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**Nasal and tracheal cytological changes after total laryngectomy in long-term survivors**

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**Abstract**

**Objective.** Complete separation of upper and lower respiratory tract after total laryngectomy results in permanent effects on nasal cavities and tracheo-bronchial airways. Aim of this study is evaluating nasal and tracheal cytological alterations of mucosa in laryngectomy long-term survivors, analyzing the feasibility of scraping for cytological examination of tracheal mucosa.

**Methods.** Twenty-five laryngectomy patients underwent symptoms' evaluation, endoscopic fiber optic examination, prick tests and nasal and tracheal scraping for cytological exam. Twenty-five healthy subjects underwent the same assessment, except for tracheal scraping. Eleven laryngectomy patients accepted inferior turbinate biopsy for histological examination.

**Results.** Nasal cytological analysis demonstrated mucous cell metaplasia in 20% of laryngectomized patients, but it was absent in all healthy subjects; no squamous cell metaplasia was found in both groups. In 15 patients (60%) bacteria were present, without inflammatory infiltrate. Tracheal cytological analysis demonstrated a quite high rate of squamous cell metaplasia (24%), neutrophilic infiltrate (32%) and presence of bacteria (40%). Histological examination of inferior turbinate showed submucosal stromal fibrosis in all patients and submucosal inflammatory infiltrate in one case (9%).

**Conclusion.** Nasal cavities and trachea of laryngectomy patients undergo long-term cytological and histological changes of mucosa and submucosa, probably due to airflow modifications.

**Keywords:** Nasal cytology; Tracheal cytology; Laryngectomy; Histological changes; Cytological changes; Total laryngectomy

## Introduction

Complete separation of upper and lower respiratory tract after total laryngectomy results in permanent effects on nasal cavities and tracheo-bronchial airways. In laryngectomy patients the air comes directly into trachea. Therefore, nasal cavities are excluded from respiration and cannot carry out their physiological functions, such as filtering, heating and moistening of the inspired air and smell. Hyposmia, impaired mucociliary function, cytological and histological alterations of nasal mucosa arise.<sup>1-4</sup> Moreover, the "unconditioned" inspired air may induce chronic inflammation of trachea and bronchi, with cough, excessive secretions and sometimes crusting, especially in the first three months after total laryngectomy.

Very few studies evaluated cytological and histological changes of nasal mucosa in laryngectomy individuals<sup>1-2</sup> and none investigated such alterations in tracheal mucosa. Previous studies focused on smell disorders of these patients.<sup>4</sup>

Nasal airflow has been proposed to be necessary for normal functioning of nasal epithelium. However, there is no convincing evidence that airflow changes are a causative factor in nasal pathology.<sup>5</sup> Epidemiological studies have showed an increased incidence of lower respiratory tract infections in patients with a tracheostomy.<sup>6,7</sup> Conversely, rhinosinusitis has not been generally mentioned as associated with laryngectomy.<sup>5</sup> Previous studies have showed a microbial colonization of nasal cavities and trachea after total laryngectomy, without clinical signs of infection.<sup>1,8,9,10</sup>

Aim of this study was to evaluate nasal and tracheal cytological alterations of mucosa in laryngectomy long-term survivors, due to airflow changes. Furthermore, we analyzed the feasibility of scraping for cytological examination of tracheal mucosa.

## Materials and methods

Twenty-five healthy subjects and twenty-five consecutive long-term survivor patients who underwent total laryngectomy for advanced laryngeal cancer between 2005 and 2011 at our Department were evaluated in this retrospective cross-sectional comparative study. All laryngectomy patients included in the study were disease free at the time of engagement. Moreover, they had no clinically evident viral, bacterial and/or mycotic diseases involving nasal cavities or lower respiratory tract. No antimicrobial therapy was administered to patients for at least one week before the evaluation. Enrolled patients had at least a 2-year follow-up period (mean  $52.97 \pm 28.96$  months, range 26-97 months), in order to evaluate long-term mucosal alterations. No patient had tracheal cannula and/or tracheo-esophageal voice prostheses at enrolment. They were previously evaluated for nasal and tracheal microbial colonization at our Department.<sup>10</sup> Healthy subjects were patients with vocal fold nodules or polyps and without any clinical and endoscopic sign of sinonasal disease. Written informed consent was obtained in every case. Institutional Review Board approval was obtained.

