ORIGINAL CONTRIBUTION

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Olfactory mucosa in nasal polyposis: Implications for FESS outcome*

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SUMMARY

Objective: *This study investigates olfactory epithelium biopsies from patients with nasal polyposis, in correlation with olfactory test results and ratings of olfactory dysfunction pre- and postoperatively.*

Material & Methods: *Twenty-seven patients with nasal polyposis were included. Olfactory function was tested with the "Sniffin Sticks" test. Biopsies from the olfactory region performed at the end of endoscopic surgery and studied with immunohistochemistry. Patients used a visual analogue scale to report their olfactory dysfunction when re-examined one year postoperatively.*

Results: *Subjects with little or no inflammation in olfactory and respiratory biopsies had shorter duration of disease and better olfactory function. Pathological changes of olfactory mucosa included replacement by respiratory metaplasia, degenerated epithelium, rupture of epithelial surface and infiltration by inflammatory cells.*

Postoperative olfactory test results showed improvement of olfaction in 74% of patients. Although this improvement seemed to be better in subjects with little or no inflammation, this was not significant. Preoperative and postoperative olfactory test results were positively correlated.

Conclusions: *The disease process includes loss of structural organization and inflammatory infiltration of the olfactory epithelium. The inflammation severity is related to the olfactory test results; however, it cannot be the only predictive factor postoperatively.*

Key words: olfaction, nasal polyposis, anosmia, inflammation, biopsy

INTRODUCTION

Nasal polyposis is one of the most common causes of olfactory dysfunction [(1,2)]. The disease is characterized by a prolonged inflammatory response of the nasal and sinus mucosa [(3)]. Pathology studies demonstrated that nasal respiratory mucosa in nasal polyposis presents an infiltration by inflammatory cells with fibrosis and thickening of the lamina propria [(4)]. However a few studies assessed olfactory mucosa in patients with chronic sinusitis [(5-7)]. These studies were not prospective and thus correlations with their surgical outcome were difficult. Patients with sinusitis and anosmia often do not respond favourably to surgery in terms of the improvement of olfactory loss, although they report satisfactory resolution of other complaints [(6,8,9)]. Although nasal polyposis is considered as a subgroup of chronic rhinosinusitis [(10)] differences between them in cellular, mediator and clinical profiles, [(11)] suggest that nasal polyposis should be assessed regarding olfactory function as a distinct entity. These facts raise questions regarding the relation of histological changes of the olfactory epithelium and the clinical presentations of olfactory dysfunction in patients with nasal polyps. An additional question relates to the idea whether olfactory mucosa biopsy can provide useful information in order to predict the surgical outcome.

The adult olfactory epithelium typically exhibits a mixed representation of olfactory receptor cells and patches of respiratory epithelium [(12,13)]. The degree of variability in neuroepithelial distribution may be related to various factors, such as age [(14)], sex [(14)] and the nasal or other pathology [(15-17)]. The aim of this study was to investigate histological changes of the olfactory epithelium in a group of patients with the same chronic nasal disease and correlate these findings with the preoperative and the postoperative olfactory function. It should be stated that although olfactory biopsy studies contributed to better understand the pathophysiology of olfactory disorders, a biopsy is

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not informative on an individual basis and thus cannot be used in clinical practice.

MATERIALS AND METHODS

Patients

This study was performed in accordance with the Declaration of Helsinki regarding biomedical research on human subjects and approved by the Ethics Committee of the Aristotle University of Thessaloniki. Every patient enrolled in this study gave a signed consent, being informed that biopsy of olfactory epithelium has no negative effect on the ability to smell [18].

A total of 27 patients were included, 17 men and 10 women, who underwent endoscopic sinus surgery for nasal polyposis. The mean age was 41.8 years with a range from 21 to 60 years. Recordings obtained from patients' case-notes and included smoking habits, years of the nasal disease based on patients' history at the initial appointment and previous nasal surgery. Exclusion criteria were history of craniofacial trauma, neurological and metabolic disorders, or olfactory dysfunction due to viral infection. None of the patients had a history of asthma or aspirin intolerance.

