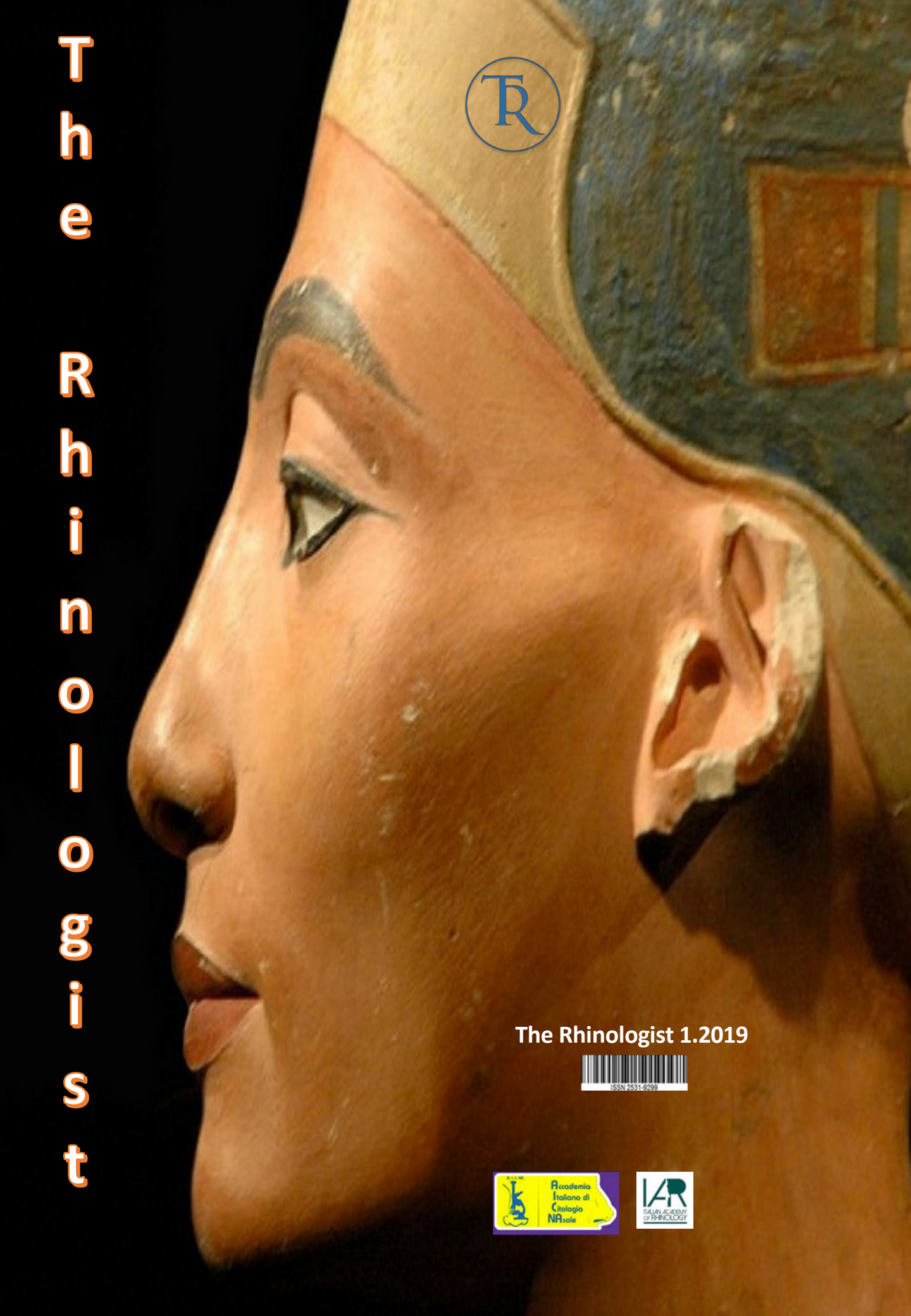
The Rhinologist



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Diagnostic tools in rhinology: the importance of the stratification

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Rhinitis and rhinosinusitis represent a significant health problem in modern society cause their increasing frequency as well as their substantial financial burden on society [1,2]. An accurate investigation of upper airways disorders is extremely important for several reasons [3,4]. The first one is related to the quality of life of patients; the second is that a late diagnosis can lead to severe disorders. The third is associated with the fact that an upper respiratory tract may extend to the lower tract if not correctly treated.

An accurate and precise diagnosis of nasal disease should be fundamental for ENT surgeon as well as for allergist, chest physicians, and pediatricians. A lot of diagnostic tools have been described, and their applicability, specificity, and sensitivity were classified in 2011 with a Position Paper by the EAACI [5].

The ideal diagnostic tool should be straightforward to use, have high specificity and sensitivity and a low cost.

In this paper, we will describe the diagnostic techniques available in rhinology considering their utility and applicability. Not all the diagnostic techniques must be used in every patient, but the diagnostic route should be planned on the basis of the symptoms and the efficacy of the therapeutic strategies using the concept of stratification to avoid unnecessary e and expensive exams [1]. For these reasons, we divided the exams into different levels of complexity.

A) LEVEL ONE

This is considered the basic level in rhinology and should be the armamentarium of all allergist, chest physician and pediatrics as well as ENT surgeons. This first step is vital to understanding, and diagnosing the disease and gives preliminary therapeutic strategies and orient for further diagnostic investigations.

HISTORY OF THE PATIENT

An accurate medical history of the patient is fundamental in every field of medicine. A one to one interview evaluates the presence, severity and duration of symptoms and can

orient the diagnostic route. To assess the severity of symptoms is useful to use a visual analogue scale (VAS).

QUALITY OF LIFE INSTRUMENTS

Sinonasal diseases can have a significant impact on quality of life and the effects of disease on daily activities as perceived by the patient are considered as an essential characteristic of rhinitis severity [4]. Health-related quality of life has been defined as "the functional effects of an illness and its consequent therapy upon a patient, as perceived by the patient". Data are collected on questionnaires that can be generic or specific. Quality of life instruments are used most for clinical trials but can be useful, associated with standard medical measures, to quantify clinical outcome[6].

NASAL EXAMINATION

The evaluation of a patient with sino-nasal symptoms should start with inspection and palpation of the nose and face. An evaluation of the shape of the external nose and nasal valve can give information about anatomical anomalies and post-traumatic diseases as well as a widened dorsum of the nose can indicate the presence of polyps that cause deformation of nasal bones (Woakes Syndrome). After an inspection of the shape of the nose, we can obtain some functional information visualizing nasal valve both during inspiration and expiration.

Anterior rhinoscopy allows an evaluation of the anterior compartment of the nasal cavity and gives the first data that can orient the diagnosis such as septal deviation or congestion of the turbinates. Anterior rhinoscopy is limited in its evaluation of the entire nose bust represent the first step in the rhinological diagnostic route.

ENDOSCOPY

Nasal endoscopy allows visualization and a global evaluation of the nasal cavities. Endoscopy is performed by flexible or rigid endoscope attached to a strong light source by glass fibre and generally connected to a camera.

The exam is generally preceded by local administration of anesthetic and decongestion drugs to improve the visualization end decrease patient discomfort. Rigid endoscopy has proven to be more patient-friendly, supplies a better image than flexible endoscopy and significantly more structures were visualized with the rigid scope than the flexible scope [7]. It is advisable to be meticulous and systematic performing a rigid endoscopy. The exam, using a 0 or 30 degree angled telescope, begins with the inspection of the whole inferior meatus, until the nasopharynx is reached and a good evaluation

of tubaric ostium is obtained . Presence of secretions or polyps from the spheno-ethmoid recess can be seen, a sign of posterior ethmoid or sphenoid pathology. Then, with a backward movement, the inspection of the middle meatus begin again from the nasal valve, avoiding changing the angle of the scope inside the nose to prevent damage and discomfort. This second step aims to search for signs of pathology of the anterior ethmoidal complex by the presence of pathology of the osteo-meatal-complex (OMC) or the posterior one, given by secretions or polyps above the middle turbinate. The third and last step is performing an accurate inspection of the head and the upper portion of the middle turbinate with the exploration of olfactory cleft.

B) LEVEL TWO

Once assessed the medical history of the patients including his symptoms, their severity and a full endoscopic examination of the nasal cavities, some tools are available to achieve a differential diagnosis between and within three main roads: rhinitis, rhinosinusitis with/without polyps and sino-nasal neoplasms.