At enrolment, laryngectomy patients underwent symptoms evaluation (rhinorrhea, hyposmia, cough), endoscopic fiber optic nasal and tracheal examination, skin prick tests and nasal and tracheal scraping for cytological exam. Healthy subjects underwent the same assessment, except for tracheal cytological, because they had no tracheostomy. Eleven laryngectomy patients accepted inferior turbinate biopsy for histological examination. **No control subject underwent nasal biopsy.**

Cytological samples were obtained by scraping with a Rhino-pro® Curette (ASI Arlington Scientific Inc, Springville, UT, USA). Nasal samples were collected from the medial portion of the inferior turbinate, while tracheal samples from posterior wall of the

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4 trachea 2 cm below the tracheostomy, preventing contamination from the tracheostomy  
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6 border. After sampling, the material is laid on a microscope slide, fixed in 95° alcohol for 4 s  
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8 and stained by the May-Grünwald-Giemsa method. Observation is performed by a common  
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10 optical microscope, provided it is able of a 1000x magnification, analyzing at least 50  
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12 microscopic fields. Scraping and cytological analysis were performed by an otolaryngologist  
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14 expert in these procedures and not knowing whether the subjects underwent laryngectomy or  
15  
16 not. A pathologist re-analyzed and interpreted the tissue samples. All subjects underwent skin  
17  
18 prick tests to correctly evaluate cytological data, in particular inflammatory infiltrate, when an  
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20 allergic aetiology is suspected. All cytological samples were collected in November, when the  
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22 majority of pollens were absent. Specimen evaluation was performed using a checklist for  
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24 cytological findings.<sup>11</sup> Respiratory tracheal mucosa is similar to nasal mucosa, therefore we  
25  
26 used the same staining method. Mucous cell metaplasia was defined as the presence of  
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28 mucous cells > 25% of epithelial cells, while squamous cell metaplasia as the presence of  
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30 squamous cells. Cytological atypias were defined as nuclear enlargement associated with  
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32 increased nuclear-cytoplasmic ratio, hyperchromatism, chromatin clumping with moderately  
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34 prominent nucleoli, irregular nuclear membranes and multinucleation, and variation in size  
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36 and/or shape of the cells and nuclei.<sup>11</sup>

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42 Statistical Package for Social Sciences (SPSS), version 17.0 was employed for data  
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44 analysis. A descriptive analysis of all data was performed and they were reported as means or  
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46 percentages and standard deviations. The Kolmogorov-Smirnov test demonstrated a non  
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48 Gaussian distribution of variables, so non parametric tests were used. The chi-square test was  
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50 used to assess differences between groups in the mean of categorical variables. A p value less  
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52 than 0.05 was considered statistically significant.  
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## Results

### *Patients*

Mean age was  $62.64 \pm 9.53$  years (range 48-76 years) for healthy subjects and  $68.76 \pm 8.10$  years (range 50-83 years) for laryngectomy patients. Table 1 reports socio-demographic and clinical characteristics, such as age and sex, tobacco, alcohol consumption, previous exposure to irritant substances (i.e. asbestos dust) and allergies, and tumor related factors, such as histological type, TNM classification, grade and stage. There were no clinical or demographic differences (sex, tobacco, alcohol consumption, previous exposure to irritant substances and allergies) between control group and treatment group ( $p > 0.05$ ).

All patients underwent bilateral selective neck dissection, associated to total laryngectomy. Total laryngectomy was performed as salvage surgery for local recurrence in 20% of cases: in 3 patients after previous laser cordectomy and in 2 patients after partial supracricoid laryngectomy. Adjuvant radiation therapy or chemo-radiotherapy was administrated in 8 and 2 patients respectively, because of histopathological adverse features (extracapsular nodal spread, positive margins, pT4 primary, N2 or N3 nodal disease, perineural invasion, vascular embolism).

Concerning symptoms evaluation, anterior rhinorrhea was reported by 56% of patients, dry or productive cough by 48% and hyposmia by all patients.

