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Changing in nasal cellularity in post-acute covid patients

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Abstract

Introduction

The sudden and storming onset of coronavirus 2 infection (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) was associated by severe acute respiratory syndrome. Corona virus disease 19 (COVID-19) has appeared as a pandemic throughout the world. The mutational nature of the virus, along with the different means of entering and spreading throughout the body has involved different organs. Thus, patients are faced with a wide range of symptoms and signs.
SARS-CoV-2 enters the human airway through aerosol or fomite transmission and comes into contact with the mucous membranes lining the oropharynx, nose and eyes. The nasal mucosa can be considered one of the first targets of SARS-CoV-2 and ciliated cells are primary targets for SARS-CoV-2 replication. Although the nose plays a crucial role in the transmission and multiplication of the virus and nasal symptoms such as anosmia/hyposmia may represent the first manifestation of SARS-CoV-2 infection, the changes in nasal mucosa induced by COVID-19 have not yet been well characterized.

Objective

The aim of this study was to evaluate cytological changings of the nasal mucosa caused by SARS-CoV-2, in patients who were still positive for the infection but who had passed the acute phase.

Material and Methods

The study involved the analysis of 26 nasal cytological samples taken from patients that had already overcome the acute phase of the disease but well still positive for COVID-19. Samples were evaluated after 21 days after sampling, to avoid an infective risk.

Discussion

The presence of both neutrophils and eosinophils underlines an inflammatory reaction in the nasal mucosa. In fact, neutrophils play a critical function in the clearance of bacteria with specific mechanisms to combat viruses. The role of eosinophilic inflammation in COVID-19 is still debated. Probably, these cells could directly affect the course of COVID-19 by being involved in killing bacteria and viruses and acting by producing antiviral molecules, but also by serving as antigen-presenting cells.

Conclusion

This study shows that SARS-CoV-2, not only causes cytopathic damage at the ciliated cells level, but also induces an important inflammatory response, responsible for symptoms commonly associated with COVID-19. Not only neutrophils but also eosinophils would appear to be involved in the inflammatory defense mechanisms activated to fight the infection.

Key words: Sars-Covid 19, Cytology, Ciliocytophthoria, nasal cell, eosinophils, neutrophils

All the Authors declare no conflict of interest in this work.

Authors may declare any patents related to the published work, either those pending or already obtained.

“Written informed consent has been obtained from the patients to publish this paper”

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Introduction

In December 2019 the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified in Wuhan, China, and quickly spread all over the world[1]. After causing an outbreak in China, coronavirus disease (COVID-19) has developed into a pandemic that rapidly spread worldwide, resulting in over 60 million cases and more 1.000.000 deaths within a few months.
Among the European countries, Italy was the first place to be hit by the COVID-19 disease[2]. COVID-19 mainly affects lower respiratory tract causing symptoms such as fever, cough, dyspnea, and chest tightness that could progress rapidly to acute respiratory distress syndrome[3]. However, the disease also causes upper respiratory tract symptoms including nasal congestion, sore throat, and olfactory dysfunction[4]. As a matter of fact, SARS-CoV-2 enters the human airway through aerosol or fomite transmission and comes into contact with the mucous membranes lining the oropharynx, nose and eyes[5]. Then the virus binds with angiotensin-converting enzyme 2 (ACE2) receptors, enters inside cell, multiply there and manifests itself. Thus, the nasal mucosa can be considered one of the first targets of SARS-CoV-2. This mucosa is characterized by a pseudostratified ciliated epithelium, with active ciliary movement, interspersed with mucus-secreting globet cells and covered with a thin layer of mucus, that traps and removes pathogens, including SARS-CoV-2, and particulate matter from inspired air. While this epithelium represents a front line in respiratory defense against lower airway infection, it can also be a target tissue for infection, multiplication, and propagation of pathogens. In particular, ciliated cells have been shown to be primary targets for SARS-CoV-2 replication, since ACE2 receptor is highly abundant in the apical surface membrane in fully differentiated ciliated cells of the nasal cavity[6]. During the first stage, the primary target cells are infected and consumed rapidly. The viral load experiences a substantial increase to the peak level. This agrees with the clinical data that onsets of ARDS are observed around this time. Following the viral peak, the viral load declines slightly and enters a plateau phase in which the viral load remains approximately unchanged or declines slowly. The source of this plateau phase is the infection of lymphocytes as a secondary target. Without the infection of lymphocytes, SARS-CoV-2 would continue to infect and deplete pneumocytes. Therefore, viral load would decline quickly, and the infected individual would lose the transmissibility. After viral load persists for a period of time (e.g. a week in some patients or month in other one), the next phase follows as the adaptive immune response emerges. Seroconversion was also detected about a week after acute respiratory disease (ARDS) onset in COVID-19 patients. During this stage, viral load declines rapidly to an undetectable