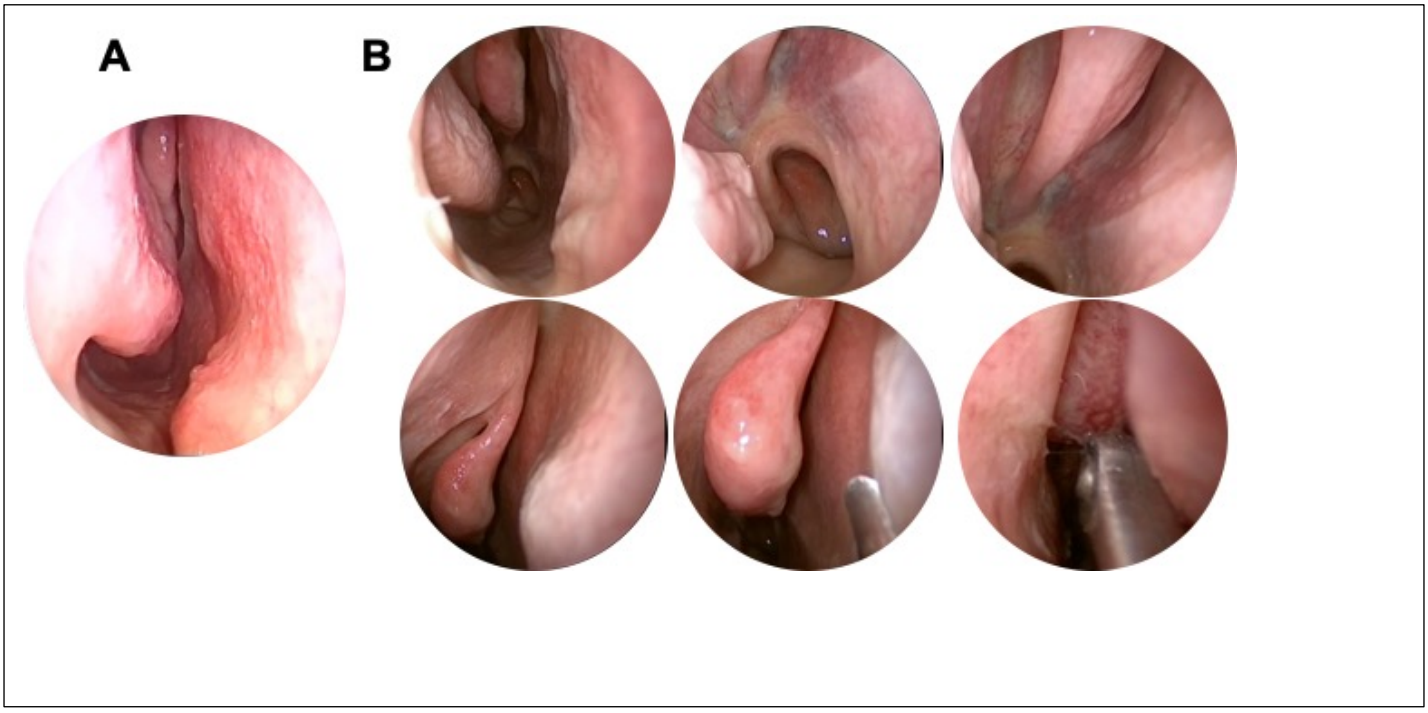

Fig.1 : Different region explorable with A: anterior rhinoscopy. B: endoscopy.

ALLERGY TEST

As the allergen-specific IgE is the triggering factor of symptoms of allergic rhinitis, the goal of diagnostic tests is to demonstrate the presence and activity of such IgE. In vivo skin prick test (SPT) is the gold standard for the detection of allergic sensitizations for its efficiency, safety, and low costs. Second level tests as serum allergen-specific IgE detection, basophil degranulation test and specific nasal provocation test are available to confirm a diagnosis or useful if symptoms and SPT disagree. To remember the need for discontinuation of antihistamines at least five days before the SPT to avoid false negative results.

CYTOLOGY

To better detail the phenotypic characteristics of rhinitis, nasal cytology is a useful, cheap and easy-to-apply diagnostic tool that allows identifying the normal cells (ciliated and mucinous), the inflammatory cells (lymphocytes, neutrophils, eosinophils, mast cells), bacteria, or fungal hyphae/spores. This test aims to discriminate the different pathological conditions and to evaluate the effect of various stimuli (allergens, infectious, irritants, physical-chemical) and it is a crucial tool for the differential diagnosis among rhinitis (Table 1). Evaluation of mucociliary clearance by ciliary beat frequency analyzed with phase-contrast microscopy can also be assessed [8,9].

RADIOLOGY

Imaging of the nose and sino-nasal cavity is used in selected cases in which is necessary to provide anatomic details, define a diagnosis in case of unilateral pathology and/or symptoms, persistent or severe symptoms or doubt of the presence of complications. Standard X-ray and ultrasonography (USG) are usefulness compared to computed tomography (CT) and magnetic resonance imaging (MRI) that provide the best anatomic and pathology details for this region.

CT scanning is the modality of choice for the paranasal sinuses due to the optimal display of air, bone and soft tissue.

Table 1: Examples of differential diagnosis at nasal cytology [8]

	Eosinophils	Mast cells	Neutrophils	Bacteria	Fungal spores
Healthy	0	0	0/1+	0	0
Allergic rhinitis	2+/4+	2+/4+	2+/4+	0	0
NARES	2+/4+	0	Variable	0	0
NARESMA	2+/4+	2+/4+	Variable	0	0
NARNE	0	0	3+/4+	0	0
Common cold	0	0	1+/4+	0	0
Bacterial	0/1+	0	3+/4+	3+/4+	0
Fungal	0	0	Variable	0	2+/4+
Atrophic	0	0	Variable	0	0

Much attention has recently been given to the radiation exposure associated with CT scans, whose use have increased 20 fold in the last 30 years.[10,11] Thus several protocols have been developed to decrease radiation exposure with comparable or improved resolution, and cone beam technology is becoming increasingly available and is associated with lower radiation exposure than conventional imaging having a higher spatial resolution.

MRI does not have the radiation risk and has improved soft tissue definition over CT scan with an ability to differentiate between soft tissue masses and retained/obstructed secretions. Thus, MRI compliments CT in the workup of suspected neoplastic processes [12] or suspected meningoencephalocele.

It should be reminded that incidental abnormalities are found on scanning in up to a fifth of the 'normal' population, so the use of imaging without an appropriate question should be avoided [3].

C) LEVEL THREE

Planning a surgical or medical treatment or managing a patient refractory to a first line empiric therapy, often needs for further exams. Microbiology tests are advised to plan a targeted therapy, histologic tools to confirm a suspected diagnosis and schedule the correct treatment and some blood tests can help to discern ambiguous diagnosis or to monitor the response to therapy.

MICROBIOLOGY

The evaluation of the presence of virulent pathogens inside the nasal and/or sinus cavities is an available diagnostic tool, but its use should be considered in selected cases. From literature data, there is robust evidence, proved by randomized double-blind placebo controlled trials, that advise the use of antibiotics as treatment of acute rhinosinusitis but there is no evidence that antibiotic therapy based on microbiological sampling gives better outcomes compared to empiric antimicrobial therapy in non-complicated acute rhinosinusitis[3,5]. Thus, nasal and sinus samples for microbial assessment should be used in patients affected by acute rhinosinusitis non-responsive to empirical antimicrobial treatment, in patients affected by complicated acute rhinosinusitis or in patients affected by fungal rhinosinusitis [12] .

HISTOLOGY

Histopathological examination of tissue sampled by the nasal cavity or paranasal sinus is mandatory in four main scenarios: the confirmation of a suspected autoimmune disease whose onset often is a nasal pathology (e.g. Wegener granulomatosis, Churg-Strauss disease); the pathological diagnosis of chronic rhinosinusitis with nasal polyps; the definition of characters of fungal

rhinosinusitis and for the diagnosis and evaluation of complete resection of sinonasal neoplasm. Before performing a biopsy in local or general anesthesia of masses of nasal cavity or paranasal sinus it is necessary to get crucial information from CT scans and also perform a MRI with FLAIR and/or fat suppression sequences if bone interruption between skull base or orbit and nasal cavities are suspected or if a suspected neoplastic or unilateral lesion is being studied. A biopsy of a meningoencephalocele, of an orbital herniation or a juvenile angiofibroma should never be performed.

BLOOD TESTS

Some blood and biochemical analysis can help to confirm or refute some specific diagnosis. Among rhinitis, they are useful to discriminate allergic etiologies from autoimmune ones or also to confirm a suspected CFS leak (Beta-2 transferrin or Beta-trace measure). A definitive diagnosis of rhinosinusitis could be needed for a full blood analysis including inflammatory markers as the C Reactive Protein, the presence of acquired immunodeficiency and c-ANCAs and p-ANCA measure.

D) OTHERS

To evaluate some the main nasal functions as the respiratory one, the sense of smell and the ciliary activity, some specific tests are available; most of them are not routinely used in clinical practice and are limited to research fields, but in particular cases they can be useful to answer specific clinical questions.

EVALUATION OF NASAL PATENCY

Currently available techniques to evaluate the nasal respiratory airflow are the peak nasal inspiratory flow measurement, the active anterior rhinomanometry, the posterior rhinomanometry, and the acoustic rhinometry.

Nasal inspiration correlates most with the subjective feeling of obstruction; peak nasal inspiratory flow evaluation represents a physiologic measure of the air flow through both nasal cavities during forced inspiration expressed in liter per minute, and it is the best-validated technique for the evaluation of nasal flow through the nose[5].

The active anterior rhinomanometry is the technique used most for the evaluation of nasal airflow resistance and in general to have a functional measure of nasal patency. It represents a physiologic measure of nasal airflow and pressure during normal inspiration and expiration giving information on each nostril separately and being an easy technique and not time-consuming although it is not feasible in case of total obstruction of one nostril.

The posterior rhinomanometry evaluates both nasal cavities at the same time; its execution is more laborious, but it is reported a good correlation with a subjective feeling of nasal obstruction.

Acoustic rhinometry is a non-physiologic technique that gives a measure of nasal obstruction analyzing reflections of a sound pulse introduced via the nostrils. This technique is rapid, reproducible, non-invasive and requires minimal cooperation from the subject as it does not need airflow; anyway, a weak correlation with subjective nasal congestion has been seen[13].