At nasal fiber optic endoscopic evaluation, no statistically significant difference was observed between laryngectomy patients and healthy subjects ( $p > 0.05$ ). The main findings were turbinate hypertrophy (40%), pale nasal mucosa (40%) and serous nasal secretions (68%). Polypoid degeneration of nasal mucosa was seen in one case (4%). Regarding tracheal endoscopic exam, 64% of patients had dry mucosa, while one case (4%) had hyperaemia. Table 2 shows symptoms and endoscopic findings of laryngectomy patients and healthy



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4 subjects. No patient reported specific treatments for nasal and/or tracheal complaints. We did  
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6 not observe endoscopic findings of acute or chronic rhinosinusitis. All patients wore a  
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8 protective bib anteriorly to tracheostomy.  
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### 10 11 12 *Nasal cytology*

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15 Nasal cytological analysis demonstrated a mucous cell metaplasia (Figure 1A) in 20%  
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17 of laryngectomy patients, but it was absent in all healthy subjects ( $p < 0.05$ ); no squamous cell  
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19 metaplasia was found in both groups (Table 3). No statistically significant difference between  
20  
21 healthy subjects and laryngectomy patients was observed for neutrophilic and eosinophilic  
22  
23 **infiltration** ( $p > 0.05$ ). One healthy subject had rare eosinophils, due to allergic perennial  
24  
25 rhinitis. No mast cell and lymphocyte were found in both groups. In 15 patients (60%)  
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27 bacteria were present, without inflammatory infiltrate (Figure 1B). Figure 1C shows normal  
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29 nasal cytology from a control subject.  
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33 Nasal cytology did not show atypia. Concerning laryngectomy patients,  
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35 hyperchromatic supranuclear stria was absent in ciliated cells.<sup>12</sup>  
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38 Analyzing nasal cytological changes of treated patients, statistical analysis did not  
39  
40 show significant correlation with symptoms, endoscopic findings (turbinate hypertrophy,  
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42 mucosal hyperemia, nasal secretions), patients' age, tobacco smoking, tumor stage and  
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44 adjuvant radiotherapy ( $p > 0.05$ ).  
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### 48 49 *Tracheal cytology*

50  
51 Tracheal cytological analysis demonstrated a quite high rate of squamous cell  
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53 metaplasia (24% of patients), neutrophilic infiltrate (32%) and presence of bacteria (40%)  
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55 (Figure 2A-B). Four out of ten patients with bacteria had no inflammatory infiltrate. Mucous  
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4 cell metaplasia was observed in 12% of cases. We did not observe tracheal cytological atypia.  
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6 Sample material was insufficient for examination in 20% of cases, due to dry tracheal mucosa  
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8 (Table 4). Statistical analysis did not show significant correlation with symptoms, endoscopic  
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10 findings (mucosal hyperemia, tracheal secretions), patients' age, tobacco smoking, tumor  
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12 stage and adjuvant radiotherapy ( $p>0.05$ ). Insufficient sampling was associated with dry  
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14 tracheal mucosa ( $p<0.05$ ). A normal tracheal cytology in a patient without laryngectomy  
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16 showing ciliated cells was added from an independent bronchoscopy patient whose tracheal  
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18 brushing was banked in the pathology department (Figure 2C).  
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22 Normal nasal and tracheal cytology was observed in only 4 (16%) and 2 (8%)  
23  
24 laryngectomy subjects (8%), respectively.  
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### 26 27 28 **Histology**

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31 Histological examination of inferior turbinate showed submucosal stromal fibrosis in  
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33 all laryngectomy patients and submucosal neutrophilic and eosinophilic infiltrate in one case  
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35 (9%) (Figure 3).  
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### 37 38 39 **Discussion**