level.[30] Although the nose plays a crucial role in the transmission and multiplication of the virus and nasal symptoms such as anosmia/hyposmia may represent the first manifestation of SARS-CoV-2 infection, the changes in nasal mucosa induced by COVID-19 have not yet been well characterized[7]. Therefore, the aim of this study was to evaluate cytological changings of the nasal mucosa caused by SARS-CoV-2, in patients who were still positive for the infection but who had passed the acute phase[31]. Unlike viruses such as influenza, smallpox, and polio, coronaviruses have only recently been discovered to infect the human population. When they were first discovered in the 1960s, there was almost no epidemiological, genomic, or pathogenic information about these viruses – only that they contained RNA surrounded by a membrane composed of 'spike'-shaped proteins [39]. The crown-like appearance of these surface 'spike' proteins gave the virus family the name – 'corona' is Latin for the crown. Viruses with that specific shape and structure belong to the family of Coronaviridae, which are grouped into four genera using their phylogeny: alpha-CoV, beta-CoV, gamma-CoV, and delta-CoV [40]. As of 2020, the US-based Centers for Disease Control and Prevention (CDC) recognizes seven coronavirus strains that can infect humans. In general, they are classified as single-stranded, positive-sense RNA genome-bearing viruses. Their genome is estimated to be around 26–32 kilobases (for comparison, the human genome is 3 000 000 kilobases). The first identified coronaviruses in the human population were human CoV-229E (HCoV-229E) and HCoV-OC43. These viruses were found to cause upper respiratory tract diseases, such as the common cold, and infections caused by the viruses have low levels of severity. After the emergence of the first two coronavirus strains, two other strains were identified: HCoV-HKU1 and HCoV-NL63. Three other coronavirus strains that have been identified in the human population since are SARS-CoV, MERS-CoV, and SARS-CoV-2. All three of these coronavirus strains vary from the four common strains as they can cause severe illnesses that may result in death.[41]

According to the World Health Organization, COVID-19 has been confirmed in more than 113 million cases across 223 countries, leading to more than 4.1 million deaths. Recent estimates indicate that up to 98% of individuals diagnosed with COVID-19 developed forms of chemosensory disorders, most prominently smell loss. Data collected before the COVID-19 pandemic showed that up to 49% of the population report an episode of olfactory loss over their lifetime, with 5% of them reporting complete smell loss (anosmia). Population-based epidemiological studies before COVID-19 provide prevalence estimates of smell loss ranging from 2.7 to 24 and taste disorders ranging from 0.6 to 20%. Moreover, in older adults the prevalence of olfactory impairment increases. Reports to date reveal that the COVID-19 pandemic has already significantly

increased the prevalence of chemosensory disorders worldwide, especially among younger cohorts, yet the global estimates on chemosensory disorders may be markedly underestimated.