ASSESSING SENSE OF SMELL

In patients reporting smell dysfunction, once a complete medical history of the patient and a full examination of head and neck region are undergone, some specific olfactory tests are available to fully determine the disease burden and clinical impact of interventions[14].

In general, three different types of olfactory testing can be undertaken: subjective, patient-reported olfactory assessment; psychophysical olfactory assessment and olfactory assessment using electrophysiological studies or magnetic resonance imaging.

Among patient reported olfactory assessment, where possible, validated questionnaires should be used; where this is not possible, a recognized form of assessment, possibly quantitative and/or anchored, such as a visual analogue scale, should be used. Psychophysical assessment tools used in clinical and research settings should include tests of odor threshold, and/or one of odor identification or discrimination; psychophysical assessment tools should be reliable and validated for the target population[14].

Olfaction can also be assessed in a less subjective way using electrophysiological and imaging studies. Electrophysiological studies include electroencephalography (EEG) and electro-olfactography (EOG - the recording of generator potential via an electrode in contact with the olfactory neuroepithelium) [15,16]. EEG, in particular, is useful in medico-legal assessment as well as in patients who might not be able to comply with psychophysical testing while EOG testing is limited to the research setting. Functional imaging allows for the identification of brain activity in response to odorous stimuli and includes positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) [17].

EVALUATION OF MUCOCILIARY CLEARANCE

The mucociliary transport mechanism ensures the clearance of entrapped particles in the mucus lining the nasal mucosa towards the hypopharynx. Severe dysfunction of

mucociliary transport, like in primary ciliary dyskinesia, is often diagnosed early in the childhood, while the diagnosis of secondary ciliary dyskinesia is less easy and needs specific exams to clarify an unexplained chronic rhinosinusitis refractory to treatments. Some specific tests permit the assessing of such function, including noninvasive procedures and invasive ones[5].

Although the evaluation of ciliogenesis in vitro with a biopsy of the nasal mucosa is the gold standard for diagnosis of PCD, allowing the differentiation between primary and secondary ciliary dyskinesia, several non-invasive procedures are available to reach a reliable differential diagnosis.

Test evaluating the mucociliary clearance time are non-invasive procedures and the most common used are the saccharine test that assesses the time a patient needs to have a sweet taste after placement of a 1-2 mm particle of saccharine on the inferior turbinate mucosa and the blue methylene test, in which it is evaluated the time of a dye-like methylene blue to be transported from the mucosa of the anterior third of the nasal cavity towards the hypopharynx. The mucociliary clearance time is considered to be normal below 15 minutes and should be less than 1 hour. Although these tests are non-invasive and quick to perform, they require the cooperation of the patients and patency of nasal cavities with the absence of severe mucosal disease, so their sensitivity and specificity are poor[5].

With a sterile cytology brush it is possible to harvest epithelial cells to perform further analysis as either structural investigation of the cilia with electron microscopy or for measuring ciliary beat frequency (CBF). Electron microscopy permits to investigate several abnormalities in the dynein structures of the epithelial cilia [18]; CBF measurement consists of the analysis in detail, by digital high-speed video imaging, of the frequency of the beating of cilia as well as of their coordinated movement. The demonstration of normal CBF and beat pattern excludes the diagnosis of PCD.

CONCLUSION

A wide amount of tests are available to evaluate sino-nasal morphology, pathology, and function. The correct choice of the diagnostic route to achieve the final diagnosis is crucial for many reasons, first of all, to begin the right therapy quickly, if needed, also to avoid useless and invasive procedures, if not recommended, and last to take advantage better of the limited resources available.

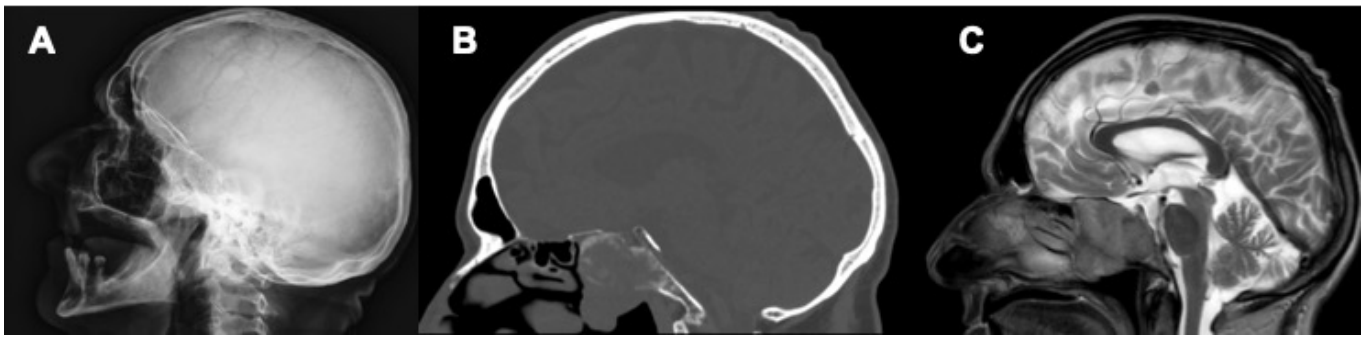

Fig. 2 : Sagittal scan of three different radiological exames. A RX scan: result an opacification of sphenoid sinus without any detailed anatomical informations. B TC scan: result an opacification of sphenoid sinus and the bone erosion of sphenoidal sinus is clear. C RM scan T2: result a sphenoidal mass with a low vascularization, strictly in contact with the skull base without a meningeal infiltration

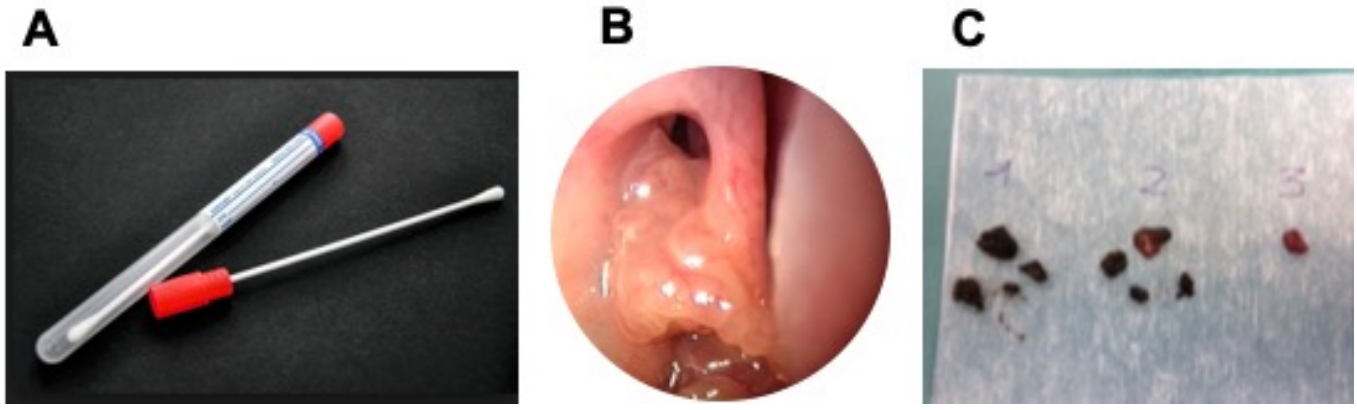

Fig 3. : Biological material can be collected A: for microbiological exam. B: for histopathology C: the combination of microbiology and histopathology such as a case of fungus ball.

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Nasal cell vitality: the pidotimod activity in elderly with recurrent respiratory infection

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Key words: Nasal Cell Vitality, SISS, Pidotimod, Recurrent respiratory Infections

Abstract

Recurrent respiratory infections (RRI) are a widespread, increasing problem especially for elder age.

Nasal cell functionality represents the first-line defense against pathological agents coming from external enviroment and cellular morphology and activity can be easily demonstrated by a simple examination like microscopic nasal citology, and this can give important informations on patients' clinical status. The possibility of acting on behalf of repairing or enhancing cellular damages, expecially of the nasal mucosa, allows to create an effective barrier against external assaults.

The present study has shown that an immunostimulant like Pidotimod can interact at a cellular level, enhancing cellular functionality and cellular vitality inside nasal mucosa, allowing a significant reduction on the number of recurrent respiratory tract infection in elders age.

INTRODUCTION

Despite the use of antibiotics and vaccines, the frequency of respiratory tract infections is still high and these infections interest a wide range of patients, from children to aged people, including in particular these extreme

categories because of the deficiency of their immune system, due to immaturity in the former case and to "immunosenescence" in the latter. [1]

Indeed in the elderly, wear and tear of the human body results in reduce organ function, overstretches the system [2], and places grater demand on normally redundant elements of host defence to infection [3]. For that reason, immunostimulant drugs are getting more important to prevent and to attenuate infections [1].

Moreover the socio-economic burden of RRI is very high considering direct costs for drugs and hospitalization [1].

Pidotimod (3-l-Pyroglutamyl-1-tiazolidine-4-carboxylic acid used in subjects 3 years of age or older) is a synthetic dypetide immunostimolant acting on both adaptative and innate immune systems [4] inducing dendritic cells maturation, increasing NK, neutrophilic and macrophagic cells activity (4). Pidotimod activates Th1 response increasing gamma-IFN production, and it disables the Th2 response. Furthermore it increases production and secrection of salivary IgG and secretory IgA. Studies conducted since the 90's on animal and human models have shown that Pidotimod is able to modulate the inflammatory cascade, to up-regulate the expression of HLA-DR and different co-stimulatory molecules and to balance the intestinal flora [1].