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42 Permanent changes of airflow in nasal cavities and trachea, due to complete separation  
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44 of upper and lower airways in laryngectomy patients, result in loss of physiological nasal  
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46 functions and presence of "unconditioned" inspired air in lower airways. Therefore,  
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48 laryngectomy patients represent a model to study nasal mucosal reaction to inactivity and  
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50 tracheal reaction to unconditioned inspired air. In literature, there is little evidence that  
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52 cessation of nasal airflow after laryngectomy has any harmful effect on nasal cavities. Only  
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54 two studies have evaluated cytological and histological changes on nasal mucosa.<sup>1,2</sup> In this  
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4 study we reported cytological alterations of nasal and tracheal mucosa, likely due to airflow  
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6 changes after total laryngectomy.  
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9 Experiments on unilateral naris closure in the rabbit demonstrated an increase of  
10 goblet cells number and changes of transitional epithelium into ciliated epithelium.<sup>13,14</sup>  
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12 Human respiratory nasal epithelium after laryngectomy appears to undergo similar changes to  
13 those observed in rabbits with unilateral naris closure. An early hypersecretory phase with an  
14 increased speed of mucociliary clearance occurs after total laryngectomy, while on the  
15 contrary a long term decrease of mucociliary clearance appears.<sup>3,13,15,16</sup> Early findings could  
16  
17 be explained in some patients abolishing the harmful effects of cigarette smoke on the nasal  
18 epithelium, as many laryngectomy subjects are heavy smokers before surgical treatment. Late  
19 alterations may be due to atrophy of nasal mucosa, chronic inflammatory changes and  
20 secondary infections by saprophytic bacteria.<sup>3</sup> Similar studies concerning lower airways in  
21 laryngectomy patients are not present in literature. Moreover, the presence of tracheal mucosa  
22 alterations in these subjects has never been studied.  
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35 Nasal cytology is a safe, not invasive, low-cost method for evaluating mucosal  
36 changes. It is already a currently used tool for the diagnosis of allergic and non-allergic  
37 rhinitis and other pathological alterations of nasal mucosa. The nasal mucosa of healthy  
38 subjects is constituted by four cytotypes (ciliated, goblet, striated and basal), which are  
39 arranged in a pseudostratified pattern on an underlying basement membrane. It does not show  
40 other cells except, rarely, neutrophils; therefore, the detection of a cell type different from  
41 these is a sign of possible pathology.<sup>11,17</sup>  
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51 Skoloudik et al. showed that main cytological alterations of nasal mucosa after total  
52 laryngectomy were hyperplasia of the basal zone cells and presence of bacteria without any  
53 inflammatory changes.<sup>1</sup> Karaca et al. showed a high percentage of histopathologic alterations  
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4 in laryngectomy subjects: destruction of goblet cells, destruction of cilia, destruction of  
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6 submucosal glands and fibrosis in stroma, focal or total atrophy of the mucosa, myxoid  
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8 degeneration, neovascularization and congestion.<sup>2</sup> Signs of damage, consisting mainly of  
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10 various degree of epithelial degeneration, were also observed for olfactory neuroepithelium,  
11  
12 associated to structural disorders of Bowman's glands.<sup>18</sup>  
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15 According to literature, our findings confirmed a high rate of stromal fibrosis in nasal  
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17 submucosa (all laryngectomy patients in our series). On the contrary, we did not see any  
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19 hyperplasia of the basal zone cells at nasal cytological examination and we found an higher  
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21 percentage of laryngectomy patients with mucous cell metaplasia, compared to healthy  
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23 subjects. Moreover, there was not a statistically significant difference for inflammatory  
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25 infiltrate between laryngectomy patients and healthy subjects. No squamous cell metaplasia  
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27 was observed in both groups. Based on these results, we could hypothesise that the absence of  
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29 nasal airflow may induce some cytological and histological long-term changes, not only in the  
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31 mucosa, but also in submucosa, with the tendency to a mucous cell metaplasia in nasal  
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33 mucosa and to a fibrotic status in submucosa. Probably different molecular mechanisms are  
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35 the reason why our findings are divergent from the previously published works on this topic.  
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39 Different molecular mechanisms could be related to a different individual genetic  
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41 predisposition to fibrosis and mucous cell proliferation, e.g. production of Fibroblast Growth  
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43 Factor (FGF) and/or mucins. Then, further studies on larger samples are necessary to  
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45 overcome bias due to different genetic predisposition and/or to better understand the  
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47 molecular reasons for the findings observed in our and other studies, such as mucous cell  
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49 metaplasia, submucosal fibrosis, and hyperplasia of the basal zone cells.  
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53 Nasal cytological examination demonstrated the presence of bacteria without  
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55 inflammatory infiltrate, according to previous studies that analyzed the bacterial flora of nasal  
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4 cavities in laryngectomy patients. Their nasal cavities and trachea are generally colonized by  
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6 non-pathogenic and/or potentially pathogenic bacteria, without signs and symptoms of  
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8 infection.<sup>1,8,10</sup> The absence of a statistically significant difference in cytological findings  
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10 between patients who underwent adjuvant radiotherapy and patients without such treatment  
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12 can be explained because the nose was not in the radiation fields.