All patients had a preoperative computed tomography (CT) scan of the nasal sinuses. One of the authors who was blinded for the patients' symptoms, assessed the CT scans with the use of the Lund – Mackay system score, which is a well validated staging system in chronic rhinosinusitis (CRS) [19]. Each sinus is graded between 0 and 2 (0 no abnormality, 1 partial opacification, 2 total opacification), whereby the maximum score for each side is 12. The osteomeatal complex is scored with 0 (not obstructed) or 2 (obstructed).

Olfactory assessment

The patients were tested for their olfactory ability preoperatively and retested in a follow up appointment at least 12 months after surgery (range 12-24 months). The severity of olfactory dysfunction was assessed by means of the validated and reliable "Sniffin' Sticks" test battery (Burghart, Wedel, Germany) [20-22]. Odorants were presented in commercially available felt-tip pens. For odour presentation the cap was removed by the experimenter, and the pen's tip was placed approximately 2 cm in front of both nostrils for approximately 2 sec before the pen was capped again. The examination involved tests for phenyl ethyl alcohol odour thresholds, odour discrimination, and odour identification [23].

R esults of the three subtests were presented as a composite "TDI score" which was the sum of individual scores for threshold, discrimination, and identification measures. Based on a multicentric investigation in more than 1000 patients with olfactory loss, patients with a score below 15 are considered functionally anosmic, and with a score between 15 and 31 considered hyposmic [21]. The olfactory ability is measured uninasally as each nostril is considered to have an independent contribution in olfaction. The other nostril is blocked with a silicone tape. Postoperative significant improvement was considered when the TDI score changed by 6 points and more. Similarly a decrease of the TDI score with more than 6 points was considered as deterioration of olfactory function [24].

Using visual analogue scales (length 10 cm; scales from 0 to 100 units) patients rated the degree of olfactory dysfunction and nasal obstruction both pre- and postoperatively. In these scales, 0 represented the absence of symptom and 100 the highest possible presence of a symptom.

Surgery

Endoscopic surgery involved removal of the diseased mucosa and polyps as well as opening of the anatomical compartments, as described by Stammberger [25]. In all cases surgery was performed by the same surgeon and a standardized surgical technique. This technique included uncinectomy, middle meatal antrostomy and anterior and posterior (if required) ethmoidectomy. The middle turbinate remained intact when not distorted; otherwise the inferior part was removed. Patients did not use topical or oral steroids for at least three months prior to the time of surgery. Postoperative treatment included a two weeks course of oral antibiotics and oral steroids (prednisolone 0.5mg/kg/day), and then a long-term course of nasal steroids (budesonide nasal spray for 12 weeks, 256 g/nostril/day). Patients did not receive further treatment until their last follow-up assessment. During this appointment one year postoperatively, patients were assessed with the same olfactory test and they rated the degree of their symptoms.

Olfactory biopsies

Unilateral biopsy of the postero-superior septum or superior concha from the best side regarding olfaction was obtained at the time of endoscopic sinus surgery using a Blakesley forceps. The location of the biopsies was in accordance with the suggestion by Feron et al. [26] that the site of the highest probability of finding olfactory epithelium is the dorsoposterior region of the nasal cavity. Specimens were immediately placed in formalin (10%) using a needle to gently manipulate the olfactory mucosa. Olfactory tissue from 5 normosmic subjects was obtained incidentally at the time of endoscopic pituitary surgery. Control subjects were considered as normosmics when they had a computed tomography (CT) scan without evidence of sinusitis and a TDI score greater than 30.

Immunohistochemistry

All samples were fixed in formalin, embedded in paraffin and sectioned at 6 m. Sections processed for immunohistochemistry were deparaffinized and blocked with normal sera diluted 1:1 with phosphate-buffered saline (PBS).

In all cases the presence of functional olfactory mucosa was identified with immunohistochemistry using an avidin-biotin-peroxidase technique [27]. Sections were incubated with antibodies against 1) Olfactory marker protein (OMP; polyclonal goat; 1:6000, kindly provided by F. Margolis, University of Maryland, Baltimore), 2) Beta-III-tubulin (polyclonal rabbit;

Table 1. Descriptive statistics of the study group.