Nasal cytology has been an important diagnostic test and has been increasingly used in the rhinological field to differentiate between different types of rhinologic diseases. It helps us discriminating between different citotypes (eosinophils, mast cells, neutrophils, bacterias, spores) in order to calculate their percentages comparing to the number of total leukocytes. A direct index of cell integrity is represented by the hyperchromic stria present in ciliated cells, immediately above the nucleus, called "hyperchromatic supranuclear stria" (SNS). This stria is selectively present in healthy cells (not affected by cellular alterations of the

nucleus, of the cytoplasm and the ciliary apparatus).

Aim of this study is to observe Pidotimod efficacy in reducing RRI recurrence and to state its improvement on the nasal mucosa's inflammation stating cytological differences between treated and non-treated patients, based on the cellular types, the SNS cellular status and cilia motility.

METHODS AND STUDY POPULATION

The study was performed in accordance with the declaration of Helsinki and the patients gave their written consent to participate.

From September 2018 to November 2018, a single-blind randomized parallel groups study was carried on to 35 patients, aged between 70 and 85 years old (mean age 76 years old). 18 were males and 17 females. All the patients were enrolled after a specialist outpatient examination in the Otorhinolaringology Clinic at A.S.S.T. Sette Laghi in Varese.

In order to investigate if the treatment with Pidotimod could result in less airway inflammations, patients were randomized: 20 patients (10 male and 10 female) received the drug (Case Group) and were treated with Pidotimod in oral solution once daily for 20 days every month for 3 consecutive months; the other 15 who did not receive the drug represented the Control Group.

All patients were examined twice: at Visit 0 (before treatment start) and after the third month of treatment (Visit 1). During both visits nasal cytology was performed (after at least 10 days drug wash-out from antihistamines and steroids), clinical history of IRR and the need for antibiotics therapy were collected.

Cytological sampling was performed by scraping with Rhino-Probe in the middle part of the lower turbinate, an area characterized by a significant number of cells, in particular ciliated cell. After air fixation the slides were coloured with May-Grünwald-Giemsa staining method. The microscopic observation with a

400X enlargement allowed us to check coloration quality and cells distribution and the 1000X enlargement, in immersion, allowed us to discriminate between different cytotypes (eosinophils, mast cells, neutrophils, bacteria, spores) in order to calculate their percentages comparing to the number of total leukocytes and to study the intracellular components and identify the hyperchromatic supranuclear stria (SNS). (9)

RESULTS

The nasal cytological examination before and after the treatment in both groups of treated and not-treated patients gave the following results:

Neutrophils in nasal inflammation: no difference was recorded in the Control Group in neutrophilic inflammation. In the Case Group, neutrophilic inflammation rate was lower than before treatment, resulting in a reduction of the cell count and in a total absence in 20% after therapy with Pidotimod. The low percentages make this result not statistically relevant.

Eosinophil in nasal inflammation: 15% of Case Group had no eosinophil cells at Visit 1. In the Control Group no differences were observed at Visit 0 and 1 .

Mast-cells in nasal inflammation: in 25% of Case Group mast-cells inflammation rate was lower after therapy and in 25% was completely absent after therapy. In 10% of the Control Group mast-cell counts were reduced. These results are not statistically relevant.

Lymphocytes in nasal inflammation: only 10% of treated patients had lymphocytes cells at the control but these values are not statistically relevant.

Bacterial inflammation: 90% of treated patients and 100% of not-treated patients showed no difference before and after the treatment. 10% of treated patients showed a reduction of bacterial inflammation and other 20% patients showed absence of bacteria after treatment.

SNS expression in ciliated cells: it showed to be improved in 8 patients treated with Pidotimod; the same result was not found in not-treated children (**Table 1**). Anyway the small number of patients makes the result not statistically relevant.

	NOT TREATED GROUP (PLACEBO)		TREATED GROUP (PIDOTIMOD)	
	Before treatment	After treatment	Before treatment	After treatment
N° patients	15	15	20	20
SNS +	5	7	10	18
SNS -	10	8	10	2
X^2	P = 1.0 ns		P = 0.191 ns	

Table 1: *SNS expression in ciliated cells. No differences were noticed in SNS expression into the group of not-treated patients. On the other hand, in the group of patients who received the treatment there was an increase of SNS expression in 8 patients. Anyway this result is not statistically relevant (p=0.191).*

Cells motility: All not-treated patient showed no differences before and after treatment; in the treated group there was an improvement of cellular motility in 55% patients while in 45% no differences were found (**Table 2a and 2b**).

	NOT TREATED GROUP (PLACEBO)		TREATED GROUP (PIDOTIMOD)	
	Before treatment	After treatment	Before treatment	After treatment
N° patients	15	15	20	20
Cellular Motility 1’	6	6	8	17
Cellular Motility 2’	3	3	3	2
Cellular Motility 3’	6	6	9	1
X^2	P = 1.0 ns		P = 0.007*	

Table 2a: Cells motility. This table shows cellular motility noticed at 1,2 and 3rd minutes. No differences were found into the not-treated group of patients, while an increase of cellular motility was noticed in the second group. The result is *statistically* significant (p = 0.007).

	NOT TREATED (PLACEBO)	TREATED (PIDOTIMOD)	X^2
N° improved	0	11 (55%)	P = 0.000***
N° not improved	20 (100%)	9 (45%)	

Table 2b: *Improvement of cellular motility shown by percentages.* 45% of treated patients proved to have an increased cellular motility.

These values are statistically significative as shown by the P value.

Clinical history of IRR and need for antibiotics: Pidotimod showed to be effective reducing the number of IRR and the need of antibacterial treatments as shown in the following tables.

3% of treated patients showed no difference in the number of IRR before and after therapy as well as 60% of not-treated children. 35% of treated patients reported no more IRR after therapy and the same result was found in 5% of not-treated patients (**Table 3a, 3b and 3c**)

N° EPISODES	BEFORE STUDY	DURIGN STUDY
NOT-TREATED (PLACEBO)	102	70 (-31%)
TREATED (PIDOTIMOD)	98	21 (-78%)
χ^2	P=0.000 ***	

Table 3a: *Clinical History of IRR.* This table shows the number (and the relative percentages) of acute respiratory infections' episodes recorded before and during the study. It seems that in the not-treated group there was a reduction of the number of episodes of only 31%, while inside the group of patients who received Pidotimod there was a reduction of 78% of acute episodes. These values are statistically significative as shown by the P value.

	NOT TREATED (PLACEBO)	*TREATED (PIDOTIMOD)*	χ^2
PZ IMPROVED	6 (40%)	18 (90%)	P=0.006**
PZ UNCHANGED	9 (60%)	2 (10%)	

Table 3b: *Number of patients who did and who did not showed to have an improvement in the number of IRR.*

Inside the not-treated group 55% of patients did not change the number of IRR episodes before and after the study. The group of patients who received Pidotimod showed to have an improvement during the study (in terms of reduction of the IRR episodes) in the percentage of 90%: only 2 patients out of 20 did not improve.

	NOT TREATED (PLACEBO)	*TREATED (PIDOTIMOD)*	χ^2
PZ RECOVERED	2 (5%)	7 (35%)	P=0.008 *
PZ UNCHANGED	13 (95%)	13 (65%)	

Table 3c: *Number and percentages of patients who did not have any other IRR episode during the study period.* This table shows that those patients who did receive treatment with Pidotimod completely recovered in 65% of cases, while those who did not get the treatment unchanged in 95% of cases.

A statistically significant decrease of antibacterial treatments was observed in 85% of treated patients and, in the same way, 50% of treated patients did not need any antibacterial treatment after therapy as well as only 5% of not-treated patients (**Table 4a and 4b**):

	NOT TREATED (PLACEBO)	*TREATED (PIDOTIMOD)*	χ^2
PZ IMPROVED	6 (40%)	18 (85%)	P=0.009 **
PAZ UNCHANGED	9 (60%)	2 (15%)	

Table 4a: *Use of antibiotics during IRR episodes.* 85% of paatients who received Pidotimod showed an improvement in IRR number and therefore in need for antibiotics treatments. On the other hand, those who did not get Pidotimod did not show any improvement in 60%.

	NOT TREATED (PLACEBO)	*TREATED (PIDOTIMOD)*	χ^2
NO NEED FOR AB	2 (5%)	10 (50%)	P=0.005 **
UNCHANGED	13 (95%)	10 (50%)	

Table 4b: *No need for antibiotics during IRR episodes*. In the group on not-treated patients only 2 patients did not need any antibiotic treatment during the study. Inside the group of treated patients there were 10 patients who did not need to take any antibiotic treatment during the study period.

DISCUSSION

Recurrent respiratory infections (RRI) represent a huge problem among elderly. The use of pidotimod as immunostimulant has been shows to be effective in pediatric patients (7-13). Moreover, no differences in absorption, excretion and pharmacokinetic parameters was evident between old volunteers and the young of a previous work [8].

The benefits of the pidotimod was also documented by cytologic exam in the pediatric patients.

Nasal cytology is a simple, economic, repeatable, and a safe diagnostic procedure that allows to assess the normal and pathological aspects of the nasal mucosa by identifying and counting the different cell types and their morphology (12).

