for at least six months, and the patient should be asked to communicate any adverse events by telephone or email. It is also advisable to reduce post-operative clinical checks by organising telephone or video call contacts, where possible. This editorial aims to share the experience acquired in a short time in a Lombardy cancer hub, pointing out the pitfalls and difficulties that head and neck surgeons may encounter daily, and indicating how to deal with them throughout this health emergency.

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Head and neck

Stem cell markers in oral and oropharyngeal squamous cell carcinomas in relation to the site of origin and HPV infection: clinical implications

Marker di staminalità nei carcinomi squamocellulari del cavo orale e orofaringe in relazione al sito di origine e a infezione da HPV: implicazioni cliniche

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SUMMARY

The expression of potential stem cell markers in HNSCCs was investigated to assess their potential clinical role. 69 primary, previously untreated oral (OSCC) and oropharyngeal squamous cell carcinomas (OPSCC) were enrolled; personal, clinical and follow-up data were collected. HPV infection and expression of 5 potential stem cell markers (CD44, CD133, Oct-4, Nanog, and Sox-2) were evaluated. HPV+ OPSCC showed lower expression of Nanog. The cytoplasmic expression of Nanog was associated with significantly worse prognosis in OPSCC, but not in OSCC. Sox-2 staining was more intense among OPSCCs. Sox-2 nuclear staining was associated with worse prognosis. Nanog expression was associated with HPV- OPSCC and may have a role as a surrogate diagnostic marker. In general, the expression profile of some stem cell markers in HNSCC seems to vary according to the site of origin and HPV infection. Nanog and Sox-2 may also have prognostic value.

KEY WORDS: molecular markers, prognosis, HPV diagnosis, Sox-2, Nanog

RIASSUNTO

In questo studio è stata esaminata l'espressione di potenziali marcatori di staminalità nei carcinomi della testa e collo (HNSCC) per valutarne il loro possibile ruolo clinico. Sono stati arruolati 69 carcinomi squamocellulari del cavo orale (OSCC) e dell'orofaringe (OPSCC) primitivi e non precedentemente sottoposti a trattamento, raccogliendo i dati anagrafici, clinici e sul follow up. Abbiamo valutato l'eventuale infezione da HPV e l'espressione di 5 potenziali marker di staminalità (CD44, CD133, Oct-4, Nanog, and Sox-2). Gli OPSCC positivi per HPV hanno mostrato minor espressione di Nanog, mentre la sua espressione citoplasmatica è stata associata con una prognosi significativamente peggiore negli OPSCC ma non in OSCC. La colorazione di Sox-2 si è rivelata più intensa tra gli OPSCC, e la sua espressione nucleare è associata con una peggiore prognosi. L'espressione di Nanog è associata a OPSCC HPV-negativi e può avere un ruolo come marker diagnostico surrogato. In conclusione il profilo di espressione di alcuni marker di cellule staminali nei HNSCC sembra essere differente a seconda del sito di origine del tumore e dell'infezione da HPV. Inoltre Nanog e Sox-2 potrebbero presentare un significato prognostico.

PAROLE CHIAVE: *markers molecolari, prognosi, diagosi di HPV, Sox-2, Nanog*

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Introduction

Various malignant tumours are considered to originate from a typical cell of origin. Nevertheless, within the same tumour, cancer cells often exhibit functional heterogeneity, exhibiting distinct proliferative and differentiation potentials (referred to as tumour heterogeneity) [1,2]. The cancer stem cell (CSC) model is a carcinogenic theory, demonstrated primarily for haematologic malignancies - although evidence is also accumulating in solid neoplasms - that coherently accounts for such heterogeneity within the tumour cell population [3,4]. The CSC model proposes a hierarchical organizsation of cells within the tumour, in which a subpopulation of tumour cells displays some characteristics that are similar to normal stem cells. These so-called cancer stem cells (CSCs) have the ability to give rise to all cell types in a particular neoplasm. Thus, these cells are responsible for sustaining tumour growth as well as for local relapse and metastasis. CSCs share important properties with normal tissue stem cells, including self-renewal (by symmetric and asymmetric division) and differentiation capacity, albeit aberrant, but this does not imply that the cell of origin of a given tumour was necessarily a stem cell.

From a clinical perspective, the CSC concept has significant implications as these cells, which are thought to be more resistant to chemotherapy and targeted therapy, should be the primary target of every non-surgical therapeutic approach in order to provide long-term disease-free survival.

The isolation of CSCs from different malignancies has been aimed, on a speculative level, at confirming that the CSC model is valid for a certain neoplastic disease. Moreover, identification of a population of cells, on which the effectiveness of different therapeutic approaches could be tested, would also be highly relevant from a clinical perspective. A number of cell surface markers have been demonstrated to be useful for identification of CSCs, while it is not yet known whether these merely represent surrogate markers or have a meaningful role in regulating CSC function. In head and neck oncology, the CD44 protein (CD44) has been proven to be the most reliable surface marker [5,6], even if measurement of the activity level of some enzymes has been demonstrated as a potentially reliable approach, as in the case of aldehyde dehydrogenase (ALDH) [7,8].

Other cellular markers, such as octamer-binding transcription factor 4 (OCT-4), homeobox protein NANOG (Nanog) and SRY (sex determining region Y)-box 2 (SOX-2), are not suitable for easy isolation of the CSCs as they are either not expressed on the membrane surface or lack detectable enzymatic activity. Nevertheless, such markers have been reported to be associated with stem cells and to have a possible clinically predictive role in head and neck cancers [9-12].

Head and neck squamous cell carcinomas (HNSCCs) represent most of the malignancies arising from the mucosal lining of the upper aero-digestive tract. They are an extremely heterogeneous group of tumours from both molecular [13,14] and clinical points of view. The main clinical heterogeneity factor is the site of origin, which substantially defines different diseases, each with their own typical risk factors, presentation at diagnosis, tendency to local and distant metastasis, chemo- and radiosensitivity as well as prognosis. In this context, high risk HPV infection, whose role in oropharyngeal carcinogenesis is well established [15], defines a group of oropharyngeal squamous cell carcinomas with peculiar clinical [16-18] and molecular [19] features.

The aims of the present work were to study the expression of different potential stem cell markers in HNSCCs arising from the oral cavity and oropharynx in relation with the above-cited heterogeneity factors, namely, site of origin and HPV infection as well as to assess their potential clinical utility as prognostic markers.

Materials and methods

Patient characteristics

We retrospectively collected data from 69 patients affected by primary, previously untreated oral (OSCC) and oropharyngeal squamous cell carcinomas (OPSCC) and treated between March 2008 and December 2011, at Policlinico Agostino Gemelli - Università Cattolica del Sacro Cuore, Rome, Italy. All patients had been examined at the same institution by a multidisciplinary head and neck tumour board, which provided therapeutic recommendations following histological diagnosis and staging according to TNM classification, VII edition [20]. FFPE tumour samples adequate for immunohistochemistry (IHC) and DNA extraction were available. All 39 patients with OSCC underwent primary surgery ± radiotherapy ± chemotherapy, while all 30 patients with OPSCC underwent primary radiochemotherapy, reserving surgery for the salvage setting.

Authorisation for this retrospective study was obtained by the local ethics committee.

HPV detection

For HPV detection in FFPE samples, we used previously described and validated methods [17,18]. FFPE samples were sectioned for DNA extraction and collected in 1.5 ml micro-tubes. One ml of xylene was then added to each micro-tube and incubated for 30 min at room temperature. The samples were then centrifuged at 14,000 rpm for 3 min, and the supernatant was discarded; this procedure

was repeated twice. The pellet was then washed twice with absolute ethanol (5 min at room temperature). The samples were then incubated overnight with 1 ml of Lysis Buffer (BioMérieux, Rome, Italy) at 37°C.
Nucleic acid extraction was performed using the NucliSens easyMAG platform (BioMérieux, Rome, Italy), according to the manufacturer's protocols. Detection of HPV DNA was performed using the Digene Hybrid Capture 2 (HC2) assay (Qiagen Inc., Valencia, CA, USA), which allows for detection of 18 HPV genotypes and differentiation between high risk (HR) (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, and 68) and low-risk (types 6, 11, 42, 43, and 44) (LR) HPV.

Immunohistochemistry (IHC) for stem cell markers

FFPE tumour specimens were evaluated by IHC for the expression of 5 potential stem cell markers: CD44, CD133 protein (CD133), Oct-4, Nanog and Sox-2. Tissue sections were cut at lengths of 2 to 4 mm and deparaffinised. After antigen unmasking for 10 ± 1 minutes at 95 to 99°C in Tris buffer, pH 9.0, slides were allowed to cool to room temperature in the solution for 20 ± 1 min. Endogenous peroxidases were blocked with 3% hydrogen peroxide for 5 ± 1 minutes. The IHC Vectastain® Abc Kit (Vector Laboratories, Inc., Burlingame, CA) was used according to the manufacturer's protocol. The slides were stained with corresponding primary antibodies, namely, Anti-CD44 (Monoclonal Mouse, Phagocytic Glycoprotein-1, Clone DF1485. Code n. M7082) at a 1:50 dilution, Anti-CD133 (CD133/1 (AC133) pure human, monoclonal Myltenyi Biotec) at a 1:10 dilution, anti-OCT4 (C52G3, rabbit, cod. 2890 Cell Signaling Technology), anti-NANOG (C52G3, rabbit; cod. 4903 Cell Signaling Technology) and anti-SOX2 (D6D9 XP, rabbit; cod. 3579 Cell Signaling Technology), and incubated overnight at 4°C. Biotinylated secondary antibodies and VECTASTAIN® ABC Reagent were applied for 45 and 30 min, respectively. After development using a substrate-chromogen solution (AEC, Dako, Copenhagen, Denmark) for 2 min, the immunostained slides were counterstained using haematoxylin (Dako). Four "blinded" histopathologists evaluated the immunohistochemistry in independent readings. The cases that varied among the readers were re-evaluated to obtain a consensus.
The rate of cells with immunoreactivity (from 0 to 100%) was evaluated from 5 different fields and a total of at least 100 cancer cells.
Staining intensity was scored from 0 (no staining) to 3 (strong staining). For CD44, membrane and cytoplasmic staining were evaluated. For OCT-4, NANOG and SOX2, which are considered to be transcription factors with prominent nuclear expression, both cytoplasmic and nuclear expression patterns were specifically evaluated.

Statistical analysis

Statistical analysis was performed using JMP in software, release 7.0.1, from the SAS Institute (Cary, NC, USA). Confidence intervals for hazard ratios were determined by Cox multivariate analysis using STATA version 10, by StataCorp LP.
Correlations between categorical and numerical variables were evaluated by a Wilcoxon test, as most of the numerical variables in the present work did not display a normal distribution.
The oncological endpoint in prognostic evaluation was disease-specific survival (DSS). Univariate survival analysis according to nominal variables was performed by drawing Kaplan-Meyer curves and by evaluating statistical significance using a Wilcoxon test. Multivariate analysis was performed using Cox regression.

Results

Characterisation of the tumours and presence of HPV

Patient and tumour characteristics are shown in Table I. All patients were available for follow-up; the median length of follow-up was 40 months.
The most frequent subsite from which the SCCs originated was the mobile tongue (33%), followed by the tonsil (29%). We observed a marked prevalence of advanced cases (stage III and IV) (approximately 80%). More than 65% of patients in our study cohort presented with clinically positive lymph nodes at diagnosis.
Within the subgroup of OPSCC, the frequency of HR HPV infection was 33% (10/30), and all but one HPV-positive case originated from the tonsil. No HR HPV infection was detected in OSCCs. As expected and as previously described [21], HR HPV infection was associated with a markedly better survival among OPSCCs ($p = 0.045$ for Wilcoxon test).
Clinical TNM staging displayed a prognostic value in the entire series ($p = 0.016$ for Wilcoxon test) as well.

Description of the distribution of markers among HPV+ OPSCC, HPV-OPSCC and OSCC

In Table II, the IHC results for the different stem cell markers in the entire series, OSCC and OPSCC patients, are shown.
In most tumours, a distinct population of CD44+, usually representing approximately 10% of cancer cells, was identifiable. Most of these cells displayed membrane staining (Tab. II, Fig. 1A) in both OPSCCs and OSCCs. Nevertheless, the intensity of membrane staining for CD44 was significantly higher among OSCCs ($p = 0.0035$ for Wilcoxon test). More interestingly, such significance was

Table I. Descriptive statistics of the main variables concerning patients and tumour parameters.

Characteristic		69 patients
Age at diagnosis		
Median		62
Range		45-79
Follow-up period in months		
Median		40
Range		8-87
Smoking habits		
Non-smoker		20 (29%)
Current smoker		38 (55%)
Former smoker		11 (16%)
Alcohol consumption		
More than 4 glasses/day		23 (33.3%)
Less than 4 glasses/day		46 (66.7%)
Sex, no. (%)		
Male		53 (76.8%)
Female		16 (23.2%)
Site of origin, no. (%)	**Subsite of origin, no. (%)**	
Oral cavity 39 (56.5%)	Mobile tongue	23 (33.3%)
	Hard palate	2 (2.9%)
	Floor of mouth	10 (14.5%)
	Retromolar trigone	4 (5.8%)
Oropharynx 30 (43.5%)	Tonsil	20 (29%)
	Base of tongue	8 (11.6%)
	Soft palate	2 (2.9%)
AJCC stage, no. (%)		
I		3 (4.3%)
II		11 (16%)
III		14 (20.3%)
IVa		37 (53.6%)
IVb		4 (5.8%)
cT classification, no. (%)		
T1		7 (10.1%)
T2		22 (31.9%)
T3		10 (14.5%)
T4a		26 (37.7%)
T4b		4 (5.8%)
cN classification, no. (%)		
N0		24 (34.8%)
N1		16 (23.2%)
N2a		2 (2.9%)
N2b		12 (17.4%)
N2c		15 (21.7%)
Grading, no. (%)		
G1		20 (29%)
G2		26 (37.7%)
G3		23 (33.3%)
HPV DNA in FFPE samples, no. (%)		
Negative		59 (85.5%)
High risk HPV		10 (14.5%) (All in the oropharynx)

lost when excluding HPV positive OPSCCs, even if the comparisons of the expression of stem cell markers between the HPV positive (n = 10) and HPV negative (n = 20) OPSCCs did not demonstrate significant differences.

CD44 staining did not show any correlation with prognosis in our series.

As for CD133 staining, its expression was detected in only one sample, and with a low staining intensity.

Among the other markers evaluated, Oct-4 and Nanog were found to be expressed in less than 50% of HNSCCs, with prominent cytoplasmic expression (Tab. II; Figs. 1B, C, D, E, F, G, H, I, L). They did not display different expression profiles according to the site of origin of the tumour. Nevertheless, HPV positive cancers, and especially HPV+ OPSCC, showed significantly (in the Wilcoxon test) lower expression of Nanog in the cytoplasm ($p = 0.0041$ for intensity of staining, $p = 0.0054$ for the percentage of stained cells). Interestingly, the cytoplasmic expression of Nanog was associated with significantly worse prognosis in OPSCC ($p = 0.0012$ for Wilcoxon test, Fig. 2), but not in the OSCC subgroup when analysed separately.

Sox-2 staining was prevalently localised in the nucleus (Fig. 1M, N) and was significantly more intense and frequent among OPSCCs ($p = 0.0006$ for intensity of staining, $p = 0.0001$ for rate of stained cells), while it did not show any significant correlation with HPV infection.

Survival analysis

Sox-2 nuclear staining was associated with worse prognosis when evaluated within the entire series (Fig. 3).

Cox multivariate analysis for DSS took into account age, gender, tumour site, clinical stage, CD44 membrane staining, Oct-4 staining, Nanog cytoplasmic staining and Sox-2 nuclear staining (but not HPV infection, due to its strong correlation with cytoplasmic Nanog staining). To improve the readability and potential clinical applicability of the results, we transformed all of the numerical variables (namely age, CD44 membrane staining, Oct-4 staining, Nanog cytoplasmic staining, and Sox-2 nuclear staining) into nominal variables using the medians as cut-off values. The only parameter retaining prognostic significance at multivariate analysis was Nanog cytoplasmic staining ($p = 0.043$), while age at diagnosis, clinical stage and Sox-2 nuclear staining showed significant trends (Tab. III).

Discussion

Research on stem cell markers, in oncology in general and in HNSCCs in particular, may be interesting for at least two aims [2]: definition of the subpopulation of cancer stem cells, which should be specifically targeted by treatments, and the molecular characterisation of tumours for outcome prediction and treatment selection.

Table II. IHC for stem cell markers.

Marker	Entire series (n = 69)	OPSCC (n = 30)	HPV+ OPSCC (n = 10)	HPV- OPSCC (n = 20)	OSCC (n = 39)
CD44					
Membrane staining intensity					
0	5 (7.3%)	4 (13.3%)	2 (20%)	2 (10%)	1 (2.6%)
1	9 (13%)	5 (16.7%)	2 (20%)	3 (15%)	4 (10.3%)
2	18 (26.1%)	11 (36.7%)	4 (40%)	7 (35%)	7 (17.9%)
3	37 (53.6%)	10 (33.3%)	2 (20%)	8 (40%)	27 (69.2%)
Cytoplasmic staining intensity					
0	20 (29%)	11 (36.7%)	3 (30%)	8 (40%)	9 (23.1%)
1	35 (50.7%)	12 (40%)	5 (50%)	7 (35%)	23(59%)
2	13 (18.8%)	7 (23.3%)	2 (20%)	5 (25%)	6 (15.4%)
3	1 (1.5%)	0	0	0	1 (2.5%)
CD133					
Staining intensity	1 case w weak staining	No staining	No staining	No staining	1 case w weak staining
Oct-4					
Site of staining					
Nuclear	2 (2.9%)	2 (6.7%)	1 (10%)	1 (5%)	0
Cytoplasmic	27 (39.1%)	13 (43.3%)	5 (50%)	8 (40%)	14 (35.9%)
None	40 (58%)	15 (50%)	4 (40%)	11 (55%)	25 (64.1%)
Staining intensity					
0	40 (58%)	15 (50%)	4 (40%)	11 (55%)	25 (64.1%)
1	9 (13%)	6 (20%)	2 (20%)	4 (20%)	3 (7.7%)
2	14 (20.3%)	6 (20%)	3 (30%)	3 (15%)	8 (20.5%)
3	6 (8.7%)	3 (10%)	1 (10%)	2 (10%)	3 (7.7%)
Rate (%) of stained cells					
Mean	15.3	19.3	23.7	17.7	12.3
SD	25	27.9	29.7	27.76	22.5
Nanog					
Site of staining					
Nuclear	5 (7.2%)	3 (10%)	3 (30%)	0	2 (5.1%)
Cytoplasmic	28 (40.6%)	13 (43.3%)	0	13 (65%)	15 (38.5%)
Nuclear and cytoplasmic	1 (1.5%)	1 (3.4%)	0	1 (5%)	0
None	35 (50.7%)	13 (43.3%)	7 (70%)	6 (30%)	22 (56.4%)
Staining intensity					
0	35 (50.7%)	13 (43.3%)	7 (70%)	6 (30%)	22 (56.4%)
1	7 (10.2%)	3 (10%)	2 (20%)	1 (5%)	4 (10.3%)
2	12 (17.4%)	5 (16.7%)	1 (10%)	4 (20%)	7 (17.9%)
3	15 (21.7%)	9 (30%)	0	9 (45%)	6 (15.4%)
Rate (%) of stained cells					
Mean	22.6	30	12.5	36.6	17
SD	29.3	33.7	28.1	33.8	24.4
Sox-2					
Site of staining					
Nuclear	35 (53.7%)	23 (76.7%)	7 (70%)	16 (80%)	14 (36.8%)
Cytoplasmic	12 (17.9%)	4 (13.3%)	3 (30%)	1 (5%)	8 (21.1%)
Nuclear and cytoplasmic	4 (6%)	2 (6.7%)	0	2 (10%)	2 (5.3%)
None	15 (22.4%)	1 (3.3%)	0	1 (5%)	14 (36.8%)
Staining intensity					
0	15 (22.1%)	1 (3.3%)	0	1 (5%)	14 (36.8%)
1	10 (14.7%)	5 (16.7%)	2 (20%)	3 (15%)	5 (13.2%)
2	16 (23.5%)	6 (20%)	2 (20%)	4 (20%)	10 (26.3%)
3	27 (39.7%)	18 (60%)	6 (60%)	12 (60%)	9 (23.7%)
Rate (%) of stained cells					
Mean	42.4	61.72	65	60.5	27.6
SD	32.5	26.1	25	26.9	29.4

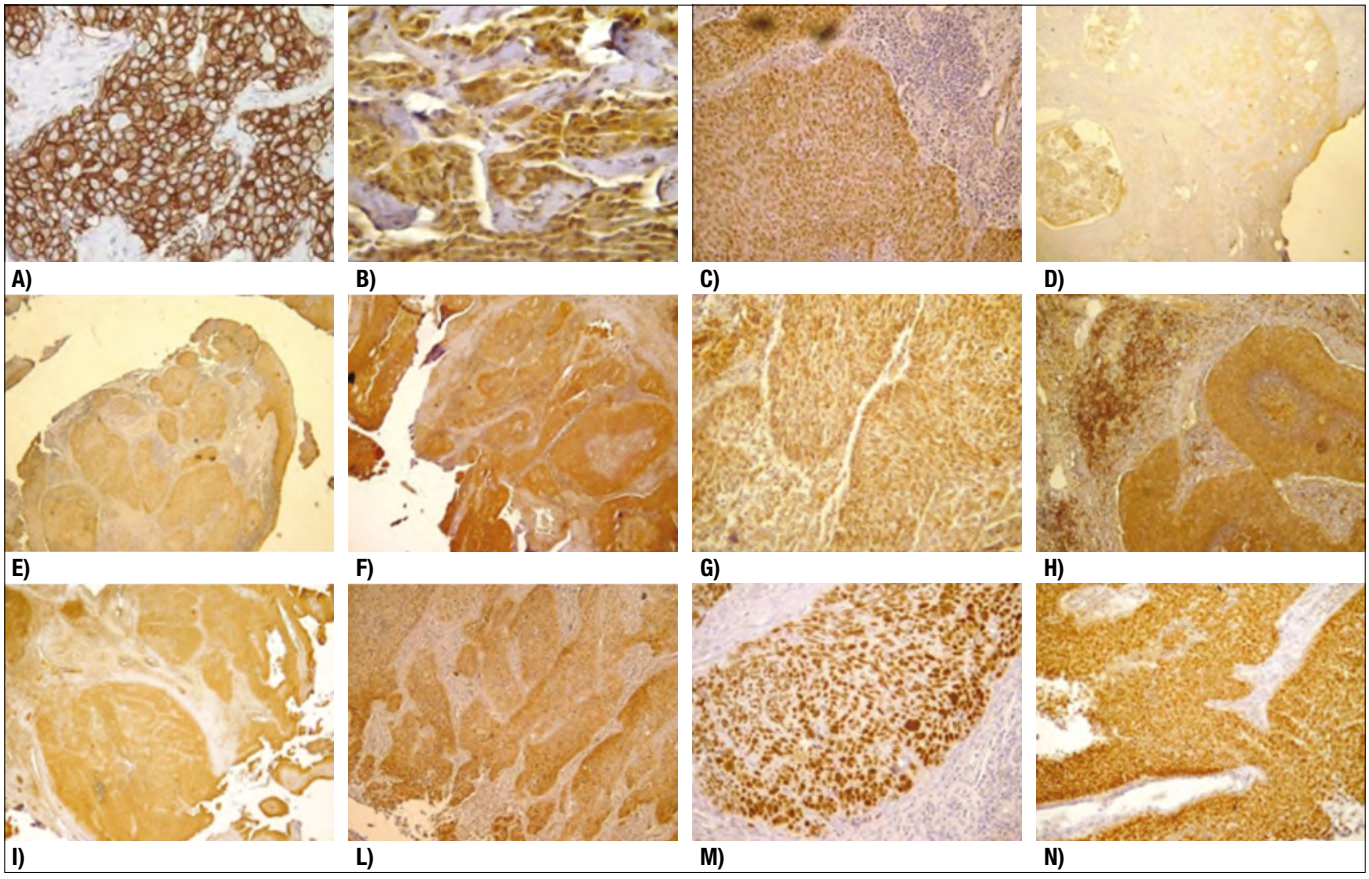

Figure 1. Immunostaining for CD44, Oct-4, NANOG and Sox-2 is shown. (**A**) membrane (3+) and cytoplasmic (2+) immunostaining for CD44 in a case of squamous cell carcinoma of the oropharynx, G2, T4bN2cM0, stage IV; (**B**) nuclear Oct-4 immunostaining, in a case of squamous cell carcinoma of the oral cavity (mobile tongue), T4bN0M0, stage IVb, G2, staining intensity of 3, 90% diffusion of staining; (**C**) nuclear Oct-4 immunostaining from a patient with squamous cell carcinoma of the oropharynx, tongue base, G2, T4N2cM0, stage IV; (**D**) cytoplasmic Oct-4 immunostaining from a case of squamous cell carcinoma of the oral cavity, T3N2bM0, stage IVa, G3, staining intensity of 2, 10% diffusion of staining; (**E**) cytoplasmic Oct-4 immunostaining from a case of squamous cell carcinoma of the oropharynx, T4N2cM0, stage IVa, G3, staining intensity of 2, 70% spread; (**F**) cytoplasmic Oct-4 immunostaining from a case of squamous cell carcinoma of the oral cavity, T4bN1M0, stage IVa, G3, staining intensity of 2, 70% spread; (**G**) nuclear Nanog immunostaining from a case of squamous cell carcinoma of the oropharynx, T4aN2cM0, stage IVa, G3, staining intensity of 3, 80% spread; (**H**) nuclear Nanog immunostaining from a case of squamous cell carcinoma of the oral cavity, T4aN1M0, stage IVa, G3, staining intensity of 2, 70% spread; (**I**) cytoplasmic Nanog immunostaining from a case of squamous cell carcinoma of the oral cavity, T3N1M0, stage III, G2, staining intensity of 3, 80% spread; (**L**) cytoplasmic Nanog immunostaining from a case of squamous cell carcinoma of the oropharynx, T3N2cM0, stage IVa, G3, staining intensity of 3, 70% spread; (**M**) nuclear Sox-2 immunostaining from a case of squamous cell carcinoma of the oropharynx (tonsil), G3, T4aN2cMx, stage IV; (**N**) nuclear Sox-2 immunostaining from a case of squamous cell carcinoma of the oral cavity, T3N2cM0, stage IVa, G3, staining intensity of 3, 100% diffusion of staining.

From the first perspective, the present work confirms the potential utility of CD44 localised on the cell membrane, almost constantly expressed in approximately 10% of cancer cells, consistent with observations in previous reports [5,6,21]. CD44 membrane or cytoplasmic expression did not influence DSS in the present series. CD44 was differentially expressed on the cell membranes of OSCCs and OPSCCs, suggesting, as plausible, that molecular differences associated with the different sites of origin in head and neck [22] also involve the subpopulations of CSC. Excluding HPV-related OPSCC from the analysis eliminated the statistical significance of such differences, confirming that HR-HPV has a role in determining the phenotype of OPSCCs stem cells.

CD133 was substantially undetectable and therefore does not appear to be a valuable stem cell marker in HNSCC. However, we cannot definitively rule out its role as a stem cell marker in HNSCC since the inability of the antibody utilised to detect CD133 molecule in FFPE samples might also be responsible for the results obtained.

The impact of HPV infection on the phenotype of HNSCC cells is even more evident when analysing Nanog cytoplasmic expression, which was always absent in HPV-related OPSCC, while it was frequent in the others.

To our knowledge, such negative correlation between HR-HPV infection and Nanog expression in HNSCC has not been previously described. Nanog is a transcriptional factor that plays a critical role in regulating the cell fate of the pluripotent inner cell mass during embryonic development [23]. Nanog cytoplasmic expression was demonstrated to be a strong prognostic predictor in OPSCC and was the only prognostic marker retaining its significance at Cox multivariate analysis in the entire series. A previous study on OSCC showed correlation of Nanog expression with stage at diagnosis, and, when associated with other markers, with prognosis [24]. In the present study, we show a prognostic role of Nanog expression, but apparently limited to the oropharynx, and probably correlated with HPV infection. No prognostic significance was detected either for the expression of Oct-4, which is a member of the family of POU domain transcription factors, expressed in pluripotent embryonic stem and germ cells [25-27] and functionally related to Nanog [28]. Furthermore, differently from previous hypotheses [28] and descriptions in OSCC [24], both proteins in the present series were prevalently localised in the cytoplasm.

Conversely, Sox-2 displayed the expected nuclear localisation and was shown to have prognostic value at univariate analysis in the entire series, as previously described [11], even if such significance was not retained at multivariate analysis in the present work. Nuclear expression of Sox-2 was significantly higher among OPSCCs, reconfirming the phenotypic differences among CSCs from different sites in the head and neck.

In conclusion, in the present study, CD44 appears to be a reliable marker for identification of the CSC subpopulation in HNSCC. Nevertheless, when evaluating the expression of membrane CD44 itself, and also nuclear

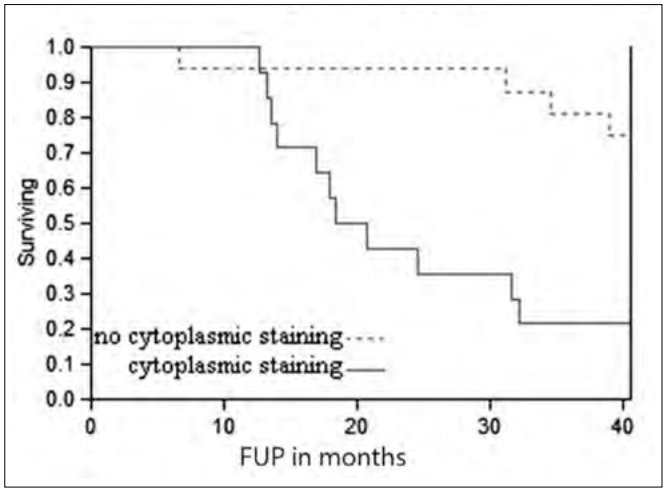

Figure 2. In the OPSCC group, the absence of Nanog cytoplasmic staining was associated with significantly better prognosis (p = 0.0012 for Wilcoxon test).

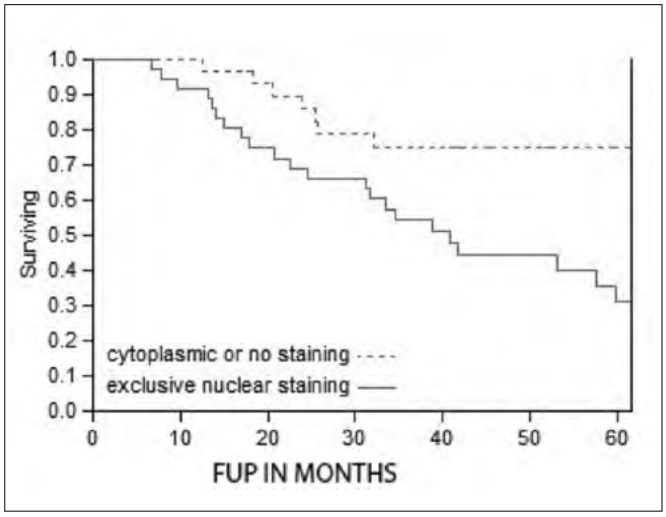

Figure 3. In the entire series of OSCCs and OPSCCs, nuclear staining for Sox-2 was associated with worse prognosis (p = 0.012 for Wilcoxon test).