Materials and Methods

Our study involved the analysis of 26 cytological samples taken from patients admitted to a subacute COVID-19 Center for rehabilitation after an acute viral infection caused by SARS-CoV-2. All the patients had a Covid-Sars 19 infection that causes hospitalization and began oxygen therapy and steroids. Various ventilation methods were used during hospitalization, from orotracheal intubation to the use of a helmet for proper ventilation, to the use of a venturi mask with various oxygen levels. 20 patients had ventilation in intensive care and use of a helmet, and 6 patients had only high ventilation with venturi mask. The patients had all overcome the acute phase of the disease and were hospitalized to continue their rehabilitation pathway. Inclusion criteria were arranged as follows: genders: both; pathology: documented SARS-CoV-2 infection, assessed by at least one positive molecular test for SARS-CoV-2 and one positive nasopharyngeal (NP) swab, with the first positive test within the previous 8 to 21 days. Specific exclusion criteria were ongoing local or systemic corticosteroid treatments; non-authorization to perform the nasal cytological sampling. All recruited patients agreed to undergo nasal cytology and signed informed consent. No patients had an endoscopic exam of the nasal fossa due to the infection of coronavirus and the impossibility to do an exam in an isolated area. We have done only anterior rhinoscopy with Kilian to evaluate the nasal fossa and to do the nasal scarping. All the patients had an inflammation of the mucosa, only 5 patients had turbinate hypertrophy, and all the patients has consistent secretion inside the nose, due to the use of oxygen therapy.

Nasal cytology (NC) represents a useful and easy diagnostic tool to study rhinitis, because it allows to detect and measure the cell population within the nasal mucosa at a given instant, to better discriminate different pathological conditions and to evaluate the effects of various stimuli (allergens, infectious, irritants, physio-chemicals) or treatments. At the end of the 1800s, Gollash and Von Mihalkovics, firstly depicted the microscopic aspects of nasal mucosa, but this remained only an anatomical and morphological description. In 1927, Eyermann firstly identified eosinophils in the nasal secretion of patients suffering from hay fever. Although the pathogenesis of allergic reactions was still over the horizon, these authors clearly underlined the relationship between a specific cell population and a specific clinical disease. After decades of scarce interest, the study of nasal cytology had a rapid and progressive development during the 1970s, when the technique was

used to assess the effects of various drugs and stimuli [6–8]. The use of nasal scrapings was further developed, with no standardized techniques, during the last decades. The technique of NC was better systematized and investigated in depth starting from 2006. The NC approach subsequently provided relevant contributions to the knowledge of rhinitis from a pathophysiological point of view, allowing also to identify different phenotypes.

The nasal mucosa is a pseudo-stratified ciliated epithelium, containing also mucinous cells that are responsible for the continuous mucus secretion. The ciliated cell Fig. 1 is the most differentiated cell type in the nasal mucosa. Ciliated and mucinous cells both contribute to the mucociliary clearance that is part of the innate and first-line defense of airways. The normal ciliated/mucinous cell rate is around 4:1. In normal conditions (healthy individuals without nasal diseases), only four cytotypes can be identified at NC: ciliated cells, mucinous cells, basal cells/striated cells; only sparse neutrophils can be found occasionally. The perinuclear halo or hyperchromatic supranuclear stria in ciliated cells is a hallmark of normal function. On the contrary, the detection of eosinophils, mast cells, bacterial or fungal hyphae clearly identifies a pathological condition. NC is easy to perform, not invasive, cheap, and repeatable in the same subject also at short time intervals. For these reasons, it represents an affordable diagnostic technique that can be applied in all age ranges, also at the physician's office. In detail, the technique involves sampling, processing and microscope reading. Sampling requires the collection of cells from the surface of nasal mucosa. This can be made by a common sterile cotton tip or, better, with a sterile disposable curette (9Rhino-Probe, Arlington Scientific, Springville, UT, USA). Cotton tips can be used in infants when an anterior rhinoscopy may be considered more difficult to perform. Nonetheless, in our experience, due to the conformation of nostrils and accessibility, there is no special problem for the procedure even in very young children. It must be considered that this procedure does not require a biopsy (histological sample), but a simple surface cytological collection. Samples should be collected from the middle portion of the inferior turbinate where the rate ciliate/ mucinous cells are expected to be well balanced. The procedure can be easily performed under anterior rhinoscopy, with an appropriate light source. No application of anesthetic is required because the procedure is totally painless. Obviously, the operator should be well trained, to ensure a proper sampling. The presence of squamous cells usually indicates a contamination from the skin epithelium of nares, thus a not optimal sampling. When the curette is used, the sample is immediately smeared on a glass slide and air-dried. Then, the slide is stained with the common May–Grunwald–Giemsa (MGG) procedure. This staining method allows to easily identify all the cellular components (neutrophils, eosinophils, lymphocytes,