Ciliated cells are highly differentiated compared to other cellular types in the upper airways, and they are the most affected by degenerative process during inflammatory diseases (11). The hypercromatic supranuclear stria (SNS) is detected only in ciliated cells because this type of cell demands a significant amount of ready-to-use and reserve energy, and it represents a special accumulation of proteins. The SNS is therefore considered to be a marker for the anatomical and functional integrity of ciliated cells. Its' absence is a prognostic sign of the disease themselves [9]. To confirm this, a statistically significant decrease in the percentage of positive streaks (SNS +) was observed in vasomotor, inflammatory and infectious rhinitis, and it was found that this percentage correlates with the severity of the disease. [11]

Pidotimod (Axil) is a well-tolerated and safe immunostimolant. According to recent studies

it is able to reduce the number of respiratory infections, the days of fever, the severity of symptoms and the use of antibiotics [1]. In Vitro animal and human models showed that Pidotimod is able to modulate both innate and adaptive immune responses.

Considering the nasal inflammation as an index of welfare of upper and lower respiratory airways, we conducted a randomized controlled study was conducted in order to evaluate Pidotimod efficacy in reducing nasal inflammation and improving elderly quality of life too.

35 elderly patients affected by RRI were enrolled and randomized into two groups: one treated with Pitodimod and the other with placebo. Both groups were observed for three months. In order to determine the level of nasal inflammation, nasal citology and cell motility of each patient was studied at the beginning and at the end of the period of observation. The number of respiratory infections and the need for antibiotics use have also been evaluated.

According to patients' rhinocytograms there was no significant reduction of eosinophils, neutrophils, mast-cells and lymphocytes in the two groups at visit 1. However we observed a stastically significant improvement of nasal cells motility ($p = 0.007$). Nasal mucociliary clearence is essential for the defense of respiratory airways by protecting the lung against organic and inorganic particles and it is known to depend strictly on the protein reserve of the cell itself: the more healthy the cell is, the more proteins it has stored inside the cytoplasm, the more it moves; and the protein reserve is represented by the supranuclear hypercromatic stria, which therefore represents an indirect sign of wellness of nasal ciliated cells. [8]. In our study elderly treated with Pidotimod showed an increase of the SNS wich anyway was not statistically relevant ($p>0.0191$ ns).

A significant decrease of the number of respiratory tract infections ($P < 0.006$) and of use of antibiotics after the treatment with

Pidotimod ($p < 0.009$) was also shown to be statistically relevant.

CONCLUSIONS

Nasal cell functionality represents the first-line defense against pathological agents coming from external enviroment and cellular morphology and activity can be easily demonstrated by a simple examination like microscopic nasal citology, and this can give important informations on patients' clinical status.

The possibility of acting on behalf of repairing or enhancing cellular damages, especially of the nasal mucosa, allows to create an effective barrier against external assaults.

The present study has shown that Pidotimod can interact at a cellular level, enhancing cellular functionality and cellular vitality inside nasal mucosa, allowing a significant reduction on the number of recurrent respiratory tract infection in elderly patients.

Since RRI have an important impact on elderly quality of life, new ways of treatment are needed. It has been widely demonstrated that Pidotimod is an effective immunostimolant. By our study we can confirm its ability in reducing the number of respiratory tract infections and the need for use of antibiotics.

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Endoscopic endonasal approach for resection of a large olfactory schwannoma with extension to the anterior skull base

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Compliance with Ethical Standards

The Authors declare that they have no conflict of interest. All procedures performed in studies involving human participants were in accordance with the ethical standards of our institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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ABSTRACT

Objective. The achievement of a fully endoscopic endonasal approach for the resection of a large olfactory schwannoma with extension to the anterior skull base. Study design. Case report. Methods**.** A case of a large benign schwannoma originating from the olfactory groove is reported. Surgical treatment is considered the treatment of choice to achieve long-term success. In this report we describe the positive surgical outcome after a fully endoscopic endonasal procedure for large tumor and the advantages and disadvantages of this kind of approach. **Conclusion.** We report the case of a large olfactory schwannoma completely resected with an endoscopic endonasal approach. Reconstruction was performed with a double nasoseptal flap. To date this is one of the largest olfactory schwannoma that has been resected with a fully endoscopic endonasal approach.

Keywords: olfactory groov schwannoma, endonasal approach, endoscopic approach, skull base

INTRODUCTION

Schwannomas or neurilemmomas are benign tumors deriving from the nerve sheet. They rarely involve the nose and paranasal sinuses; in fact, 25-45% of all schwannomas are seen in the head and neck region and only 4% occur in the nose and paranasal sinuses [1]. They are believed to origin from the ophthalmic and maxillary branches of the trigeminal nerve; the specific nerve of origin is rarely identified preoperatively or during tumor resection. The definitive treatment is surgical, with complete removal of the lesion. The surgical approach, depending on size and location of the tumor, can be a traditional external approach or an endoscopic endonasal approach. Traditional approaches are performed for larger lesion and endoscopic endonasal approaches are possible with smaller lesions[2]. In this article

we report the case of a large frontoethmoidal schwannoma extended to the anterior cranial fossa of the left side treated exclusively with an endoscopic endonasal resection.

CASE REPORT

History and examination. In December 2017 a 72-year old woman presented with a history of worsening proptosis of the left eye, left nasal obstruction and hyposmia; she also reported frequent headaches. She did not report signs of visual impairment. An MRI with contrast media enhancement showed a large hourglass shape left fronto-ethmoidal mass with a prevalent solid component, situated in the left cribriform plate of the lamina mediana that appeared completely eroded. The lesion was well-defined, heterogeneously hypointense in T1 and hyperintense in T2, and had small central necrotic areas. The maximum diameters were 55x28x42 mm (CC-LL-AP). From the cribriform plate it reached inferiorly the ethmoidal cells, laterally it dislocated the lamina papyracea towards the orbital content with no clear signs of orbital infiltration, and superiorly it elevated and compressed the frontal lobe with falcine midline shift from left to right, with no surrounding edema (Fig. 1 and 2). The patient also underwent an endoscopic endonasal biopsy in local anesthesia that diagnosed the lesion as a schwannoma. A CT scan obtained at our hospital showed erosion of the cribriform plate and partial erosion of the lamina papyracea on the left side. Clinical endoscopic examination showed a smooth large mass occupying the upper portion of the left nasal fossa.

Surgical procedure. The surgery was performed by a two-surgeon multidisciplinary team composed of a skull base neurosurgeon and an otolaryngologist specialized in endoscopic sinus and skull base surgery. Because of the inferior extension of the mass and to avoid frontal lobe retraction, surgeons planned to start with an exclusive endoscopic endonasal approach to achieve complete resection of the tumor and subsequent reconstruction of the skull-base defect. At the

same time an informed consent was obtained from the patient in case of need to switch from an exclusively endoscopic endonasal approach to a traditional external approach: for this reason the patient was prepared for an eventual bifrontal craniotomy.

After anesthesia induction, cotton patties, soaked in 2ml of 1:1000 adrenaline, were placed into the nasal cavities to allow for mucosal decongestion. After fixation of the patient's head, the head was slightly extended and rotated to the right. The patient's abdomen and right leg were prepped and draped in a sterile fashion in case fat or fascia grafts were needed.

The tumor occupied entirely the left nasal fossa so it was impossible to visualize the choana posteriorly and the superior and posterior portion of the septum, from which the lesion seemed to be separable. Under a 0° degree endoscopic visualization a debulking of the inferior portion of the tumor was necessary to visualize the choana, followed by a medial maxillectomy, total ethmoidectomy and sphenoidotomy. The tumor anterior boundary was posterior to the posterior wall of the frontal sinus, that in this patient appeared hypoplasic, so it was not necessary to perform a frontal sinusotomy. Eventual bleeding from the turbinal branches of the sphenopalatine artery was controlled with cauterization using the bipolar forceps. Medially the tumor compressed the nasal septum shifting it from left to right, but there was no infiltration of the mucosa. With a 45 °degree endoscope smooth dissection of the lesion was continued laterally from the medial portion of the orbit content, that appeared exposed since the tumor had partially eroded the lamina papyracea. The anterior ethmoidal artery was contained within the tumor: it was carefully identified, coagulated and sectioned. Dissection and debulking continued towards the ethmoidal roof until an erosion of the bone and the dura mater were encountered through which the tumor attached itself to the anterior portion of the left frontal lobe. We proceeded with smooth dissection of the

tumor from the frontal lobe. For reconstruction after tumor resection two vascularized naso-septal flaps were harvested: one on the left side based upon blood supply from the sphenopalatine artery and one on the right side based upon blood supply from the anterior ethmoidal artery. Autologous fascia lata graft and fat graft was taken from the right thigh. Reconstruction was then performed using a triple layered technique with autologous fascia lata, adipose tissue and both the vascularized pedicle flaps (Fig. 3). At the end of the procedure the patient was moved to an intensive care unit where she spent the first night. Nasal package was removed after 2 days. Instructions were given to the patient on how to avoid maneuvers that place undue pressure on the reconstruction. A soft bowel regimen was instituted for a period of 6 weeks and a post-operative CT scan was obtained to assess for sequelae such as subdural hematomas and tension pneumocephalus (Fig.4). The patient was dismissed after 7 days of hospital stay. A post-operative MRI was obtained after 6 months showing no signs of recurrence (Fig.5).