Table III. Univariate and multivariate analysis of prognostic covariates for disease-specific survival.

Characteristic	Multivariate analysis		
	HR[a]	CI (95%)[b]	p
Age at diagnosis			
Over 65	1		
Under 65	0.48	0.22-1.03	0.06
Sex			
Female	1		
Male	1.52	0.61-3.8	0.365
Clinical stage			
I-II-III	1		
IVa-IVb	2.31	0.88-6.1	0.089
Primary site			
Oral cavity	1		
Oropharynx	1.67	0.65-4.26	0.283
CD 44 membrane staining			
Staining intensity 0, 1, 2	1		
Strong staining (score 3)	0.78	0.32-1.9	0.584
OCT-4 staining			
No staining	1		
Presence of stained cells	1.15	0.52-2.52	0.733
Nanog cytoplasmic staining			
No staining	1		
Presence of stained cells	2.45	1.02-5.84	**0.043**
Sox-2 staining			
No nuclear staining	1		
Presence of nuclear staining	2.24	0.9-5.56	0.083

[a]: hazard ratio; [b]: 95% confidence intervals.

Sox-2, clear differences emerged between different sites in the head and neck. Previous approaches in the study of CSCs have sometimes grouped HNSCCs together, but our results suggest that different markers could be used in the future for isolation as well as for targeting of CSCs in SCCs arising from different head and neck sites.

Other markers, such as Nanog, are influenced by HR-HPV infection. HPV infection is currently considered the most promising molecular marker in head and neck oncology, and has also been included by NCCN in the diagnostic work up for oropharyngeal SCC [29]. Debate about the standard detection method for HPV in FFPE samples is still ongoing, and the reliability of p16 expression as surrogate marker is questioned [18,30]. The absence of Nanog may be useful in this situation, being another effective indicator of HPV infection, which deserves to be evaluated in combination with other parameters (p16 and pRb, for example) to define the HPV related phenotype in OPSCCs, with potentially relevant clinical implications. In fact, Nanog might become an alternative, or more probably, an integration to p16 IHC, for diagnosis of HPV driven carcinogenesis in the oropharynx. At a cellular level, such differences in Nanog expression, still awaiting a consistent explanation, may turn out to be a useful clue to explain the clear phenotypic differences between HPV+ and HPV- SCCs.

As prognostic stratification, currently relying on clinical parameters only, is considered unsatisfactory, the definition of molecular predictive factors aimed to delineate homogeneous groups of patients for prognostic stratification and treatment selection (molecular characterisation) is potentially one of the most relevant areas of translational research in the head and neck. From this perspective, both Sox-2 and Nanog look promising as prognostic markers, although larger studies, also evaluating additional head and neck sites, are required before confirmation of this hypothesis and introduction into daily clinical practice.

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Laryngology

Modified Isshiki's arytenoid adduction without separating cricothyroid and cricoarytenoid joints

Intervento di adduzione aritenoidea sec. Isshiki modificata, senza separazione cricotiroidea e cricoaritenoidea

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SUMMARY

Modified methods of arytenoid adduction (AA) have been reported to keep the cricothyroid (CT) joint intact. However, postoperative laryngeal oedema and long-term vocal function have not been compared with those after AA with CT joint separation. We refined AA to combine it with nerve-muscle pedicle (NMP) flap transfer for preservation of the CT joint. Eight patients with unilateral laryngeal paralysis underwent the procedure (Group 1). Postoperative oedema at membranous vocal fold (MVF), arytenoid mound (AM) and pyriform sinus (PS) was assessed using a 4-point ordinal scale: none (0) to severe (3). Laryngeal oedema in Group 1 was compared with that of 19 patients who had AA with CT joint separation (Group 2). Maximum phonation time (MPT), jitter and voice handicap index-10 (VHI-10) were measured before surgery and one year postoperatively. Vocal function in Group 1 was compared with 58 patients who underwent AA + NMP flap transfer with CT joint separation (Group 3). The degree of oedema from postoperative days 1 to 6 in Group 1 was relatively invariable: 1.2~1.6 at MVF, 1.3~1.7 at AM, and 1.4~1.7 at PS. The scores at 3 and 4 days postoperatively at MVF and PS in Group 1 were significantly lower than in Group 2 (P = 0.0032 and 0.0317 at day 3, and 0.0224 and 0.0182, at day 4, respectively). The degree of oedema at day 3 at AM in Group 1 was significantly less than in Group 2 (P = 0.0260). One year after surgery, there were no significant differences in MPT, jitter and VHI-10 between Groups 1 and 3 (P = 0.660, 0.111 and 0.556, respectively). Preservation of the CT joint might be beneficial in reducing the maximum degree of laryngeal oedema after AA. Vocal function after AA + NMP flap transfer with CT joint preservation is comparable to that after AA + NMP flap transfer with CT joint separation.

KEY WORDS: modified arytenoid adduction, preservation of the cricothyroid and cricoarytenoid joints, location of the muscular process, postoperative oedema, vocal function

RIASSUNTO

Gli interventi modificati di adduzione aritenoidea (AA) sono finalizzati a preservare l'integrità dell'articolazione cricotiroidea. Non ci sono tuttavia studi comparativi che confrontino tali metodiche con interventi in cui vi sia la separazione cricotiroidea in termini di edema laringeo postoperatorio e funzione fonatoria a lungo termine. In questo studio si valuta un intervento di AA con allestimento di lembo peduncolato neuromuscolare (+ NMP) e preservazione dell'articolazione cricotiroidea. Sono stati valutati otto pazienti con paresi cordale monolaterale sottoposti a tale procedura (Gruppo 1). È stato valutato l'edema post operatorio a livello delle corde vocali, cappucci aritenoidei e seni piriformi con una scala, da 0 a 3 (0: nessun edema - 3: edema grave). Il gruppo controllo (Gruppo 2) è rappresentato da 19 pazienti sottoposti a AA con separazione dell'articolazione cricotiroidea. Il tempo massimo di fonazione (MPT), il jitter e il voice hadicap index (VHI) sono stati misurati prima e 1 anno dopo la chirurgia. I risultati funzionali fonatori del Gruppo 1 sono stati comparati con quelli di un gruppo controllo formato da 58 pazienti sottoposti a AA + NMP (Gruppo 3). L'edema a livello delle corde vocali, cappucci aritenoidei e seni piriformi del

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Conflict of interest
The Authors declare no conflict of interest.

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Gruppo 1 è risultato relativamente stabile dal primo al sesto giorno post operatorio. L'edema a livello delle corde vocali e seni piriformi nel Gruppo 1, a 3 e 4 giorni dalla chirurgia è risultato inferiore rispetto al Gruppo 2. Ad un anno dopo la chirurgia, non sono risultate differenze in termini di MPT, Jitter e VHI fra il Gruppo 1 e il Gruppo 3. La preservazione dell'articolazione cricotiroidea può risultare vantaggiosa nel ridurre l'edema laringeo post operatorio dopo AA. La funzione vocale dopo AA + NMP con preservazione o separazione dell'articolazione cricotiroidea sono comparabili.

PAROLE CHIAVE: *adduzione aritenoidea modificata, preservazione dell'articolazione cricotiroidea e cricoaritenoidea, processo vocale, edema post operatorio, funzione vocale*

Introduction

Arytenoid adduction (AA), often combined with type I thyroplasty, has been applied to improve hoarse voice in patients with unilateral vocal fold paralysis. The original method reported by Isshiki et al. [1] included separation of the cricothyroid (CT) joint to open the paraglottic space, as well as opening of the cricoarytenoid (CA) joint for identification of the muscular process of the arytenoid cartilage.

Opening of the CA joint destabilises the arytenoid cartilage and predisposes its prolapse anteriorly with suture traction [2,3]. Thus, several modifications to avoid CA joint opening have been reported [4,5]. We also did not open the CA joint while basically following Isshiki's method [6]. Recently, AA without CA joint separation has prevailed. On the other hand, Zeitels et al. observed that, after CT joint separation, the thyroid lamina became retrodisplaced with relation to the cricoid, resulting in the vocal fold shortened and reduction of the thyroarytenoid muscle tension [7]. The routes to locate the muscular process reported by Maragos [4] and Tokashiki et al. [5] also avoided CT joint separation. However, these authors did not assess postoperative laryngeal oedema and did not compare vocal outcomes of their methods with that of AA with separation of the CT joint [4,5].

We have modified Isshiki's method to perform AA by preserving the CT as well as CA joints. In the present paper, we describe a key technique of our refined AA procedure and preliminary evaluation of postoperative laryngeal oedema and vocal function to compare them with those following AA with separation of the CT joint.

Materials and methods

This study was approved by the institutional review board of Kumamoto University Hospital.

Surgical procedures

The difference of the surgical procedure from our previous report [6] is that the CT joint was not separated. Isshiki's original method was that, after separating the CT joint and the thyrohyoid ligament, the thyroid lamina is rotated as a whole to open the paraglottic space [1]. In our modified method, following separation of the thyrohyoid ligament, the pyriform sinus (PS) mucosa and inner perichondrium are elevated from the superior cornu and thyroid ala medially and inferiorly. Next, the thyroid ala is rotated anteriorly from the cranial side to open the paraglottic space for location of the muscular process (Fig. 1). Creation and transfer of a nerve-muscle pedicle (NMP) flap was performed as reported previously [6]. Intravenous hydrocortisone sodium succinate (500 mg) was administered. Intravenous steroids were given at 300 mg on postoperative day (POD) 1 and tapered down to 100 mg on PODs 2 to 3, with minor dose modifications depending on the patient's baseline and perioperative airway anatomy.

Subjects

Nine consecutive patients underwent AA combined with NMP flap transfer between May 2014 and July 2016 at Kumamoto University Hospital. One patient received a tracheostomy after the operation because of poor pulmonary function and was excluded from the study. Thus, eight patients were enrolled (Group 1).

The degree of oedema at each subsite during the period from POD 1 to 6 in Group 1 patients was compared with that of 19 patients after AA with the CT joint separated (Group 2). The data reported by Narajos et al. [8] were used because they separated the CT joint to perform AA. Their patients underwent either of AA alone, AA with recurrent laryngeal nerve-ansa cervicalis nerve anastomosis, or AA with NMP flap transfer. Vocal function of Group 1 patients

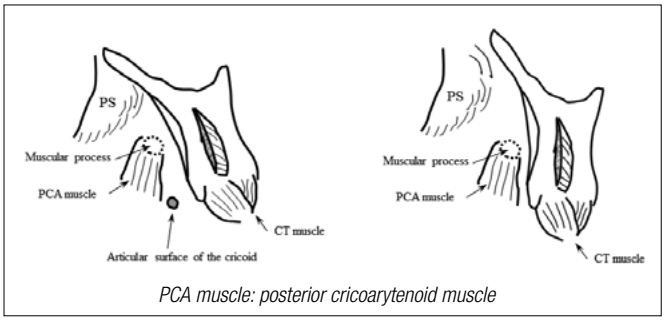

Figure 1. Surgical approach to locate the muscular process of the arytenoid (right side). Left: after separating the cricothyroid (CT) joint and thyrohyoid ligament, the thyroid lamina is rotated anteriorly as a whole to open the paraglottic space for location of the muscular process. Right: the CT joint is not separated. Following separation of the thyrohyoid ligament, the pyriform sinus (PS) mucosa and inner perichondrium are elevated from the superior cornu and thyroid ala. Next, the cranial side of the thyroid ala is rotated anteriorly to locate the muscular process.

was compared with that of 58 patients after AA + NMP flap transfer with CT joint separation (Group 3). They were operated on between July 2002 and April 2014 at Kumamoto University Hospital. The same operator (EY) performed the procedures under general anaesthesia in all patients in Groups 1 to 3. In Group 2, only AA was performed when recurrent laryngeal nerve-ansa cervicalis nerve anastomosis and NMP flap transfer were not feasible. Patients in Groups 1 and 3 were not randomly assigned because Group 1 patients underwent AA + NMP flap transfer after May 2014, while those in Group 3 underwent the operation before April 2014.

Evaluation of postoperative laryngeal oedema

All recordings were made under videolaryngoscopy using a flexible transnasal videoendoscope (VNL-1171K, EPK-1000; PENTAX, Tokyo, Japan) and fed to a computerised data filing system (Claio; Findex Inc., Tokyo, Japan). Postoperative laryngeal findings were recorded daily from POD 1 to 10. Patients were observed during quiet breathing.

Three experienced laryngologists rated postoperative laryngeal oedema. Each examiner was given a digital versatile disc copy of the postoperative laryngeal recordings. The videolaryngoscopic images without voice signal were ordered randomly by patient and the POD on which the recordings were taken. Each examiner rated postoperative laryngeal oedema at the three subsites; membranous vocal fold (MVF), arytenoid mound (AM), and pyriform sinus (PS). A four-point ordinal scale was used to score the degree of oedema as none (0), mild (1), moderate (2), or severe (3) (Tab. I) [8].

Vocal function measurements

The vocal function of each patient in Groups 1 and 3 was assessed twice; within two weeks before surgery and one year after surgery. To measure maximum phonation time (MPT), patients were instructed to produce sustained phonation of the vowel /a/ for as long as possible at comfortable pitch and loudness. MPT was measured twice for each patient and the greater value was recorded. Voice recording was performed in a sound-treated room using a digital recorder (Model PMD 670; Marantz, Sagamihara, Japan) connected to a microphone (Model WM-421; Panasonic, Kadoma, Japan). The microphone was held at a distance of 20 cm from the mouth during recordings. Recording samples of sustained vowel /a/ at comfortable pitch and loudness digitised at 45 kHz through antialiasing filter were stored with a pulse-code modulation format. A stable portion of the vowel of 0.5 to 1 second duration was trimmed and utilised for acoustic analysis. Jitter, shimmer and noise-to-harmonics ratio were obtained with the use of Multi-Dimensional Voice Program Model 5105 (version 3.1.7; KayPentax, Lincoln Park, NJ). Further, all patients in Group 1 were asked to rate each of the 10 statements in a validated Japanese version [9] of VHI-10 query sheet on a scale of 0 to 4 (0 = strongly disagree, 4 = strongly agree), for a possible worst total score of 40. Since we started to evaluate patients' subjective difficulty in their daily life due to unilateral vocal fold paralysis in November 2008, 40 of 58 Group 3 patients were asked to fill out a VHI-10 query sheet.

Statistical analysis

Spearman correlation coefficients were calculated to examine reliability of the three judges' evaluations. Wilcoxon signed rank test was used to examine whether there was a significant difference in the degree of oedema at each subsite according to the postoperative time course. Wilcoxon signed rank test was also used to examine changes in VHI-10 during a follow-up period in Groups 1 and 3. Paired t test was used to examine changes in MPT and jitter measurements during follow-up.

The degree of oedema at each subsite during the period from POD 1 to 6 was compared with that of Group 2 (AA with the CT joint separated); Mann-Whitney U test was used. Measurements of MPT and jitter, and VHI-10 scores before operation and one year after the operation were compared between Groups 1 and 3, respectively, using Mann-Whitney U test because the number of patients in Group 1 was small. The significance level was set at $P < 0.05$.

Results

Table II presents the demographic characteristics of Group 1 including age, gender, side and cause of paralysis, history

Table I. Videolaryngoscopic scoring scheme for the degree of laryngeal oedema at three subsites.

Score	Membranous vocal fold	Arytenoid mound	Pyriform sinus
0	No oedema	No oedema	No oedema
1	Confined oedema (no contact with the opposite vocal fold)	Confined oedema (only to the affected arytenoid mound)	Visible space (but more shallow than the unaffected side)
2	Expanding oedema (contacts ≤ one half of the opposite vocal fold)	Expanding oedema (extends to the aryepiglottic fold)	Some visible space (mucosa obliterates ≥ one half of the pyriform sinus)
3	Expanding oedema (contacts > one half of the opposite vocal fold)	Expanding oedema (extends to the aryepiglottic fold and base of the epiglottis)	No visible space (mucosa completely obliterates pyriform sinus)

Table II. Patient demographics in Group 1.

No.	Age	Gender	Side	Cause of paralysis	History of neck surgery	Duration of paralysis
1	53	F	L	Post heart op	Absent	11 months
2	77	F	L	Idiopathic	Absent	unknown
3	34	F	L	Post heart op	Absent	32 years
4	74	M	L	Idiopathic	Absent	9 months
5	30	M	L	Post aortic aneurysm op	Absent	9 months
6	54	F	R	Epithelioid angioendothelioma in the internal jugular vein	Present	3 months
7	64	F	L	Clipping of intracranial aneurysm	Present	8 months
8	14	M	L	Post-intubation	Absent	71 months

F: female; M: male; L: left, R: right.

of neck surgery and duration of paralysis until surgery. No postoperative complications, such as haematoma or wound infection, were encountered during hospitalization. No patients complained of dyspnoeic sensation and no episodes of airway obstruction requiring a tracheostomy occurred. Table III presents demographic characteristics of the patients in Groups 2 and 3 including age, gender, side and cause of paralysis, and duration of paralysis until surgery. Because duration of paralysis until surgery ranged widely with a skewed distribution in the Groups 2 and 3 patients, mean and median values together with the ranges were listed.

Spearman correlation coefficients of three pairs of examiners for scoring the degree of oedema at each laryngeal subsite in Group 1 patients ranged between 0.679 and 0.746 ($P < 0.0001$). Since the scores of the three judges agreed significantly, the degree of oedema was assessed in terms of the mean of the three scores. Figure 2 illustrates time course of laryngeal oedema at each subsite following AA with the CT and CA joints preserved (Group 1, indicated by continuous line) and following AA with the CT joint separated [8] (Group 2, indicated by dotted line). The degree of oedema at each subsite from PODs 1 to 6 in Group 1 was relatively invariable: 1.2~1.6 at MVF, 1.3~1.7 at AM, and 1.4~1.7 at PS. Significant decreases in the degree of oedema at each subsite were detected when the scores for degree of oedema at PODs 1 to 6 were compared with those at POD 10.

Table III. Patients demographics in Groups 2 and 3.

	Group 2	Group 3
Number of patients	19	58
Age (average ± SD, range)	56.0 ± 15.3, 32~75	59.3 ± 13.3, 22~82
Gender (male/female)	14/5	28/30
Side of paralysis (left/right)	16/3	44/14
Duration of paralysis (months)		
Mean (range)	41.0 (4~360)	35.0 (1~612)
Median	11.0	11.5
Cause of paralysis:		
Iatrogenic:		
Thyroid cancer	7	17
Graves' disease		1
Vagal nerve neurinoma of neck		2
Subarachnoid haemorrhage		4
Meningioma		1
Mediastinal tumour	2	6
Aortic aneurysm	3	11
Heart valvular disease		1
Lung cancer	1	8
Esophageal cancer	4	0
Non-iatrogenic:		
Pulmonary tuberculosis		1
Idiopathic	2	6

SD: standard deviation.

The scores at PODs 3 and 4 at MVF in Group 1 were significantly lower than those in Group 2 ($P = 0.0032$ and 0.0317, respectively). The degree of oedema at POD 3 at AM in Group 1 was also significantly less than that in Group 2 ($P = 0.0260$). Furthermore, the scores at PODs 3 and 4 at PS in Group 1 were significantly lower than those in Group 2 ($P = 0.0224$ and 0.0182, respectively).

Figure 3 illustrates changes in MPT, jitter and VHI-10 before surgery and one year after surgery in Groups 1 (dotted bar) and 3 (oblique-lined bar). MPT measurements and VHI-10 scores in Group 1 improved one year after surgery, but, the improvements did not reach significance. Jitter in Group 1 showed a significant improvement after surgery ($P = 0.01$), and MPT, jitter and VHI-10 showed a significant improvement after surgery in Group 3 ($P < 0.001$ in all parameters).

Preoperatively, differences in jitter and VHI-10 between Groups 1 and 3 were not significant ($P = 0.182$ and 0.156, respectively), and MPT in Group 3 was significantly smaller than in Group 1 ($P = 0.041$). One year after surgery, there were no significant differences in MPT, jitter and VHI-10 between Groups 1 and 3 ($P = 0.660$, 0.111 and 0.556, respectively).

Discussion

Arytenoid adduction often combined with type I thyroplasty reported by Isshiki et al[1] has been the prevailing treatment for paralytic dysphonia. Their method included separation of the CT joint to open the paraglottic space, as well as opening of

the CA joint for identification of the muscular process of the arytenoid cartilage. Preservation of the CA joint contributes to relatively stable support of the arytenoid and impedes its anterior shift. Additionally, CT joint preservation avoid posterior displacement of the thyroid lamina [7].

Postoperative laryngeal oedema is a major complication of AA. The frequency of the necessity of tracheostomy after AA ranged between 1.6% and 4.2% [10,11]. It has been believed that postoperative oedema of the AM relates directly to the precision and extent of management around the CA joint and length of time to perform it [12]. However, relation of the operative management around the CT joint to laryngeal oedema has not been evaluated.

The superior thyroid artery has a branch that projects to the larynx (superior laryngeal artery (SLA)) and then runs inferiorly to the thyroid gland and CT muscle. Iimura et al. further studied the branches of the SLA (Fig. 4) [13]. Its first branch, the superoposterior branch, projects to the aryepiglottic fold, while the anterior branch goes to the front wall of the larynx, the medial posterior branch projects to the posterior wall of the arytenoid and front wall of the pharynx, the medial branch runs medially to the laryngeal wall and, finally, the SLA separates into anteroinferior and posteroinferior branches. The two final divisions project to the lower part of the anterior and posterior walls of the larynx. They also reported that the superoposterior and medial posterior branches show marked meandering in their courses. In addition, some branches anastomose one another. In the process of exposure and separation of the CT joint during AA, the CT branch of the superior thyroid artery and medial posterior, anteroinferior and posteroinferior branches of the SLA might be injured. Such injuries could be a factor which cause exacerbation of laryngeal oedema. Thus, preservation of the CT joint might be beneficial in reducing the maximum degree of laryngeal oedema after AA. Actually, in Group 1, the degree of oedema at each subsite was significantly lower at PODs 3 and 4 versus those in Group 2 (Fig. 2). However, the number of Group 1 patients was too small to draw any definitive conclusion regarding the effect of AA with CT joint preservation on postoperative oedema.

Vocal function assessed with the use of MPT, jitter and VHI-10 did not show a significant difference between Groups 1 and 3 at one year after surgery. Thus, vocal function one year after surgery in Group 1 is highly comparable to that of Group 3.

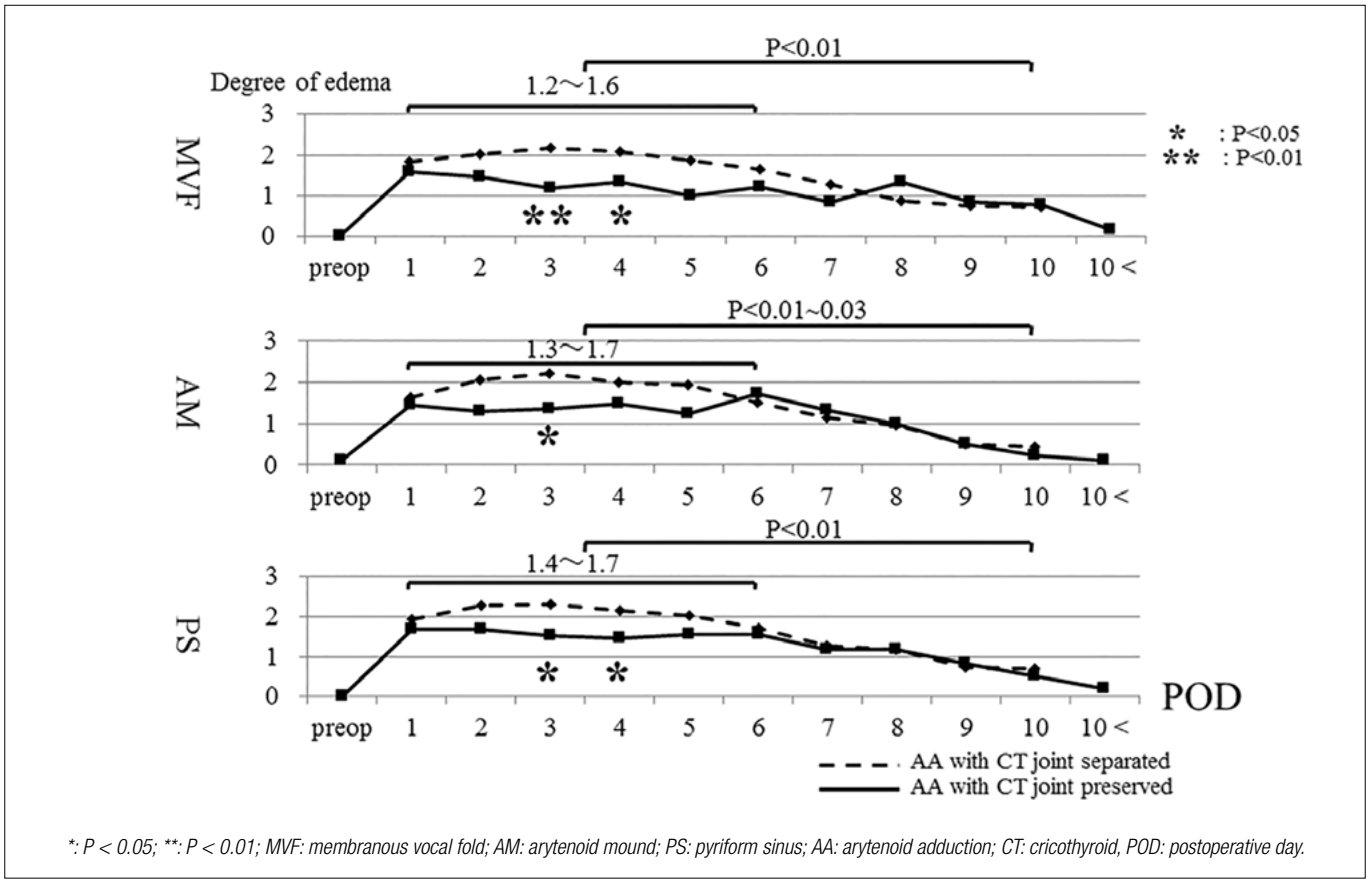

Figure 2. Time course of laryngeal oedema at each subsite following AA with the CT joint preserved (Group 1, continuous line) and following AA with the CT joint separated (Group 2, dotted line), as reported in our previous paper (from Narajos et al., 2012 [8]).

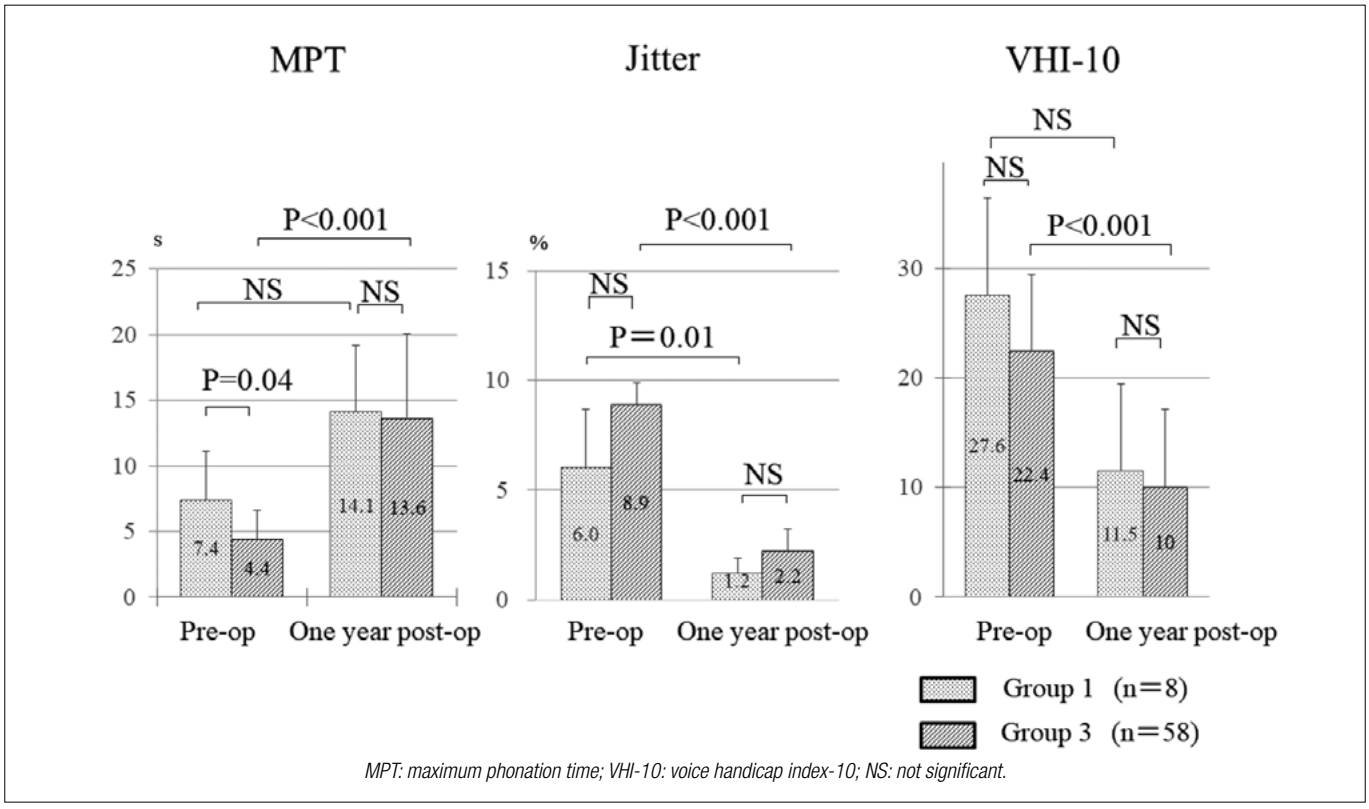

Figure 3. Changes in MPT, jitter and VHI-10 before surgery and one year after surgery in Group 1 (AA + NMP transfer with the CT joint preserved) and Group 3 (AA + NMP transfer with the CT joint separated). Dotted bars represent measurements in Group 1 and oblique-lined bars represent measurements in Group 3. The number in a bar shows the mean value of each parameter.

Although there was no significant difference, jitter in Group 1 was nearly half that of Group 3. In addition, as reported by Kodama et al. [14,15] MPT, jitter and VHI-10 after AA combined with NMP flap transfer continued to improve over the course of 2 years postoperatively. Therefore, vocal function after AA without separation of the CT joint might continue to improve over a 2-year period.