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14  
15 Bronchoalveolar lavage and bronchial brushing under flexible bronchoscopy are  
16  
17 generally used for cytological examination of lower respiratory tract mucosa in several  
18  
19 patients and represent minimally invasive techniques. Since the tracheal mucosa is similar to  
20  
21 respiratory nasal one, we evaluated the feasibility of scraping with a curette for cytological  
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23 examination of tracheal mucosa in laryngectomy patients, using the same staining method.  
24  
25 Transient cough during procedure was observed in only five patients (20%) and was the  
26  
27 unique slight complaint reported by patients. No bleeding due to procedure occurred. May-  
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29 Grünwald-Giemsa staining method allowed a good identification of all cell types, epithelial  
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31 and inflammatory ones. Therefore, tracheal scraping could be considered a safe, not invasive,  
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33 low-cost method to assess alterations of tracheal mucosa in laryngectomy subjects. However,  
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35 it is important to take into account that sampling material could be insufficient in 20% of  
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37 cases, because of dry tracheal mucosa.  
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42 Tracheal cytological examination showed squamous cell metaplasia in 24% of cases,  
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44 and mucous cell metaplasia in 12% of patients. Possible causes of these alterations could be  
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46 the passage of unconditioned air with exposure to irritants and cold temperature, radiotherapy  
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48 effects on tracheal mucosa, and smoke. However, our statistical analysis did not show  
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50 significant correlation with tobacco smoking and adjuvant radiotherapy. Similarly to nasal  
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52 cavities and according to previous studies,<sup>7,9,10,19</sup> trachea was colonized by bacteria in 40% of  
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54 cases without clinical signs of infection. Only a few cases recognized the presence of bacteria  
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4 as a cause of neutrophilic inflammation; nevertheless, these patients had not endoscopic  
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6 findings of tracheitis. Further studies on larger samples are necessary to investigate reasons of  
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8 cytological tracheal alterations in these patients, assessing the role of airflow and bacterial  
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10 colonization. Moreover, stability or change of nasal and tracheal cytology over time after total  
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12 laryngectomy should be analyzed in future studies in order to better understand how airflow  
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14 alterations interacts with upper end lower respiratory tract in laryngectomy patients.  
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17  
18 In conclusion, the present study demonstrated that nasal cavities and trachea of  
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20 laryngectomy patients undergo long-term cytological and histological changes of mucosa and  
21  
22 submucosa, probably due to modifications of airflow. These changes must be kept in mind  
23  
24 when a laryngectomy patient is evaluated for nasal or tracheal complaints. Possible roles of  
25  
26 tracheal cytology in laryngectomy patients in clinical daily practice should be assessed with  
27  
28 further studies on larger samples. Our data set is small and has current little clinical relevance,  
29  
30 but it should be a foundation for future works. In future, detailed knowledge of cytological  
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32 changes in patients' tracheal mucosa could represent a key prerequisite for the choice of  
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34 effective interventions for tracheal pathology, such as saline solution aerosol, antibiotics,  
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36 corticosteroids and/or hyaluronic acid. Further studies are necessary in order to investigate  
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38 whether different treatments for tracheal complaints in patients who underwent total  
39  
40 laryngectomy may be effective for different cytological findings.  
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47  
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4 **Tables**  
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8 **Table 1.** Patients and tumor characteristics  
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Characteristic	Number of subjects (%)	
	Laryngectomy group (25 subjects)	Control group (25 subjects)
<b>Sex</b>		
Male	22 (88)	19 (76)
Female	3 (12)	6 (24)
<b>Smoker or former smoker</b>		
Yes	22 (88)	18 (72)
No	3 (12)	7 (28)
<b>Previous alcohol consumption</b>		
Yes	21 (84)	20 (80)
No	4 (16)	5 (20)
<b>Previous exposure to irritant substances</b>		
Yes	1 (4)	0 (0)
No	24 (96)	25 (100)
<b>Allergies</b>		
Yes	4 (16)	6 (24)
No	21 (84)	19 (76)
<b>Tumor site</b>		
Supraglottic larynx		7 (28)
Glottic larynx	17 (68)	-
Subglottic larynx	1 (4)	-
<b>Histological type</b>		
Squamous cell carcinoma	25 (100)	-
<b>Tumor (pTNM VI ed.)</b>		
T1	0 (0)	-
T2	3 (12)	-
T3	16 (64)	-
T4	6 (24)	-
<b>Nodes (pTNM VI ed.)</b>		
N0	16 (64)	-
N1	4 (16)	-
N2	5 (20)	-
N3	0 (0)	-
<b>Distant Metastasis (pTNM VI ed.)</b>		
M0	25 (100)	-
M1	0 (0)	-
<b>Grade</b>		
G1	1 (4)	-
G2	7 (28)	-
G3	17 (68)	-
<b>Stage</b>		
I	0 (0)	-
II	1 (4)	-
III	13 (52)	-
IV	11 (44)	-