Gender	17 Male	10 Female
	mean	***range***
Age *(n = 27)*	41.8± 3.2 years	21 - 60
Duration of the disease *(n = 27)*	4.5 ± 0.8 years	0.5 - 12
Smoking smokers (n = 9) non smokers (n = 18)	6.3 ± 1.2 pack years	1 - 12
Previous surgery *(n = 7)*	1.8 ± 0.2 operations	1 - 3
Lund Mackay score *(n = 27)*	13.8± 2.5	8-24
Preoperative olfactory function *(n = 27)*	17.8± 2.4	2 – 29.5
Postoperative olfactory function *(n = 27)*	26.31± 2.6	10.8 - 39
Subjective preoperative olfaction *(n = 27)*	73.3± 7.2	20 - 99
Subjective postoperative olfaction *(n = 27)*	30.3± 5.4	0 - 75
Subjective preoperative Nasal obstruction *(n = 27)*	73± 8.7	30 - 96
Subjective postoperative Nasal obstruction *(n = 27)*	12.6± 4.2	0 - 35

Sigma #T2200; 1:4000) and 3) Protein Gene Product 9.5 (PGP 9.5; Biotrend, Cologne, Germany, #7863-0504, 1:2000).

OMP is a 19-kD protein found almost exclusively in the cell bodies and peripheral processes of olfactory receptor neurons [28,29]. However OMP is only expressed in mature olfactory neurons [30]. Immunoreactions for beta-tubulin were also chosen to identify all olfactory neuronal profiles (non-OMP-ir, presumably immature) in the superficial olfactory epithelium [7]. Antibody against PGP 9.5 was additionally used as a neurospecific peptide for the detection of olfactory receptor neurons [31].

A pathologist, blinded to the study, evaluated the degree of inflammation. All specimens were reviewed and scored according to a 4-point severity scale, in which 0 represents normal findings, 1 mild, 2 moderate and 3 severe inflammation. Criteria for the assessment of inflammation were the infiltration of epithelium and lamina propria by inflammatory cells (lymphocytes, plasma cells, neutrophils, and eosinophils), the presence of oedema, fibrosis and dilated capillaries in the lamina propria. This qualitative grading system was used similarly with other authors [32,33] as inflammation assessment includes non-numerical parameters e.g. oedema.

Statistical analysis

For statistical analysis, SPSS for WindowsTM was used (Statistical Package for the Social Sciences, Version 15.0, SPSS Inc. Chicago, IL, USA). Descriptive statistics are presented as means ± standard errors of the means (± SEM). Correlations were performed using the Pearson coefficient. The clinical assessment with the Sniffin Sticks had been performed uninasally, thus correlation analysis with the histological findings was done with the best side pre- and postoperatively. Comparisons of means between groups with no or mild inflammation and moderate or severe inflammation performed with the use of t-tests. The minimum alpha level was set at 0.05.

RESULTS

The descriptive statistics of the study group are presented in details in Table 1. The mean preoperative olfactory function of the study group as expressed on the basis of the TDI score was 17.2 ± 2.1 in the right and 18.4 ± 1.8 in the left side.

Olfactory function of the right nasal cavity was found to negatively correlate with the right Lund-Mackay score ($r = -0.53$, $p = 0.005$). However, this was not the case with the left side.

In addition, the duration of the disease correlated negatively with the olfactory performance of the best nostril ($r = -0.41$, $p = 0.036$). Patients with previous surgery had significantly lower olfactory function ($p < 0.001$) than those who did not, however, this was not the case in postoperative results. Smoking did not correlate with the olfactory performance in nasal polyposis patients pre- and postoperatively. In addition, smoking does not present any correlation with the other assessed parameters in this study.

Interestingly, the subjective ratings assessment showed that the lower subjects rated their olfactory dysfunction, the higher it was found in the olfactory testing ($r = -0.67$, $p < 0.001$). Similarly, subjective ratings of nasal obstruction correlate negatively with olfactory function of the best nostril ($r = -0.665$, $p < 0.001$).

The percentage of recognized olfactory mucosa was 56% (15 from a total of 27 biopsies). Ten specimens were recognized as respiratory epithelium and two specimens contained no specific epithelium. Further analyses indicated that there was no significant correlation (Spearman) between age or number of previous surgeries and presence or absence of olfactory mucosa ($r27 < -0.23$, $p > 0.26$). In addition, there was no significant difference between subjects with or without present olfactory mucosa in terms of age ($t[df\ 25] = 1.18$, $p = 0.25$) or the number of previous surgeries ($Chi^2 = 1.49$, $df\ 3$, $p = 0.69$).