Since Maragos reported the "Posterior window approach" to reach the muscular process [4], this approach has become commonplace in the United States to perform AA [16]. The posterior window was a half circle with radius of 10 mm and the centre of the circle was set at the intersection of the posterior border of the thyroid ala and the horizontal line at the estimated vocal fold level. When AA through the posterior window approach is combined with type I thyroplasty, it is preferable to make a window size small enough to maintain the framework of the thyroid ala. Actually, Maragos reported that three patients had fractures of either the inferior cornu or the lateral ala secondary to forward retraction on the thyroid ala when combined with type I thyroplasty [4]. McCulloch et al inserted Gore-Tex from below the thyroid cartilage when AA through the posterior window approach is combined with type I thyroplasty [16]. In addition to type 1 thyroplasty, injection laryngoplasty is also a useful adjunct after AA to augment the paralysed vocal fold. Kimura et al. reported significant improvements in vocal function with collagen injection for those who did not achieve satisfactory glottal competence with AA alone [17]. They stated that collagen injection has the advantage of being minimally invasive outpatient office procedure over type 1 thyroplasty.

When AA is combined with NMP flap transfer, a window

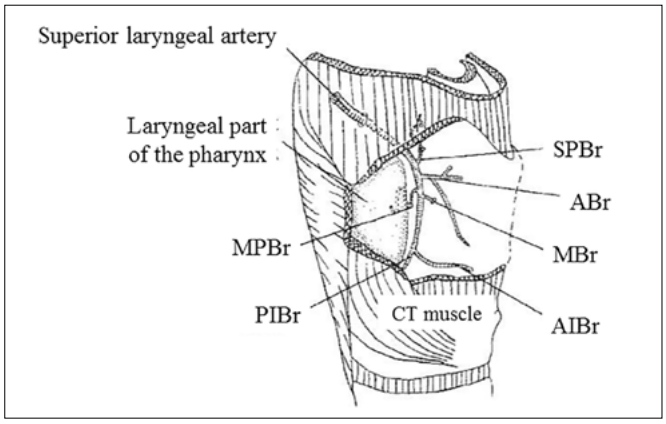

Figure 4. Distribution of the six intralaryngeal branches of the superior laryngeal artery (from Iimura et al., 2004 [13]).

is opened in the thyroid ala to expose the thyroarytenoid muscle. The size of the window ranges 10-12 mm in a horizontal direction and 5-7 mm in a vertical direction. The medial edge of the window is set 5-7 mm laterally from the midline [6]. Hiramoto studied anatomy of the thyroid cartilage using 28 male and 23 female adult larynges in the Japanese population [18]. The horizontal length of the thyroid ala at the vocal fold level was reported to range from 29 mm to 44 mm with a mean of 37 mm in men and from 24 mm to 32 mm with a mean of 28 mm in women [18]. Therefore, the posterior window approach is not an appropriate route to locate the muscular process while keeping the framework of the thyroid ala intact when AA is combined with NMP flap transfer.

Whether or not AA with the CT joint preserved is feasible in all patients remains to be clarified. We were able to perform AA without separation of the CT joint in all subjects in the present series, including two who had a history of neck surgery on the paralysed side. However, it is highly likely that cicatricial tissue interferes with the location of the muscular process unless the CT joint is separated. Moreover, the thyroid laminae in the male larynx often make a steep angle with each other, and separation of the CT joint might be required for location of the muscular process. Therefore, further studies with a greater number of patients are necessary to confirm the preliminary results of the present study.

Conclusions

We modified Isshiki's method to perform AA by preserving the CT as well as CA joints. In our method of AA combined with NMP flap transfer, it is essential to elevate the inner perichondrium from the superior cornu and thyroid ala towards medially and inferiorly. Next, the thyroid ala is rotated anteriorly from the cranial side to open the paraglottic space for location of the muscular process. Preservation of the CT joint might be beneficial in reducing the maximum degree of laryngeal oedema after AA. Vocal function one year after AA + NMP flap transfer with the CT joint preserved is comparable to that after AA + NMP flap transfer with the CT joint separated. This is the first report to suggest the relation of the operative management around the CT joint to postoperative laryngeal oedema. Further studies with a larger number of patients are required to better assess the significance of preservation of the CT joint.

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LARYNGOLOGY

The thyro-cricoarytenoid space (TCAS): clinical and prognostic implications in laryngeal cancer

Lo spazio tiro-crico-aritenoideo (TCAS): implicazioni cliniche e prognostiche nel carcinoma laringeo

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SUMMARY

The recent literature on laryngeal surgical oncology has increasingly focused on the negative prognostic impact of neoplastic involvement of the posterior portion of the inferior paraglottic space, which we refer to as the "thyro-cricoarytenoid space" (TCAS). We retrospectively considered the prognostic significance of TCAS involvement in a cohort of 84 patients treated with open partial horizontal laryngectomy for glottic squamous cell carcinoma. Univariate analysis was conducted on the prognostic value of several clinical and pathological parameters. Cases with TCAS involvement experienced a higher recurrence rate and shorter disease-free survival. Neoplasms involving the TCAS should be considered and treated as extralaryngeal malignancies. Posterior glottic tumours with TCAS invasion have worse prognosis when managed with conservative surgery. Total laryngectomy should be considered in cases of locally-advanced glottic carcinoma with TCAS involvement.

KEY WORDS: TCAS, thyrocricoarytenoid, space, posterior, glottic carcinoma

RIASSUNTO

La recente letteratura riguardante la chirurgia oncologica laringea si sta sempre più focalizzando sul significato prognostico negativo del coinvolgimento da parte della neoplasia della porzione posteriore dello spazio paraglottico inferiore, che può essere definito spazio "tiro-crico-aritenoideo" (TCAS). Abbiamo valutato retrospettivamente il significato prognostico del coinvolgimento di tale sito anatomico in una coorte di 84 pazienti trattati con laringectomia parziale orizzontale open. È stato inoltre valutato mediante analisi univariata il significato prognostico dei parametri clinici e patologici. I casi con coinvolgimento del TCAS hanno avuto un maggior tasso di recidiva ed una minore sopravvivenza libera da malattia, rispetto ai casi senza coinvolgimento dello stesso. In conclusione, le neoplasie coinvolgenti questo sito laringeo dovrebbero essere considerate e trattate come tumori extra-laringei. I carcinomi glottici posteriori con invasione del TCAS hanno una prognosi peggiore quando gestiti mediante chirurgia conservativa. Nei casi di carcinoma glottico localmente avanzato con coinvolgimento del TCAS la laringectomia totale dovrebbe essere considerata il trattamento di scelta.

PAROLE CHIAVE: *TCAS, tirocricoaritenoideo, spazio, posteriore, carcinoma glottico*

Introduction

The recent literature in the sphere of laryngeal surgical oncology has increasingly focused on the anatomical space delineated by the thyroid, cricoid and arytenoid cartilages [1-7], which we refer to as the "thyro-cricoarytenoid space" (TCAS).

The inferior paraglottic space (PGS) is a submucosal compartment bound laterally by the laryngeal cartilaginous framework and medially by the

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Conflict of interest
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vocal muscle [8]. We use the acronym TCAS to define the anatomical site that includes the posterior portion of the inferior PGS and adjacent muscles, located dorsally to a coronal plane and lying tangential to the vocal process of the arytenoid cartilages. It is limited laterally by the thyroid lamina and pyriform sinus, and medially by the arytenoid and cricoarytenoid joint (CAJ), as well as by the ipsilateral hemicricoid. Caudally, it borders on the cricoid and the lower edge of the thyroid lamina (Fig. 1). The TCAS consists of adipose tissue, glands and vessels, and contains the thyro-arytenoid and the lateral cricoarytenoid muscles. The lateral cricothyroid muscle identifies a side boundary.
Endoscopically, TCAS involvement by a tumour may cause impaired motility or fixation of the vocal cord and arytenoid due to infiltration of the vocal muscle, the lateral cricoarytenoid muscle extending from the arytenoid cartilage, or the CAJ [5,9]. Contrast-enhanced CT and MRI can reveal direct signs of infiltration, or suspected infiltration, such as sclerosis of the arytenoid [10] (Fig. 2).

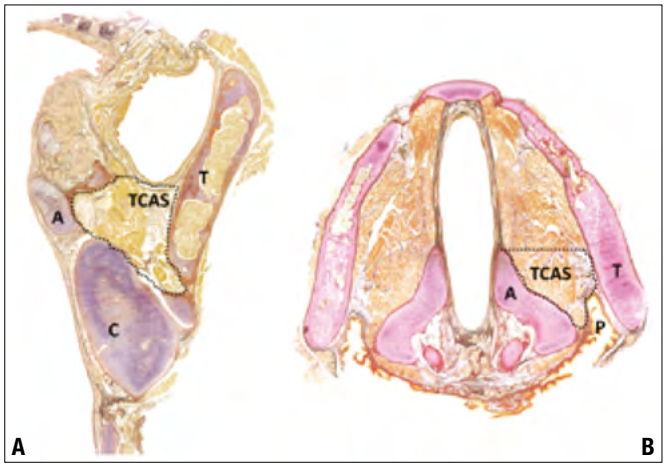

Figure 1. Healthy larynx: coronal (**A**) and axial (**B**) histological sections showing the TCAS. A: arytenoid cartilage; C: cricoid cartilage; P: pyriform sinus; T: thyroid cartilage.

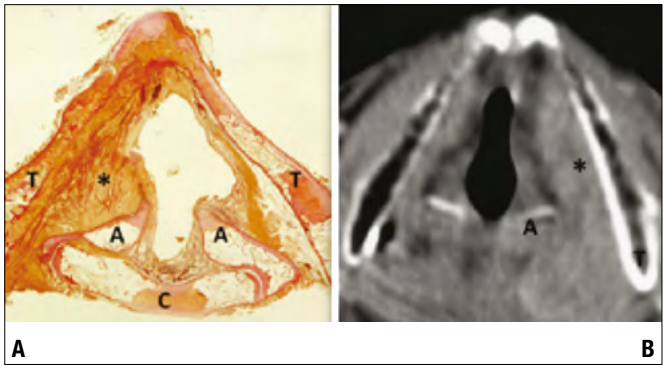

Figure 2. Glottic cT3 carcinoma involving the left TCAS: (**A**) axial histological section; (**B**) axial contrast-enhanced CT scan. A: arytenoid cartilage; C: cricoid cartilage; T: thyroid cartilage; * : tumour.

The TCAS is always involved when laryngeal cancer spreads posteriorly through the PGS, and this event coincides with dramatically worsening prognosis [5].
The primary aim of the present study was to retrospectively investigate the prognostic meaning of TCAS involvement by laryngeal squamous cell carcinoma (LSCC) treated with open partial horizontal laryngectomy (OPHL).

Methods

Patients

From 2013 to 2016, 106 patients consecutively underwent OPHL at the Otolaryngology Service of Vittorio Veneto Hospital (Italy). The present study involved a cohort of 84 LSCC patients (67 men and 17 women; mean age 60.1 ± 9.4 years, median 63) who met the inclusion criteria.
Exclusion criteria were: i) supraglottic cancer treated with supraglottic laryngectomy (OPHL I), since the primary aim of our study was to examine the posterior portion of the inferior PGS; ii) patients who underwent salvage OPHL; iii) a follow-up < 24 months; iv) locally-advanced disease making it impossible to establish the anterior vs posterior compartmentalisation of the tumour; v) final histology other than LSCC.
Clinical charts were retrospectively reviewed and any radiological/pathological evidence of TCAS involvement was recorded.
Laryngeal tumours were staged according to the 8th classification of the Union Internationale Contre le Cancer and the American Joint Committee on Cancer.
All patients completed preoperative diagnostic work-up with laryngeal indirect flexible video-endoscopy, contrast-enhanced neck CT scan or MRI, chest X-ray, and oesophagoscopy. In all cases, TCAS involvement was preoperatively evaluated with contrast enhanced CT scans or MRI of the larynx. Laryngoscopy was then performed under general anaesthesia using rigid 0°, 30° and 70° telescopes in white light and narrow band imaging to complete the diagnostic work-up.
All procedures performed were in accordance with the ethical standards of the institutional Ethics Review Board and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Surgery

An OPHL was adopted in selected cases of early glottic cancer (cT1-2) not amenable to transoral laser microsurgery (TLM), and selected cases of intermediate or advanced disease (cT3-4a), with at least one safe cricoarytenoid unit,

no massive extralaryngeal spread (i.e. a limited diffusion to prelaryngeal tissues without invasion of the thyroid gland or of the infrahyoid muscles), and acceptable general conditions and comorbidities. General patient-related contraindications to OPHL included alcohol and drug abuse, or major comorbidities such as heart failure, lung diseases, mellitus diabetes, or severe neurocognitive decay.
The open partial procedures consisted of supracricoid laryngectomy with cricohyoidoepiglottopexy (OPHL type IIa), or cricohyoidopexy (OPHL type IIb), and supratracheal laryngectomy with tracheohyoidoepiglottopexy (OPHL type IIIa), or tracheohyoidopexy (OPHL type IIIb), according to the ELS classification.
Pathological examination of the surgical margins was routinely performed on intraoperative frozen sections during OPHL. All margins were also checked postoperatively by final histology.
A radical or modified radical neck dissection (RND and MRND, respectively) were performed in the event of clinically or radiologically proven lymph node involvement. Selective neck dissection (SND) of levels II-III-IV was performed electively for cT3-4a N0 disease, or with curative intent for clinically or radiologically limited node metastases. Bilateral neck dissection was routinely performed in cases of supraglottic spread. An ipsilateral paratracheal neck dissection was used in the event of disease extending to the hypoglottis.

Pathological assessment

All laryngectomy specimens were opened postoperatively, and analysed for tumour site and extent. All sections were examined by the same team of experienced head and neck pathologists. Slides stained with haematoxylin and eosin, and photographs of gross specimens were reviewed and assessed in terms of TCAS involvement. All examinations were performed at ×1, ×5, ×10, ×20, and ×40 magnification with a Leica DM LB microscope (Meyer Instruments, Inc., Houston, TX) connected to a personal computer. For each case, 2 to 10 full-length longitudinal sections containing the tumour were obtained, depending on the tumour's size.

Statistical analysis

Fisher's exact test was used to calculate the association between different clinical and pathological parameters and the disease recurrence rate. The log-rank test and Kaplan-Meier survival function were used to calculate disease-free survival (DFS) for patients stratified by the selected variables.
The multivariate logistic model (Wald test) was applied to the same parameters (Fisher's exact test, $p < 0.20$) to identify independent prognostic factors in relation to recurrence rate, and the relative 95 % confidence intervals were calculated. A p-value < 0.05 was considered significant. The STATA 14 statistical package (Stata Corp., College Station, TX) was used for all analyses.

Results

Open partial horizontal laryngectomies

OPHL type IIa was performed in 46 cases, type IIb in 3, and type IIIa in 35; none of the patients had OPHL type IIIb. Eighteen patients (21%) experienced disease recurrence after 13.8 ± 10.6 months.
The cases of LSCC were classified as follows: cT1 in 1 patient; cT2 in 29; cT3 in 47; and cT4a in 7. Regional node status was classified as: cN0 in 68 cases; cN1 in 5; cN2 in 10; and cN3 in one. The pathological classification was: pT1 in 3 cases; pT2 in 10; pT3 in 52; and pT4a in 19. The pathological classification of cervical nodes was: pNX-0 in 69 cases; pN1 in 3; pN2 in 3; and pN3 in 9.
Ipsilateral neck dissection was performed in 70 cases, and bilateral neck dissection in 11. There was evidence of extranodal dissemination in 11 cases. Twenty-seven patients received postoperative radiotherapy or chemo-radiotherapy (CRT).

Pathological findings

In 49 cases, the tumour involved the posterior glottis with TCAS invasion, while in 35 cases the TCAS was uninvolved. Table I shows the distribution of the main pathological findings by presence or absence of TCAS invasion.
At pathology, 27 patients had positive surgical margins, while 57 had free or close surgical margins. Vascular and perineural invasion were detected in 43 and 27 cases, respectively. As for pathological grade, this was well differentiated in 19 cases, moderately differentiated in 33, poorly differentiated in 22 and indeterminate in 10.

Table I. Main pathological findings in patients with or without TCAS invasion.

	TCAS invasion (No. of patients)	No TCAS invasion (No. of patients)	p*
pN+	6	7	0.92
Vascular invasion	24	19	0.68
Perinerural invasion	15	11	0.75
Delphic node metastasis	2	0	0.75
Extranodal extension	6	5	0.82
Positive margins	15	12	0.77
Total	49	35	

**: Mann-Whitney U-test.*

Oncological outcomes

The mean follow-up was 50.1 ± 44.5 months (range 26-71 months). Seven patients were lost to follow-up. At latest follow-up, 70 patients (82%) were alive and disease-free, 10 (12%) were alive with disease, 2 (3%) died of their disease and 2 (3%) died with no evidence of disease. The final overall and disease-specific survival rates were 95% and 97%, respectively. Considering only cases with TCAS involvement, 15 patients experienced disease recurrence, and 2 died of their disease. Among the patients with no TCAS invasion, 3 suffered a relapse, 2 died of other causes and none died of their LSCC.

Univariate and multivariate analysis

Details of the results of univariate analysis are shown in Table II. Higher recurrence rates and shorter DFS rates were seen in patients with TCAS invasion, those who underwent OPHL III, and those with younger age (Fig. 3), although no significant p values emerged from our statistical analyses. Patients with locally intermediate or advanced disease (pT3-4) and positive nodes (pN+) had a higher recurrence rate and shorter DFS (Fig. 3), although statistical analysis identified no significant p values. Patients with positive surgical margins had a significantly higher recurrence rate and shorter DFS (Fig. 3) than those with negative margins.

Multivariate analysis confirmed that only the status of surgical margins was an independent prognostic factor in terms of recurrence rate (Tab. III)

Discussion

Several recently published reports corroborate the poorer prognosis associated with the posterior spread of glottic

Table II. Recurrence and disease-free survival rates (months) by main clinical and pathological parameters.

	No. of patients (%)	Recurrence rate (%)	p*	Disease-free survival (mean ± SD)	p**
Age					
Age ≥ 65	31 (37)	5 (16)	1.00	28.4 ± 25.7	0.83
Age < 65	53 (63)	9 (17)		27.0 ± 26.5	
Pathological T classification					
pT1	3 (4)	2 (15)	1.00	21.5 ± 25.4	0.92
pT2	10 (12)				
pT3	52 (61)	12 (16)		28.6 ± 26.2	
pT4a	19 (22)				
Pathological N classification					
pN0	69 (81)	10 (14)	0.43	29.1 ± 27.3	0.16
pN1	3 (4)				
pN2	3 (4)	4 (26)		19.9 ± 18.4	
pN3	9 (11)				
Resection margins					
Negative	57 (67)	5 (8)	0.00	29.3 ± 29.4	0.00
Positive	27 (33)	9 (33)		23.7 ± 17.2	
Vascular invasion					
Negative	41 (48)	6 (14)	0.77	28.5 ± 29.9	0.85
Positive	43 (52)	8 (18)		26.4 ± 21.9	
Perineural invasion					
Negative	58 (68)	10 (17)	1.00	29.4 ± 29.4	0.80
Positive	27 (32)	4 (14)		23.5 ± 17.1	
Type of OPHL					
II	56 (67)	7 (12)	0.20	25.6 ± 23.6	0.21
III	27 (33)	7 (25)		28.6 ± 27.8	
Infiltration of TCAS					
Negative	35 (41)	3 (8)	0.13	31.9 ± 28.6	0.06
Positive	49 (59)	11 (22)		24.1 ± 23.8	

**: Fisher's exact test; **: Log-rank test.*

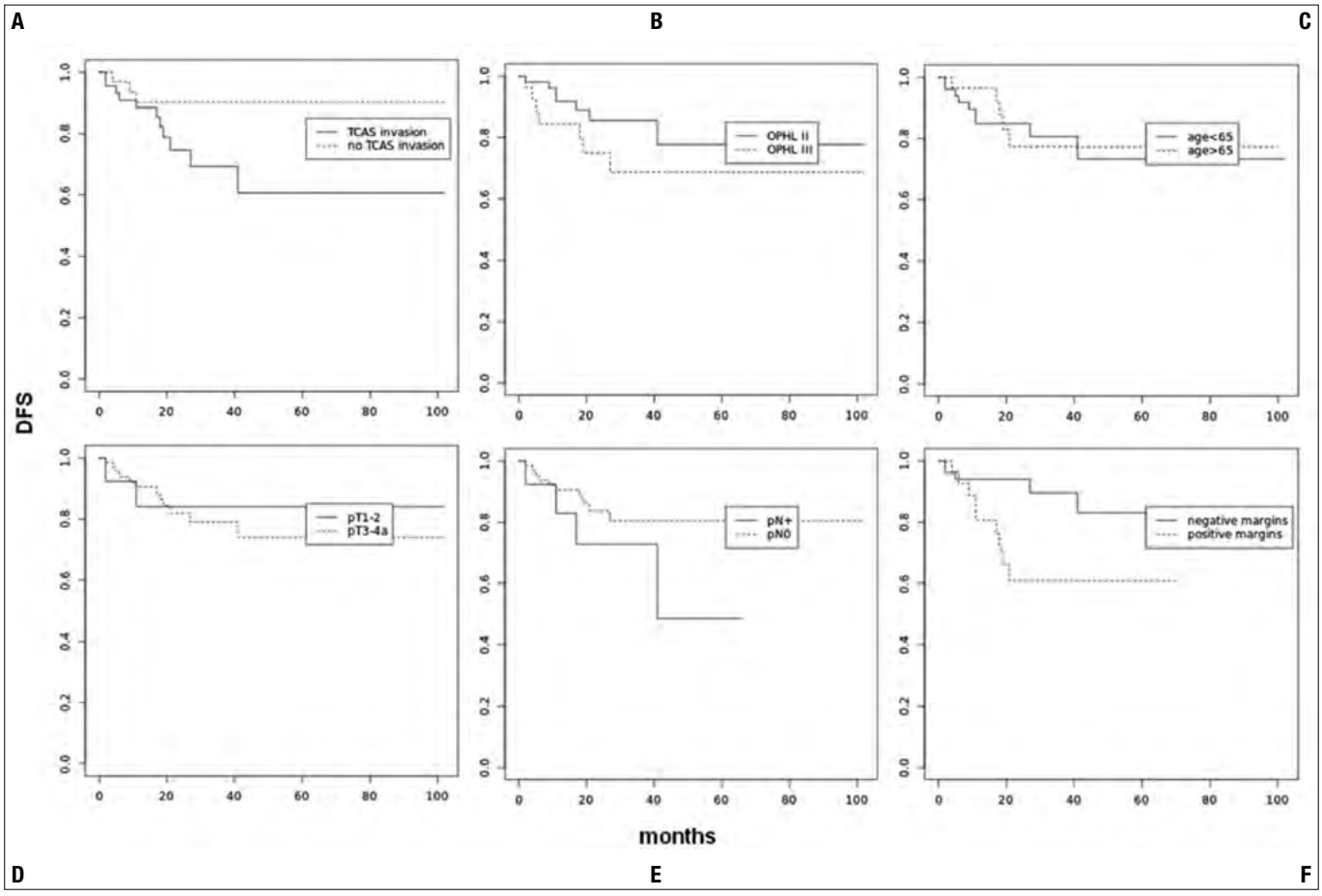

Figure 3. Kaplan Meier disease-free survival curves for patients stratified by TCAS invasion (**A**); surgical procedure (**B**); age (**C**); pT classification (**D**); pN classification (**E**); and status of surgical margins (**F**).

carcinoma. TCAS involvement is a negative prognostic factor for intermediate-advanced glottic carcinomas in terms of locoregional control rates after both surgical and nonsurgical treatments [5,7,11,12].

Lee and coworkers considered primary radiation treatment with 6-MV photons for the treatment of early glottic cancer, finding that patients with posterior third involvement had a poor local control rate, and suggesting that alternative approaches should be considered [12].

Using TLM, the deep muscle plane (the lateral cricoarytenoid and cricothyroid muscles) may not be manageable endoscopically with sufficient radicality [9], and thus TCAS involvement represents a clear contraindication to TLM.

In a cohort of patients with pT3N0 glottic disease and arytenoid fixation treated with OPHL IIa, Luna-Ortiz et al. reported that neoadjuvant chemotherapy produced an oncological benefit in patients awaiting surgery [13].

In 2018, Succo and coworkers analysed oncological outcomes of OPHL for locally-advanced LSCC by glottic compartmentalisation. The authors distinguished between pT3 glottic carcinomas that were "anterior" as opposed to "posterior" (subcategories I and II, respectively) to an ideal coronal plane tangential to the vocal process of the arytenoid cartilages. They found that anterior pT3 tumours (subcategory I) had better OS, DSS, DFS and locoregional control rates than posterior pT3 tumours (subcategory II) [5]. Our results confirm these findings, since TCAS invasion correlated with a worse prognosis in terms of recurrence rate and DFS. We might hypothesise an anatomical explanation: while the thyroid lamina is an excellent barrier to the tumour's anterior diffusion, posteriorly it can proceed towards the pyriform sinus, retrocricoid region and through the thyro-cricoid membrane towards the paralaryngeal spaces.

The TCAS serves as the postero-lateral resection margin in OPHL, and preoperative misdiagnosis of tumour spread at this level can be responsible for locally relapsing disease. However, in the present study, statistical analysis failed to recognise a correlation between the status of margins and TCAS invasion ($p = 0.77$)

Table III. Multivariate analysis of the main clinical and pathological parameters.

	Odds ratio	p*	95% confidence interval	p**
Pathological N classification				
pN0	1.00	0.43	Reference group	0.35
pN+	2.25		0.11-1.22	
Resection margins				
Negative	1.00	0.00	Reference group	0.00
Positive	3.25		0.13-0.72	
Type of OPHL				
II	0.95	0.20	Reference group	0.30
III	2.05		0.95-4.95	
Infiltration of TCAS				
Negative	0.90	0.13	Reference group	0.10
Positive	2.00		0.62-4.80	

**: Fisher's exact test; **: Wald test.*

(Tab. I). On the other hand, if independently considered, these two parameters were related to worsening of the oncologic outcome, in terms of recurrence rate and DFS. We hypothesised that this finding could be related to lymphatic neoplastic embolisation, which, due to the anatomical peculiarity, is more likely to interest the posterior rather than the anterior glottis.

When the tumour infiltrates the TCAS, conservative surgery should very carefully be considered. The tumour's contiguity to the CAJ often means that the cricoarytenoid unit has to be sacrificed. Supratracheal laryngectomy (OPHL type III) thus becomes the only available conservative surgical approach in cases of TCAS involvement, but local control is unsatisfactory [5]. Such cases should be managed with total laryngectomy.

The literature also provides evidence to suggest that carcinoma spreading to the posterior glottis from the various primary laryngeal and pyriform fossa sites may follow differing pathways of invasion, but always reveals a direct extension and connection to the subglottis [14].

Given the absence of anatomical barriers, the tumour can spread through the TCAS into the retrocricoid region (hypopharynx) and extralaryngeal neck compartment. Invasion of the fibres of the previously mentioned intralaryngeal muscles could represent a preferential pathway for the spread of tumour cells. Given the higher concentration of lymphatic vessels in the PGS than in the vocal cord, neoplastic involvement of the TCAS could also prompt early diffusion of tumour cells into the lateral and paratracheal neck regions, increasing the risk of neck metastases.

In 2018, Lucioni and coworkers investigated paratracheal lymph node involvement in LSCC extending into the subglottis. They found a significant correlation between paratracheal and laterocervical lymph node involvement in patients with posterior subglottic extension [15].

In a previously mentioned study, Succo et al. found tumours with a posterior glottic localisation were at higher risk of neck metastases to both the laterocervical and anterior levels (OR 1.69, and 3.77, respectively). The rate of node metastases with extracapsular dissemination was also significantly higher in posterior than in anterior pT3 glottic carcinomas (OR 2.03) [5].

Tumour invasion of the TCAS can significantly impair vocal cord motility due to a more limited arytenoid mobility, or to complete fixation of the vocal cord as a result of invasion of the crico-arytenoid muscles or cricoarytenoid joint. In a recently published study, our group investigated the clinical and radiological signs of posterior glottic tumour dissemination (through the TCAS). We found that it significantly related to impaired vocal cord motility and radiological evidence of sclerosis of the arytenoid cartilage [4]. The recent literature confirmed the negative prognostic meaning of such posterior spread of glottic cancer to the cricoarytenoid joint and associated arytenoid fixation [6]. A change of TNM classification (from T3 to T3b) have already been suggested as a result [1]. Involvement of the cricoarytenoid joint, with fixation of the arytenoid, would preclude many conservative laryngeal surgical approaches [1].

Conclusions

The TCAS is a critical issue in the case of laryngeal cancer dissemination, and neoplasms involving this site should be considered and treated as extralaryngeal malignancies. Posterior glottic tumours with TCAS invasion have poorer prognosis when managed with conservative surgery or CRT. Given the poor oncological results, TLM should be avoided in such cases, and the feasibility of OPHL should also be carefully assessed.

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Rhinology

Prognostic value of the Sinonasal Outcome Test 22 (SNOT-22) in chronic rhinosinusitis

Valore prognostico del Sinonasal Outcome Test 22 (SNOT-22) nella rinosinusite cronica

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SUMMARY

Previous studies have highlighted that baseline Sinonasal Outcome Test 22 (SNOT-22) score affects surgical outcomes in chronic rhinosinusitis (CRS) and suggested that a SNOT-22-based approach might ameliorate patients' understanding of expectations after treatment. Our study aimed at verifying this hypothesis in an Italian CRS population. In 457 CRS patients treated with endoscopic sinus surgery after failure of maximal medical therapy, the percentage of achieving a minimal clinically important difference (MCID) and the percentage of relative improvement after surgery were calculated. Moreover, the impact of several factors on preoperative and postoperative SNOT-22 score was investigated. Symptom improvement occurred in the majority of patients and was directly proportional to baseline SNOT-22. 79,7% of patients achieved the MCID and the percentage of relative improvement was 50,1%. Psychological and social-functioning implications significantly affected SNOT-22 scores. Multiple regression analysis showed that history of previous surgery, asthma, preoperative endoscopic and SNOT-22 scores predicted the postoperative SNOT-22 score (R2 = 0,298). Submitting CRS patients to SNOT-22 prior to surgical treatments might help to inform about probable outcomes, although it is strongly influenced by individual perception. Further studies are needed to identify an effective set of subjective and objective parameters for evaluation of outcomes.