and mast cells), plus bacterial and fungal spore/hyphae. The traditional MGG staining procedure requires about 30 min, but pre-mixed compounds, the stained sample is read at optical microscopy, with a 1000X objective with oil immersion. At least 50 fields should be read, to obtain a mean value of the differential cellular count. The count of each cell type can be expressed as a percentage of the total cells (including mucinous and ciliated cells), as an absolute value, or by a semi-quantitative grading. It is obviously essential that the same count method is always used in reporting the results within clinical studies or routine activity. This aspect remains one of the major drawbacks of NC, because the reporting of cellular count varies from author to author and from a laboratory to another. Despite this limitation, the differential cell count and the microscopic appearance of nasal smears usually allow to discriminate different pathological aspects Nasal cytology samples were taken from the middle part of the inferior turbinate under anterior rhinoscopy, according to validated criteria, using a Nasal Scraping. The samples were then smeared on a glass slide and air-dried. Since SARS-CoV-2 has been shown to maintain a dangerous viral load on inanimate surfaces, such as glass, for up to 21 days, nasal cytology samples were stained with May-Grunwald-Giemsa only after 21 days[9]. Stained samples were then read at optical microscopy, with a 100x objective with oil immersion. A minimum of fifty fields is considered necessary to identify a sufficient number of cells[10]. The presence of any cytopathological alterations as well as inflammatory cells infiltrating the nasal mucosa was evaluated.[35,36,37,38]

Results

Twenty-six consecutive patients were recruited, including 15 males (58%). The patients mean age was 69 years (age range= 29-92 years). All patients were still positive for SARS-CoV-2 infection when underwent nasal cytology after written consent. Of the 26 patients, 18 (69%) were still receiving supplemental oxygen administered with nasal cannulas (flows varying from 2 l/min to 4 l/min), none with a venturi mask. 7 (27%) patients had a history of allergies: 5 (19%) had allergies to pollen and 2 (8%) to drugs (amoxicillin, ASA). The analysis of the cytological samples showed that all patients had a variable degree of neutrophilic infiltration and an abundant eosinophilic infiltrate. The presence of biofilm was found in 10 (38%) samples. Interestingly, rarefaction of the ciliary apparatus was found in all the cytological samples, since all the ciliated cells showed the disappearance of the hyperchromatic “Supranuclear Stria” (SNS), corresponding to the Golgi apparatus, which is considered a marker of wellness of ciliated cells[11]. Moreover, demonstrating cellular distress, all ciliated cells showed signs of “Ciliocytophthoria” (CCP), such as condensed nuclear chromatin,

marginalization of nucleoli, inclusion bodies, presence of intranuclear halo and cytoplasmic vacuoles[12].

Discussion

The central role played by the nose in SARS-CoV-2 infection has already been 8demonstrated, as viral entry-associated genes have been detected in nasal epithelial cells together with genes involved in innate immunity, highlighting the cells' potential role in initial viral infection, spread and clearance. As a matter of fact, SARS-CoV-2 entry receptor ACE2 and viral entry-associated protease TMPRSS2 are highly expressed in nasal goblet and ciliated cells. This finding implicates these cells as loci of original infection and possible reservoirs for dissemination within and between individuals. Moreover, the co-expression of these receptors in superficial conjunctival cells could explain the ocular symptoms observed in COVID-19 patients, in which the virus could potentially spread from the nose to the eyes through the nasolacrimal duct[13-29].