Olfactory biopsies from 5 patients (olfactory mucosa was recognized in 4 of them) who underwent pituitary surgery used as reference of normal olfactory mucosa. These patients demonstrated normal “Sniffin’ Sticks” scores (> 31) and normal CT findings.

Normal human olfactory mucosa is shown in Figure 1 with a thin basement membrane, olfactory receptor neurons, basal cells, and sustentacular cells. The epithelium presents many

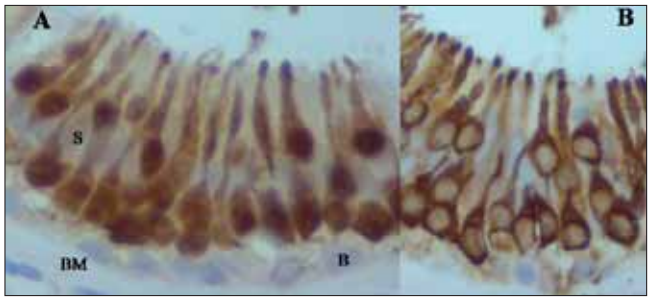

Figure 1. Normal olfactory epithelium with olfactory receptor neurons reacting positively with A) PGP 9.5 and B) B-Tubulin immunohistochemistry (stained brown). A) The epithelium presents a thin basement membrane (BM), basal cells (B), and sustentacular cells (S). B) In contrast to PGP 9.5, B -Tubulin does not label the nuclei of neuronal cells. Note that normal epithelium presents many olfactory receptors with straight dendrites, a sufficient thickness and continuity of epithelial surface. (PGP 9.5, B-Tubulin x 1000).

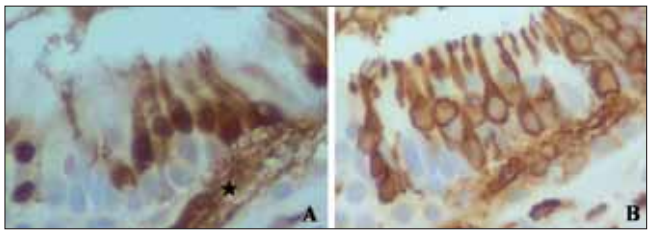

Figure 2. Olfactory epithelium of a patient with hyposmia. A) No major structural changes of the epithelium, various thicknesses and a nerve fiber bundle which is partly intraepithelial (asterisk) (PGP 9.5 x 2000). B) More olfactory neurons are reacting with Beta -Tubulin antiserum staining as immature neurons are also stained (x 2000). The number of olfactory receptor neurons is decreased.

Table 2. Comparison of subgroups divided according the degree of inflammation in the biopsy specimens.

	no/mild inflammation *n: 9 patients*	Moderate/severe inflammation *n: 18 patients*	Significance *a level 0.05*
Duration of disease *(Years)*	1.78 ± 0.27	5.94 ± 0.80	p<0.001*
Preoperative olfactory function *(TDI score of best nostril)*	25.05 ± 3.24	17.69 ± 1.70	p= 0.036*
Lund Mackay score	12.56 ± 1.93	14.39 ± 1.29	p= 0.430
Postoperative olfactory function *(TDI score difference of best nostril)*	11.04 ± 2.82	7.25 ± 1.67	p= 0.231

olfactory receptor cells, a sufficient thickness and continuity of epithelial surface. Light microscopic evaluation of the olfactory mucosa from patients with nasal polyposis demonstrated certain pathological changes such as infiltration by inflammatory cells (15 specimens), degeneration – disorganized epithelium