KEY WORDS: Sinonasal Outcome Test-22 (SNOT-22), chronic rhinosinusitis, endoscopic sinus surgery, outcome prediction, quality of life

RIASSUNTO

Studi in letteratura hanno evidenziato che il punteggio basale del Sinonasal Outcome Test 22 (SNOT-22) influenza l'outcome chirurgico nella rinosinusite cronica (CRS) ed hanno suggerito che un approccio SNOT-22-mediato potrebbe migliorare la comprensione delle aspettative dei pazienti dopo il trattamento. Il presente studio mirava a verificare questa ipotesi in una popolazione italiana di CRS. In 457 pazienti con CRS, trattati con chirurgia endoscopica endonasale dopo fallimento della terapia medica massimale, sono stati calcolati la percentuale di raggiungimento della differenza minima clinicamente rilevabile (MCID) e la percentuale di miglioramento relativo dopo l'intervento chirurgico. Inoltre, è stato studiato l'impatto di diversi fattori sul punteggio dello SNOT-22 preoperatorio e postoperatorio. Il miglioramento dei sintomi si è verificato nella maggior parte dei pazienti ed era direttamente proporzionale alla SNOT-22 basale. Il 79,7% dei pazienti ha raggiunto l'MCID e la percentuale di miglioramento relativo è stata del 50,1%. Le implicazioni psicologiche e sociali hanno influenzato significativamente i punteggi dello SNOT-22. Un'analisi di regressione multipla ha mostrato che la storia di precedenti interventi chirurgici, asma, score endoscopico preoperatorio e SNOT-22 basale hanno statisticamente predetto il punteggio dello SNOT-22 postoperatorio (R2 = 0,229). Sottoporre i pazienti con CRS a SNOT-22 prima dei trattamenti chirurgici potrebbe quindi aiutare ad informarli sui probabili esiti, sebbene sia fortemente influenzato dalla percezione individuale. Sono necessari ulteriori studi per identificare un set efficace di parametri soggettivi e oggettivi per la valutazione dei risultati.

PAROLE CHIAVE: *Sinonasal Outcome Test-22 (SNOT-22), rinosinusite cronica, chirurgia endoscopica nasosinusale, previsione dell'outcome, qualità della vita*

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Introduction

Since the advent of nasal endoscopy, the evaluation of treatment outcomes in patients affected by chronic rhinosinusitis (CRS) has been a matter of debate. The pioneers of endoscopic sinus surgery (ESS) demonstrated surgical success rates of around 90-95% [1]. However, these results are far from the actual rates reported in literature. This is easily explained in two ways. As ESS was introduced recently in the 1980s and it was not in widespread practice, there was a lack of long-term follow-up studies that described the actual surgical effects [2]. Moreover, evaluation method of outcomes was based on qualitative scales, often estimating changes only on one or a few items of the CRS symptoms criteria, lacking a global assessment of improvement [3,4]. Finally, and this is partly also a current issue, the cohorts of patients were inhomogeneous, including cases of acute rhinosinusitis, massive nasal polyposis, or recurrent sinusitis after external procedures [5]. The 1990s witnessed the clinical application of the biopsychosocial model [6]. This theory supported that, in order to understand and respond adequately to patient suffering, clinicians should consider the biological, psychological and social dimensions of illness simultaneously. In practice, this was a way of considering the patient's subjective experience as an essential contributor to accurate diagnosis, health outcomes and humane care. In accordance with this philosophy, a "quality of life revolution" was observed in different areas of medicine [7] and several quality of life (QoL) questionnaires have been developed to quantify the individual and societal burden of chronic diseases. This paradigm shift also occurred for CRS. Since then, rhinologists have used several specific symptom-based scores to evaluate treatment outcomes in CRS patients, such as the Sinonasal Outcome Test 22 (SNOT-22) [8]. Applying these tools, it emerged that around 20-30% of CRS patients do not experience significant improvement after surgery, although the impact of ESS on QoL is generally reported as positive [9]. Moreover, other studies have quantified the 5-year risk of revision surgery to be 10-20%, while the presence of certain comorbidities, such as asthma and aspirin sensitivity, along with other factors like high baseline CT stage or incomplete sinus dissection, have been associated with elevated revision rates of 25-40%. However, despite the presence of known risk factors for revision surgery, evidence for several of these clinical characteristics has failed to reliably predict ESS outcomes [9]. Contrarily, it seems from previous regression studies that baseline SNOT-22 is one of the most important factors affecting the outcome [10] and several studies suggested its prognostic role in terms of achievement of improvement and risk of revision surgery [11]. In light of these observations, the presented study aimed at verifying in an Italian CRS population whether SNOT-22 could assist physicians in predicting surgical outcomes, improving shared decision-making process and ameliorating patients' understanding of their QoL expectations after treatment. The primary outcomes included measurement of the percentage of patients receiving a minimal clinically important difference (MCID) and the percentage of relative improvement (RI) after surgical treatment.

Materials and methods

This prospective study was conducted according to the declaration of Helsinki and was previously approved by the Institutional Review Board of the hospital (n. 109/2016).

Clinical data were obtained from a population of 457 patients affected by CRS operated in the same tertiary care centre in the period 2015-2018.

Enrolled patients were adult subjects affected by bilateral CRS undergoing ESS as a primary procedure after failure of maximal medical therapy [12]. All study participants had completed previous medical therapy including, but not limited to, at least two courses of topical steroid (60 days each). Oral steroid or culture-directed antibiotics were added when necessary (at least one course of 15 and 10 days respectively). However, medical therapy was not suspended until surgery.

Exclusion criteria were previous trauma, congenital facial malformations, systemic autoimmune diseases, cystic fibrosis, ciliary dyskinesia, head and neck malignancies or history of previous radiotherapy, any other nasal surgery performed concomitantly.

All surgical procedures were performed by the same 4 surgeons with more than 10 years of experience in ESS.

Postoperative medical therapy consisted in nasal irrigation with saline solution and intranasal corticosteroid [12], delivered with a high-volume squeeze bottle device [13]. A perioperative short-term of oral corticosteroid was also administered. Non-standardised oral steroid or culture-directed antibiotic therapy were added in cases of recurrent infection or uncontrolled symptoms. Patients were followed at 15 days, 1, 3, 6 and 12 months after surgery.

Each patient was evaluated about 15 days before surgery and during follow-up visits using a set of objective and subjective (self-assessed) measurements. Data obtained in the preoperative assessment and during the last follow-up visit (12 months) were collected for analysis.

Concerning the objective evaluation, the Lund-Kennedy (LK) [14] and the Lund-Mackay (LM) [15] scales were used. The evaluation between preoperative and postoperative LM

scores was not possible, because CT scan is not routinely performed after surgery unless required for particular clinical conditions.

For subjective evaluation, the Italian version of the Sino-Nasal Outcome Test-22 (I-SNOT-22) [16] was used. It is the most frequently employed in clinical practice because it is simple, intuitive and takes only a few minutes to complete [17]. It represents a questionnaire structurally composed of 22 CRS-related items scored from 0 to 5 (total score range 0-110, higher scores represent worse symptoms), which evaluates the severity of complaints that patients have been experiencing over the past weeks due to CRS [18]. SNOT-22 items can be divided into 2 categories: questions about physical symptoms (items 1-12) which cover rhinologic as well as ear and facial symptoms, and questions about health and QOL (items 13-22) which cover sleep function and psychological issues [19].

Similar to Rudmik [20], the cohort of patients was divided into 10 groups according to baseline SNOT-22 score. These groups were based on 10-point increments of the SNOT-22 score (patients who scored less than 10 were excluded since they had no chance to receive an MCID). The percentage of patients reaching at least an MCID, which in SNOT-22 is defined as a reduction of around 9 points after ESS [21], was estimated. The percentage of RI for each preoperative SNOT-22 group was then calculated with the formula [(mean postoperative SNOT-22 score - mean preoperative SNOT-22 score)/mean preoperative SNOT-22 score] x 100 [20].

Statistical analysis

Results are given as arithmetic mean ± standard deviation. The Kolmogorov Smirnoff test was used to test the normality of distribution. Parametric tests were used to evaluate differences between groups. In particular, ANOVA test with Tukey post-hoc test and Chi-square test were used when appropriate to compare groups. A multiple regression analysis was run to predict the SNOT-22 postoperative score from age, sex, smoking habit, asthma, allergy, aspirin intolerance, LK score, LM score, history of previous surgery for CRS and preoperative SNOT-22 score. A significance level of 0.05 for all testing was used. Statistical analyses were performed using the SPSS 25.0 package.

Results

A total of 457 CRS patients were consecutively enrolled. Among these, 34 patients were lost to follow-up. The remaining 423 patients attended the scheduled follow-up visits for 12 months and were considered eligible for analysis. The mean age of the cohort was 47.4 ± 13.5 years (range 18-86 years). 112 patients were asthmatic (26.5%) and 156 patients were allergic to common inhalants (36.9%), while 31 patients complained aspirin intolerance (7.3%). 225 patients were affected by CRS with nasal polyps (CRSwNP) (53.2%), while the remaining 198 (46.8%) were affected by CRS without nasal polyps (CRSsNP).

The mean preoperative SNOT-22 score was 48.9 ± 20.8 (range 13-106), and the mean preoperative SNOT 1-12 score was 30.8 ± 10.3 (range 9-56). The preoperative SNOT 1-12 score accounted for the total SNOT-22 for 67.4% (percentage of the SNOT-22 related to rhinologic symptoms). The mean preoperative LK score was 5.6 ± 2.8 (range 0-12), while the mean preoperative LM score was 11.5 ± 6.6 (range 0-24). The mean postoperative SNOT-22 score was 22.9 ± 17.9 (range 1-75), and the mean postoperative SNOT 1-12 score was 14.3 ± 9.5 (range 1-41). The postoperative SNOT 1-12 score accounted for the total SNOT-22 for 70.7%. The mean postoperative LK score was 1.7 ± 2.1 (range 0-10). These differences were significant at Student's t test ($p = 0.001$ for SNOT-22 score; $p = 0.001$ for SNOT 1-12 score; $p = 0.001$ for the percentage of SNOT-22 related to rhinologic symptoms, and $p = 0.001$ for LK score).

Based on baseline SNOT-22 score, 10 different groups of patients were defined. The sample sizes for each preoperative SNOT-22 group appeared to follow a normal distribution ($p = 0.132$ at Kolmogorov-Smirnoff test), with the largest groups composed of patients with baseline SNOT-22 scores between 20-69 (Fig. 1). Clinical characteristics, as well as preoperative subjective and objective scores, are depicted in Table I.

Postoperative SNOT-22 score was significantly improved in each of the 10 groups at paired Student's t test ($p = 0.001$ for all comparisons). 79.7% of the total cohort achieved a MCID improvement after ESS. Among the patients who achieved a MCID, the percentage of RI was 62.7%. When considering the total cohort (including also those who did not achieved a MCID) the percentage of RI was 50.1%. The MCID and the percentage of RI obtained from each of the 10 groups, as well as pre- and postoperative SNOT-22 scores are reported in Table II. A clear distinction of behaviour was observed between patients with baseline SNOT-22 score greater or less than 30. In particular, the mean percentage of achieving a MCID in groups 3-10 is 91.6% with an average 56.8% of RI. Contrarily, the mean percentage of achieving a MCID in groups 1-2 is 44.2% with an average of 38.9% of RI.

Significant differences in the number of patients achieving the MCID were demonstrated by the chi-square test ($p = 0.001$). In detail, patients in groups 1-2 achieved a

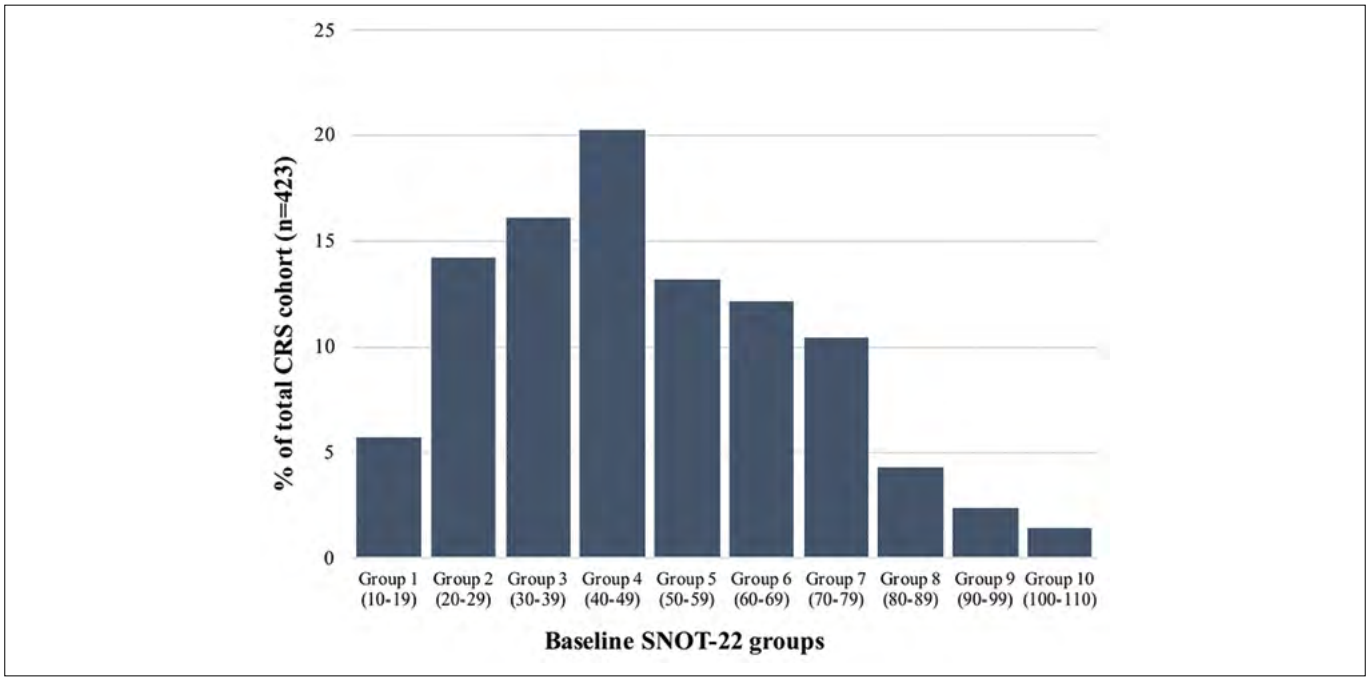

Figure 1. Distribution of the study population according to baseline SNOT-22 score.

Table I. Pre-treatment clinical features of the study population classified in 10 groups based on baseline SNOT-22 score.

	Group 1 (10-19)	Group 2 (20-29)	Group 3 (30-39)	Group 4 (40-49)	Group 5 (50-59)	Group 6 (60-69)	Group 7 (70-79)	Group 8 (80-89)	Group 9 (90-99)	Group 10 (100-110)
N (%)	24 (5.7)	60 (14.2)	68 (16.1)	86 (20.3)	56 (13.2)	51 (12.1)	44 (10.4)	18 (4.3)	10 (2.4)	6 (1.4)
Age	48.3 ± 16.6 (18-73)	49.1 ± 15.4 (18-71)	47.7 ± 16.5 (18-80)	50.4 ± 12.3 (21-86)	47.1 ± 12.7 (22-77)	46.2 ± 11.1 (27-67)	43.6 ± 10.9 (24-65)	42.1 ± 11.1 (24-61)	42.2 ± 6.6 (34-53)	47.3 ± 9.1 (41-59)
Sex (M:F)	16:8	52:26	44:24	54:32	28:28	33:18	22:22	8:10	2:8	2:4
Asthma, n (%)	10 (41.7%)	14 (23.3%)	18 (26.5%)	18 (20.9%)	18 (32.1%)	8 (15.7%)	14 (31.8%)	4 (22.2%)	8 (80%)	0 (0%)
Allergy, n (%)	8 (33.3%)	25 (41.7%)	22 (32.6%)	18 (20.9%)	26 (46.4%)	23 (45.1%)	20 (45.5%)	10 (55.6%)	4 (40%)	0 (0%)
Aspirin intolerance, n (%)	2 (8.3%)	4 (6.7%)	2 (2.9%)	8 (9.3%)	6 (10.7%)	5 (9.8%)	0 (0%)	4 (22.2%)	0 (0%)	0 (0%)
CRSwNP, n (%)	14 (58.3%)	33 (55%)	36 (52.9%)	36 (41.9%)	30 (53.6%)	26 (50.9%)	26 (59.1%)	12 (66.7%)	6 (60%)	6 (100%)
LK score	4.9	4.9	6	6.1	5.8	5.4	5.3	5.4	5.6	5.7
LM score	11.3	10.6	11.5	13.6	11.6	11.3	7.8	12.4	14.6	10.3

M: male; F: female; CRSwNP:chronic rhinosinusitis with nasal polyps; LK: Lund-Kennedy; LM: Lund-Mackay.

MCID with a significant less frequency than those in the other groups. Furthermore, the percentage of RI among the 10 groups was also significantly different at ANOVA test ($p = 0.002$) and patients in group 2 scored significantly lower than those in group 6, 8 and 10 ($p = 0.013$, $p = 0.022$ and $p = 0.039$, respectively, Tukey post-hoc test). Interestingly, the percentage of the SNOT-22 score related to nasal symptoms was significantly different among the 10 groups in both the pre- and post-treatment conditions ($p = 0.001$ and $p = 0.001$, respectively, ANOVA test). In particular, at baseline, the SNOT 1-12 score accounted for 88.5% of the SNOT-22 total score in group 1, while it accounted for the 51.9% in group 10. These differences were significant with Tukey's post-hoc test. In the post-treatment assessment, the SNOT 1-12 score ranged from 88.0% of the SNOT-22 total score in group 1 to 54.5% in group 9. These differences were significant by Tukey's post-hoc test.

Each of the 10 groups was further divided into two subgroups according to the presence of polyps. The results of SNOT-22 scores obtained before and after the surgery,

Table II. Probability of patients with CRS achieving MCID after ESS based on preoperative SNOT-22 score group.

	Preop. SNOT-22 score	% SNOT 1-12 over preop. SNOT-22	Postop. SNOT-22 score	% SNOT 1-12 over postop. SNOT-22	Probability of achieving MCID (%)	RI (%)
Group 1 (10-19) n = 24	16 ± 2.2	88.5%	8.7 ± 3.8	88.0%	33.3% (n = 8)	- 44%
Group 2 (20-29) n = 60	24.8 ± 2.9	82.1%	16.3 ± 12.2	75.6%	55% (n = 33)	- 33.8%
Group 3 (30-39) n = 68	34 ± 2.7	76.4%	17.5 ± 13.6	79.9%	82.4% (n = 56)	- 49%
Group 4 (40-49) n = 86	44.5 ± 3.1	67.2%	22.9 ± 16.8	66.8%	86.1% (n = 76)	- 48.9%
Group 5 (50-59) n = 56	54.2 ± 2.8	58.9%	25.9 ± 15.8	63.8%	85.7% (n = 48)	- 52%
Group 6 (60-69) n = 51	65.4 ± 3.2	57.5%	23.1 ± 19.2	69.9%	92.1% (n = 47)	- 64.6%
Group 7 (70-79) n = 44	73.9 ± 2.8	55.8%	35.6 ± 21.9	62.4%	86.3% (n = 38)	- 51.6%
Group 7 (80-89) n = 18	82 ± 2	53.7%	32.8 ± 21.4	68.6%	100% (n = 18)	- 60.2%
Group 9 (90-99) n = 10	94.6 ± 3.6	53.5%	43.8 ± 21.6	54.5%	100% (n = 10)	- 53.8%
Group 10 (100-110) n = 6	104 ± 2.4	51.9%	26.7 ± 14.5	69.4%	100% (n = 6)	- 74.3%
Total n = 423	48.9 ± 20.8	67.4%	22.9 ± 17.9	70.7%	79.7% (n = 338)	- 50.1%

MCID: minimal clinical important difference; RI: relative improvement.

as well as the probability of achieving a MCID and the percentage of RI are reported in Table III and Table IV. No differences between CRSwNP and CRSsNP patients in the postoperative SNOT-22 score ($p = 0.177$), the percentage of the SNOT-22 score related to rhinologic symptoms in the pre- ($p = 0.366$) and post-treatment ($p = 0.300$) conditions, and the percentage of the RI ($p = 0.162$) were demonstrated by Student's t test. Moreover, no difference in the probability of achieving a MCID was demonstrated at chi-square test ($p = 0.215$). On the contrary, a significant difference in the baseline SNOT-22 score was found with the Student's t test ($p = 0.010$). In particular, patients affected by CRSsNP scored significantly better than those affected by CRSwNP. A multiple regression analysis was run to predict the postoperative SNOT-22 score from gender, age, smoke, asthma, LK, LM, previous surgery, allergy, aspirin intolerance and preoperative SNOT-22 score. Some of these variables predicted the postoperative SNOT-22 score, $F(9, 423) = 6.423$, $p = 0.001$, $R2 = 0.298$. A history of previous surgery for CRS was the most important predictor ($B = 6.277$, $p = 0.009$). Other factors predicting ESS outcomes included the presence of asthma ($B = 5.286$, $p = 0.045$), preoperative LK score ($B = 0.937$, $p = 0.040$) and preoperative SNOT-22 score ($B = 0.326$, $p = 0.001$).

Discussion

Chronic rhinosinusitis affects a large portion of the world population leading to significant impairment of QoL [12]. Current studies report that about half of CRS patients remain symptomatic despite first-line pharmacological therapy [22]. Consequently, patients and physicians have to make a decision as whether to continue with medical therapy alone or undergo ESS followed by pharmacological therapy. On

Table III. Probability of patients with CRSwNP achieving MCID after ESS based on preoperative SNOT-22 score group.

	Preop. SNOT-22	Postop. SNOT-22	Probability of achieving MCID (%)	RI (%)
Group 1 (10-19) n = 14	16.9 ± 2.1	7.7 ± 4.5	42.9% (n = 6)	- 54.7%
Group 2 (20-29) n = 33	25.6 ± 2.7	16.9 ± 15.1	54.6% (n = 18)	-33.7%
Group 3 (30-39) n = 36	33.1 ± 2.5	17.1 ± 9.1	77.8% (n = 28)	- 48.1%
Group 4 (40-49) n = 36	44.5 ± 3.3	23.8 ± 18.1	88.9% (n = 32)	- 46.8%
Group 5 (50-59) n = 30	54.3 ± 2.7	27.4 ± 18.2	80% (n = 24)	- 48.9%
Group 6 (60-69) n = 26	64.3 ± 3.4	21.4 ± 19.2	92.3% (n = 24)	- 66.6%
Group 7 (70-79) n = 26	74 ± 2.9	36.6 ± 26.1	76.9% (n = 20)	- 50.1%
Group 7 (80-89) n = 12	81.5 ± 2.1	23.2 ± 16.2	100% (n = 12)	- 71.6%
Group 9 (90-99) n = 6	92 ± 3.8	39.7 ± 18.1	100% (n = 6)	- 56.8%
Group 10 (100-110) n = 6	104 ± 2.2	26.7 ± 14.5	100% (n = 6)	- 74.3%
Total n = 225	48.9 ± 20.7	22.9 ± 18.4	78.2% (n = 176)	- 50.9%

MCID: minimal clinical important difference; RI: relative improvement.

Table IV. Probability of patients with CRSsNP achieving MCID after ESS based on preoperative SNOT-22 score group.

	Preop. SNOT-22	Postop. SNOT-22	Probability of achieving MCID (%)	RI (%)
Group 1 (10-19) n = 10	14.8 ± 2.1	10.0 ± 1.9	25.0% (n = 2)	- 30.6%
Group 2 (20-29) n = 27	23.9 ± 2.7	15.4 ± 7.6	55.6% (n = 15)	- 33.8%
Group 3 (30-39) n = 32	35.1 ± 2.9	18.0 ± 17.5	87.5% (n = 28)	- 50%
Group 4 (40-49) n = 50	44.4 ± 3.1	22.2 ± 15.9	84.0% (n = 42)	- 50.3%
Group 5 (50-59) n = 26	54.2 ± 2.2	24.1 ± 12.7	92.3% (n = 24)	- 55.6%
Group 6 (60-69) n = 25	66.4 ± 2.5	24.9 ± 19.5	92.0% (n = 23)	- 62.5%
Group 7 (70-79) n = 18	73.8 ± 3.5	34.0 ± 14.9	100% (n = 18)	- 53.8%
Group 7 (80-89) n = 6	83 ± 3.1	52.0 ± 17.6	100% (n = 6)	- 37.4%
Group 9 (90-99) n = 4	98.5 ± 0.6	50.0 ± 27.7	100% (n = 4)	- 49.4%
Group 10 (100-110) n = 0	/	/	/	/
Total n = 198	47.6 ± 19.3	23.1 ± 17.3	82.8% (n = 162)	- 49.2%

MCID: minimal clinical important difference; RI: relative improvement.

one hand, Steele et al. showed that 57% of patients electing continued medical therapy failed to improve 1 MCID with a mean relative score improvement of 16%. Moreover, 1 in 5 patients experienced deterioration by > 1 MCID [23]. On the other hand, although surgical benefits are much more remarkable [1,8,9,24], the decision to face surgical cannot disregard evaluation of related risks and costs. To date, a tool that is able to identify patients who might benefit from surgery and the expected degree of improvement is still lacking. This is a natural consequence for not having a standardised staging system that drives treatment choices.

Many reports have investigated a number of factors that might influence the outcomes of CRS surgery. These include both patient-related factors (baseline SNOT-22, radiological extent of disease, presence of polyps, asthma or other comorbidities, gender, previous surgery) and surgical factors (experience of surgeon, timing of surgery, postoperative management) [25]. It seems from previous regression studies, and partly confirmed by our work, that baseline SNOT-22 is one of major factors affecting outcomes [10]. In this sense, the advantage of submitting CRS patients to SNOT-22 prior to any surgical treatment could, in theory, help physicians to inform them about their probable outcomes after ESS. For simplicity, explaining to a patient that he/she is likely to receive a 50% reduction in symptom load will aid informed consent and optimise preference-based decisions.

The fact is that, luckily, the majority of patients experience an improvement in symptoms after ESS, intended as a reduction of the SNOT-22 score after treatment ($p = 0.001$) [1,8,9,24]. We have shown that improvement of symptoms occurs in all groups and that the improvement is

directly proportional to the baseline SNOT-22 value. In other words, patients with worse preoperative symptomatology obtain the greatest range of score reduction after treatment. However, this statistical significance might not imply a clinical benefit. Indeed, the MCID has been proposed to combat this conceptual vice by defining a threshold value by which a statistically significant result may also offer a clinically meaningful result. The MCID is the lowest degree of change that a patient will notice, which for SNOT-22 score has previously been defined as 8.9 points in a 3-month postoperative score [18]. However, what represents a clinically important change may vary from one individual to another and may not necessarily reflect the patients' expectation for improvement after treatment. As an example, a patient reaching a MCID of 9 points in the postoperative SNOT-22 may not be satisfied with this outcome due to a persistent measurable burden of disease, despite achieving a noticeable improvement. To overcome the intrinsic limitations of MCID, a clinically significant change should be also outlined by a parameter expressing the true magnitude of postoperative improvement, i.e. the percentage of RI. Hence, integrating these measurements might optimise patient understanding and counselling. Rudmik et al. demonstrated that 80% of patients with a SNOT-22 score > 30 improved by an average of 48% following ESS [20]. Similarly, in our series, patients with SNOT-22 score >30 showed a 91.6% chance of achieving MCID with a mean 56.8% of RI. Also, a larger UK cohort showed a 66% chance of achieving a MCID with baseline SNOT-22 score > 30 [26]. On the other hand, patients with SNOT-22 < 30 have less than half the probability of achieving the MCID and a reduced degree of RI. That was evident in all the above-mentioned studies and confirmed in our series (44.2% mean MCID achievement, 38.9% mean RI). Therefore, although the baseline SNOT-22 score and chance of achieving the MCID is not intended to be used as an absolute threshold for eligibility for surgery, these global results suggest that a patient with low preoperative score might be less likely to benefit from surgery and caution should be paid when operating on patients with a score < 10. It is also true that only the categories of patients with lower baseline SNOT-22 values are likely to achieve a normal or near-normal status. Indeed, prior studies submitting SNOT-22 to patients with no sinus disease resulted in an average score of around 10 [16,18]; conversely, patients with higher baseline SNOT-22 values, despite a good RI, are still left with a significant burden of disease and remain more symptomatic than healthy controls. To be honest, SNOT-22 groups on either extreme of the scoring scale contained small sample size in all studies, which makes it difficult to provide accurate statistical results and introduce larger degrees of uncertainty around the means of these groups. Therefore, larger collaborative CRS databases should be developed to better define these categories of patients [26,27] and understand their behaviour.

Although our results are in line with the current literature, they should be interpreted with caution. First, though few in number, CRS patients with baseline SNOT-22 score < 10 were excluded from the analysis because of their near-normal status. Moreover, since all surgical procedures have been performed in the Day Surgery division, patients with severe comorbid asthma are not included in the study population. This choice obviously affected the overall mean values of percentage of MCID achievement and RI. Second, all surgeries were performed by specialist rhinologists, minimising the unfavourable outcomes due to surgical inexperience. Third, CRS is a dynamic disease characterised by fluctuating trends from quiescence to outbreaks. A one-off administration of a self-assessed questionnaire might not be enough reliable to assess the overall burden of the disease, especially considering a limited follow-up of 12 months.

In light of the above, two reflections arise. If we assume that a patient with a low baseline SNOT-22 score has a low probability of reaching the MCID and that a patient with a high baseline SNOT-22 score has a high probability of reaching the MCID, but not enough RI to become asymptomatic, either we are far from having an ideal treatment for CRS or SNOT-22 (in general QoL-based questionnaires) may not be a sufficiently effective tool to evaluate treatment outcomes. While, on the one hand, basic research efforts are aimed at discovering innovative targeted therapies [28], on the other, clinical practice efforts are focused on defining new comprehensive method of outcomes evaluation. In particular, the attempt is to incorporate subjective and objective parameters since symptom-based items are influenced by psychological habitus and show a wide inter-individual variability. Indeed, our data show that in groups with low baseline SNOT-22 almost all of the SNOT-22 score is given by rhinologic symptoms, while in groups with high baseline SNOT-22 score rhinologic symptoms account only for about 50% of the global SNOT-22 value, suggesting that psychological and social-functioning aspects significantly affect the SNOT-22 score. Furthermore, Hopkins et al. [18] demonstrated that when the sleep-psychological domain items dominate the total SNOT-22 score, ESS outcomes may be suboptimal. In fact, CRS patients that showed a moderately-severe total SNOT-22 score with high burden from sleep-psychosocial items may have less durable benefit after treatment, showing a statistically and clinically improvement at 3 months after ESS, followed by a worsening of symptoms at 6 months.

For this reason, these patients may be counselled to expect less benefit than those in whom nasal subdomain scores predominate [29].