Given the central role of the nasal mucosa in the transmission and spread of SARS-CoV-2, research has focused on the possibility of using administered drugs/vaccines to limit the spread of the virus[14,15]. However, cytological changes in nasal mucosa of patients affected by COVID-19 have not been clearly defined. Before us, Gelardi and colleagues were the only ones to have performed Nasal Cytobrush Cytology to evaluate possible alterations of the nasal mucosa of 18 COVID-19 patients. This study showed that all patients had a mild lymphocytic infiltrate and a rarefaction of the SNS, while none of the patients had Ciliocytophthoria or other signs of cell injury[16]. Thus, our study is the first to describe the cytopathic effects of the virus on nasal cells. Indeed, as shown in the results, all patients examined showed signs of cellular injuries, such as the rarefaction of SNS and Ciliocytophthoria. Interestingly, we also found a mixed neutrophilic-eosinophilic inflammatory infiltrate. The presence of both neutrophils and eosinophils underlines an inflammatory reaction in the nasal mucosa. In fact, polymorphonuclear neutrophil granulocytes are the immune system's first responders to threats by invading microorganisms, as they combat the intruders by phagocytosis and externalization of granules containing lytic and microbicidal factors[17]. Since neutrophils play a critical function in the clearance of bacteria with specific mechanisms to combat viruses, the role of neutrophils in SARS-CoV-2 infection has been widely investigated. In this context, a rising neutrophil count and a falling lymphocyte count during the severe phase of the infection have been correlated with a worse prognosis and treatments using neutrophil extracellular traps (NETs)-targeting approaches have been considered to decrease the damage caused by hyperinflammation[18]. In

support of this, in influenza pneumonia neutrophils have already been shown to induce NET generation and increased endothelial damage, causing acute lung injury and alveolar-capillary damage[19]. Therefore, the neutrophilic nasal and subsequently pulmonary infiltrate in COVID-19 could be related to the severity of the disease and the associated symptoms. While the finding of neutrophils in cytological samples can be explained by the role intrinsically played by neutrophils, the reasons for the presence of eosinophils in the nasal inflammatory infiltrates appear less clear, as they are typically involved in parasitic infections, allergies and in several Type-2 diseases including asthma, chronic rhinosinusitis with nasal polyps, eosinophilic gastrointestinal disorders, and hyper eosinophilic syndromes[20]. The role of eosinophils in SARS-CoV-2 infection is still debated since several studies have shown that decreased eosinophil and lymphocyte count and increased neutrophil count are associated with a worse clinical outcome of COVID-19[21,22]. Nevertheless, other studies have shown that the lungs of COVID-19 patients can exhibit eosinophil-mediated inflammation, together with an elevated NKT cell response, which is associated with COVID-19 pneumonia[23]. Indeed, although eosinophil blood count could be reduced in COVID-19 patients, a more detailed analysis of eosinophils in primary care patients with COVID-19 has revealed increased eosinophilic expression of CD11, indicating baseline eosinophilic activation. These data suggest that the reactive eosinophils disappear from the blood during severe COVID-19 disease, leaving behind the resident eosinophils, but these cells show increased responsiveness and higher sensitivity or priming for (ex vivo) activation. [33] A hyper-responsive eosinophilic compartment could hint toward a more immune-regulatory function of these cells in primary care patients with COVID-19 disease. [32]Thus, eosinophils could directly affect the course of COVID-19 as they have also been described to be involved in killing bacteria and (respiratory) viruses and act by producing antiviral molecules, but also by serving as antigen-presenting cells [24,26,26]. The nasal mucosa has traditionally been considered a mere physical barrier between the host and its environment. Nowadays, it is becoming increasingly clear that nasal mucosa is metabolically active and plays a crucial role in maintaining the immunological barrier, producing various inhibitory substances and secretory IgA. While histological samples allow to directly detect any defects in the integrity of the nasal mucosal barrier, nasal cytology samples allow to indirectly detect such alterations. In particular, the Hyperchromatic Supranuclear Stria (SNS) is considered a specific cytological marker for the anatomic and functional integrity of ciliated cells and, therefore, for the mucosal barrier integrity. Indeed, since diseases affecting nasal mucosa epithelium determine its rearrangement, ciliated cells manifest distress phenomena including the disappearance of the hyperchromatic SNS. Therefore, the absence of the

SNS is considered a useful prognostic sign of nasal disorders. Furthermore, among the mechanisms responsible for epithelial toxicity during allergic inflammation, the predominantly eosinophilic inflammatory infiltrate has been shown to be responsible for barrier damage. As a matter of fact, eosinophils secrete several cationic proteins, including major basic protein (MBP), which induces a marked decrease in the number of desmosomes and exfoliation of epithelial cells. The noteworthy role that eosinophils play in epithelial barrier damage has been clearly described in asthmatic patients, in which a strong correlation has been found between the eosinophilic infiltrate, MBP and the presence of "Creola bodies," defined as clusters of apoptotic epithelial cells resulting from MPB-induced exfoliation. In AR, the increased permeability of the epithelium, due to damage of the cell junctions, as well as the exfoliation of the epithelial cells allows allergens and other noxious substances to penetrate the barrier and cause the main symptoms of Sino-nasal disease.