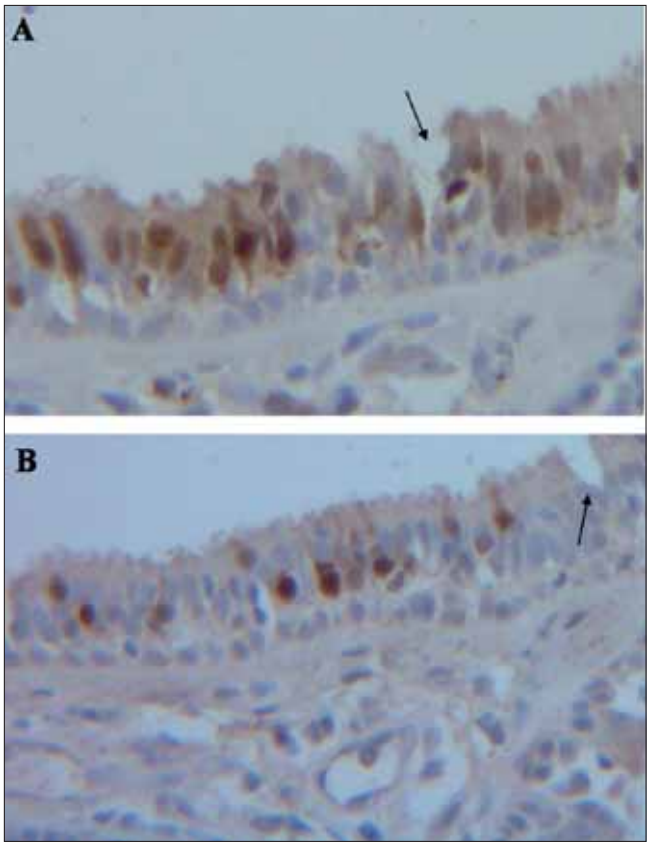

Figure 3. A) Microphotograph from a patient with hyposmia demonstrating the olfactory mucosa after reaction with OMP antibody. Note the degenerative area of an epithelium with rupture of epithelial surface (arrow) and different shapes of olfactory neurons with tortuous dendrites. B) Degenerative area of olfactory mucosa from a patient with anosmia. Note the sparse presence of OMP positive neurons, the rupture of epithelial surface (arrow) and the mild inflammatory infiltration of the lamina propria. (OMP x 500).

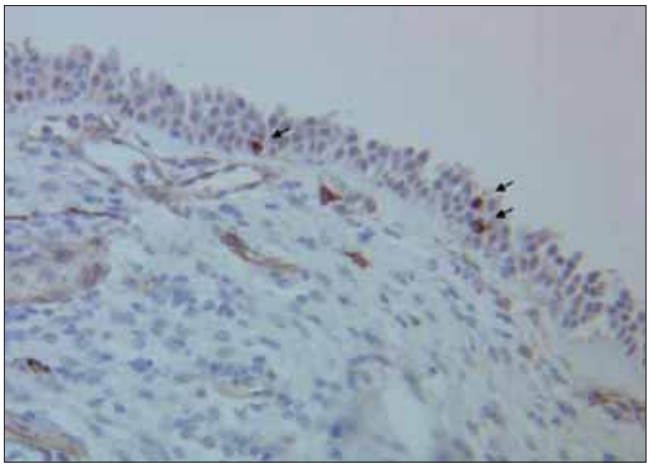

Figure 4. Microphotograph from a patient with anosmia: Complete replacement of olfactory epithelium by metaplastic epithelium. Note the sparse presence of OMP positive olfactory neurons (arrows). The lamina propria is moderately infiltrated by inflammatory cells. (OMP x 150).

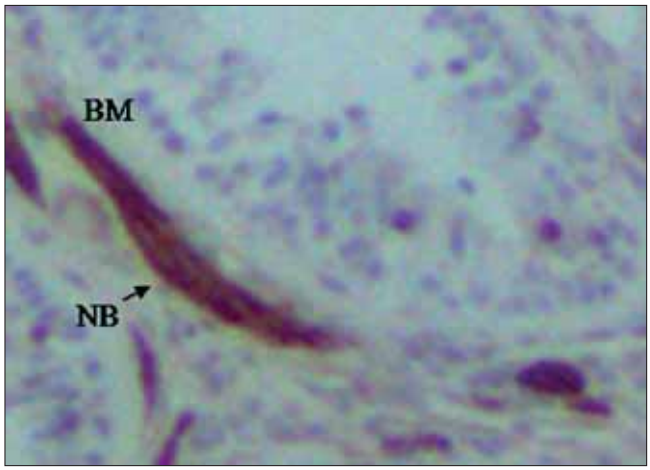

Figure 5. Degenerated olfactory epithelium with only a few OMP positive olfactory neurons. Note the nerve fiber bundle (NB) in the lamina propria (arrow), and the thickened basement membrane (BM). (PGP 9.5 x 200).