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**Table 2.** Symptoms and endoscopic findings

Characteristic	Number of subjects (%)		p value
	Laryngectomy group (25 subjects)	Control group (25 subjects)	
<b>Symptoms</b>			
<b>Rhinorrea</b>			
Anterior	14 (56)	5 (20)	<b>0.02</b>
Posterior	2 (8)	1 (4)	0.26
<b>Hyposmia</b>			
Yes	25 (100)	2 (8)	<b>0.03</b>
No	0 (0)	23 (92)	
<b>Cough</b>			
Dry	3 (12)	3 (12)	<b>0.03</b>
Productive	9 (36)	1 (4)	
No	13 (52)	21 (84)	
<b>Endoscopic findings - Nasal cavity</b>			
<b>Nasal septal deviation</b>			
Yes	17 (68)	15 (60)	0.82
No	8 (32)	10 (40)	
<b>Turbinates</b>			
Hypertrophic	10 (40)	7 (28)	0.33
Normotrophic	14 (56)	18 (72)	
Atrophic	1 (4)	0 (0)	
<b>Nasal mucosa</b>			
Pale	10 (40)	8 (32)	0.67
Pink	15 (60)	17 (68)	
Hyperemic	0 (0)	0 (0)	
<b>Secretions</b>			
Dry nose	6 (24)	1 (4)	0.24
Serous	17 (68)	22 (88)	
Thick	2 (8)	2 (8)	
Purulent	0 (0)	0 (0)	
<b>Polypoid degeneration of mucosa</b>			
Yes	1 (4)	0 (0)	0.85
No	24 (96)	25 (100)	
<b>Endoscopic findings - Trachea</b>			
<b>Tracheal mucosa</b>			
Pale	2 (8)	-	
Pink	22 (8)	-	
Hyperemic	1 (4)	-	
<b>Secretions</b>			
Dry trachea	16 (64)	-	
Serous	4 (16)	-	
Thick	5 (20)	-	
Purulent	0 (0)	-	

**Table 3.** Nasal cytology findings.

Characteristics	Number of subjects (%)		p value
	Laryngectomy group (25 subjects)	Control group (25 subjects)	
<i>Normal nasal cytology</i>	4 (16)	21 (84)	0.02
<i>Squamous cell metaplasia</i>	0 (0)	0 (0)	0.99
<i>Mucous cell metaplasia</i>	5 (20)	0 (0)	0.03
<i>Neutrophilic infiltration</i>	2 (8)	3 (8)	0.95
<i>Eosinophilic infiltration</i>	0 (0)	1 (4)	0.64
<i>Presence of bacteria</i>	15 (60)	0 (0)	0.02

**Table 4.** Tracheal cytology findings.

Characteristics	Number of patients (%) (25 subjects)
<i>Normal tracheal cytology</i>	2 (8)
<i>Squamous cell metaplasia</i>	6 (24)
<i>Mucous cell metaplasia</i>	4 (12)
<i>Neutrophilic infiltration</i>	8 (32)
<i>Eosinophilic infiltration</i>	0 (0)
<i>Presence of bacteria</i>	10 (40)
<i>Insufficient sampling</i>	5 (20)

**Figure legends**

**Figure 1.** Nasal cytology (May-Grünwald-Giemsa staining): (A) mucous cell metaplasia (black arrows) in laryngectomy patients (magnification x400); (B) presence of bacteria (white arrows) without inflammatory infiltrate in laryngectomy patients (magnification x1000); (C) normal ciliated cells in control patients (black stars), without mucous cells, inflammatory cells and bacteria (magnification x400).