In this context, Hopkins et al. obtained a long list of potential parameters revising the current literature. After intricate statistical analysis, the 54 initial items were distilled down to a final core set of 15 items, over 4 domains, including the SNOT-22 repeated over time with some additional questions and the Lund-Kennedy score [30]. This core outcome set (COS) represents the first "prototype" of an evaluation tool for CRS that is able to integrate subjective and objective parameters, but further work is still necessary to make it relevant for clinical practice. In this regard, a recent study highlighted a close correlation between symptoms and burden of inflammation. A cohort of CRSsNP patients undergoing ESS was clustered in 4 preoperative SNOT-22-based groups. These groups were significantly different with respect to primary versus revision ESS status, number of previous sinonasal surgeries, asthma prevalence and total SNOT-22 scores. More interestingly, the cluster of subjects with the highest total preoperative SNOT-22 score had the highest tissue eosinophilia compared to the other symptomatic groups and a more frequent diagnosis of asthma, suggesting that a high burden of inflammation correlates with worse symptomatology [29].

Conclusions

Submitting CRS patients to SNOT-22 prior to surgical treatments might help to inform about their probable outcomes, although it is strongly influenced by individual perception. Based on recent preliminary observations, the integration of SNOT-22 scores and tissue histopathology could represent an innovative method to predict treatment outcomes in CRS patients. Further studies are needed to define a simple and effective evaluation tool by implementing the knowledge of pathophysiological mechanisms underlying the different expressions of this disease. Eventually, this will lead to identify new histopathological-biomolecular pathways that are able to classify the CRS patients into homogeneous subgroups, to establish endotype-driven treatments and possibly provide objective predictors of response to therapy.

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OSAHS

Evaluation of neurocognitive abilities in children affected by obstructive sleep apnea syndrome before and after adenotonsillectomy

Valutazione delle abilità neurocognitive in bambini affetti da sindrome delle apnee ostruttive in sonno prima e dopo adenotonsillectomia

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SUMMARY

Obstructive sleep apnoea syndrome (OSAS) is the most severe form of sleep-related disordered breathing (SRDB) and is characterised by snoring, apnoeas, and/or hypopnoeas associated to hypoxia, hypercarbia, or repeated arousals from sleep. OSAS has three major categories of morbidities: neurobehavioural, cardiovascular and somatic growth failure. The gold standard for objective diagnosis of obstructive-SRDB severity is polysomnography (PSG). The indication for surgical treatment in children is moderate-severe OSAS (AHI, apnoea hypopnoea index > 5/h) and in mild OSAS (AHI 2-5/h) with complications or morbidity. The entire spectrum of PSG-defined SRDB (ranging from Primary Snoring to severe OSAS) may correlate with behavioural, attentional and executive function deficits relating to hypoxia and sleep disruption: in some cases, these alterations may mimic attention deficit hyperactivity disorder (ADHD). The aim of this research was to evaluate visuoperceptual and constructional abilities, paediatric sleep questionnaire and polysomnographic scores before and 6 months after adenotonsillectomy with objective and subjective information. We included 59 children who underwent neuropsychiatric and otolaryngologist clinical evaluation and the Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI); children parents were asked to fill in the Paediatric Sleep Questionnaire (PSQ); each child underwent PSG. At 6 months after adenotonsillectomy, all patients were evaluated again. There is a significant difference in PSQ parameters, VMI standard, visual tests scores and PSG parameters before and after adenotonsillectomy in children affected by OSAS. These results showed the achievement of therapeutic benefits with improvement of the quality of life for both children and their parents.

KEY WORDS: OSAS, adenotonsillectomy, pediatric OSAS, neuropsychological nonverbal skills

RIASSUNTO

La Sindrome delle Apnee Ostruttive del Sonno (OSAS, Obstructive Sleep Apnea Syndrome) è la forma più severa di Disturbo Respiratorio Sonno-correlato (SRDB, Sleep-related Disordered Breathing) ed è caratterizzata da russamento, apnee, e/o ipopnee associate a ipossia, ipercapnia, o ripetuti risvegli dal sonno (arousals). L'OSAS include tre categorie di morbidità: neurocomportamentali, cardiovascolari, disturbi della crescita somatica. Il gold standard per la diagnosi oggettiva di severità del SRDB ostruttivo è rappresentato dalla polisonnografia (PSG). L'indicazione al trattamento chirurgico nei bambini è la diagnosi di OSAS moderata-severa (indice di apnea-ipopnea > 5/h) e di OSAS lieve (indice di apnea-ipopnea 2-5/h) se sono presenti complicanze o comorbidità. Lo spettro intero di SRDB definiti mediante PSG (dal Russamento Primario all'OSAS severa) potrebbe correlare con deficit comportamentali, attentive e delle funzioni esecutive dovuti all'ipossia ed alla frammentazione del sonno: in alcuni casi, queste alterazioni possono mimare il Disturbo da deficit di Attenzione con Iperattività (ADHD, Attention Deficit Hyperactivity Disorder). Lo

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scopo di questa ricerca è stato quello di valutare le abilità visuopercettive e costruttive, i punteggi al Pediatric Sleep Questionnaire ed alla PSG prima e dopo l'intervento di adenotonsillectomia fornendo informazoni oggettive e soggettive. Abbiamo incluso in questo studio 59 bambini sottoposti a valutazione clinica otorinolaringoiatrica e neuropsichiatrica e poi al test di Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI) ed ai genitori veniva chiesto di compilare il Pediaric Sleep Questionnaire (PSQ); ogni bambino veniva sottoposto a PSG. Dopo 6 mesi dall'intervento di adeotonsillectomia sono state ripetute tutte le valutazioni come al tempo 0. L'analisi statistica evidenzia risultati significativi al PSQ, VMI standard e visivo ed ai parametri polisonnografici se confrontati prima e dopo l'intervento chirurgico di adenotonsillectomia nei bambini affetti da OSAS moderata-severa. Questi risultati hanno mostrato il raggiungimento dei vantaggi terapeutici attraverso il miglioramento della qualità della vita dei bambini e dei loro genitori.

PAROLE CHIAVE: *OSAS, adenotonsillectomia, OSAS pediatrica, abilità neuropsicologiche non verbali*

Introduction

Sleep-related disordered breathing (SRDB), pathologic nocturnal respiratory functioning, includes clinical conditions that range from primary snoring (PS) to obstructive sleep apnoea syndrome (OSAS) and represents one of the most common sleep disorders in childhood affecting up to one-third of children (prevalence up to 34.5%) [1,2].

OSAS is believed to be present in about 1% to 3% in children aged 2 to 18 with no gender predominance; furthermore, chronic snoring may be present in more than 10% of children [3-5]. PS and OSAS are the two extreme conditions of a wide spectrum of increased resistance in the upper airways [3-5].

PS is the mildest form of SRDB and is defined as habitual snoring without discrete respiratory events, gas exchange abnormalities, or evidence of sleep fragmentation; OSAS is the most severe form of SRDB and is characterised by snoring, apneoas, and/or hypopnoeas associated with hypoxia, hypercarbia, or repeated arousals from sleep [6].

OSAS has three major categories of morbidities: neurobehavioural, cardiovascular and somatic growth failure [7,8]. Symptoms in children with OSAS include snoring, breathing difficulty and/or breathing pauses during sleep, excessive sweating and enuresis; daytime symptoms may include oral breathing, headaches and excessive sleepiness, behavioural and neurocognitive changes such as attention deficit, hyperactivity, irritability, learning difficulties, memory loss and intelligence alterations [9-12]. In adults but less so in children, OSAS is associated with arterial systemic hypertension due to alterations in the renin-angiotensin axis secondary to hypoxia during sleep; left ventricular wall thickness; pulmonary vascular hypertension [13,14]. The gold standard for diagnosing OSAS is overnight polysomnography (PSG) which gives information about the frequency and severity of respiratory events and associated blood gas changes [15].

The European Respiratory Society (ERS) Task Force on diagnosis and management of obstructive SRDB in childhood (2 to 18 years old) analysed selected evidence from 362 articles since prospective cohort studies describing its clinical topics and randomised, double-blind, placebo-controlled trials, regarding its treatments, are scarce [16]. Kaditis et al. summarised the conclusions of the ERS Task Force indicating seven steps for diagnosis and management of SRDB in children (Tab. I) [16,17].

Some clinical conditions can be considered as factors predicting long-term persistence of obstructive SRDB: obesity and increasing BMI percentile, male sex, severity of OSAS (AHI > 5 episodes/h), African-American Ethnicity and persistent tonsillar hypertrophy with a narrow mandible [18,19].

The gold standard for objective diagnosis of obstructive-SRDB severity is PSG and is indicated in children candidate to adenotonsillectomy especially in the presence of obesity, craniofacial deformities, neuromuscular disorders, complex abnormalities such as Chiari malformation, Down syndrome and Prader-Willi syndrome or when the need for treatment is unclear [20,21]. Furthermore, PSG is indicated before and after rapid maxillary expansion or application of oral appliances, continuous positive airway pressure (CPAP) or noninvasive positive pressure ventilation (NPPV) treatments and when symptoms of OSAS persist after therapy [20].

The AHI, or the number of mixed, obstructive or central apnoeas and hypopnoeas per hour of total sleep time, is the most commonly used parameter to describe the severity of SRDB [22].

In children without SRDB symptoms or associated morbidities, the 90th percentile for the AHI according to the American Academy of Sleep Medicine (AASM 2007 scoring rules) is 3.2 episodes/h for the second year of life, up to 2.5 episodes/h for the ages > 2 and ≤ 6 years, and up to 2.1 episodes/h for the ages > 6 and < 18 years [23]. OSAS is defined when AHI ≥ 2 episodes/h or in presence of SRDB symptoms when AHI ≥ 1 episode/h: mild OSAS if AHI 2-5 episodes/h; moderate-severe OSAS if AHI > 5 episodes/h (Tab. II) [18,23].

Indications for treatment in children is moderate-severe OSAS (AHI > 5/h) or mild OSAS (AHI 2-3/h) only when morbidities are present [18]. The need for combined treatments (adenotonsillectomy when needed, weight loss, CPAP,

Table I. A stepwise approach to the diagnosis and management of obstructive SRDB in 2-18-year-old children (from Kaditis et al., 2016 [16]; Kaditis et al., 2012 [17]).

Step 1 **Child at risk of SDB if (one or more)**

1.1. Symptoms of upper airway obstruction (snoring, apnoea, restless sleep, oral breathing)

1.2. Finding on exam (tonsillar hypertrophy, obesity, midface deficiency, mandibular hypoplasia, neuromuscular disorders, Down syndrome, Prader-Willi Syndrome)

1.3. Objective findings related to SDB (lateral neck radiography, flexible nasopharyngoscopy, cephalometry, upper airway MRI or CT)

1.4. Prematurity or family history of SDB

Step 2 **Recognition of morbidity and conditions coexisting with SRDB**

2.1. Morbidity

- Cardiovascular system:
  - elevated blood pressure
  - pulmonary hypertension and cor pulmonale
- Central nervous system:
  - excessive daytime sleepiness
  - inattention/hyperactivity
  - cognitive deficits/academic difficulties
  - behavioural problems
  - enuresis and somatic growth delay or growth failure
  - decreased quality of life
- Conditions coexisting with SRDB (probably common pathogenesis):
  - history of recurrent otitis media or tympanostomy tube placement
  - recurrent wheezing or asthma
  - metabolic syndrome
  - oral-motor dysfunction

Step 3 **Recognition factors predicting long-term persistence of SDB**

3.1.

- Obesity and increasing BMI percentile
- Male sex
- Obstructive AHI > 5 episodes/h
- African-American ethnicity
- Untreated tonsillar hypertrophy, narrow mandible

Step 4 **Objective diagnosis and assessment of SDB severity**

4.1. PSG or polygraphy if child at risk for SDB (step 1 and 2)

4.2.

- OSAS-definition 1: SDB symptoms in combination with obstructive AHI ≥ 2 episodes/h or obstructive apnea index ≥ 1 episodes/h
- OSAS-definition 2: SDB symptoms and AHI ≥ 1 episodes/h (including central events)

4.3. If AHI ≥ 5 episodes/h SDB unlikely to resolve spontaneously and child at risk for morbidity

4.4. if PSG or polygraphy not available: ambulatory PSG or polygraphy, nocturnal oximetry, Paediatric Sleep Questionaire or Sleep Clinical Record

Step 5 **Indication for treatment of SDB**

5.1.

- AHI > 5 episodes/h irrespective of the presence of morbidity
- Treatment may be beneficial if AHI 1-5 episodes/h especially in the presence of morbidity from the cardiovascular system (see 2.1.); from the central nervous system (see 2.1.); enuresis; somatic growth delay or growth failure; decreased quality of life; risk factors for SDB persistence (see step 3)
- If at risk for SDB and PSG or polygraphy not available, treatment is considered when positive oximetry or SDB questionnaire (see 4.4.) or morbidity present

5.2. Unclear whether should treat primary snoring (evaluation annually)

5.3. OSAS treatment is a priority in the presence of: major craniofacial abnormalities; neuromuscular disorders; achondroplasia; Chiari malformation; Down syndrome; mucopolysaccharidoses; Prader-Willi syndrome

Step 6 **Stepwise treatment approach to SDB**

6.1. A stepwise treatment approach (from 6.2. to 6.9.) is usually implemented until complete resolution of SDB

6.2. Weight loss if the child is overweight or obese

6.3. Nasal corticosteroids and/or montelukast

6.4. Adenotonsillectomy

6.5. Unclear whether adenoidectomy or tonsillectomy alone are adequate

6.6. Rapid maxillary expansion and orthodontic appliances

6.7. CPAP or NPPV (for nocturnal hypoventilation)

6.8. Craniofacial surgery

6.9. Tracheostomy

Continues

Table I. *Follows.*

Step 7	**Recognition and management of persistent SDB**
	7.1.
	• Outcomes monitored after intervention (6 weeks - 12 months): symptom, PSG, quality of life, cardiovascular or central nervous system morbidity, enuresis, growth rate
	• If PSG not available: polygraphy, oxymetry/captography
	• PSG after 6 weeks after adenotonsillectomy (persistent SDB symptom or at risk of persistent OSAS preoperatively); after 12 weeks of montelukast/nasal steroids
	• PSG after 12 months of rapid maxillary expansion (earlier if symptoms persist) and after 6 months with an oral appliance
	• PSG for titration of CPAP, NPPV and then annually; PSG as predictor of successful decannulation with tracheostomy
	• Airway re-evaluation by nasopharyngoscopy, drug-induced sleep endoscopy, MRI

MRI: magnetic resonance imaging; CT: computed tomography; BMI: body mass index; AHI: apnoea-hypopnoea index; PSG: polysomnography; OSAS: obstructive sleep apnoea syndrome; CPAP: continuous positive airway pressure; NPPV: noninvasive positive pressure ventilation.

Table II. Definition of OSAS from AHI parameter (from Marcus et al., 2013 [18]; Iber et al., 2007 [23]).

Normal values or OSAS definition	AHI (episodes/h)
No SRDB symptoms or morbidity	3.2 AHI (2 years old) (90th percentile) 2.5 AHI (2-6 years old) (90th percentile) 2.1 AHI (6-18 years old) (90th percentile)
SRDB symptoms and/or morbidity	
Mild OSAS	2-5 AHI
Moderate-severe OSAS	> 5 AHI

NPPV) is a priority in these clinical conditions: major craniofacial abnormalities, neuromuscular disorders (i.e. Duchenne muscular dystrophy), achondroplasia, Chiari malformation, Down syndrome, mucopolysaccharidoses, Prader-Willi syndrome [24-26].

The entire spectrum of PSG-defined SRDB (ranging from PS to severe OSAS) may correlate with behavioural, attentional and executive function deficits relating to hypoxia and sleep disruption [27].

It is important to emphasise the risk of greater impairment of both behavioural and cognitive functions in children with milder forms of SRDB so that PS is not a universally benign condition [28]. Preschool children with obstructive SRDB are more vulnerable in their adaptative and behavioural function vs. cognitive function: this age has a sort of '*window of opportunity*' for early treatment by preventing cognitive deficits arising later in childhood [28].

Some authors analysed neurocognitive function in children with OSAS after CPAP-treatment (5-6 months): significant improvements in their abilities to analyse and synthesise abstract information, multitasking, simultaneous processing, divided attention and in recognition and recall for visual information were all shown [29].

Sleep disruption, even without respiratory compromise, may induce neurocognitive alterations, and for this reason the severity of SRDB, from PS to OSAS, does not correlate with neurocognitive deficits [30].

Cognitive functions are correlated with sleep fragmentation and thus to SRDB: arousals seem to be an important defensive mechanism against sleep fragmentations induced by SRDB [31]. Children with high levels of arousal seem to present a high degree of protection against cognitive consequences of SRDB [32].

The aim of the present study is to analyse changes in neurocognitive and behavioural functions in children 4 to 11 years old affected by moderate-severe OSAS, after surgical treatment with adenotonsillectomy to investigate the role of obstruction in SRDB symptoms.

We evaluated visuoperceptual and constructional abilities, PSQ and full overnight polysomnographic values, before and 6 months after adenotonsillectomy, giving objective and subjective information about sleep disturbances in OSAS.

Materials and methods

We enrolled 86 children with obstructive SRBD, aged between 4 to 11 years old, referred by primary care paediatricians for snoring and suspected apnoeas to the Clinic of Child and Adolescent Neuropsichiatry and then to the Ear Nose and Throat Unit of the University of Campania "Luigi Vanvitelli" between October 2015 and April 2018.

Each child underwent neuropsychiatric and otolaryngologic clinical evaluation and VMI test, and parents were asked to fill in the PSQ.

Three overnight full sleep PSG were performed, and obstructive AHI were identified as normal, mild, moderate, or severe OSAS.

Inclusion criteria were: history of habitual snoring and/or apnea; frequent and continuous breathing pauses referred by parents; nocturnal difficult breathing; daytime hyperactivity; attentive deficit, poor school performance. Moreover, children with moderate (respiratory disturbance index of 5-10 episodes/h with average SaO_2 > 95%) or severe (respiratory disturbance index >10 episodes/h with an average < 95% SaO_2) OSAS were included; tonsillar and adenoidal grading 3-4, clinical febrile episodes (FE) and frequent acute pharyngotonsillitis[2]; age between 4 and 11 years.

Children were excluded if they had any of the following: sensorineural hearing loss; tube-tympanic alterations (OME

otitis media with effusion, acute otitis media recurrent AOM); nasal obstruction due to nasal septal deviation or hypertrophy of inferior and middle turbinates, obesity (BMI ≥ 90th percentile); cardiac and pulmonary metabolic disorders; craniofacial anomalies; neuromuscular disorders and genetic syndromes.

During the first step of enrollment, we excluded 27 children:

- 15 did not meet inclusion criteria (4 obese children, BMI ≥ 90th percentile; 2 affected by otitis media with effusions associated to conductive hearing loss; 1 affected by craniofacial anirmalities, Pierre Robin Sequence and 8 children affected by mild OSA (AHI < 2);
- 10 children declined to participate;
- 2 children for other reasons.

At the end of this evaluation, we included 59 children affected by moderate-severe OSA (AHI > 5) with adenotonsillar hypertrophy.

The second step of treatment included adenotonsillectomy performed in the Ear Nose and Throat Unit of University of Campania "Luigi Vanvitelli".

The third step included follow-up at 6 months after surgery: children underwent sleep PSG, VMI test and their parents filled in the PSQ. During this step, 22 patients refused to continue.

The last step of the analysis of data included 37 children mean age 8.44 ± 2.26 (Fig. 1).

Paediatric Sleep Questionnaire

PSQ is a SRBD scale questionnaire for children parents containing 22 items, indicating the presence of apnoeas. These items regard frequency, loud snoring, observed apnoeas, alterations in breathing during sleep, daytime symptoms and signs such as sleepiness, inattentive and hyperactive behaviour. Responses are "yes" (= 1 score), "no" (= 0 score), and "do not know" (= missing). A cut-off value of 0.33, which would be most effective in identifying pediatric OSA, was used.

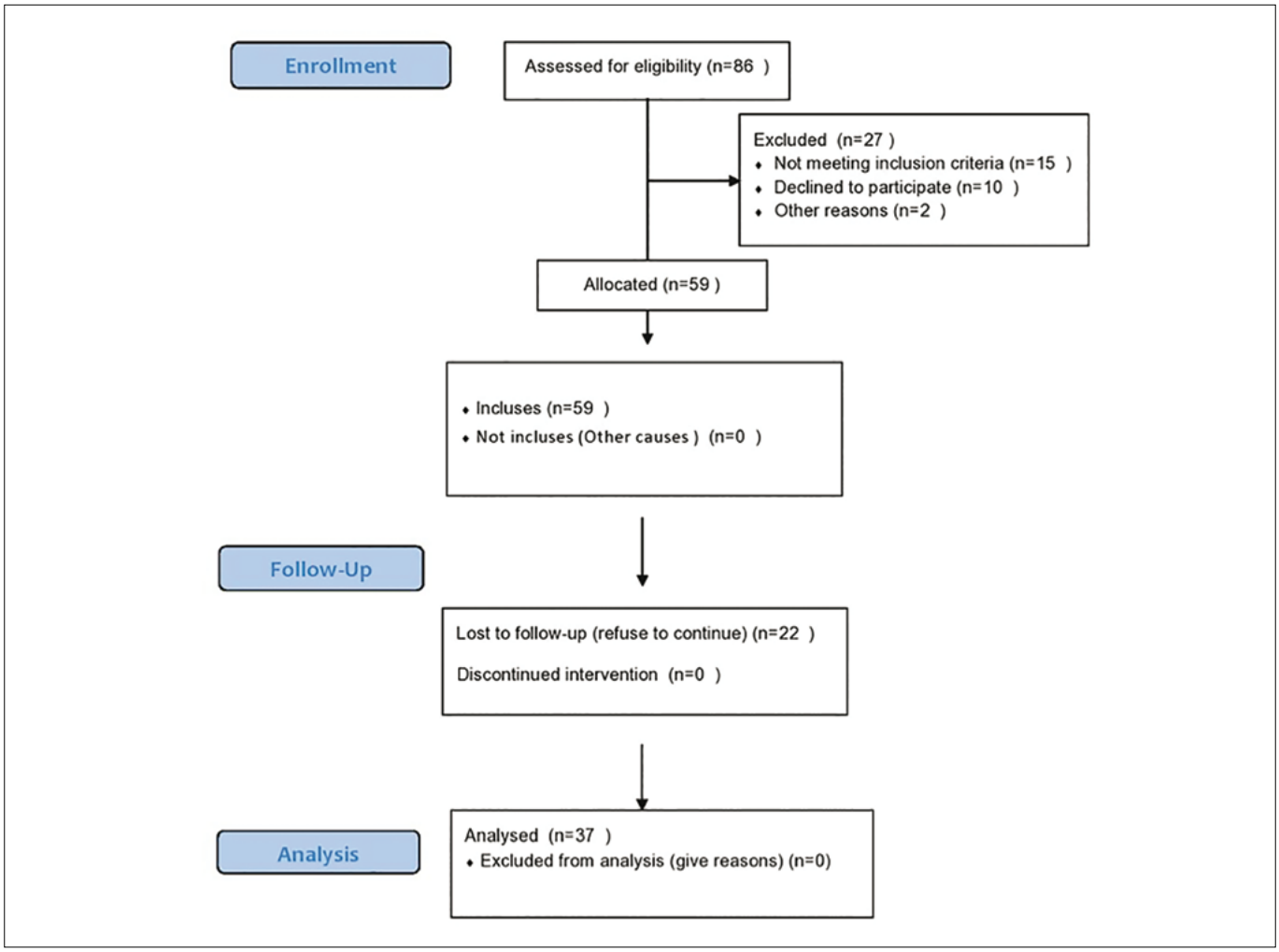

Figure 1. Enrollment and study flow.

Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI)

The Beery-Buktenica Developmental Test of Visual-Motor Integration Performances in Children (VMI task) is a paper-and-pencil test in which children have to imitate or copy up to 27 geometric forms with increasing complexity using paper and pencil [32]. The test was stopped when a child made more than two errors in a row [32]. Copying errors were marked if they reflected problems in fine motor coordination and a pure visuospatial problem. The Beery VMI task is specifically designed for children and takes about 10 minutes to complete [32]. The Beery VMI scores were standardised for age and gender using normative data for the Italian general population [32].

The percentile scores were used for diagnosing the visuomotor abnormalities in our sample. A value less than or equal to the 5th percentile was considered to indicate VMI impairment.

Overnight full polysomnography recordings (PSGs)

The polysomnographic recording was performed using a videorecorder connected to probes, electrodes and bands suitable to the patients' age and weight. The exam is computerised and uses the Embletta System, a system recognised and appointed by the American Academy of Sleep Medicine for the study of sleep apnoea. The recording time is 6-8 hours, and supervised by medical and technical staff, who interpreted the data collected. We analysed sleep and respiratory parameters:

- Sleep Latency, SL (min);
- REM Latency, RL (min);
- Total Sleep Time, TST (min);
- Sleep Period Time, SPT or TIB-SL (min);
- Wake After Sleep Onset, WASO (min);
- 1-2-3 NREM, N1-N2-N3 (%TST);
- REM (%TST);
- Sleep Efficiency, SE or TST/TIB% (%);
- Arousal Index, AI (n Arousal/h sleep);
- Periodic Limb Movement Index, PLMI (n PLM/ h Sleep);
- AHI (number of AH/h sleep)
- Nadir SaO_2 during sleep;
- Nadir SaO_2 mean during sleep;
- Oxygen Desaturation Index, ODI (n desaturation/h sleep);
- TST % with $SaO_2 < 90\%$;
- TST % with $SaO_2 < 80\%$.

ENT evaluation

Each child underwent ear, nose and throat evaluation characterised by otoscopy, anterior rhinoscopy and oropharyngoscopy with particular attention to febrile episodes per year in the last 3 years. We evaluated haemochrome, antistreptolysin test, erythrocyte sedimentation rate, and creatine phosphokinase for all children. These clinical parameters are important to understand the indication for surgical treatment in children with adeno-tonsillar hypertrophy.

The tonsils were subjectively measured using a grading system. In grade I, the tonsils were hidden in the tonsillar fossa and were barely visible behind the anterior pillars. In grade II, the tonsils were visible behind the anterior pillars and occupied up to 50% of the pharyngeal space (the distance between the medial borders of the anterior pillars). In grade III, the tonsils occupied between 50 and 75% of the pharyngeal space. In grade IV, the tonsils occupied more than 75% of the pharyngeal space.

Adenoids were analysed during rhinofiberoptic evaluation: a grading system for adenoid hypertrophy was created based on the anatomical relationships between the adenoid tissue and the vomer, soft palate, and torus tubaris. The grading is based on the relationship of the adenoids to adjacent structures when the patient is at rest (i.e., when the soft palate is not elevated).

Adenotonsillectomy

Patients underwent general anaesthesia in oral intubation maintaining supine in the Rose position. Adenoidectomy was performed followed by extracapsular tonsillectomy using cold/hot technique for dissection and cold/hot for homeostasis [33,34].

This study was conducted according to the World Medical Association Declaration of Helsinki and was retrospectively registered with number 28/2018.

Statistical analysis

Nonparametric analyses using the Wilcoxon Test were used to evaluate the effects of treatment (pre- and post-adenotonsillectomy) in the variables examined: PSQ test scores, VMI test standard scores (VMI, test motor and visual test), PSGs parameters (TIB, SPT, TST, SOL, FRL, SS-h, AWN-h, SE%, WASO-min, N1-min, N2-min, N3-min, REM-min, WASO spt, N1-spt, N2-spt, N3-spt, REM spt, N1-tst, N2-tst, N3-tst, REM-tst, PSQ, AHI, ODI, OD%, PLMI).

Pearson's correlation was used to assess the mean preoperative and postoperative VMI scores with preoperative and postoperative polysomnographic parameters. The threshold for statistical significance was $p < 0.05$. All statistical analyses were performed with a statistical software package (STATISTICA 8.0, StatSoft Inc.)

Results

The data collected before and 6 months after adenotonsillectomy are indicated in Tables III and IV. Table III shows the results of PSQ and VMI tests. There was a significant difference for PSQ parameters, VMI standard and VMI visual test ($p < 0.05$), but not for the VMI motor test.

In Table IV PSG parameters before and after adenotonsillectomy are shown. There was a significant difference for macrostructural sleep and respiratory parameters TIB, SPT, TST, SOL, SS-h, AWN-h, SE%, WASO-min, N1-min, N2-min, N3-min, REM-min, WASO spt, N1-spt, N2-spt, N3-spt, N1-tst, N2-tst, N3-tst, PSQ, AHI, ODI, OD%, PLMI ($p < 0.05$).

Table III. Comparison of PSQ and VMI scores before and after adenotonsillectomy.

	Pre	Post	Wilcoxon Test		
	Mean (DS)	Mean (DS)	U	Z	p
PSQ	0.37 (0.15)	0.15 (0.07)	0.00	5.011926	0.000001
VMI st	106.42 (17.84)	111.24 (13.32)	61.50000	4.375023	0.000012
VMI motor test st	103.87 (25.39)	114.35 (17.67)	60.00000	1.679970	0.092964
VMI visual test st	97.78 (15.82)	107.43 (14.61)	24.00000	4.854568	0.000001

Table IV. Comparison of PSG parameters before and after adenotonsillectomy.

	Pre		Post		Wilcoxon Test		
	Mean	DS	Mean	DS	U	Z	p
TIB-min	386.4057	83.37142	589.1892	86.07090	0.00	5.302829	0.000000
SPT-min	342.3486	64.93097	555.4730	75.34052	0.00	5.302829	0.000000
TST-min	269.0622	56.76741	529.8784	70.22715	0.00	5.302829	0.000000
SOL-min	44.0570	43.74958	24.6757	18.74656	211.0000	2.119623	0.034039
FRL-min	104.2919	31.16452	130.0405	54.27404	220.0000	1.983847	0.047274
SS-h	9.7838	2.59235	7.5243	3.41121	162.0000	2.858851	0.004252
AWN-h	8.7324	2.60502	1.6486	1.86467	0.00	5.302829	0.000000
SE%	70.3054	9.19560	90.2514	5.60063	3.000000	5.257571	0.000000
WASO-min	73.2865	23.48791	25.5946	26.47479	23.50000	4.862423	0.000001
N1-min	57.1135	23.98776	17.3514	21.95572	46.00000	4.608860	0.000004
N2-min	88.5378	33.91517	231.5946	46.38110	0.00	5.302829	0.000000
N3-min	57.7568	25.12663	162.1622	69.83563	1.000000	5.287743	0.000000
REM-min	65.6541	23.08347	118.6892	32.43141	19.00000	5.016190	0.000001
WASO-spt	21.5716	6.21865	4.4541	4.44607	2.000000	5.272657	0.000000
N1-spt	16.7676	6.79174	3.1730	3.93211	9.000000	5.167053	0.000000
N2-spt	25.6962	8.50912	42.1000	7.81466	12.00000	5.121794	0.000000
N3-spt	16.8414	6.54840	28.6946	9.68389	31.00000	4.835155	0.000001
REM-spt	19.1227	6.26117	21.5595	5.89545	245.0000	1.606689	0.108124
N1-tst	21.7743	9.65647	3.4189	4.38209	8.000000	5.182139	0.000000
N2-tst	32.3411	9.17601	44.0432	7.74441	63.00000	4.352393	0.000013
N3-tst	21.4795	8.15687	30.0432	10.01681	106.0000	3.703683	0.000213
REM-tst	24.4041	7.89300	22.5108	5.83423	284.0000	1.018324	0.308525
AHI	10.3378	3.01786	5.9216	1.30110	11.00000	5.136880	0.000000
ODI	5.8270	1.81974	3.5027	0.55752	12.00000	5.043095	0.000000
Mean OD%	94.4865	1.97120	96.8000	0.73749	7.000000	5.197225	0.000000
Lowest OD%	89.3405	2.96287	94.3568	0.93290	0.00	5.231621	0.000000
Average OD%	5.1459	2.34338	2.4432	0.73543	5.000000	5.227398	0.000000
PLMI	6.5062	2.31283	3.1746	1.14721	29.00000	4.865327	0.000001

Clinical evaluation at PSQ six months after adenotonsillectomy showed a reduction in values compared with those before surgery (Fig. 2).