(10 specimens), rupture of epithelial surface (4 specimens) and respiratory metaplasia (7 specimens) (Figures 2-4). In one specimen, although there were some positive OMP nuclei, the main finding of olfactory mucosa was the presence of an olfactory nerve fiber bundle in the lamina propria (Figure 5).

Patients were assigned to two groups according to the degree of inflammatory infiltration of respiratory and/or olfactory specimens: the first group consisted of those with no or mild inflammation (9 patients) and the second group of those with moderate to severe inflammation (18 patients). The distribution of patients according the severity of inflammation in their specimens is shown in Figure 6.

Comparisons of means between groups showed that patients with mild or no inflammation in olfactory and respiratory mucosa biopsies had significantly shorter duration of disease and significantly better olfactory function preoperatively than those with moderate or severe inflammation. While the improvement after surgery seemed to be better in patients with little or no inflammation, this was also not significant. All the above-mentioned comparisons are presented in details in Table 2.

The mean postoperative TDI score of the study group was 26.8 ± 2.5 in the right and 27.3 ± 2.3 in the left side. Postoperative olfactory test results showed significant improvement of the sense of smell in 74% of the patients (20 patients improved, 6 patients no change, 1 patient worsened). Only one patient experienced significant decrease of his olfactory function. It should be noted that preoperative olfactory function was positively correlated with the postoperative olfactory function ($r = 0.59$, $p > 0.001$).

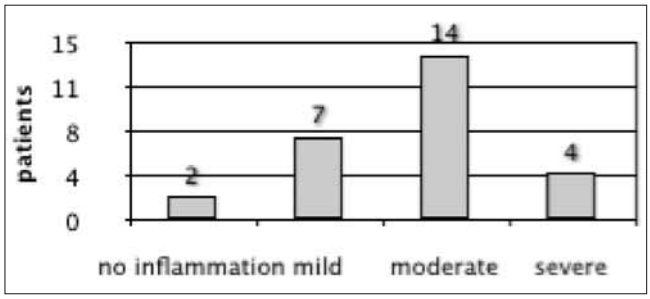

Figure 6. The distribution of patients according the severity of inflammation in their specimens.

Three patients who required partial resection of the middle turbinate (inferior part) due to distortion presented significant improvement in olfactory function (TDI change > 6). This increase although not comparable (mean TDI change 11.4) was similar with that of patients having intact middle turbinate and improved olfactory function postoperatively (17 patients, mean TDI change 12.1).

DISCUSSION

This study confirms that olfactory loss in chronic rhinosinusitis with polyps is a more complex process involving both transport and sensory pathology as seen in similar studies [5,6,8,34]. Our data showed that: 1) structural changes of the olfactory epithelium and 2) inflammatory infiltration are involved in a sensory deficit of olfaction at the level of the neuroepithelium.

Regarding the first main finding, olfactory bipolar cells appeared at a low frequency in pathological specimens. Moreover when olfactory bipolar cells were identified, the disorganisation of the neuro-epithelium was a frequent finding. In our study only 15 of the 27 patients presented olfactory mucosa on the biopsy sample (55.5%), which is lower than the rate reported by Feron et al. regarding normal human olfactory mucosa [26]. Specifically, Feron et al. reported a rate of 73% for the dorsoposterior septum and 76% for the superior turbinate in normosmic subjects for the same sites of biopsies. The olfactory epithelium is gradually replaced by respiratory mucosa during the lifespan, although this is probably part of the normal aging process, the low rate of recognized olfactory mucosa in our study is mainly related to nasal polyposis, as no correlation was found between the presence or not of olfactory mucosa and aging or previous surgery. This is in agreement with a study by Kern et al. suggesting increased apoptosis of olfactory epithelium in sinusitis patients [17]. However the low percentage found may be due to the small sample size and this should be considered in the interpretation of the results.

Microscopic assessment of biopsies often showed patches of olfactory bipolar cells interrupted by respiratory metaplasia or intense inflammatory infiltration. In some specimens the presence of an olfactory nerve bundle in the lamina propria was the main finding of pre-existing olfactory epithelium. Another frequent finding was ruptures of the olfactory epithelium. The significance of this finding is still unclear; however it may be related with the presence of mucosal inflammation [6].