**Figure 2.** Tracheal cytology (May-Grünwald-Giemsa staining): (A-B) squamous cell metaplasia (black arrows), neutrophilic infiltrate (white stars) and presence of bacteria (white arrows) in laryngectomy patients (magnification x1000); (C) tracheal cytology in a patient without laryngectomy showing normal ciliated cells (black stars) (magnification x400).

**Figure 3.** Histological examination of inferior turbinate (Hematoxylin-Eosin staining, magnification x100): submucosal stromal fibrosis (black arrows) in laryngectomy patients.

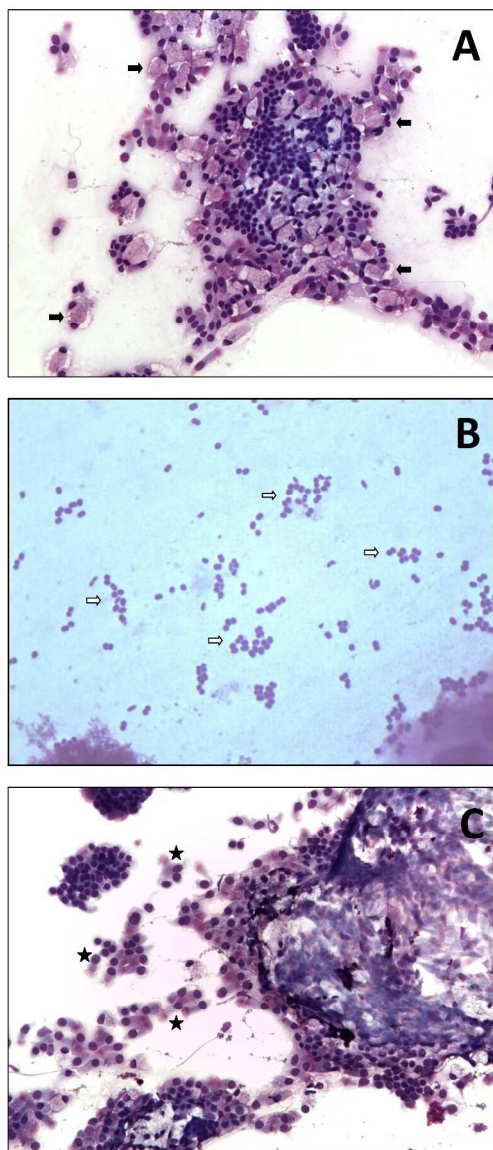


Figure 1. Nasal cytology (May-Grünwald-Giemsa staining): (A) mucous cell metaplasia (black arrows) in laryngectomy patients (magnification x400); (B) presence of bacteria (white arrows) without inflammatory infiltrate in laryngectomy patients (magnification x1000); (C) normal ciliated cells in control patients (black stars), without mucous cells, inflammatory cells and bacteria (magnification x400).