Conclusion

This study shows that SARS-CoV-2, which is a particular tropism for hair cells, not only causes cytopathic damage at the ciliated cells level but also induces an important inflammatory response, responsible for symptoms commonly associated with COVID-19, such as rhinorrhea and nasal obstruction. In this context, not only neutrophils but also eosinophils would appear to be involved in the inflammatory defence mechanisms activated to fight the infection. In particular, SARS-CoV-2 infection may drive specific innate immune responses, including eosinophil-mediated inflammation, and subsequent pulmonary pathogenesis via enhanced Th2-biased immune responses, which might be crucial drivers of critical disease in COVID-19 patients[26]. Further studies are needed to better define the characteristics of the cellular damage induced by the virus as well as to understand the inflammatory pathways activated by SARS-CoV-2 infection, with the aim of identifying new preventive and therapeutic strategies.

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Figure 1. Ciliar Cell with SIS. Magnification 100x objective with oil immersion. Stained with May Grundwald Giemsa

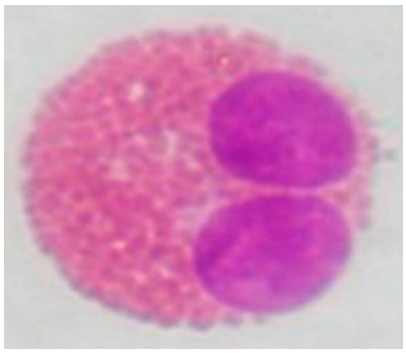

Figure 2. Eosinophilic cell. Magnification 100X objective with oil immersion Stained with May Grunwald Giemsa

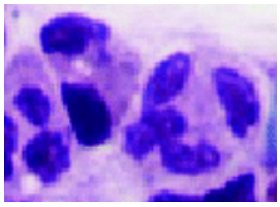

Figure 3 Neutrophil cell. Magnification 100X objective with oil immersion Stained with May Grundwald Giemsa

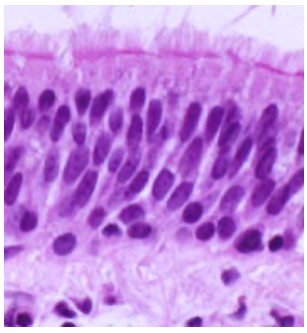

Figure 4 Pseudostratified epithelium ciliated cell objective with oil immersion Stained with May Grundwald Giemsa. Magnification 100x with optical microscope

SOLITARY FIBROUS TUMOR OF THE SINONASAL TRACT: A RARE CASE INVOLVED THE NASAL SEPTUM

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ABSTRACT

Solitary fibrous tumor (STF) is a mesenchymal neoplasm with fibroblastic differentiation that usually develops in the pleura, but can also affect extrapleural sites. Sinonasal localization is very rare. A 60-year-old man complained right-sided nasal obstruction and bilateral epiphora for 5 years. Based on MRI and CT, the tumor was surgically removed. Final histology examination evidenced diffuse spindle-shaped cells proliferation and less cellular areas with collagenized stroma. The immunohistochemistry showed positivity for STAT6, vimentin, CD34 and CD99. The definite diagnosis was Solitary Fibrous Tumor.

INTRODUCTION

Solitary fibrous tumor (STF) is a spindle cell mesenchymal neoplasm with fibroblastic differentiation that usually develops in the pleura, but can also affect extrapleural sites [1]: in 6% of cases it involves the head and neck region [2]. Sinonasal localization is very rare where it can lead to nonspecific symptoms such as unilateral nasal obstruction. It has a slight prevalence for the male sex compared to the female sex (M:F 5:4). The etiology is unknown although the possible pathogenesis lies in the NAB2-STAT6 gene fusion [3,4].
We describe the case of a tumor with implantation at the level of the right nasal septum, which on preoperative biopsy was classified as an anthrochoanal polyp.
Thanks to CT and MRI study, final histological examination, and positivity for vimentin, CD34, and CD99 on immunohistochemical examination, we were able to reach the correct diagnosis of solitary fibrous tumor.
At 24 months, the patient is free of disease.