VMI standard scores, VMI motor test and VMI visual test scores showed improvements in performance at 6 months after surgery, which was significant for standard and visual scores (Figs. 3, 4) and not significant for motor scores (Fig. 5).

Spearman rank order correlation between preoperative and postoperative VMI scores and preoperative and postoperative PSG parameters was statistically significant $p < 0.05$ (Tab. V): there was no linear correlation. There was a significant difference for PSQ parameters, VMI standard, visual tests scores and PSG parameters, but not for motor test scores before and after adenotonsillectomy in children affected by OSAS. When comparting VMI standard, motor and visual tests score with PSG parameters, the difference before and after surgery was statistically significant.

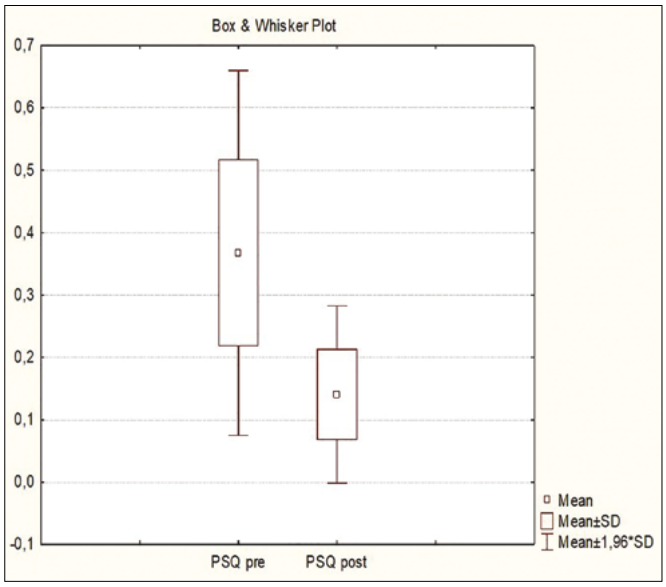

Figure 2. Comparison of PSQ scores before and six months after adenotonsillectomy.

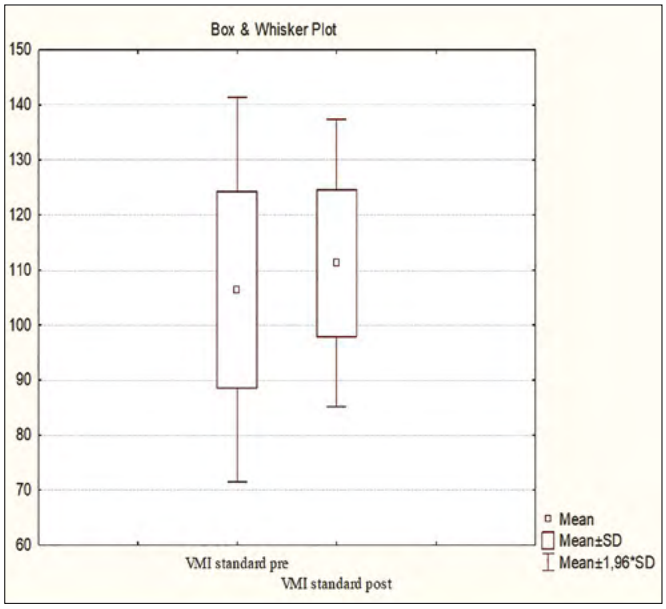

Figure 3. Comparison of VMI standard scores before and six months after adenotonsillectomy (statistically significant).

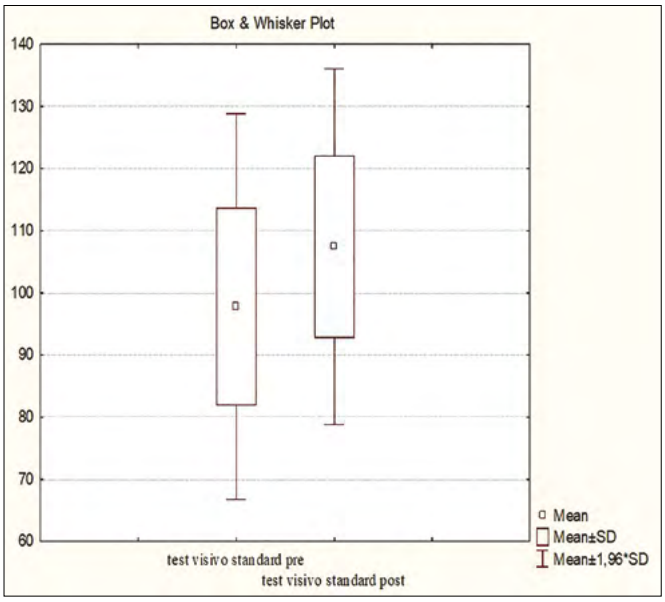

Figure 4. Comparison of VMI visual test scores before and six months after adenotonsillectomy (statistically significant).

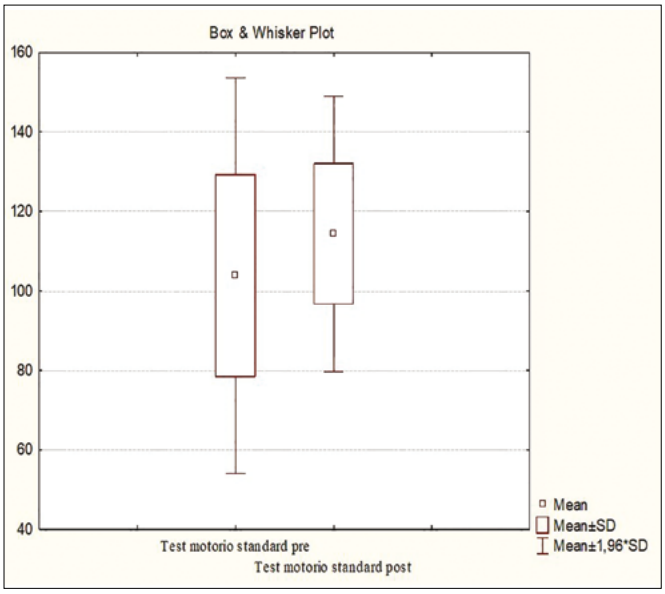

Figure 5. Comparison of VMI motor test scores before and six months after adenotonsillectomy (not statistically significant).

Table V. Correlation between VMI scores and PSG parameters before and 6 months after adenotonsillectomy.

	Spearman Rank Order Correlations MD pairwise deleted Marked correlations are significant at p < 0.05000		
	VMI st	VMI motor test st	VMI visual test st
TIB-min	0.144274	-0.097321	-0.116585
SPT-min	0.211697	-0.059812	-0.108225
TST-min	0.167254	-0.058115	-0.099394
SOL-min	-0.132756	-0.016344	-0.042546
FRL-min	-0.068619	-0.072612	0.044218
SS-h	0.097221	-0.171699	-0.155499
AWN-h	0.110438	-0.014303	-0.214789
SE%	0.063701	0.049596	0.152116
WASO-min	0.132909	-0.046434	-0.173408
N1-min	-0.018648	0.093144	-0.037976
N2-min	0.099488	0.025531	0.027575
N3-min	0.197135	-0.080507	-0.121304
REM-min	-0.039059	-0.070151	0.111569
WASO-spt	0.023792	-0.038759	-0.101054
N1-spt	-0.053335	0.119138	0.011890
N2-spt	-0.035937	0.064147	0.116147
N3-spt	0.170595	-0.085442	-0.132817
REM-spt	-0.203929	-0.072725	0.220432
N1-tst	-0.055982	0.096213	0.001882
N2-tst	-0.040447	0.066204	0.088461
N3-tst	0.194365	-0.082518	-0.158518
REM-tst	-0.173607	-0.081733	0.147272
PSQ	-0.121546	0.035007	-0.021625
AHI	-0.084110	0.144959	0.085684
ODI	-0.011723	0.134956	-0.076610
Mean OD%	-0.006353	0.062628	-0.027591
Lowest OD%	0.012973	-0.005733	0.034711
Average OD%	-0.019340	0.040488	-0.044393
PLMI	0.026528	-0.054086	-0.021249

Discussion

Diagnosis and treatment of suspected mechanical OSAS in children represent a highly controversial issue for the differences in terms of diagnostic resources and therapeutic approach. Only careful evaluation of medical history and clinical presentation together with polysomnography, can provide the right diagnostic classification and, consequently, the correct choice of treatment [35-38]. The resolution of mechanical obstruction must be as fast as possible in order to reduce the risk of cardiac, metabolic and neurological complications [7,8]. Our attention is focused on the close relationship between OSAS and neurocognitive and neurobehavioral disorders in very young children with a mean age of 8.44 ± 2.26. The set of symptoms referred by patients or by children's parents together with respiratory distress is often characterised by attention deficit and hyperactivity, irritability and learning disorders [9-12]. Recent studies have shown a clear relationship of cause and effect between nocturnal hypoxia leading to respiratory events (obstructive apnoeas and hypopnoeas), alteration of the structure of sleep with fragmentation and arousals and neurocognitive deficit [9-12]. In particular, it has been established that in OSAS patients the brain area mostly involved in the hypo-anoxic stress is the prefrontal cortex [9,38]. It presents a considerable reduction in activity in all stages of sleep and seems to be disconnected from other cortex areas [9,37]. Many authors have shown considerable improvement after adenotonsillectomy in children's cognitive performance (memory, learning, IQ) and in quality of life (reduced daytime sleepiness, irritability and mood alteration) [15,38]. These authors have used: Differential Ability Scales (similar to I.Q.), Non Verbal Cluster [36]; Behavioral Assessment System for Children [39] (BASC), to evaluate the behaviour (mood, hyperactivity and somatisation); Epworth sleepiness Stairs and Osler Test (to evaluate semantic, episodic and work memory) [15]; the Standfort Binet Intelligence Scale 5th edition and the Developmental Neuropsychological Assessment (NEPSY) for neuropsychological skills [40].

VMI test has never been performed to evaluate visual-motor performances in children with OSAS.

The Beery-Buktenuica Developmental Test of Visual-Motor Integration (VMI; Beery & Beery, 2004) was developed to assess visuoperceptual and constructional abilities in children and adolescents and is among the most widely administered neuropsychological tests [32,41,42]. This test has been used to evaluate children with traumatic brain injury and attention-deficit/hyperactivity disorder (ADHD) [42].

We evaluated children with OSAS using VMI standard scores, motor and visual test scores, PSQ and PSG parameters before and at 6 months after adenotonsillectomy. Visuoperceptual and constructional performances were improved in all children after surgical treatment; analysis of sleep quality (respiratory parameters and neurological ones) showed better sleep macrostructural architecture and better respiratory scores after adenotonsillectomy than before.

The results showed the achievement of therapeutic benefits. Six months after the mechanical removal of the obstruction there was an improvement of cognitive performance and quality of life.

Conclusions

Dealing with a subject so controversial for the lack of homogeneity in terms of diagnostic and therapeutic

resources, such as OSAS in children, we chose a multidisciplinary approach. Nowadays there are no studies in the literature specifically correlating mechanical OSAS and visual-spatial skills. We first assessed the deficiencies and then the improvements in visual-spatial skills in children affected by OSAS before and after adenotonsillectomy. Our idea has been confirmed by the results obtained from the 6 month postoperative VMI tests. At any starting level, all children showed improvement in performance. Thus, at 6 months after adenotonsillectomy important therapeutic benefits have been demonstrated both in visual-motor performance and in the child's quality of life according to the objective data of PSG.

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OSAHS

Risk factors for otitis media with effusion in children with adenoid hypertrophy

Fattori di rischio per l'otite media effusiva nei bambini con ipertrofia adenoidea

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SUMMARY

The aim of this study was to determine the most important risk factors in the development of otitis media with effusion (OME) in children with adenoid hypertrophy. A total of 539 patients undergoing surgery for adenoid hypertrophy (AH Group) (n = 429) or adenoid hypertrophy and otitis media with effusion (AH + OME Group) (n = 110) between February 2012 and February 2018 constituted the study group. Data were obtained on neonatal history (breastfeeding, bottle feeding), past health and medical history (presence of atopy or allergic rhinitis, snoring at night, cough, tonsillitis in the past 12 months), environmental factors (presence of pets, attending to daycare centers, district of school), family history (passive smoking at home, number of siblings, family size, parental education), and family income. The groups did not differ from each other for age ($p = 0.684$) and gender ($p = 0.728$). Our data support the presence of atopy or allergic rhinitis ($p < 0.001$), frequent (> 5) tonsillitis ($p < 0.001$), attending to daycare centers ($p < 0.001$), exposure to smoke ($p < 0.001$), having 3 or more siblings ($p < 0.001$), and 4 or more people in the household ($p < 0.001$) as the main risk factors for OME. Comprehensive knowledge of modifiable risk factors found in this study could help to minimise the complications of OME in children.

KEY WORDS: otitis media with effusion, predictors, risk factors

RIASSUNTO

Lo scopo di questo studio è determinare i fattori di rischio più importanti per l'otite media effusiva (OME) nei bambini con ipertrofia adenoidea. 539 pazienti sono stati sottoposti a chirurgia per ipertrofia adenoidea o ipertrofia adenoidea + OME, fra febbraio 2012 e febbraio 2018. Sono stati valutati i dati circa la storia clinica neonatale e l'anamnesi remota, fattori ambientali, anamnesi familiare e lo status economico familiare. I gruppi sono risultati omogenei per età e genere. Dai nostri dati si evince che i fattori di rischio per l'OME sono la presenza di rinite allergica/atopica, tonsilliti ricorrenti, frequentazione dell'asilo nido, esposizione al fumo passivo, la presenza di 3 o più fratelli, la presenza di 4 o più persone conviventi. La correzione di tali fattori di rischio modificabili potrebbe contribuire a diminuire le complicanze dell'OME nei bambini.

PAROLE CHIAVE: *otite media effusiva, fattori di rischio, fattori predittivi*

Introduction

Otitis media with effusion (OME) is defined as the accumulation of fluid behind the intact tympanic membrane without signs and symptoms of acute ear infection [1]. Fluid in the middle ear affect the function of the tympanic membrane and middle ear and leads to conductive hearing loss, feeling of fullness in the ear and pain due to alterations in pressure [2]. OME is a prevalent disease that is encountered in childhood and may often be overlooked because its symptoms are not always severe. It is the most frequent cause of acquired hearing loss in preschool children [2]. Hearing loss that develops in early childhood may cause irreversible sequelae that

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Conflict of interest
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may be lifelong, affecting the child's speech, language and social relations. In one study, it was found that 90% of children had at least one episode up to the age of 10 [3]. Ninety percent of cases with otitis media with effusion spontaneously resolve within three months [3]. Between 5% and 10% of attacks may last more than one year. Thirty to 40% of children experience recurrent episodes [4].

Numerous research has been performed to reveal the aetiology of OME [4]. Respiratory tract infections, adenoid hypertrophy, craniofacial malformations, mechanical obstruction of the nasopharynx, allergy and immunological factors have been suggested as the major aetiological factors in the pathogenesis of otitis media with effusion [4]. As additional factors in OME, race, gender, climatic conditions, environment, humidity, socioeconomic status, duration of breastfeeding, living in a crowded home, going to nursery or kindergarten, passive smoking and gastro-oesophageal reflux have all been suggested as possible factors [5].

The effects of adenoid tissue on the formation of otitis media with effusion are due to supranormal size, disruption of the nasopharyngeal ventilation, obstruction of the Eustachian with a mass effect, accumulation of secretion, a source of infection, oedema due to inflammation and release of allergic inflammatory mediators from adenoid mast cells [6]. In our opinion, one of the most important limitations of screening studies aiming to reveal OME risk factors is the presence of unidentified adenoid tissue during examination. Considering this, we used adenoid hypertrophy as a common factor in both groups. In this way, we aimed to minimise the effect of adenoid hypertrophy on OME formation.

Despite being a very common disease in children, the literature regarding risk factors for OME in Turkish children is lacking, and risk factors in the English literature may be culturally and/or environmentally biased. The present study aimed to determine the most important risk factors in the development of OME in children with adenoid hypertrophy.

Materials and methods

Study design

The study was conducted in accordance with the principles of the Helsinki Declaration and approved by the local Institutional Review Board. A total of 539 patients undergoing surgery for adenoid hypertrophy (AH Group) or adenoid hypertrophy and otitis media with effusion (AH + OME Group) in the Department of Otorhinolaryngology at our university, between February 2012 and February 2018, constituted the study group. Patients were divided into two groups: those with AH only (AH Group) (n = 429) and those with AH and OME (AH + OME Group) (n = 110).

A diagnosis of OME was made with history, otoscopic findings, conduction-type hearing loss in audiometry and flat tympanograms. Effusion was also confirmed during surgery. Breast-fed children were defined as those who had received breast milk for at least six months after birth.

Exclusion criteria: Children with a chronic infection requiring prophylactic antibiotic therapy, craniofacial abnormalities, or immunodeficiency syndromes and children with ventilation tubes at the time of screening. Children with adhesive otitis were also excluded from the study.

Outcome parameters

The data obtained from patient records for estimation of potential risk factors for OME were neonatal history (breastfeeding, bottle feeding), past health and medical history (presence of atopy or allergic rhinitis, snoring at night, cough, tonsillitis in the past 12 months), environmental factors (presence of pets, attending to daycare centers, district of school), family history (passive smoking at home, number of siblings, family size, parental education) and socioeconomic background (income of the family).

Statistical analysis

Data were analysed using the IBM Statistical Package for Social Sciences v21 (SPSS Inc., Chicago, IL, USA). A normal distribution of the quantitative data was checked using the Kolmogorov-Smirnov test. Parametric tests (Independent-samples t-test and posthoc Tukey test) were applied to data of normal distribution, and non-parametric tests (Mann-Whiney U-test and Kruskal-Wallis Test) were applied to data of questionably normal distribution. Continuous data were presented as mean ± standard deviation or median (minimum-maximum), as appropriate. All differences associated with a P value £ 0.05 were considered statistically significant.

Results

Of the 539 patients (286 males, 253 females) whose charts were reviewed, the mean age was 7.02 ± 3.86 (range 2 to 14) years. The AH Group included 429 patients (225 males, 204 females) with a mean age of 7.34 ± 3.49 years; the AH+OME Group included 110 patients (61 males, 49 females) with a mean age of 6.91 ± 4.02 years. Groups did not differ considering age and gender (p = 0.684, and p = 0.728, respectively).

As for information on neonatal history, the results of the present study did not reveal a significant correlation between OME and breastfeeding (p = 0.446) or bottle feeding (p = 0.284) (Tab. I).

Regarding the past health and medical history, the prevalence of atopy or allergic rhinitis was 34% in patients

Table I. Comparison of groups for factors influencing development of otitis media.

Factors		AH Group (n = 429) n (%)	AH + OME Group (n = 110) n (%)	P value
Breastfeeding	Yes	373 (87%)	97 (88%)	0.446
	No	56 (13%)	13 (12%)	
Bottle feeding	Yes	193 (45%)	53 (48%)	0.284
	No	236 (55%)	57 (52%)	
Presence of atopy, or allergic rhinitis	Yes	107 (25%)	37 (34%)	< 0.001
	No	322 (75%)	73 (66%)	
Snoring at night	Yes	395 (92%)	98 (89%)	0.645
	No	34 (8%)	12 (11%)	
Cough	Yes	163 (38%)	46 (42%)	0.244
	No	266 (62%)	64 (58%)	
Frequent tonsillitis (> 5) in the past 12 months	Yes	167 (39%)	70 (64%)	< 0.001
	No	262 (61%)	40 (36%)	
Presence of pets	Yes	51 (12%)	15 (14%)	0.446
	No	378 (88%)	95 (86%)	
Attending to daycare centers	Yes	180 (42%)	75 (68%)	< 0.001
	No	249 (58%)	35 (32%)	
District of school	Low socioeconomic level	292 (68%)	79 (72%)	0.368
	High socioeconomic level	137 (32%)	31 (28%)	
Passive smoking at home (tobacco smoke exposure)	Yes	189 (44%)	62 (56%)	< 0.001
	No	240 (56%)	48 (4%)	
Number of siblings	2 or less	197 (46%)	26 (24%)	< 0.001
	3 or more	232 (54%)	84 (76%)	
Family size (number of people in the household)	3 or less	176 (41%)	31 (28%)	< 0.001
	4 or more	253 (59%)	79 (72%)	
Parental education	Well educated	103 (24%)	24 (22%)	0.258
	Poor educated	326 (76%)	86 (78%)	
Family income	Less than 500 $/mo	150 (42%)	42 (38%)	0.284
	More than 500 $/mo	249 (58%)	68 (62%)	

AH: adenoid hypertrophy; OME: otitis media with effusion.

with OME, and 25% in those without OME ($p < 0.001$). There was no difference between groups regarding snoring at night ($p = 0.645$) and cough ($p = 0.244$). The presence of frequent tonsillitis (> 5) in the past 12 months increased the prevalence of OME (64% versus 39%), with a significant difference between groups ($p < 0.001$).

We also evaluated the association of OME with environmental factors. Attending daycare centers was significantly more common among children with OME ($p < 0.001$). No difference between groups was found according to the presence of pets ($p = 0.446$) or school district (low *vs*. high socioeconomic level) ($p = 0.368$).

As for family history, passive smoking at home was found to be a significant risk factor ($p < 0.001$). OME was significantly more common among children with parents smoking in the home. Tobacco smoke exposure was revealed in 56% of children with OME, and in 44% of children without OME. Number of siblings was found to be an important predictor of OME ($p < 0.001$); having 3 or more siblings was revealed in 76% of children with OME, and in 54% of children without OME. There was a statistically significant influence of family size on OME. Living with more than 4 persons in the household was seen in 72% of children with OME, while in 59% of children without OME ($p < 0.001$). There was no difference between groups regarding parental education ($p = 0.258$), and family income ($p = 0.284$).

Discussion

The Panel Report from the "Ninth International Research Conference on Otitis Media" suggests that the aetiology of OME is multifactorial and that many different factors are implicated in the pathophysiology of this disease.[7] However, although frequently studied, the influence of various factors

on the pathogenesis of OME has remained controversial when results of previous studies are compared, and further investigation is thus warranted. In the present study, we found that the presence of atopy or allergic rhinitis, frequent (> 5) tonsillitis, attending daycare centres, exposure to smoke, having 3 or more siblings and 4 or more people in the household were the main risk factors for OME.

While 80% of children experience at least one OME attack within the first 10 years of their lifetime, OME is most commonly observed in children aged between six months and four years [8]. Many cases of OME spontaneously resolve within three months, but 30-40% of children have recurrent episodes and 5-10% of cases last more than one year [9]. Although not a very significant factor, OME is rare in children younger than one year of age and its incidence decreases significantly in patients over 10 years of age [8].Our cases were most frequently observed in this age group. There is yet no consensus on the relationship between gender and OME. It is expected that the disease is more common in boys as mastoid pneumatization is more rapid in girls and boys experience upper respiratory infection (URI) episodes more frequently. There are also studies in the literature showing that OME is more common in males [10]. On the contrary, there are studies showing that there is no relationship between gender and the prevalence of OME [11], and our result is consistent with these studies.

Several studies have confirmed the weak protective effects of breastfeeding or the increased risk of bottle feeding in OME. In two studies on the interaction of breastfeeding with weaning and infection [12,13], the first OME episode occurred significantly earlier in children weaned before six months of age. A number of studies including ours on OME did not find breastfeeding as a significant factor.

Among the predisposing factors of OME, allergy is an important characteristic. In addition to many publications showing that OME is associated with allergy, there are also studies in which no relationship was established [14]. Allergies may predispose to OME by causing oedema around the Eustachian tube or primary mucosal disease in the middle ear. In the study of Aydoğan et al., 44.6% of children with OME had a food allergy, and allergy was suggested to be possibly effective either by nasal congestion or by determining the middle ear mucosa as a direct target organ [15]. In a study by Alles et al. in children aged 3 to 8 years with chronic or recurrent OME, and allergy symptoms of 89% of subjects were confirmed during examinations [16]. In a study by Martinez et al., skin tests of children with OME were found to be negative in 37.1% and positive in 62.9% of cases [14]. In addition, in the study published by Marseglia et al. the authors reported in 2008 that allergic rhinitis and adenoiditis were important risk factors for the development of OME and that the risk increased even further in the co-presence of both conditions [17]. In our study, we found a significantly higher association between allergic rhinitis and OME. Bergroth et al. highlighted that pet contacts during infancy may have a protective effect on respiratory tract symptoms and infections [18]. However, we did not observe any relationship between OME episodes and children who lived with pets.

Nasal and nasopharyngeal pathologies may contribute to the formation of OME by affecting the upper airway [19]. Sinusitis, septal curvature, hypertrophy of concha choanal atresia, or stenosis can all affect air passage through the nasopharynx. Nasopharyngeal malignancies may cause the development of effusion. Cases with inadequate pneumatisation constitute a risk factor in cases of tubal dysfunction. In our study, night snoring and cough were not risk factors for OME. As a result of our analysis, the incidence of acute tonsillitis in the last 12 months was found to be a significant factor for OME. Middle ear effusion is 6-7 times more frequent in the two to six-year-old group [20]. Viral infections impair tubal function, and inadequate treatment of acute otitis may result in chronic effusion.

The results of studies investigating the relationship between smoking and frequency of OME are varied. In some studies, there is no association between smoking and frequency of OME, but other studies have reported that smoking increases the frequency of OME [21]. Kitchens et al. reported that children in whom a ventilation tube was applied for chronic OME were more frequently exposed to smoking than a control group. According to this, the number of smokers and the number of cigarettes smoked are important, in addition to passive smoke in the home [22]. In our study, exposure to passive cigarette smoke and OME were significantly associated.

Our findings showed that family size was also associated with a higher percentage of OME and that living with more than 4 persons in the household was seen in 72% of children with OME, compared to 59% of children without OME. The presence of older siblings and day care attendance were both independent risk factors for OME in this population. These variables have previously been reported as predisposing to OME and are typically attributed to the increased chance of acquiring pathogens involved in OME or URIs from contact with siblings or other children [14,23].

According to Daly et al. [24], who studied maternal knowledge and practices regarding risk factors for otitis media among mothers in Minnesota, U.S. they found that lower levels of education were associated with poorer knowledge regarding otitis media, we suggest that health education regarding OME and its risk factors should be implemented from caregivers to address these lacunae especially to these illiterate conditions.

The relationship between OME and socio-economic status is not clear. There are several publications claiming that the prevalence of OME is higher in communities with higher socioeconomic level, while others have advocated the

contrary [21,25]. According to these authors, URIs are more frequently seen in societies with low socioeconomic level due to poor hygiene and other reasons. Therefore, it is believed that the prevalence of OME increases in societies with low socioeconomic level. In our study, we found that the socioeconomic level did not have a significant relationship with the prevalence of OME.

The main limitation of our study was its retrospective design. In addition, some details of history and factors that may influence outcomes may not be completely documented. Third, this was a single-institution study, and some caution should be taken before generalising our findings to other settings. Finally, the role and the importance of naso-pharyngeal microbiota was not considered due to the retrospective nature of the study. Many papers have been published in the last years about this topic, leading to a surprising new vision in the physiopathology of the nose and eustachian tube [26]. Due to these restrictions, any associations should be interpreted with caution.

This paper contributes to the understanding of the role of different risk factors in the development of OME among children. Specifically, our data support the presence of atopy or allergic rhinitis, frequent (> 5) tonsillitis, attending to daycare centres, exposure to smoke, having 3 or more siblings and 4 or more people in the household as main risk factors for OME. Comprehensive knowledge of modifiable risk factors found in this study could help to minimise the complications of OME in children.

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Maxillo facial surgery

Factors influencing CAD/CAM accuracy in fibula free flap mandibular reconstruction

I fattori che influenzano l'accuratezza del CAD/CAM nella ricostruzione mandibolare del lembo libero di fibula

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SUMMARY

Computer-aided design/computer-aided manufacturing (CAD/CAM) technology has improved the functional and morphological results of mandibular reconstructive surgery. The purpose of this study was to objectively assess this technology and factors affecting its accuracy. Fibula free flap mandibular reconstruction was performed in 26 cases using CAD/CAM technology at the Maxillofacial Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, between June 2014 and February 2018. We evaluated the technology's accuracy by comparing the virtual surgical planning STL file (planned-target mesh) with the STL file from an early postoperative CT scan (postoperative-achievement mesh) in each case. The STL files were imported into Geomagic Studio 2016 (Geomagic GmbH). According to the position of the reconstruction plate (fixed reference point), we assessed deviations at the right condyle, right gonion, gnathion, left gonion and left condyle, calculating mean, minimum and maximum error values. Mean error values ranged from 0.6 to 2.2 mm; they were ≥ 2 mm in only 2 (7.7%) cases. The midline area (symphysis-gnathion) showed the least variation (1.05 ± 0.92 mm), and the gonion area showed the greatest variation (right and left means of 1.6 and 1.46 mm, respectively). Among all possible factors that could affect CAD\CAM accuracy, nothing showed significant influence, including the timing of reconstruction, site and size of the defect and malignancy status. CAD/CAM technology has a high degree of accuracy and reproducibility for microvascular reconstruction of mandibular defects using fibula free flaps, regardless of the defect site and length, use of a single- or double-barrel graft or timing of reconstruction.