DISCUSSION

Schwannomas or neurilemmomas are benign tumors deriving from the nerve sheet. Sinonasal schwannomas typically arise from the ophthalmic and maxillary branches of the trigeminal nerve, or from autonomic nerves in and around the paranasal sinuses[3]. In our case the patient presented with an olfactory groove schwannoma. The mainstay of treatment is gross total resection of the lesion and adjuvant therapy is not typically needed[4]. The traditional approach for resection of olfactory schwannomas is the bifrontal craniotomy. This kind of approach is associated with high morbidity, due to the need for retraction of the cerebral frontal lobes, and longer hospitalization time. In 2008, Kassam et al. [5] described for the first

time the endoscopic endonasal route for resection of olfactory schwannomas. This route can be considered a more natural and direct route to reach anterior cranial fossa lesions and there is no need for brain retraction. Potential disadvantages include the removal and disruption of normal nasal anatomy, that is not involved by pathology, and the possibility of post-operative cerebrospinal fluid (CSF) fistula. The percentage of CSF-fistula can increase if the patient has previously undergone radiotherapy, transcranial surgery or intracranial infection[6]. Additional care must be taken in identifying the anterior and posterior ethmoidal arteries and coagulate them before sectioning: retraction of a bleeding artery into the orbit could cause intra-orbital hematoma[7]. In our case the anterior ethmoidal artery was involved within the tumor and it was necessary to proceed with extreme attention to identify it. When deciding between a traditional or an endoscopic approach it is important to consider size, extent and location of the tumor. Many studies have suggested that endoscopic endonasal transcribiriform approaches may be suggested for tumors less than 4 cm[8]. In our case the tumor reached more than 5 cm of maximum diameter and we were able to achieve a complete removal without need to switch to a traditional approach. Due to tumor size, reconstruction represented the most challenging part of the procedure; it was achieved with an inlay fascia lata and fat grafts and onlay vascularized double septal flaps to lower the risk of CSF-fistula. The use of local pedicle flaps has in fact shown a drop of CFS leaks after endoscopic endonasal approaches since its introduction[9].

CONCLUSIONS

We report the case of a large olfactory schwannoma completely resected with an endoscopic endonasal approach. Reconstruction was performed with a double nasoseptal flap. To date this is one of the largest olfactory schwannoma that has been

resected with a fully endoscopic endonasal approach.

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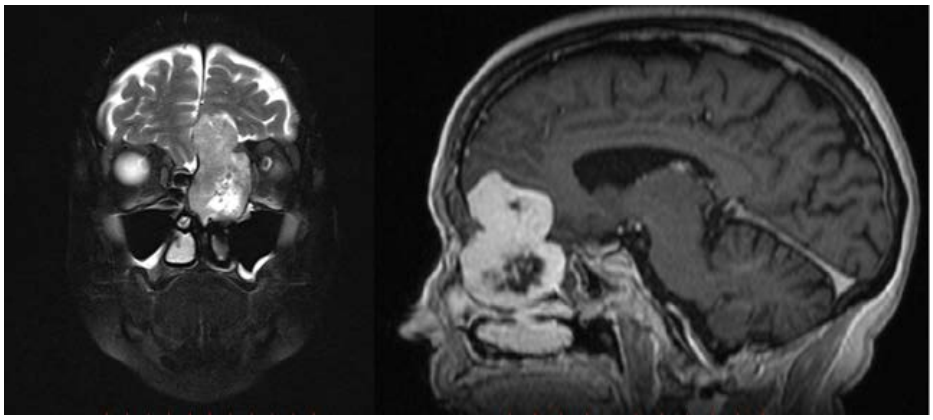

Fig. 1

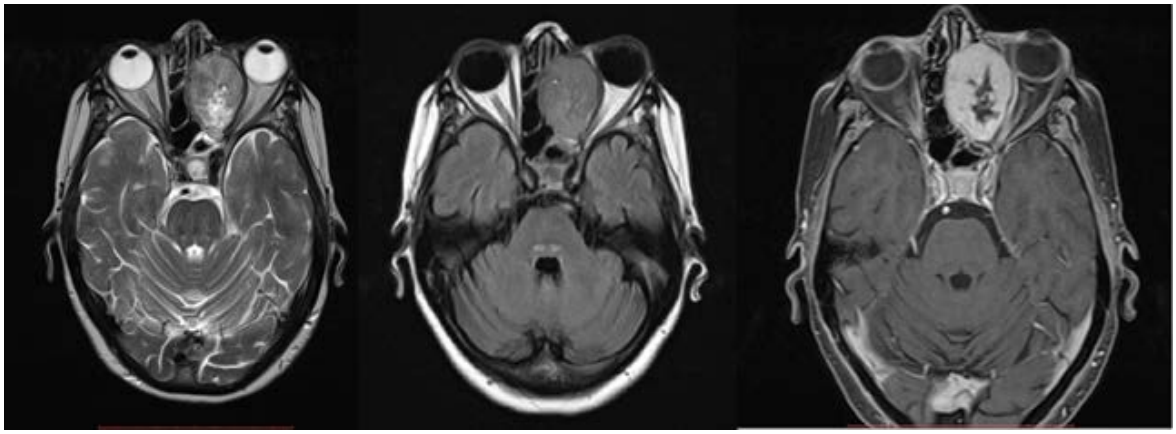

Fig. 2

Figure 1 and 2. MRI with contrast media enanchement showing a well-defined lesion, heterogeneously hypointense in T1 and hyperintense in T2, and had small central necrotic areas; maximum diameters were 55x28x42 mm (CC-LL-AP).

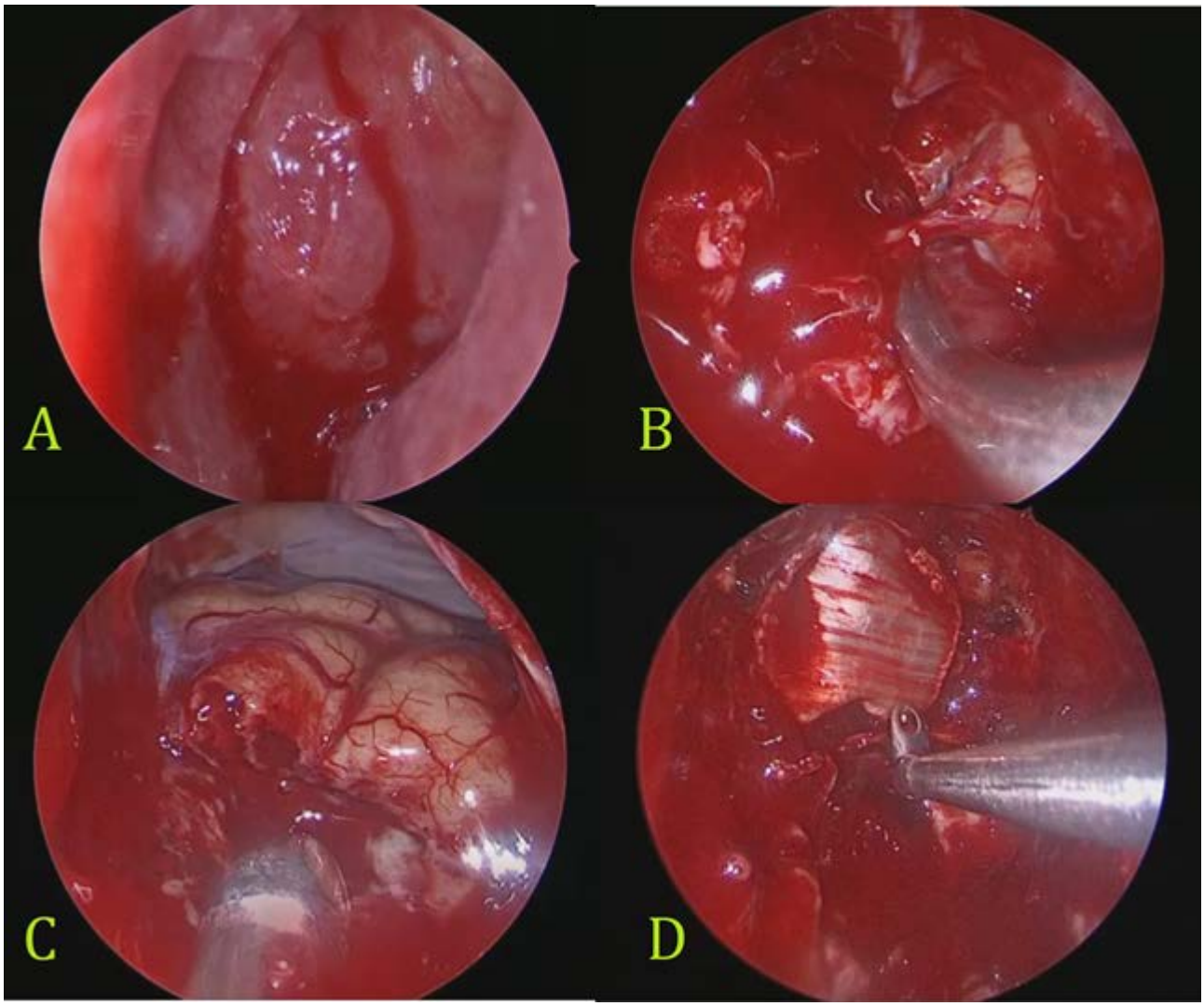

Figure 3. Surgical procedure. A: endoscopic view of the tumor; B: visualization of the anterior ethmoidal artery; C: endoscopic view of the left frontal lobe; D: multilayered technique reconstruction.