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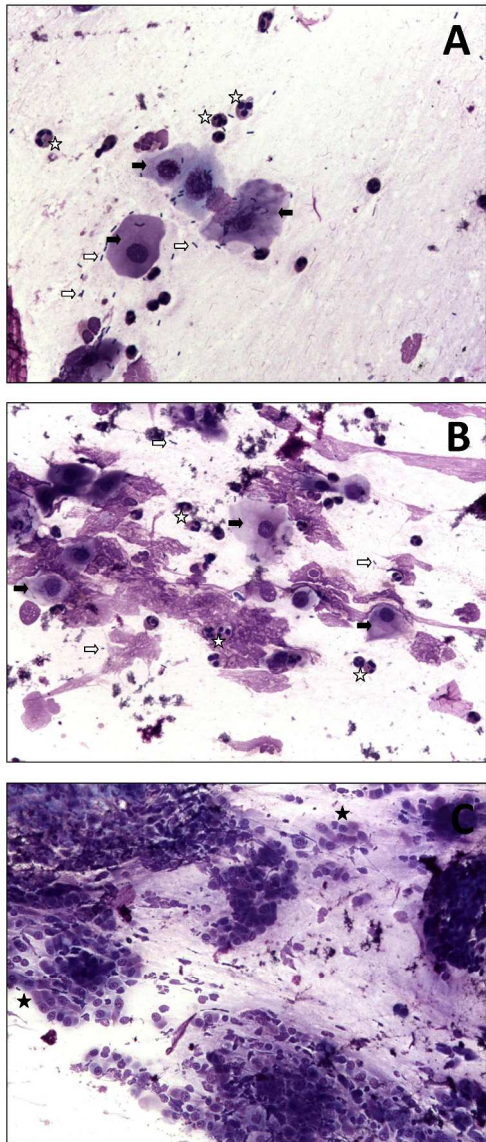


Figure 2. Tracheal cytology (May-Grünwald-Giemsa staining): (A-B) squamous cell metaplasia (black arrows), neutrophilic infiltrate (white stars) and presence of bacteria (white arrows) in laryngectomy patients (magnification x1000); (C) tracheal cytology in a patient without laryngectomy showing normal ciliated cells (black stars) (magnification x400).

329x736mm (300 x 300 DPI)



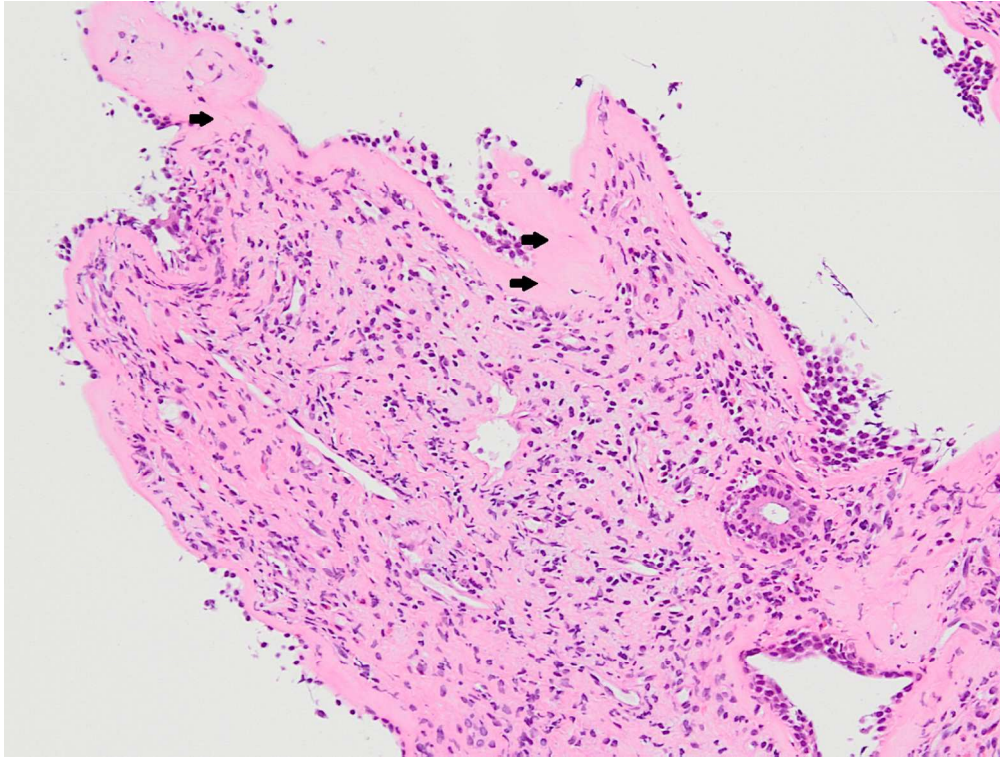


Figure 3. Histological examination of inferior turbinate (Hematoxylin-Eosin staining, magnification x100): submucosal stromal fibrosis (black arrows) in laryngectomy patients.

485x366mm (72 x 72 DPI)