CASE DESCRIPTION

A 60-year-old Caucasian man presented at our ENT unit reporting a symptomatology of right-sided nasal obstruction, anosmia, rhinorrhea and bilateral epiphora for 5 years.

His past medical history was positive for cancer: he referred previous surgery for skin melanoma. Physical examination revealed a large, lobulated, hard tumor originating from the nasal septum of the right nasal cavity, extending from the nasal vestibule to the ipsilateral choana (Fig 1).

Computed Tomography (CT) scans showed a oval mass, with lobulated margins, parenchymatous density that displaces the nasal septum to the left, the right nasal wall to the right, the right ethmoid cells superiorly, the unciform process and the right lamina papyracea superoexternally, with which it is in continuity (Fig. 2).
The tumor appeared on Magnetic Resonance (MR) with uneven intensity - mainly medium-low with hypointense and hyperintense components - in T1 and - mainly low with hyperintense components - in T2 and STIR.
A preoperative biopsy under local anesthesia was performed, which identified the mass as an anthro-coanal polyp.

The most important differential diagnosis was made with a metastasis/recurrence of melanoma because of the patient's past history. As reported in the literature, especially melanoma metastases can lose melanocyte lineage-specific markers, presenting unusual morphology and immunohistochemical features similar to SFT, emulating the same. PCR study of mutations for BRAF, NRAS, KIT, GNAQ and GNA11, which confirms the diagnosis of recurrent/metastatic melanoma, is mandatory to recognize the two forms [5].

The patient underwent surgery under general anesthesia for endoscopic exeresis of the tumor. The insertion at the level of the nasal septum was identified after dissection of the mass with a plasma blade, and then a subperoisteal removal of the septal mucosa was performed. During the surgical procedure, it was necessary to causticate the septal branch of the sphenopalatine artery. Finally, uncinectomy and anstostomy media were packed.

Final histology examination evidenced diffuse spindle-shaped cells proliferation and less cellular areas with collagenized stroma, moderate polymorphism, low mitotic index (less than 4 mitoses per 10 HPF).
The immunohistochemistry showed positivity for STAT6, vimentin, CD34 and CD99.
The definite diagnosis was Solitary Fibrous Tumor with free resection margins.
The patient underwent new examination at 3, 6, 12 and 24 months remaining free of disease (Fig. 3).

DISCUSSION

Solitary fibrous tumors are submesothelial, fibroblast-like mesenchymal tumors that typically develop in the parietal or visceral pleura [1]. However, they can be found in extrapleural site: the head-neck district is involved in only 6% of cases [2]. The peak incidence is between the 5th to 6th decade of life with a slight prevalence for the male sex compared to the female sex. In most cases it is asymptomatic or may give signs of itself by causing unilateral nasal obstruction when it totally engages the nasal fossa. MRI shows a hypo- or isointense mass at T1 weighing and hypo- or hyperintense at T2 weighing, with heterogeneous enhancement after contrast [6].
Immunohistochemical examination shows positivity for STAT-6, CD34, CD99, BCL-2 [7].
Criteria for classifying the mass as malignant have been defined by WHO: hypercellularity, increased mitoses (>4 mitoses per 10 high-power fields), cytologic atypia, tumor necrosis, and/or infiltrating margins [8,9].

STF of the sinonasal tract is very rare: only 9 cases have been described in the literature. The case history, considering those with an implantation base at the level of the nasal septum, is further reduced.

In each of them, unilateral nasal obstruction, similarly to our case, was the only sign that led the patient to a specialist examination. The diagnostic procedure consisted of endoscopic examination, imaging tests (CT and/or MRI), and preoperative biopsy. The treatment of choice was endoscopic surgery. If the mass is highly vascularized, exeresis may be preceded by embolization of the mass [10].