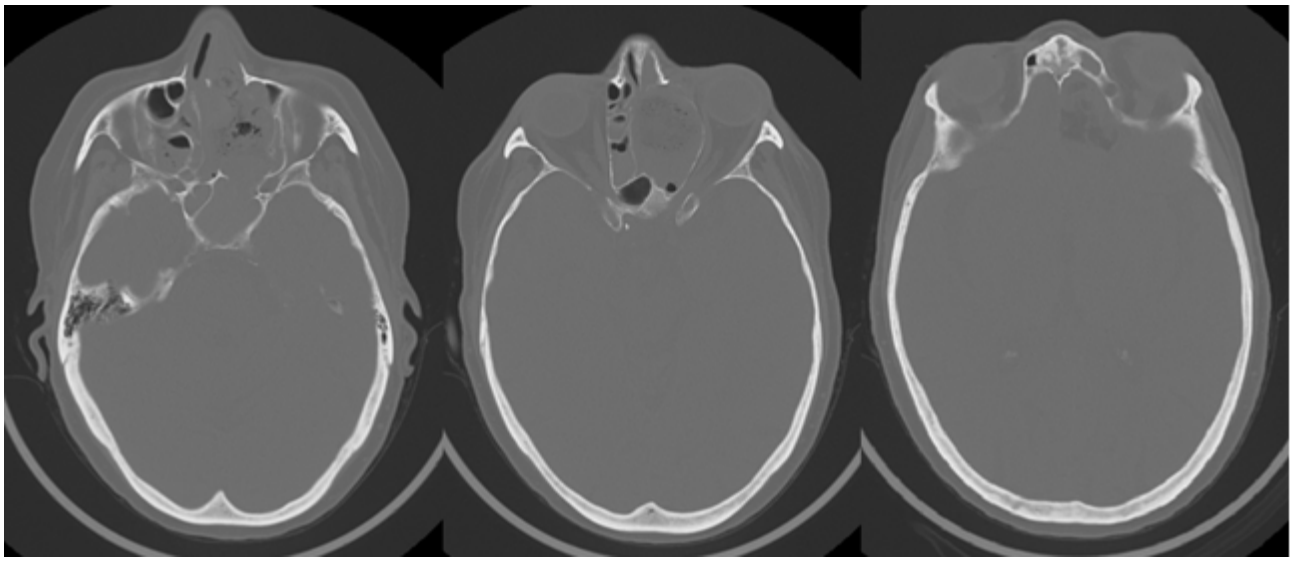

Figure 4. Post-operative CT-scan

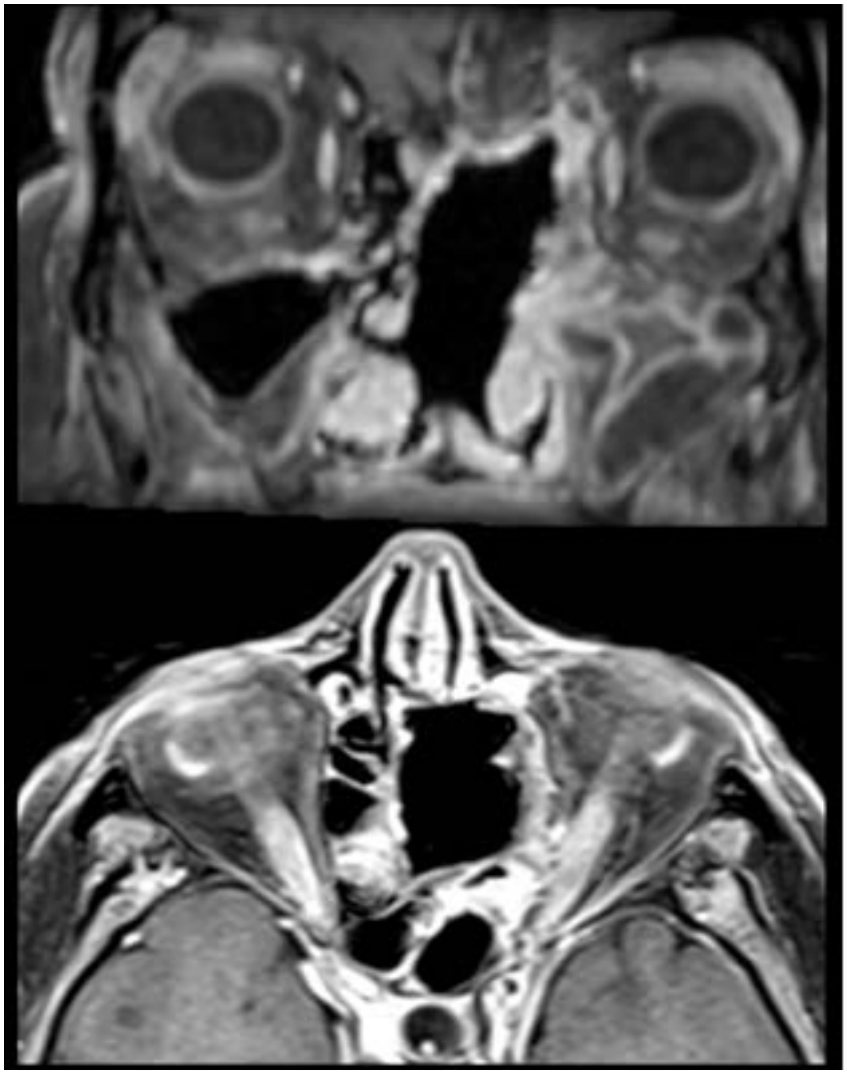

Figure 5. Six-months post-operative MRI.

New strategies for the treatment of post-radiation toxic effects in rhinopharyngeal carcinoma patients.

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ABSTRACT

Rhinopharyngeal carcinoma is a tumor with high risk of distant metastases associated with the Epstein-Barr virus. Although its aggressiveness, numerous patients are cured with standard therapy and for early stages of the disease, radiation therapy is the standard treatment. The toxic effects of radiation therapy as xerostomia, mucositis, dysphagia, hypogeusia or dysgeusia can affect the nutritional and psychological status decreasing the quality of life of patients. This study aims at evaluating the effect of the combination of *serratio peptidase, bromelain, methylsulfonylmethane and vitamin C* in the prevention and treatment of toxic effects after radiation therapy for rhinopharyngeal cancer.

The study was carried out on 32 (18 M, 14 F) outpatients (mean age 60,3±5,5) with

histologically proven rhinopharyngeal carcinoma undergoing radiation therapy.

Patients were divided in two groups, the first received nystatin, sodium bicarbonate, prednisone and a combination of serratio peptidase, bromelain, methylsulfonylmethane and vitamin C. The second nystatin, sodium bicarbonate, prednisone alone.

Patients underwent nasal endoscopy, Sf-36 questionnaire, and Visual Analogue Scale questionnaires before radiation therapy, halfway through the treatment and a month after the suspension of radiation therapy.

Our data demonstrated that the combination of *serratio peptidase, bromelain, methylsulfonylmethane and vitamin C* improves the toxic effects of radiation therapy in patients with rhinopharyngeal cancer. Moreover, this association improved the general quality of life without side effects

INTRODUCTION

Rhinopharyngeal carcinoma (RFC) is an aggressive tumor with high risk of distant metastases associated with the Epstein-Barr virus. Although its aggressiveness, numerous patients are cured with standard therapy, even in cases of loco-regionally advanced cancer. Generally, for early stages of the disease, radiation therapy (RT), due to the high radiosensitivity of rhinopharyngeal carcinoma and its deep-seated location, is the standard treatment, with a 5-year overall survival of 75%-90% (1). Conversely, for locally advanced RPC, 5-year overall survival can be increased by 4% in combination with chemotherapy (1).

Xerostomia, mucositis, dysphagia, hypogeusia or dysgeusia are significant problems affecting the quality of life (QoL) in patients treated with radiation therapy for head and neck cancer. These toxic effects of radiation therapy can affect the nutritional and psychological status and decrease the QoL of patients.

So far, treatments to alleviate acute injuries after radiation therapy are still under investigation.

This study aims at evaluating the effect of the combination of *serratio peptidase, bromelain, methylsulfonylmethane and vitamin C* in the prevention and treatment of toxic effects after radiation therapy for rhinopharyngeal carcinomas.

Bromelain is widely used in the clinical practice for its anti-inflammatory, antimicrobial and fibrinolytic property as well as for documented immunoregulatory and anti-cancer effects (2).

Serratio peptidase, an extracellular metalloprotease already used as an anti-inflammatory agent, is an antimicrobial able to inhibit the formation of biofilm by staphylococcus aureus, a bacterium that is often opportunistic during radiation therapy (3).

Methylsulfonylmethane provides protection against oxidative damage helping the organism to produce its endogen antioxidants. It also improves cell wall permeability allowing vitamin C to enter cells (4).

Vitamin C has a documented antioxidant role and does not reduce the effects of the therapy.

METHODS

The study was carried out at the University of Naples Federico II on 32 (18 M, 14 F) outpatients older than 18 years (mean age 60,3±5,5) with histologically proven RPC undergoing radiation therapy. The following criteria were eligible for this study: pathologically proven RPC with, rhinopharynx computed tomography (CT) or magnetic resonance imaging (MRI) performed before primary treatment, and receiving radical two-dimensional conventional radiation therapy (2D-CRT) or intensity-modulated radiation therapy (IMRT) at initial diagnosis. All of the included patients were staged according to the seventh edition of the American Joint Committee on Cancer (AJCC) staging system for RPC.