KEYWORDS: fibula free flap, CAD/CAM, mandibular reconstruction

RIASSUNTO

La tecnologia CAD/CAM (Computer-Aided Design/Computer-Aided Manufacturing) ha migliorato sia i risultati funzionali che morfologici nella chirurgia ricostruttiva mandibolare. L'obiettivo del nostro studio è stato quello di valutare questo tipo di tecnologia ed i fattori che possono influenzare la sua precisione. Un totale di 26 casi di ricostruzione mandibolare con lembo libero di fibula, utilizzando tecnologia CAD/CAM sono stati operati presso l'Unità Maxillofacciale della Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico di Milano, da giugno 2014 a febbraio 2018. Abbiamo valutato l'accuratezza confrontando i files STL di pianificazione chirurgica virtuale (obiettivo pianificato) con il file STL di una scansione TC postoperatoria precoce (risultato postoperatorio ottenuto). Entrambi i file STL sono stati importati su Geomagic Studio 2016 (Geomagic Gmbh). In base alla posizione della placca di ricostruzione (punto di riferimento fisso), abbiamo confrontato la deviazione sul condilo sinistro, gonion sinistro, gnathion, gonion destro e condilo destro, per calcolare l'errore medio di deviazione. L'errore medio di deviazione varia da 0,6 mm a 2,2 mm. Solo 2 dei 26 casi analizzati avevano un errore medio uguale o superiore a 2 mm (7,7%). L'area mediana (symphysis-gnathion) ha mostrato una variazione più

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bassa (1,05 ± 0,92 mm) mentre l'area di gonion ha mostrato maggiore variazione (la variazione media del gonion destro e sinistro era rispettivamente di 1,6 mm e 1,46 mm). Nessuno dei possibili fattori (tempi di ricostruzione, malignità o benignità, sito o dimensione del difetto) che potrebbero influenzare la precisione del CAD/CAM, ha mostrato un'influenza significativa. La tecnologia CAD/CAM nella ricostruzione microvascolare dei difetti mandibolari mediante lembo libero di fibula minimizza gli errori umani ed è considerato come un intervento chirurgico indipendente dall'operatore con alto grado di accuratezza e riproducibilità.

PAROLE CHIAVE: *limbo libero di perone, CAD/CAM, ricostruzione mandibolare*

Introduction

The fibula free flap (FFF) has become the gold standard for surgical reconstruction of mandibular bony defects since Hidalgo first used it in 1989 [1]. Use of the FFF has several advantages, including harvest from the longest (up to 25 cm) dense bicortical bone, the ability to employ a simultaneous two-team approach, adequate length and diameter of peroneal vessels, least donor-site morbidity and dual blood supplies from the contemporary intraosseous and segmental periosteal arterial systems, which permits the performance of multiple osteotomies (separated by as little as 2 cm) [2] and thereby optimal bone shaping without concern for bone viability [3].

Since Hirsch's description of the pioneering technique in 2009 [4], computer-assisted surgery (CAS) or computer-aided design/computer-aided manufacturing (CAD/CAM) for mandibular reconstruction has gained popularity due to its reproducibility, its role in improving surgeons' performance and patient satisfaction (aesthetic and functional), and its cost burden, which is comparable to those of traditional freehand reconstructive techniques [5,6]. CAD/CAM technology has been applied successfully even for secondary mandibular reconstruction, which is considered to be a reconstructive challenge [7].

CAS for mandibular reconstruction involves four phases: 1) preoperative planning and virtual surgery, 2) manufacturing of patient-specific devices, 3) surgical intervention and 4) postoperative evaluation. The last phase was not performed in previously reported studies [8].

van Baar et al. performed a systematic review which revealed that three methods are used for CAD/CAM evaluation: comparison of 1) preoperative and postoperative DICOM images, 2) preoperative (unrevised to the virtual plan, without segmentation or neomandible) and postoperative STL models and 3) preoperative (revised to the virtual plan, including the neomandible) and postoperative STL models [9].

Despite the widespread utilisation and proven accuracy of this technology, studies comparing it with traditional reconstructive techniques, with the analysis of variables affecting computer-aided reconstruction, are lacking [5,6]. In this study, we aimed to objectively assess the accuracy and reproducibility of CAD/CAM technology in mandibular reconstruction with vascularised FFFs, using superimposition software to assess average linear deviations and to evaluate the potential effects of various factors on the outcomes of these procedures.

Patients and methods

Study design

This retrospective study was conducted at the Maxillofacial Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, between June 2014 and February 2018. This study was approved by the facility's ethics committee and was conducted according to the ethical guidelines outlined in the Declaration of Helsinki.

Subjects

All adult patients who underwent mandibular reconstruction with FFFs using CAD/CAM technology were included, regardless of pathological aetiology, timing of reconstruction (primary vs secondary reconstruction), number of fibular segments or type and size of the mandibular defect.

After virtual surgery and preoperative planning using the Proplan CMF (Materialise, Leuven, Belgium) software programme, a patient-specific cutting guide, STL model and patient-specific plate were manufactured to guide the reconstructive surgery in each case.

Surgical procedure

A simultaneous two-team approach was employed for reconstructive and extirpative surgery. First, the FFF was harvested using the traditional[1] or minimally invasive approach [10], with or without a skin paddle according to the aetiology of the defect (benign or malignant), followed by flap reshaping using the fibula cutting guide. Next, the team performed surgical ablation of the mandibular defect using the mandibular cutting guide, as well as preparation for microanastomosis with or without neck dissection according to the defect's aetiology. The mandibular reconstruction was completed by fixing the reconstructed unit of fibula segments onto the bone defect with the aid of the patient-specific plate, and performing microvascular anastomoses between the donor and recipient vessels.

Evaluation of CAD/CAM technology accuracy

We assessed the accuracy of the CAD/CAM surgical technology by comparing the virtual surgical planning STL file (planned-target mesh) with the STL file from an early postoperative CT scan (postoperative-achievement mesh); (Third comparative model in van Baar et al. [9]).

We aligned the two meshes according to the surface of the reconstruction plate (iterative closest reference point) in software-aided superimposition. Based on the automated Hausdorff distance, used in this superimposition, the deviation between pre- and postoperative STL models was measured in relation to five constant orthognathic landmarks (right condyle, right gonion, gnathion, left gonion and left condyle). The average linear distance was estimated for calculation of minimum, maximum and average error values for each reconstruction, which minimised human error in deviation measurement. Finally, the average minimum, maximum and mean errors were determined to obtain a comprehensive outlook on the accuracy of CAD/CAM application in our patient sample. To minimise interpersonal human bias, a blind third partner separate from Proplan (Materialise, Leuven, Belgium) company and the reconstructive surgeons performed the superimposition and software processing. In addition, data interpretation was performed according to average linear distances to minimise human calculation error.

Statistical analysis

Statistical analyses were performed using the SPSS software (ver. 14.0 for Windows; SPSS Inc, Chicago, IL). The significance level was set at $P < 0.05$.

Results

This study included 25 adult patients (13 females and 12 males) and 26 reconstructive cases (patients 6 and 15 are the same patient, whose first reconstructive surgery was complicated by total flap loss, necessitating a second reconstructive surgery 1 year after the first reconstruction). The patient age ranged from 16 to 70 years (mean, 44 years). The mandibular defects had various aetiologies, with odontogenic keratocysts being the most common pathology, $n = 6$ (23.07%). The sample varied in terms of the timing of reconstruction [primary reconstruction, $n = 21$ (80.7%); secondary reconstruction, $n = 5$ (19.3%)]; mandibular defect size (mean, 8.75 cm), site and shape; and fibular segmentation (average number of fibular segments = 2.88, average number of osteotomies = 5.92, single-barrel:double-barrel FFF = 12:14; Tab. I).

Five patients underwent secondary mandibular reconstruction, due to pseudarthrosis in three cases, osteoradionecrosis that resulted in mandibular fracture (after partial mandibular resection due to squamous cell carcinoma) in one case and total flap necrosis after the first jaw reconstruction procedure in one case. The aforementioned patient was thus included twice in the study. The average follow-up period was 27 months (range, 5-48 months). During this follow-up period, total flap loss occurred in one (3.8%) patient and no sign of locoregional recurrence was observed in the six patients with malignant aetiologies.

Regarding CAD/CAM accuracy, we obtained an average minimum error of 0.37 mm (range, 0-1.7 mm), average maximum error of 2.52 mm (range, 1.6-3.4 mm) and average mean error of 1.34 mm (range, 0.6-2.2 mm). Mean errors were ≥ 2 mm in only 2 (7.7%) of 26 cases analysed. The least discrepancy between planned and achieved outcomes was observed in the midline (symphysis-gnathion) area (1.05 ± 0.92 mm), followed by the condyles (right and left means of 1.43 and 1.17 mm, respectively); the greatest variation was observed in the gonion area (right and left means of 1.6 and 1.46 mm, respectively; Tab. II).

Many variables can potentially affect the accuracy of CAD/CAM-guided reconstruction. To elaborate on the data provided in Tables I and II, we assessed the significance of the effects of the following variables on surgical outcomes: 1) defect length, 2) defect type and site, 3) number of fibular segments used, 4) reconstruction type (primary or secondary), 5) flap technique (double-barrel or single-barrel) and 6) aetiology (malignant or benign; Tab. III). Only the number of fibular segments significantly affected the accuracy of CAD/CAM-aided reconstruction; contrary to common sense, mandibular defect reconstruction with one or two fibular segments was less accurate (with a greater mean error) than reconstruction performed with three or more segments ($P = 0.0210$). Thus, reconstructive accuracy was greater for more-complex defects requiring the use of more fibular segments.

Postoperatively, total flap loss occurred in one (3.8%) case; thus, the overall FFF success rate was 96.2%. Four (15.4%) cases required re-intervention due to total flap loss, plate exposition, postoperative bleeding at the anastomosis site and condylar osteomyelitis, respectively. A donor-site complication (skin graft loss) occurred in only one (3.8%) case.

Discussion

Mandibular reconstruction represents a genuine challenge, as it should re-establish the aesthetics of the face, restore the patient's ability to eat in public, maintain the intelligibility of speech and achieve an accessible airway [11]. Reconstructive surgeons are in consensus that CAS yields outcomes superior to those of conventional surgery, with a comparable cost burden [6,12].

Despite the popularity and accuracy of CAD/CAM-aided mandibular reconstruction, few objective analyses have been performed to examine the reproducibility of virtual planning with large samples [5]. Moreover, different methods have been used to evaluate the outcomes of CAD/CAM-aided procedures, including the comparison of pre- and postoperative DICOM files, unrevised preoperative and postoperative STL files and revised virtually operated preoperative and postoperative STL files. van Baar et al. (2018) argue that the latter

Table I. Demographic data, mandibular defect data and fibula free flap data of patients.

	Sex	Age	Etiology	Mandibular defect		Fibula reshaping	
				Site/types (Urken's classification)	Length (cm)	No. of fibular segments	Double-barrelled technique
1	F	37	Ameloblastoma	R-B	8	2	No
2	F	38	Odontogenic keratocysts	B-R-C	13	2	No
3	F	53	Malignant mesenchymal tumour	B dx	5	2	Yes
4	F	61	Low grade mucoepidermoid CA	R-B	6	2	Yes
5	F	55	2ry reconstruction pseudoarthrosis	S-B	6	4	Yes
6	M	31	Ameloblastoma	R-B	8	3	Yes
7	F	70	Squamous cell carcinoma	S-B	5	4	Yes
8	M	59	Squamous cell carcinoma	B-S-B	13	3	No
9	M	62	Squamous cell carcinoma	S-B-R	14	5	Yes
10	M	60	Low grade mucoepidermoid CA	B-R	3,5	1	No
11	M	23	Odontogenic keratocysts	R-B	11,5	4	Yes
12	F	46	Ameloblastoma	B-R	9	4	Yes
13	M	65	2ry reconstruction pseudoarthrosis	B sx	7	2	No
14	F	29	Pseudoarthrosis	B dx	3,5	1	No
15	M	32	2ry reconstruction due to total flap necrosis (no.6)	C-R-B	10,5	3	No
16	M	31	Chronic slerosing osteomyelitis	C-R-B	12,5	3	Yes
17	M	67	2ry reconstruction pseudoarthrosis	B-S	5	2	No
18	M	18	Odontogenic fibromyxoma	R-B	10	5	Yes
19	F	38	Odontogenic keratocysts	B dx	7	3	Yes
20	F	32	Odontogenic keratocysts	B-R	6	3	Yes
21	M	16	Odontogenic fibromyxoma	B-S-B	15,5	4	No
22	F	55	Ossifying fibroma	B sx	6	3	Yes
23	F	55	2ry reconstruction pseudoarthrosis	R-B-S-B-R	18	3	No
24	F	58	Ameloblastoma	S-B	5,5	5	Yes
25	M	26	Odontogenic keratocysts	C-R-B	10	2	No
26	M	29	Odontogenic keratocysts	B-R	9	2	No

method is the most reliable, as it takes into consideration the remnant bony parts of the mandible and the fibular segments (neomandible) [9].

Software superimposition has yielded reliable results, but the scattering effect of the titanium plate on postoperative CT scans and human error during the conversion of DICOM files to STL files are inevitable drawbacks [13]. The choice of the reference iterative closest point may also vary according to the method of superimposition used. For example, the gold standard for superimposition is the comparison of the largest remnant mandible portion between pre- and postoperative best-fit models on a fully computerised 3D overlapping image, although a patient-specific reconstructive plate surface or screw site could also be used as a reference point for linear measurements [5-7,9].

In this study, the accuracy and reproducibility of CAD/CAM-aided reconstruction were shown by low mean error values (range, 0-2.2 mm). Mean errors were ≥ 2 mm in only 2 (7.7%) of 26 cases analysed. The least discrepancy between planned and achieved surgical outcomes was observed in the midline (symphysis-gnathion) region (1.05 ± 0.92 mm), followed by the condyles (right and left means of 1.43 and 1.17 mm, respectively); the greatest error was observed in the gonion area (right and left means of 1.6 and 1.46 mm, respectively; Tab. II). These results are in contrast to those obtained by Tarsitano et al. (2018) [5], who found that the symphysis was the site of maximum error using colour maps. We found that neither the size nor site of the mandibular defect, nor the aetiology, use of the double- or single-barrel technique or timing of reconstruction (primary vs. secondary) influenced the reproducibility and accuracy of CAD/CAM-assisted surgery (Tab. III). This finding supports the characterisation of CAD/CAM technology as an operator-independent modality that minimises human error. The only factor showing a significant influence was

the number of fibular segments used; contrary to expectations, reconstructions performed with three or more fibular segments were more accurate than those performed with one or two fibular segments ($P = 0.0210$). To our knowledge, no other statistically supported study has analysed factors affecting the use of CAD/CAM technology.

Although this technique is 'operator independent', with a high degree of reproducibility, the postoperative results never fully match the preoperative virtual plan. Inaccuracies might be introduced at various stages, including image acquisition, segmentation, 3D printing, surgery and evaluation of the postoperative results [14,15]. van Baar et al. [9]. recommended the acquisition of pre- and postoperative images with the same multidetector CT device using identical scanner parameters and a slice thickness < 1.25 mm to minimise possible deviation.

The main limitation of this study is that we were not able to evaluate human factors affecting CAD/CAM surgery, as the same bioengineer and surgical team performed all procedures. This issue could be evaluated in the future in a multi-centre study or systematic review. In addition, the number of fibular segments used should be evaluated as a potential prognostic factor for CAD/CAM accuracy in a homogenous, site-specific group to clarify its influence.

Conclusions

Our results suggest that the application of CAD/CAM technology in the microvascular reconstruction of mandibular defects using FFFs is an operator-independent approach characterised by high degrees of accuracy and reproducibility, regardless of the aetiology of the lesion (benign or malignant), site of the lesion (condyle, body, ramus or symphysis), length of

Table II. Linear distance measurements (Deviation and accuracy assessment; error value).

	Variation in 5 landmarks					Error interpretation		
	Right condyle	Left condyle	Gnathion	Right gonion	Left gonion	Mean	Minimum error	Maximum error
1	2.0	0.9	**0**	2.0	0.2	1.2	0	2
2	0.3	2.3	**0**	0.3	2.7	1.12	0	2,7
3	3.1	**0.3**	0.9	2.4	**0.3**	1.4	0,3	3,1
4	**0.4**	2.7	2.7	1.1	1.7	1.7	0,4	2,7
5	**0.3**	2.2	2.1	1.2	2.1	1.6	0,3	2,2
6	**0.3**	**0.3**	1.6	0.4	0.4	0.6	0,3	1,6
7	**0.4**	0.6	0.9	0.7	2.1	0.9	0,4	2,1
8	0.9	1.2	**0**	2.1	2.1	1.26	0	2,1
9	0.4	0.6	0	3.1	2.1	1.24	0	3,1
10	1.2	1.4	0	3.2	2.1	1.58	0	3,2
11	**0.6**	1.5	1.7	0.7	2.4	1.4	0,6	2,4
12	**0.5**	0.7	1.6	1.4	1.3	1.1	0,5	1,6
13*	1.8	2.3	**1.7**	3.1	2.1	2.2	1,7	3,1
14	1.1	2.2	**0.4**	2.7	2.7	1.8	0,4	2,7
15	1.2	**0.3**	1.2	**0.3**	2.2	1.0	0,3	2,2
16	0.4	0.6	0.6	1.7	**0.3**	0.7	0,3	1,7
17	0.7	1.2	3.1	2.1	**0.6**	1.5	0,6	3,1
18	2.1	0.6	**0.4**	**0.4**	1.2	0.9	0,4	2,1
19	3.1	1.4	**0.3**	2.1	0.6	1.5	0,3	3,1
20*	3.2	3.1	**0.7**	2.1	1.4	2.1	0,7	3,2
21	0.7	**0.4**	**0.4**	2.1	1.5	1.0	0,4	2,1
22	1.4	**0.3**	1.5	2.1	1.1	1.3	0,3	2,1
23	3.2	**0.3**	0.7	2.1	2.1	1.7	0,3	3,2
24	2.1	0.4	2.3	0.5	**0.2**	1.1	0,2	2,3
25	2.3	2.2	2.2	**0.6**	2.2	1.9	0,6	2,3
26	3.4	**0.3**	0.3	1.2	**0.3**	1.1	0,3	3,4
Mean	1,43	1,17	1.05	1,60	1,46	1,34	0,37	2,52

Bold values refer to minimum values and underlined values refer to maximum values. (*) refers to cases with error values > 2 mm.

Table III. Factors affecting accuracy of CAD/CAM technology.

	Factors affecting CAD/CAM accuracy	No. of patients	Average of error ± SD	P-value
A	Defects < 9 cm	14	1.46 mm ± 0.42	P-value = 0.0960
	Defects ≥ 9 cm	12	1.20 mm ± 0.33	
B	Number of segments < 3 cm	10	1.55 mm ± 0.36	P-value = 0.0210 *
	Segments ≥ 3 cm	16	1.21 mm ± 0.33	
C	Double barreled fibula	14	1.25 mm ± 0.41	P-value = 0.2170
	Single barreled fibula	12	1.45 mm ± 0.39	
D	Primary reconstruction	21	1.28 mm ± 0.38	P-value = 0.0931
	Secondary reconstruction	5	1.62 mm ± 0.44	
E	Malignant aetiology	6	1.35 mm ± 0.28	P-value = 0.9588
	Non-malignant aetiology	20	1.34 mm ± 0.44	
F	Defects not involving any of 5 landmarks i.e. defects located in body of the mandible	5	1.64 mm ± 0.36	P-value = 0.0654
	Defects involving any of 5 landmarks RT-LT condyle, RT-LT gonium, gnathiun	21	1.27 mm ± 0.39	
G	Defects involving midline (symphysis)	8	1.29 mm ± 0.29	P-value = 0.6501
	Defects not involving midline (symphysis)	18	1.37 mm ± 0.45	
H	Defects involving one or both angle of the mandible (ramus)	15	1.29 mm ± 0.43	P-value = 0.4638
	Defects not involving any angle of the mandible (ramus)	11	1.41 mm ± 0.37	
I	Defects involving one or both condyles of the mandible	4	1.18 mm ± 0.51	P-value = 0.3989
	Defects not involving any condyles of the mandible	22	1.37 mm ± 0.39	

the defect, timing of the reconstruction (primary or secondary) or number fibular segmentations (single- or double-barrel technique).

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VESTIBOLOGY

Incidence of unilateral and bilateral benign paroxysmal positional vertigo when the left and right Dix-Hallpike manoeuvres are positive: a model based on the sense of torsional nystagmus

Incidenza della vertigine parossistica benigna bilaterale e monolaterale in caso di positività alla manovra Dix-Hallpike a destra e sinistra: modello basato sul verso del nistagmo rotatorio

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SUMMARY

Patients presenting with nystagmus indicative of benign paroxysmal positional vertigo (BPPV) during the left and the right Dix-Hallpike manoeuvres (DHMs) are frequently seen in clinical practice. In such cases, BPPV may be unilateral or bilateral. The aim of this study is to describe the incidence of unilateral and bilateral BPPV when both DHMs are positive, taking into account the sense of the torsional component of nystagmus. This is a prospective multicentre study. BPPV patients were classified into three groups: patients with only one positive DHM (control group, CG), patients showing positive bilateral DHM with nystagmus in the same sense in both DHMs (same sense group, SSG) and patients showing positive bilateral DHM with the torsional component of nystagmus beating in opposite senses in each DHM (opposite sense group, OSG). Only one Epley Manoeuvre (EM) was performed on all patients. Based on the ipsilateral result of the EM, the contralateral result of the same EM and the BPPV resolution rate in the control group, a model was developed to predict the incidence of unilateral and bilateral BPPV in the SSG and the OSG. There were 234 patients in the control group, 20 in the SSG and 23 in the OSG. The model estimated that the percentage of unilateral BPPV would be 89.5% in SSG and 38.7% in OSG. Using these findings, we conclude that when both DHMs are positive, BPPV may be unilateral or bilateral. If the torsional components of both nystagmuses beat in the same sense, it is more likely to be unilateral BPPV. If the torsional components beat in opposite senses, both situations can be considered equally likely.

KEY WORDS: benign paroxysmal positional vertigo, multi-canal BPPV

RIASSUNTO

Frequentemente nella pratica clinica si valutano pazienti che mostrano alle manovre di Dix-Hallpike (DHM), verso destra e verso sinistra, il nistagmo tipico della vertigine posizionale parossistica benigna (BPPV). In questi casi la BPPV può essere unilaterale o bilaterale. Lo scopo del presente studio è quello di descrivere l'incidenza della BPPV unilaterale e bilaterale, quando entrambe le DHM sono positive, considerando il verso della componente torsionale del nistagmo. In questo studio prospettico multicentrico i pazienti sono stati suddivisi in tre gruppi: pazienti con solo una DHM positiva (gruppo di controllo, CG), pazienti con DHM positiva bilateralmente e con nistagmo battente nello stesso verso evocabile in entrambe le manovre (gruppo stesso verso, SSG), pazienti con DHM positiva bilateralmente e con nistagmo avente componente torsionale con verso opposto nelle due manovre (gruppo verso opposto, OSG). La manovra di Epley (EM) è stata condotta su tutti

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i pazienti. Considerando i risultati ottenuti dalla EM e il tasso di risoluzione della BPPV nel CG, è stato sviluppato un modello predittivo dell'incidenza della BPPV unilaterale e bilaterale negli SSG e OSG. Il CG comprendeva 234 pazienti, 20 erano inclusi invece nel SSG e 23 nell'OSG. Il modello ha stimato che la percentuale di BPPV unilaterale ammonterebbe a 89,5% nel SSG e 38,7% nel OSG. In conclusione, se entrambe le DHM sono positive, la BPPV può essere sia unilaterale che bilaterale. Se la componente torsionale dei nistagmi batte nello stesso verso, è più probabile che la BPPV sia unilaterale; se invece tale componente ha verso opposto alle DHM, le due condizioni possono considerarsi ugualmente probabili.

PAROLE CHIAVE: *vertigine posizionale parossistica benigna, BPPV multi-canale*

Introduction

Benign paroxysmal positional vertigo (BPPV) is the most frequent cause of vertigo [1]. The most widely accepted theory states that BPPV is caused by otoconia which become detached from the otoconial macula and reside in the semicircular canals. When changes in head position occur in the plane of these semicircular canals, gravity causes these otoconia to move, which impairs endolymph flow, thus activating the ciliary cells in ampullary receptors, a mechanism known as canalithiasis [2]. Another less frequent cause of BPPV is cupulolithiasis [3], which occurs when otoconia become attached to the cupula of a semicircular canal, thus changing the canal's sensitivity to gravity.

The Dix-Hallpike manoeuvre (DHM) [4] causes the otoconia in the posterior semicircular canals (PSC) to move. Canalithiasic BPPV of the PSC is diagnosed when a combination of torsional nystagmus and upbeating vertical nystagmus is observed [5]. It is currently accepted that BPPV may involve more than one canal, which is known as lithiasis of multiple canals (mc-BPPV) [5].

In clinical practice, patients presenting torsional nystagmus with a vertical component in both DHMs – left and right – are not infrequent [6]. Such a finding may correspond to different physiopathological situations, as the DHM is not specific to a certain PSC and may indirectly stimulate the contralateral one to a lesser degree.

Thus, when both DHMs are positive, the BPPV may be unilateral, with the DHM contralateral to the affected ear causing the otoconia to move and producing ampullopetal deflection of the cupula. This would mimic bilateral BPPV, thus producing pseudo-bilateral benign paroxysmal positional nystagmus (pb-BPPN) [7]. However, the BPPV may also be bilateral and produce hard-to-interpret nystagmuses due to the simultaneous stimulation of several canals [8].

To facilitate clinical assessment, we need to find a way to differentiate between unilateral and bilateral BPPV of the PSC using the nystagmuses observed in both DHMs and to estimate the probability that these nystagmuses are caused by unilateral or bilateral BPPV.

The aim of this study is to describe the incidence of unilateral and bilateral BPPV when both the left and right DHMs are positive by using the sense of the torsional component of the observed nystagmuses.

Materials and methods

This was a multicentric, prospective, descriptive study. The research protocol was approved by the Ethics Committee of the main participating hospital. Between April 1st 2015 and March 31st 2016 all patients presenting to the Otoneurology Unit of any of the five participating hospitals with suspected BPPV were prospectively recruited. Patients undergoing the Epley manoeuvre (EM) were required to sign an informed consent form before being included in the study.

All patients underwent anamnesis for possible risk factors that could potentially impair the success of the Epley manoeuvre [9] and were also given complete otoneurological examination including bilateral head impulse tests [10], determination of presence of nystagmus in all positions of gaze and Pagnini-McClure manoeuvres. Patients were also subjected to left and right DHMs. Subsequently, they were split into two groups: those presenting typical nystagmus in only one DHM (control group) (CG) and those presenting typical nystagmus both in the left and the right DHM. We used the term "typical nystagmus" to describe a torsional positional nystagmus, with a duration of under 60 seconds, elicited by the DHM on one or both sides, with a latency of one to a few seconds. The presence or absence of nystagmus was evaluated with the naked eye.

Patients in the CG underwent the corresponding EM. Patients who had presented nystagmus in both DHMs were then classified into two groups: those presenting torsional nystagmus in the same sense in both the left and right DHMs (same sense group, SSG) or those presenting clockwise torsional nystagmus in the left DHM and counterclockwise torsional nystagmus in the right DHM (opposite sense group) (OSG). SSG patients presenting clockwise torsional nystagmuses on both sides underwent left EM; those presenting counter-clockwise nystagmuses on both sides underwent right EM. Patients in the OSG underwent the EM to the side where the higher-intensity nystagmus had been observed. If both nystagmuses were of similar intensity, the side where the patient reported having stronger symptoms was chosen. After the EM, the physician advised patients to sleep with the head of bed elevated at 30º that night. Patients were given an appointment for a follow-up visit

7 days later. Patients were judged to have recovered from ipsilateral BPPV if nystagmus was absent in the follow-up DHM even if they presented vertigo in that position.

Patients presenting any of the following characteristics were excluded from the study: spontaneous nystagmus different from physiologic gaze-evoked nystagmus, involvement of the horizontal canal detected in the left or right Pagnini-McClure manoeuvre [11,12], absence of nystagmus observed without Frenzel glasses in both DHMs, atypical nystagmus, which is not a combination of torsional and upbeating vertical nystagmus in at least one of the DHMs, nystagmus lasting for 60 seconds or longer, patients refusing to undergo EM, patients failing to attend the follow-up visit and patients where the occurrence of nystagmus could not be verified during the follow-up visit as they closed their eyes during the DHM. Figure 1 outlines the selection process.

Using the results, a model was designed to predict the incidence of unilateral and bilateral BPPV in the SSG and OSG using three pieces of data: the ipsilateral outcome of the EM, the contralateral outcome of the same EM and the BPPV resolution rate in the CG.

Figure 1 shows the steps involved in the construction of the model. According to the model, the assumed diagnosis was unilateral BPPV in patients who show negative nystagmus in both DHMs after only one EM. The assumed diagnosis was bilateral BPPV in patients who show negative nystagmus in the DHM ipsilateral to the EM, but not in the contralateral one. In patients where the ipsilateral DHM did not become negative, it was not possible to tell whether BPPV is unilateral or bilateral; however, the number of subjects with unilateral BPPV in this group can be estimated by applying the BPPV resolution rate in the control group to patients with assumed unilateral BPPV.

The model allowed us to calculate the proportions of unilateral and bilateral BPPV in the SSG and OSG and the confidence intervals for this data were also calculated.

Results

During the study, 234 patients were included in the CG, 20 were included in the SSG and 23 were included in the OSG. Median age was 63 years; 71.5% were women. BPPV affected the right ear in 48.9% of CG patients; in the SSG and the OSG –presumably with bilateral BPPV – the right ear was the treated ear in 60 and 56.5% of cases, respectively. No significant between-group differences were found in median age ($p = 0.258$), age distribution ($p = 0.178$), gender proportion ($p = 0.133$) or side of the treated ear ($p = 0.526$).

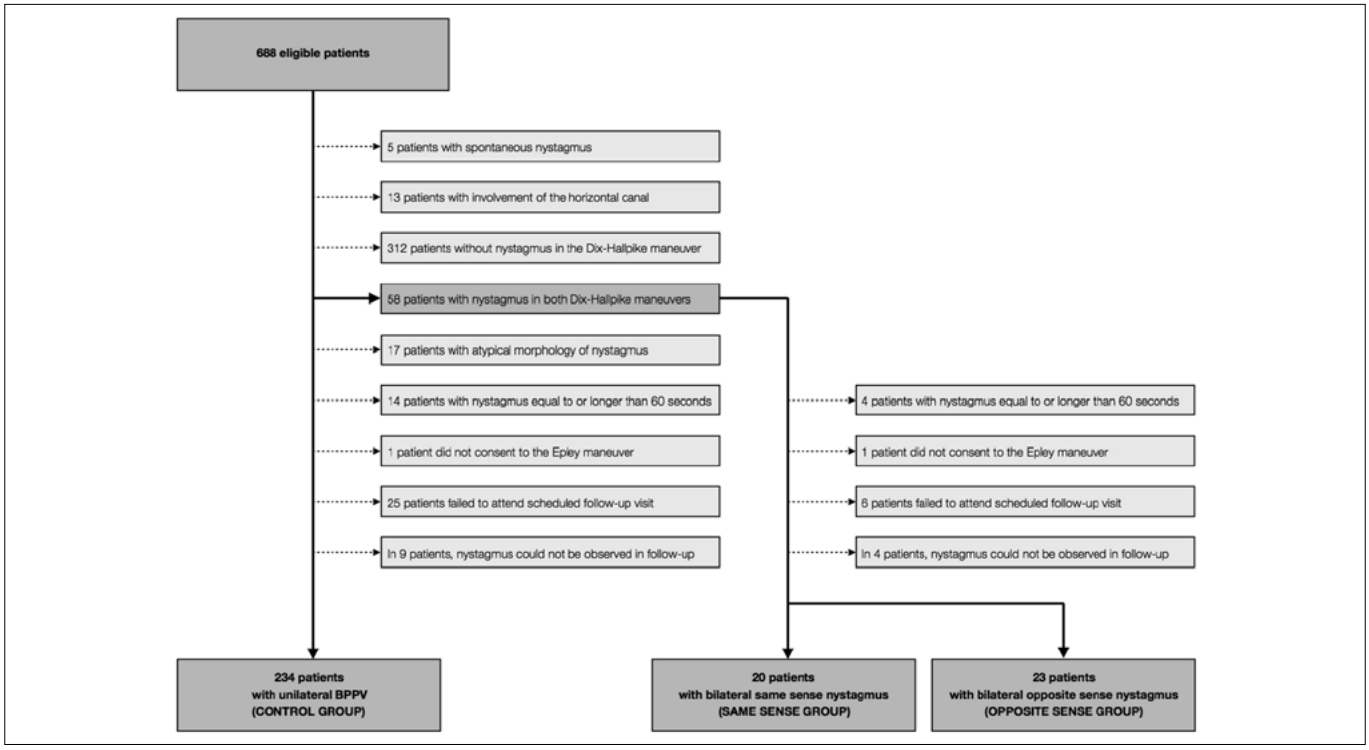

Figure 1. Selection of patients to form the three groups in the study: patients with unilateral BPPV (control group), patients showing nystagmus in both DHMs with the torsional component beating in the same sense (same sense group) and patients showing nystagmus in both DHMs with the torsional component beating clockwise in the left DHM and counter-clockwise in the right DHM (opposite sense group).