The second main finding in our study was the inflammatory changes within the olfactory mucosa of patients with nasal polyposis. It is important to note that this is the first study that is correlating the degree of smell loss with the intensity of the inflammatory response, giving evidence for a cause-and-effect relationship. Limitation of this inflammatory response assessment is that the grading of its intensity is non-quantitative, as no widely accepted staging system of inflammation in sinusitis exists [35].

Inflammatory changes in the olfactory mucosa may negatively affect olfaction with different mechanisms. In a study using a sinusitis model in rats, Ge et al. showed that inflammation and increased apoptosis of olfactory receptor neurons occurred during the infection [31]. The microenvironment of bipolar cells is disturbed because of Bowman's glands hypersecretion triggered by mediators released from lymphocytes and macrophages. The same mediators may have a direct toxic effect to olfactory bipolar cells as seen in other studies assessing the role of eosinophils and their neurotoxic mediators [36,37]. In a study by Haruna et al., the high percentage of activated eosinophils in ethmoidal mucosal specimens was significantly correlated only with olfactory dysfunction symptoms and not with other symptom categories such as nasal obstruction, nasal discharge and headache [38].

The Lund Mackay CT score is an indirect measurement of nasal inflammation and it has been used in nasal polyposis studies as a predictor of outcome [39]. In the present study, the Lund Mackay CT score did not correlate with the pathology data regarding inflammatory changes of olfactory epithelium. This is probably due to methodological problems, as first the Lund Mackay CT score additionally assesses the paranasal sinuses and second there were variable time intervals between specimens evaluation and CT scans.

Although there is a correlation between inflammatory changes of the epithelium and preoperative olfactory test results, the olfactory biopsies could not predict the postoperative results. Olfactory dysfunction in nasal polyposis appears as a multifactorial problem in which the duration of the disease and previous nasal surgery play a significant role as they negatively affect the preoperative olfactory status. However we should note that the definition of the disease duration is based on patients' history and this cannot standardize the period of the disease process.

As a result clinical images of olfactory dysfunction in nasal polyposis vary. This is probably because the factors affecting the postoperative result are working as competing forces [5,40]. Although some authors suggest that more advanced disease means more room for improvement after surgery [41], others believe that more severe disease has the risk of persistent mucosal disease or recurrence at some point after surgical treatment [42]. Our data are in accordance with the second option as it was clear that patients with better preoperative olfactory function had better postoperative outcome regarding olfaction. Although there is evidence about the negative effect of smoking on olfaction [43], it seems that initial smoking behaviour is not a significant predictor of olfactory ability in nasal polyposis patients, which is in agreement with findings of other authors [44].

The postoperative results showed improvement of olfaction in 74% of patients, which is very high considering the interval of more than one year between surgery and follow-up assessment. This is possibly due to the combination of surgery and the long-term postoperative use of nasal steroids. In addition, patients with nasal polyposis typically exhibit a very good response in terms of recovery of olfactory function [45,46] compared to other types of nasal surgery e.g. septoplasty [9]. Partial resection of the inferior part of middle turbinate in three patients did not affect the kinetics of olfactory function postoperatively as seen also in a study by Federspil et al. [47].

Interestingly our patients rated their olfactory ability similarly with their olfactory test results, although subjective ratings are not always in agreement with the olfactory testing results.

"Sniffin' Sticks" is a widely used clinical tool of olfactory assessment, examining the extent and not the nature of the disease process. Olfactory biopsies can give useful information regarding the location of the pathological process. However, the predictive value of biopsies regarding postoperative olfactory function appears to be limited. Olfactory biopsies should be considered as a useful tool for research purposes, with not established value in clinical practice, and thus should not be used at the time of routine endoscopic surgery.

In conclusion this study suggests that olfactory dysfunction in nasal polyposis apart of airflow changes to the olfactory cleft is caused by structural effects on the olfactory mucosa. The main microscopic finding was the inflammatory infiltration, which is correlated with the preoperative olfactory testing results. Further studies are needed in order to identify the contribution of each factor in the clinical presentation and thus to better predict the olfactory postoperative outcome.

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