All participants were in good performance status with a Karnofsky scale ≥ 70% (5). Patients were administered radiation therapy with or without chemotherapy with a curative intent and signed the informed consent form. Five patients dropped out of the study. Patients were divided in two groups: investigational (I) and control (C).

The I group was assigned to receive: nystatin oral suspension 6 ml once a day; mouth washing with of sodium bicarbonate 7% four times a day for the whole duration of the radiation therapy cycle; prednisone 25 mg once a day from the onset of pain and until disappearance; the combination of serratio peptidase (417 mg), bromelain (500 mg), methylsulfonylmethane (900 mg) and vitamin C (500 mg).

The C group receives nystatin oral suspension mouth washing with of sodium bicarbonate 7% and prednisone 25 mg in the same dosage as group I. All participants gave their informed consent to participate in the study, which was fully approved by the local Board of Medical Ethics. Patients underwent nasal endoscopy with a 2.7-mm 30-degree rigid endoscope (Storz, Tuttlingen, Germany) scored as previously described by Lund and Kennedy (6) the Short Form-36 (Sf-36) questionnaire and the VAS score (6).

The Sf-36 questionnaire, which measures patients' general health status, contains 36 questions that refer to 8 health concepts grouped into subgroups (physical functioning, physical role functioning, bodily pain, general health, vitality, social role functioning, emotional role functioning, and mental health). Each question was asked independently.

The Visual Analogue Scale (VAS) questionnaire, which includes questions about xerostomia (x), dysphagia (d), hypogeusia (g), pain (p) and burning (b). In addition, clinical evaluation scored the presence of mucosal edema (E), aphthous stomatitis (AS), mycosis (M), dentinal hypersensitivity (D) into absent, 0; mild, 1; moderate, 2; severe, 3.

The disease was divided into mild, moderate, and severe based on total severity VAS scores (mild, VAS=0-3; moderate, VAS=4−7; and severe, VAS=8−10). A VAS>5 affected patient QoL. (6). QoL tools, as well as nasal endoscopy, was administered at baseline before radiation therapy (T0) halfway through the treatment (T1) and (T2) a month after the suspension of radiation therapy. We also assessed the presence of adverse effects.

Statistical analysis

Continuous baseline characteristics were described as either mean standard deviation. Difference between groups were tested with the Mann-Whitney U test; $p<0.05$ was considered statistically significant. Data were tabulated in a Microsoft Excel (Microsoft Corporation, Redmond, WA) spreadsheet and imported into the Statistical Package for the Social Sciences (SPSS-PC, version 16; SPSS Inc., Chicago, IL).

RESULTS

A total of 27 patients (15 M, 12 F; mean age 61,3±6,1) were enrolled in the study and divided in two groups. The investigational group and control groups, were well matched. At baseline, no statistically significant differences were observed in the clinical parameters (Table 1).

During RT at T1 we did not observe any significant difference between two groups except for pain and burning (Table 2), whereas after RT at T2 we observed that all considered clinical parameters were significantly better in group I than in group C except for dentinal hypersensitivity (Table 3). In addition, no patients reported adverse effects.

DISCUSSION

Radiation therapy alone or in association with chemotherapy represents the primary treatment for RPC due to complex anatomical localization of the tumor and the surrounding critical structures, and to the high radiosensitivity. However, therapeutic radiation often results in acute and long-term complications, such as radiation-induced mucositis, xerostomia, dysphagia,

hypogeusia, pain, burning, stomatitis, mycosis, or dentinal hypersensitivity (7).

These toxic effects after RT are often inevitable and usually appear some days after the start of treatment and are factors that may lead to deterioration of the patients' diet, nutritional status, interruption during treatment and predisposing to late side effects. Furthermore, severe toxicity also increases the risk of infections and the hospitalization.

Although previous research studies proposed different methods to prevent and treat radiation-induced mucosal toxicity, the effect of these treatments has not been completely clarified and remains still an unsolved problem (7).

Recent literature data showed the role of *serratio peptidase, bromelain, methylsulfonylmethane and vitamin C as* anti-inflammatory, antimicrobial and antioxidant agents.

For instance, this study aims at evaluating the effect of the combination of *serratio peptidase, bromelain, methylsulfonylmethane and vitamin C* in the prevention and treatment of toxicity after RT for RPC.

Our data demonstrated that the association of *serratio peptidase, bromelain, methylsulfonylmethane and vitamin C improves* all considered clinical parameters after radiation therapy. In particular, during the treatment at T1 we did not observe any significant difference between two groups except for pain and burning, whereas after RT, at T2, we observed that all considered clinical parameters were significantly better in group I than in group C except for dentinal hypersensitivity. In addition, no patients reported adverse effects.

Our data suggested the efficacy of the therapeutic association *of serratio peptidase, bromelain, methylsulfonylmethane and vitamin C* in improving the post-RT rhinopharingeal symptomatology. The improvements are particularly evident after the suspension of RT suggesting the need to

prolong the treatment even after the suspension of RT and in particular 30 days later.

However, the improvement of pain and burning already halfway through therapy is a rather encouraging result that can improve the QoL and above all the possibility of feeding, an element of crucial importance in functional recovery after RT and in the choice of a therapy during and after RT.

This information is confirmed by the improvement of the SF-36 questionnaire that, as mentioned above, is not specific for the pathology, but explores different fields, from the purely physical to the emotional ones, of the general QoL.

The only symptom that does not improve is dentinal hysensitivity, which implies the need to find other therapeutic strategies.

Furthermore, the total absence of adverse effects on the administration of treatment is a further encouraging element.

Our data also suggested the possibility of using the association *of serratio peptidase, bromelain, methylsulfonylmethane and vitamin C* in other neoplastic pathologies of the head and neck area undergoing radiation therapy.

In conclusion, we believe that adding the association *of serratio peptidase, bromelain, methylsulfonylmethane and vitamin C* to standard therapy improves the toxic effects of radiation therapy in patients with RPC. Moreover, this association improves the general QoL in the absence of side effects.

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Baseline	I	C	*P*
Sf36	45,7±11	40,2±12	0,3
VASx	3,90±1,3	3,80 ±1,7	0,8
VASd	0,60±0,7	0,50±0,7	0,7
VASg	0,54±0,5	0,80±0,7	0,3
VASp	0,50±0,5	0,84±0,6	0,2
VASb	0,53±0,5	0,85±0,6	0,2
LK	2,2±1,1	2,9±1,1	0,1
E	0,2±0,42	0,3±0,48	0,6
AS	0,6±0,5	0,8±0,7	0,5
M	0,64±0,4	0,74±0,7	0,3
D	0,40±0,5	0,52±0,5	0,2

Table 1: At baseline, no statistically significant differences were observed in the clinical parameters. Investigational group (I), control group (C), Short Form-36 (SF-36) questionnaire, Lund and Kennedy score (LK), Visual Analogue Scale (VAS), xerostomia (x), dysphagia (d), hypogeusia (g), pain (p) and burning (b), mucosal edema (E), aphthous stomatitis (AS), mycosis (M), dentinal hypersensitivity (D).

T1	I	C	*P*
Sf36	43,4±4	36,9±11	0,2
VASx	6±1,1	5,5 ±1,7	0,4
VASd	3,1±0,9	3,5±1,2	0,4
VASg	3,2±1,1	3,9±0,7	0,1
VASp	2,5±0,5	3,1±0,5	**0,02**
VASb	2,7±0,4	3,3±0,7	**0,02**
LK	5,7±1,7	5,4±1,7	0,6
E	1,4±0,52	1,5±0,53	0,6
AS	1,2±0,7	1,3±0,5	0,4
M	1,6±0,4	1,7±0,5	0,2
D	1,4±0,4	1,5±0,5	0,2

Table 2: During RT at T1 we did not observe any significant difference between two groups except for pain and burning. Investigational group (I), control group (C), Short Form-36 (SF-36) questionnaire, Lund and Kennedy score (LK), Visual Analogue Scale (VAS), xerostomia (x), dysphagia (d), hypogeusia (g), pain (p) and burning (b), mucosal edema (E), aphthous stomatitis (AS), mycosis (M), dentinal hypersensitivity (D).

T2	I	C	*P*
Sf36	28,3±84	41±12	**0,01**
VASx	2,2±0,7	5,7 ±1,	**0,001**
VASd	2,1±0,5	3,8±0,9	**0,001**
VASg	2,4±0,5	4,2±0,6	**0,001**
VASp	1,5±0,5	3,4±0,5	**0,001**
VASb	1,5±0,5	3,5±0,5	**0,001**
LK	2,3±1,1	6,4±1,5	**0,001**
E	1,7±0,4	0,6±0,5	**0,001**
AS	1,4±0,5	0,3±0,4	**0,001**
M	1,1±0,3	1,6±0,5	**0,001**
D	1,1±0,54	1,2±0,9	0,7

Table 3: After RT at T2 we observed that all considered clinical parameters were significantly better in group I than in group C except for dentinal hypersensitivity. Investigational group (I), control group (C), Short Form-36 (SF-36) questionnaire, Lund and Kennedy score (LK), Visual Analogue Scale (VAS), xerostomia (x), dysphagia (d), hypogeusia (g), pain (p) and burning (b), mucosal edema (E), aphthous stomatitis (AS), mycosis (M), dentinal hypersensitivity (D).