One week after the EM, 157 patients in the CG showed negative nystagmus in the DHM on the treated ear, thus giving a resolution rate of 67.1%; 12 SSG patients showed negative DHMs on both sides and 2 SSG patients only a negative DHM on the treated ear (70% ipsilateral negativisation); 6 OSG patients showed negative DHMs on both sides and 6 OSG patients showed negative DHM only on the treated ear side (52.2% ipsilateral negativisation). No significant between-group differences were found in the percentage of ipsilateral negativisation ($p = 0.328$). Table I summarises the results observed.

Figure 2 shows the steps taken to create a model to estimate the incidence of unilateral and bilateral BPPV. In the third step, the rate of negativisation of nystagmus in the CG was used to calculate the number of patients with unilateral BPPV in the SSG and the OSG. According to the model, the proportion of unilateral BPPV in the SSG was 89.5% (95% CI 66.3-98.0%), while in the OSG it was 38.7% (95% CI 20.1-60.8%).

Using the model, patients were reallocated into three new groups: unilateral BPPV (252 patients; 91.0% of total), bilateral BPPV (8 patients; 2.9% of total) and undifferentiated patients, where the EM failed to make the ipsilateral DHM negative (17 patients; 6.1% of total).

Subsequently, the unilateral and bilateral groups were compared to examine two factors which have been associated with a higher risk of bilateral BPPV in the medical literature: a background of traumatic brain injury (TBI) before the start of symptoms and history of previous BPPV in any ear. Looking at the TBI background showed that no bilateral BPPV patient reported having suffered TBI in the three months before the start of symptoms, whereas 7.5% of unilateral BPPV patients did, although the difference was not statistically significant ($p = 0.420$). As for previous BPPV in any ear, 12.8% of unilateral and 50% of bilateral BPPV patients reported a history of BPPV and this difference reached statistical significance ($p = 0.015$).

Discussion

The main contribution of this study to the scientific literature is the demonstration that BPPV may be unilateral or bilateral regardless of the sense of the torsional component of DHM-elicited nystagmuses. When torsional components beat in the same sense, unilateral BPPV was the most likely diagnosis (89.5% of cases): left in the case of clockwise nystagmus and right in the case of counterclockwise nystagmus. However, although less likely, bilateral BPPV was also possible.

When torsional components beat in opposite senses – clockwise in the left DHM and counter-clockwise in the right DHM – there were more cases of bilateral BPPV cases in our sample (61.3%), although interval-based estimations did not support the claim that bilateral BPPV was more frequent than unilateral BPPV. Therefore, in this case, both types of BPPV were possible and their incidence may be the same.

Figure 3 shows that both unilateral and bilateral BPPV may cause nystagmus in both DHMs with the torsional components in opposite senses.

Pseudo-bilateral and pseudo-unilateral benign paroxysmal positional nystagmus

As hypothesised throughout herein and as demonstrated by the data from our population, nystagmuses observed in DHMs may correspond to different physiopathological situations.

At present, the only diagnostic tool available to estimate the situation of otoconia residing in a semicircular canal is the observation of nystagmus after a diagnostic positional manoeuvre such as the DHM. Thus, we found cases of pb-BPPN (pseudo-bilateral BPPN), which suggest bilateral BPPV whereas the condition is actually unilateral, as well as cases (as found in our study) of patients with pseudo-unilateral benign paroxysmal positional nystagmus (pu-BPPN), which suggest unilateral BPPV whereas the condition is actually bilateral.

Pu-BPPN has not been described in the scientific literature. It consists of bilateral positional nystagmus, compatible with canalolithiasis of only one PSC, although there is bilateral PSC BPPV. In our population, we verified only two cases of patients with pu-BPPN (0.72% of the population with BPPV of the PSC). However, given that in some patients it was not possible to determine whether the BPPV was unilateral or bilateral, this percentage might be higher, representing about 10.5% of the SSG (95% CI 1.9-33.7%). Due to the existence of pb-BPPN and pu-BPPN, it is especially important to construct a model, such as the one we offer here, for clinicians to be aware of the different possible diagnoses in clinical BPPV cases where positional manoeuvres do not offer a specific diagnosis.

Table I. Results of the EM in the three groups in the study.

	Bilateral resolution	Ipsilateral resolution only	Without ipsilateral resolution	N
CG	-	157 (67.1%)	77 (32.9%)	234
SSG	12 (60%)	2 (10%)	6 (30%)	20
OSG	6 (26.1%)	6 (26.1%)	11 (47.8%)	23

Comparison of populations

If one compares the percentage of bilateral BPPV in our population (5.8%) with that found in other publications (ranging between 8 and 10.9%) [8,13,14], our percentage seemed to be lower, although the differences were not statistically significant. This finding may be accounted for by the fact that our model can detect pb-BPPN cases, which are no longer considered as bilateral cases.

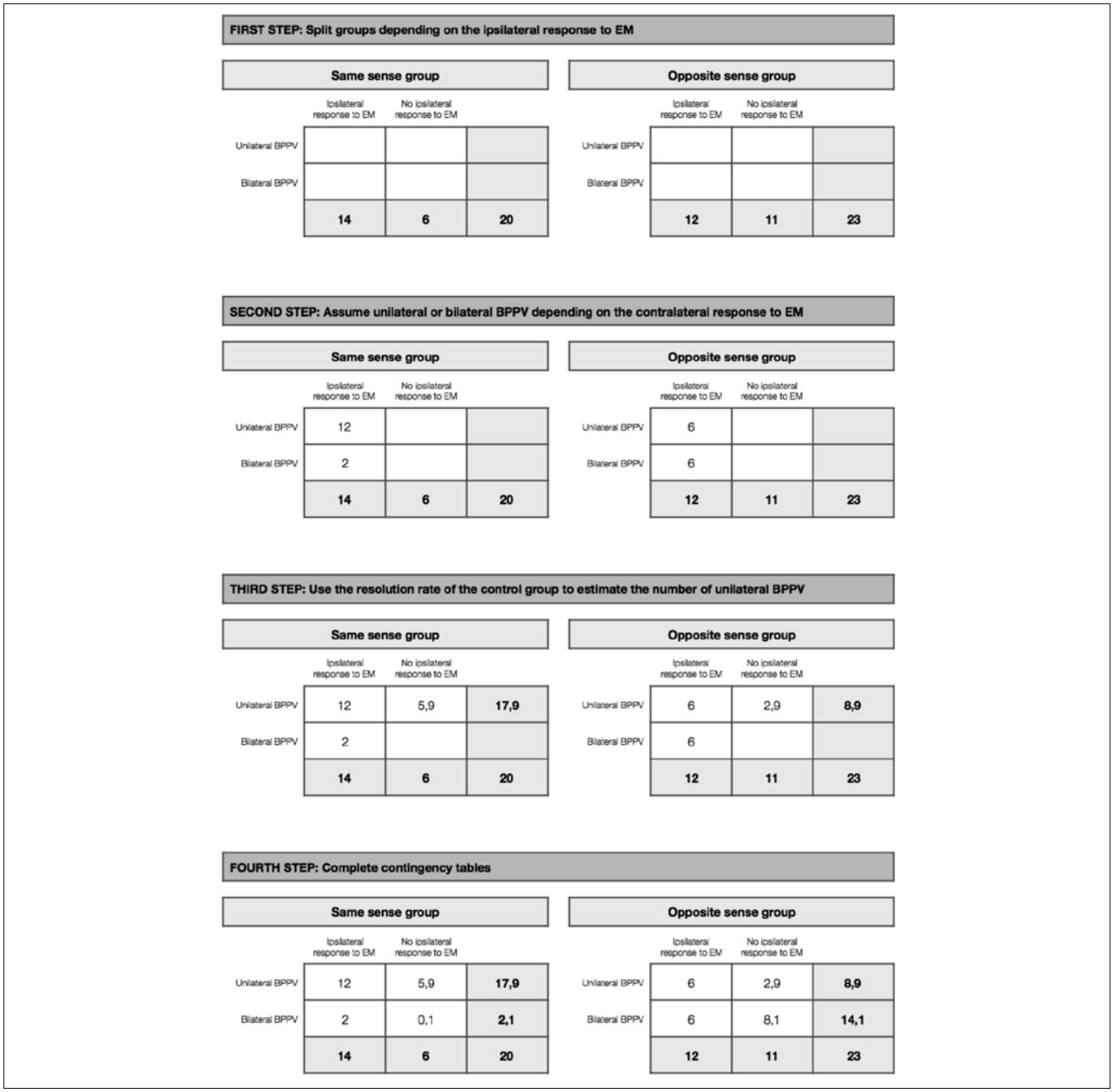

Figure 2. Steps taken to create a model to estimate the incidence of unilateral and bilateral BPPV. First step: creation of a contingency table for the SSG and another for the OSG. These tables show the ipsilateral response to the EM and the number of patients with unilateral or bilateral BPPV. Patients were classified according to their ipsilateral response to the EM. Second step: patients responding to the EM were classified as having unilateral BPPV or bilateral BPPV depending on whether the EM was successful in neutralising the nystagmus in the contralateral DHM. Note that in patients where the EM failed to neutralise ipsilateral nystagmus (second column), the contralateral response cannot be used to differentiate between unilateral and bilateral BPPV. Third step: the EM resolution rate in the control group (67.1%) was used to calculate the total number of patients with unilateral BPPV. Fourth step: contingency tables were completed.

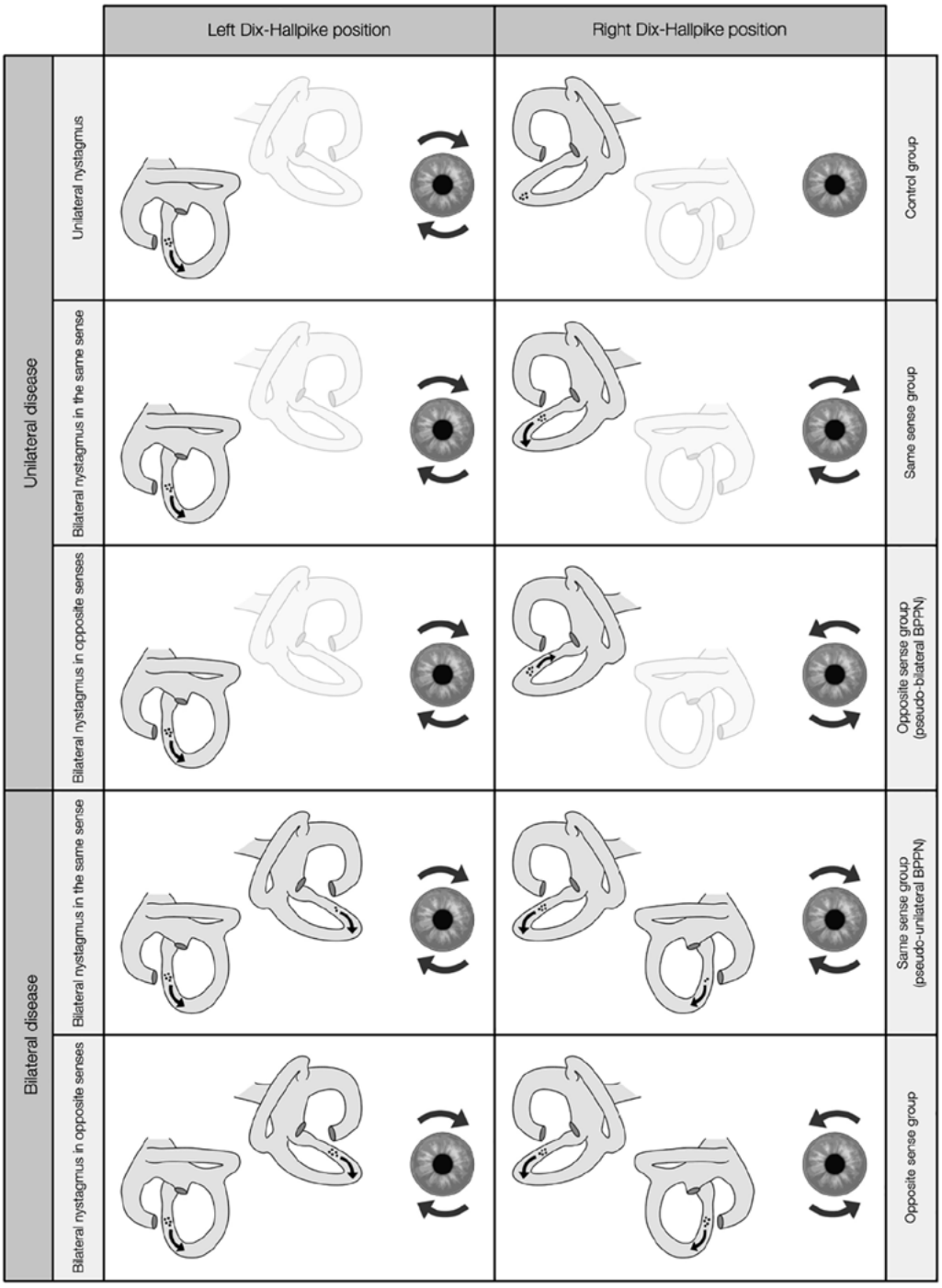

Figure 3. Explanation of how both unilateral and bilateral BPPV can produce clockwise and counter-clockwise nystagmus in the DHMs. The view of the inner ears is from the perspective of an examiner sitting behind the patient's head. The horizontal semi-circular ducts were dissected to offer an enhanced view of the PSC and their ampullae. In case of unilateral BPPV (represented as left BPPV in the figure) on the left DHM, the otoconia move ampullofugally, thus generating clockwise nystagmus. In the right DHM, either the otoconia do not move, thus not generating nystagmus, or they may move ampullofugally, thus generating clockwise nystagmus, or they may move ampullopetally, thus generating counterclockwise nystagmus (pseudo-bilateral BPPN). In the case of bilateral BPPV (higher involvement of the left ear represented in the figure) in the left DHM, the otoconia of the left ear move ampullofugally, generating clockwise nystagmus. In the right DHM, the otoconia of the right ear move ampullofugally, generating counterclockwise nystagmus. The counter-clockwise nystagmus might not inhibit the clockwise nystagmus generated by the left ear as fewer otoconia are involved (pseudo-unilateral BPPN), but it might do so as the right otoconia migrate at a faster rate than the left otoconia and that would create a more intense nystagmus (from Squires, 2004 [21], mod.).

A comparison of the different groups of patients in our population, which were classified according to the DHM outcome or the unilateral/bilateral BPPV type, revealed no significant differences in the proportion of men and women, median age, or age distribution. In a study by Soto-Varela et al. [8], no differences were found in the proportion of men and women, but differences were reported in age distribution.
Although some studies [13-15] report that a TBI background is more common in patients with bilateral BPPV than in those with unilateral BPPV, in our sample (as in the Soto-Varela study) no significant differences were found. Additionally, as in their study, we found significant differences in the percentage of patients with a history of BPPV, which was more frequent in those with bilateral BPPV [8].

Limitations of the model

Although built on physiopathological bases, this model attempted to explain an unknown underlying reality; therefore, it is subject to certain assumptions that are detailed below:

1. *Only the torsional component of nystagmus was used in the model, while the vertical component was disregarded.* The rationale behind this was the fact that the torsional component in BPPV of the PSC is more prominent than the vertical one [16], thus making it easier for clinicians to identify without Frenzel glasses. Other models have been proposed, such as that of Zhao el al., which uses the vertical component and yields mc-BPPV diagnosis for upbeating vertical components on both DHMs and unilateral BPPV for an upbeating component in one DHM and a downbeating component in the other [17] or that of Pollak et al., which uses the disappearance of the torsional component of nystagmus during the head down test to identify true bilateral BPPV [18]. Another method to differentiate between unilateral and bilateral BPPV has been proposed by Imai et al. [6], where an infrared charge-coupled device camera is used to detect the rotation vector of nystagmus in three dimensions and is used as a means of differentiating between the two types. Although this method can differentiate between unilateral and bilateral BPPV when nystagmuses in each DHM beat in opposite senses, it requires instrumental examination and a complex analysis of results.
2. *When nystagmuses in both DHM beat in opposite senses, the side which shows the most intense nystagmus following the manoeuvre is always the most affected one.* In this model, it is assumed that the ear with the most intense nystagmus is always the most affected one and that it has a larger otoconia load than the contralateral ear. The combination of Figure 3 and the third law of Ewald [19], which postulates that, in the PSC, the flow from the cupula to the duct generates a nystagmus of higher intensity than the flow in the opposite sense, makes this seem like a reasonable assumption. However, the complexity of the resulting nystagmus in the case of multicanal BPPV, raises the question whether unusual locations of the otoconia within the semicircular canals could be possible, thus invalidating this assumption.
3. *The percentage of BPPV cases involving the superior semi-circular canal is so low that it can be disregarded.* In our population, only 2% of cases, where only one canal was involved, correspond to the superior semicircular canal (95% CI 0.8-4.4%). If such a confidence interval were extrapolated to mc-BPPV cases, the superior canal would be involved in only 0 to 2% of our patients with nystagmus in both DHMs, a proportion low enough that it should not affect the results of the model in a significant way.
4. *Clockwise nystagmuses in both DHMs cannot correspond to unilateral right BPPV and counter-clockwise nystagmuses in both DHMs cannot correspond to unilateral left BPPV.* In theory, unilateral BPPV with otoconia near the *crus commune* could cause such situations due to ampullopetal flow in both DHMs. However, such a situation seems unlikely due to the fact that otoconia in that location should spontaneously descend to the utricle.
5. *The EM cannot resolve BPPV of the contralateral ear.* For this model to be valid, one must assume that the EM in one ear cannot relocate displaced otoconia in the contralateral ear. Although, as indicated in Figure 3, both DHMs may cause ampullofugal displacement of the otoconia in either ear, the EM produces a spin that would favour contralateral ampullopetal displacement of the otoconia in the PSC and thus it cannot resolve contralateral BPPV.
6. *The BPPV resolution rate of the EM in one ear is not influenced by DHM findings in the contralateral ear or the occurrence of bilateral BPPV.* No significant differences were found between the CG, SSG and OSG, regarding the percentage of ipsilateral negativisation of the DHM after the EM. However, an earlier study identified risk factors that may affect the EM outcome in bilateral BPPV in the same population [20]. In this study, the risk factors shown to affect the EM outcome were compared by looking at the three groups. No significant between-groups differences were found regarding the percentage of subjects with a history of previous BPPV. However, significant differences were found in the subjective intensity of nystagmus, which was higher in the OSG than in the CG. Such a finding could be accounted for by a

larger number of otoconia floating bilaterally and we argue that this could not affect the model in a significant way.

7. *In the 7 days between EM and the follow-up visit, there is no spontaneous recovery of the contralateral ear.* When we were designing this study, we decided that the follow-up visit should be as soon as possible after the initial visit in order to prevent any possible bias resulting from such a situation. In our population, the median time lapse between the beginning of symptoms and the application of the EM was 45 days; therefore, spontaneous recovery in 7 days seemed unlikely.
8. *In the 7 days between the EM and follow-up examination, contralateral BPPV does not develop.* The annual BPPV incidence was calculated to be 0.6% [1]; the weekly incidence must be even lower and therefore this risk was disregarded in this model.

In summary, despite all these objections, the assumptions of the model seem to be reasonable and any possible biases seem to be negligible; therefore, we postulate that this model can reasonably account for the reality of endolymph alterations when both DHMs are positive.

Conclusions

When both left and right DHMs are positive and show torsional nystagmus, both unilateral and bilateral BPPV are possible. When the torsional nystagmus components in both DHMs beat in the same sense, unilateral BPPV is more likely although bilateral BPPV is also possible. When the torsional components beat in opposite senses, unilateral and bilateral BPPV are equally likely.

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Letter to the Editor

Why Italian ENT physicians should be aware of SARS-CoV-2

Perchè gli specialisti ORL italiani devono stare in guardia contro SARS-CoV-2

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KEY WORDS: SARS-CoV-2, coronavirus, infection, otolaryngology, ENT, emergency

PAROLE CHIAVE: *SARS-CoV-2, coronavirus, infezione, otorinolaringoiatria, otorinolaringoiatri, emergenza*

Dear Editor,

at this time our country is perturbed by the outbreak of the so-called severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is imposing our government and health authorities to adopt stringent measures in order to control the disease and limit its diffusion.

This condition has been declared by the WHO the sixth public health international emergency after H1N1 in 2009, polio in 2014, Ebola in 2014 in West Africa, Zika and Ebola in 2019 in the Democratic Republic of Congo.

Since the emergence of this new infection in December 2019 in China, it rapidly diffused to other countries, with the development of new infectious foci in Lombardy and Veneto at the end of February 2020. At the time of writing, more than 101,900 cases have been confirmed worldwide with more than 3,700 new cases developing in the last 24 hours [1]. Italy is currently the first among the most affected European countries, with about 4,630 cases and more than 770 new cases detected in the last 24 hours (of 07 March 2020) [1].

Epidemiological observations suggest a constant increase in the daily number of global cases (both in China and outside it), with a decreasing trend in new cases in China but not in other countries.

Angiotensin-converting enzyme 2 (ACE2; which is mainly located on type I and type II alveolar cells in the human lungs) was found to be the receptor for SARS-CoV-2, and it has been estimated that the binding capability of SARS-CoV-2 is 10-20 times greater compared to that of SARS-CoV [2]. The binding of ACE2 would result in protein hyper-expression responsible for alveolar damage and exert the development of pathologic systemic events.

Despite the possible animal-to-human transmission previously suggested, it is now largely accepted that most cases result from human-to-human transmission by means of droplets or direct contact with a median incubation period of 3 days [3].

In addition, there is actually evidence for SARS-CoV-2 transmission by asymptomatic carriers [4]. While SARS-CoV-2 has been detected in patient stools and on environmental surfaces [5], transmission by an fecal-oral route and through inanimate objects remains unclear. A supposed hospital-related transmission has been estimated to occur in more than 40% of cases [3]. In this regard, the

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non-negligent rate of cases detected among physicians and healthcare personnel is of particular concern [6]. Among infected healthcare workers in China, Dr. Li Wengliang was an ophthalmologist who contracted SARS-CoV-2 from an asymptomatic patient with glaucoma in early January and died one month later. In addition, Chinese state media confirmed the death of Dr. Liang Wudong, a 62-year-old ENT specialist at Hubei Xinhau Hospital in Wuhan after having treated patients infected by SARS-CoV-2.

Some reports suggest that transmission from patients with subclinic infection may occur by aerosol inoculation of droplets and other infected secretions into mucous membranes of the conjunctiva and the airways [7]. Clinical manifestations include fever, cough, dyspnoea, myalgia, headache and diarrhea [3,8]. Upper airway manifestations such as rhinorrhoea and sore throat may also occur (and reported, respectively, in about 4% and 17% of cases) [3,8]. It may be speculated that in some cases milder features commonly described after influenza-like infections such as hyposmia, dyssomnia and hypogeusia may be present as well.

Some ophthalmologists recently posed attention to the risk of cross-infection between patients and physicians during ophthalmologic evaluations [9]. On the basis of these considerations, the risk of contamination of healthcare workers seems to be particularly increased for ENT physicians for several reasons. The first is related to the fact that patients need to remove a face mask in order to undergo objective clinical assessment and that clinicians are placed in close proximity to them. In addition, some clinical maneuvers (oropharyngeal inspection, nasopharyngeal and laryngeal fibre endoscopy) elicit the dispersion of aerosol particles during cough and sneezing.

Under these circumstances, the systematic resort to appropriate personal protective equipment (PPE) including gloves, medical masks, goggles or a face shield, and gowns as recently pointed out by the WHO [10] and their correct use are points of crucial importance especially for ENT physicians. In particular, ENT specialists should specifically adhere to the equipment recommended for aerosol-generating procedures performed on SARS-CoV-2 patients during each examination: i.e. N95 or FFP2 standard or equivalent respirator, gown, gloves, eye protection and apron. We need to remember that the use of gloves does not replace the need for frequent and appropriate hand washing.

Given the absence of specific antiviral treatments, strategies aimed at achieving epidemic control of SARS-CoV2 infection (including improving individual protection) are the only effective measures presently available. Greater awareness from healthcare personnel at increased risk of transmission, including ENT physicians, is also advisable in order to reduce the rate of cross-infection. There is an extremely urgent need for the national diffusion of these preventive strategies and adequate training of medical personnel.

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Letter to the Editor

Feasibility of flow cytometry in the rhinologist's clinic

Attuabilità della citometria a flusso nella pratica rinologica

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KEY WORDS: rhinology, rhinitis, cytometry, cytology

PAROLE CHIAVE: *rinologia, riniti, citometria, citologia*

Dear Editor,

The assessment of the inflammatory pattern in patients with rhinitis is conventionally performed by nasal cytology [1]. However, some studies have investigated nasal inflammation by flow cytometry, even though most aimed at evaluating issues far from conventional rhinology [2-8].

Flow cytometry allows to define a series of additional aspects in comparison with traditional nasal cytology, including cellular volume and density, the antigenic and genetic cellular pattern, and the functional state, such as activation. Moreover, flow cytometry is automated and well standardised, so it may be considered as a precise and accurate method to analyse the cellular pattern in nasal inflammation. On the contrary, it is usually considered expensive and laborious, as requires adequate machinery and well-trained staff.

The current experience was determined to evaluate the feasibility of performing flow cytometry in the rhinologist's clinic. For this purpose, we chose a real-world model such as a clinical setting: 41 consecutive patients (23 males, 18 females, mean age 38.7 years) were visited at a rhinology clinic in two consecutive days and enrolled. All had nasal complaints that need thorough otorhinolaryngological evaluation. Patients were visited, and nasal scraping, endoscopy and lavage were carried out.

Nasal scraping for traditional cytology was performed according to validated criteria [1]. Nasal lavage was performed by slowly instilling 10 mL sterile isotonic saline into each nostril using a 10 mL syringe, while the subject reclined the head and closed the soft palate. The solution was retained for approximately 10 s in the nasal cavities without swallowing. After that, it was expulsed by forward flexing the head, lightly exhaling and rinsing the lavage liquid into a sterile plastic beaker. Patients were strictly instructed to collect only secretions from the nose in the sterile beaker, whereas secretions deriving from the mouth had to be spit into the lavatory. Immediately after collection, NL-fluid was cytocentrifuged and the cell pellet was suspended in flow cytometry buffer (PBS, 0.09% sodium azide, 1% heat inactivated FBS) and stained with antibodies to CD3, CD4, CD14, CD15, CD294, CD203c, and HLA-DR, DP, DQ for 20 minutes at room temperature in the dark. Cells were washed with flow cytometry buffer, resuspended in 0.5% paraformaldehyde, and stored at 4°C in the dark. Samples were acquired within 24 hrs on a flow cytometer (Cytomics

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Table I. Frequencies of inflammatory cells recovered from nasal lavage and visualised by cytofluorimetry.

Cell type	No. of positive cells /mL [mean (SE)]			
	All patients (41)	Allergic patients (16)	Non-allergic patients (25)	P value
CD14+ cells (monocytes)	8.56 (6.80)	19.69 (17.37)	1.44 (0.45)	0.16
CD15+ cells (neutrophils)	25.73 (6.47)	27.94 (5.88)	24.32 (10.01)	0.025
CD294+/230c- cells (eosinophils)	6.46 (1.34)	9.31 (2.19)	4.64 (1.62)	0.03
CD294/203c+ cells (basophils)	0.39 (0.16)	0.81 (0.39)	0.12 (0.07)	0.042
CD3+ cells (T lymphocytes)	8.45 (1.68)	9.25 (3.33)	7.92 (1.81)	1.00
HLA DR+, DP+, DQ+ cells	12.29 (2.20)	14.19 (4.25)	11.08 (2.43)	0.99

FC 500, Beckman Coulter Diagnostics, Brea, CA, USA). Isotype-matched single colour controls were used to control for nonspecific staining and to set analysis gates. CD3 positive cells were defined as T lymphocytes, CD3-CD4 positive cells were T helper lymphocytes, CD14 positive cells were monocytes, CD15 positive cells were neutrophils, CD294 positive and CD230c negative cells were eosinophils, CD294/203c positive cells were basophils, and HLA-DR, DP, DQ positive cells were activated cells.

Table I shows the flow cytometric data. Neutrophils were the most common inflammatory cell recovered by cytofluorimetry. Patients were subdivided in two groups considering allergy: 16 were allergic and 25 non-allergic. Allergic patients had significantly more abundant cellular infiltrate, including neutrophils, eosinophils, and mast cells than non-allergic ones ($p = 0.025$, 0.03, and 0.042 respectively). Comparing outcomes from nasal cytology and cytofluorimetry, there was good agreement, especially for mast cells detectable only by cytofluorimetry as well as for activated cells (HLA-DR, DP, DQ+).

Therefore, the current real-world experience demonstrates that nasal cytofluorimetry may be considered to be a reliable test to assess inflammatory cells infiltrating the nasal mucosa in clinical practice. In addition, cytofluorimetry allows to define the activation state of cells and more precisely detect mast cells. On the other hand, cytofluorimetry needs adequate machinery, trained staff and is more expensive. For these reasons, nasal cytofluorimetry should be reserved to investigational studies at present.

On the other hand, the current experience has some limitations, including the limited number of patients, cross-sectional design and lack of symptom severity assessment. Thus, further studies should be conducted to respond to these unmet needs.

In conclusion, nasal cytofluorimetry may represent a reliable and precise tool for investigating cellular inflammation in patients with nasal disorders.

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