In our case, surgery without chemo-radiotherapy was the treatment of choice because the mass did not meet WHO histologic criteria as it was characterized by hypocellularity, low mitotic number (<4 mitoses per 10 high-power fields), absence of cytologic atypia, tumor necrosis, and/or infiltrating margins.
Specifically, endoscopic resection of the mass with excision of its attachment to the mucoperichondrium with cartilage was performed.
The study by Sireci et al., in this regard, retrospectively analyzed the database of two University Hospitals (Genoa and Palermo) on the type of treatment given to sinonasal tumors from 2012 to 2020. A cohort of 32 patients with nasal septal tumors was identified: 28 (87.5%) cases were benign neoplasms and four (12.5%) cases were malignant tumors.
The surgical approach chosen, for benign tumors, was resection of the mucocondrium/periosteum; in contrast, malignant tumors were treated with resection of all layers of the nasal septum [11].
In the case described, we preferred the transnasal endoscopic approach and the "disassembly technique," which consists of oriented anatomic decomposition of the mass to precisely locate the implant base [12].
The plasma blade was used for the dissection of the mass because, as described in the literature, the CO2 laser can produce high thermal energy that can damage the tissue, distorting the pathological examination.

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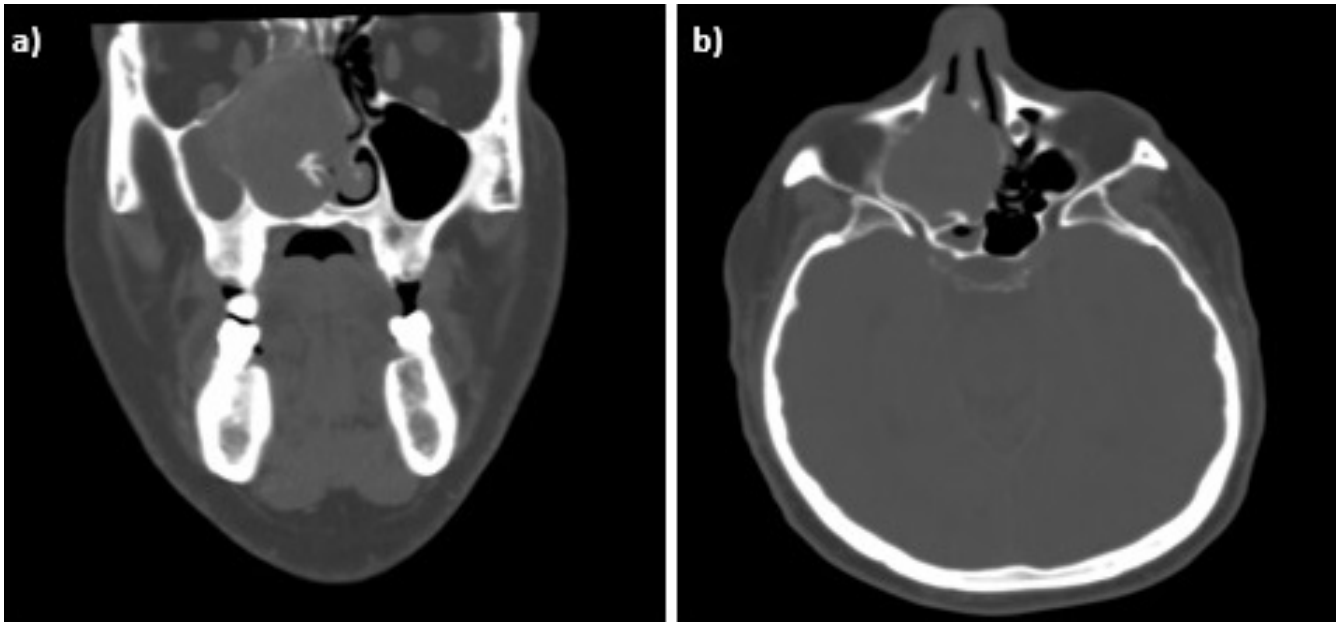

Fig.1 – CT image of the SFT in coronal section (a) and axial section (b)

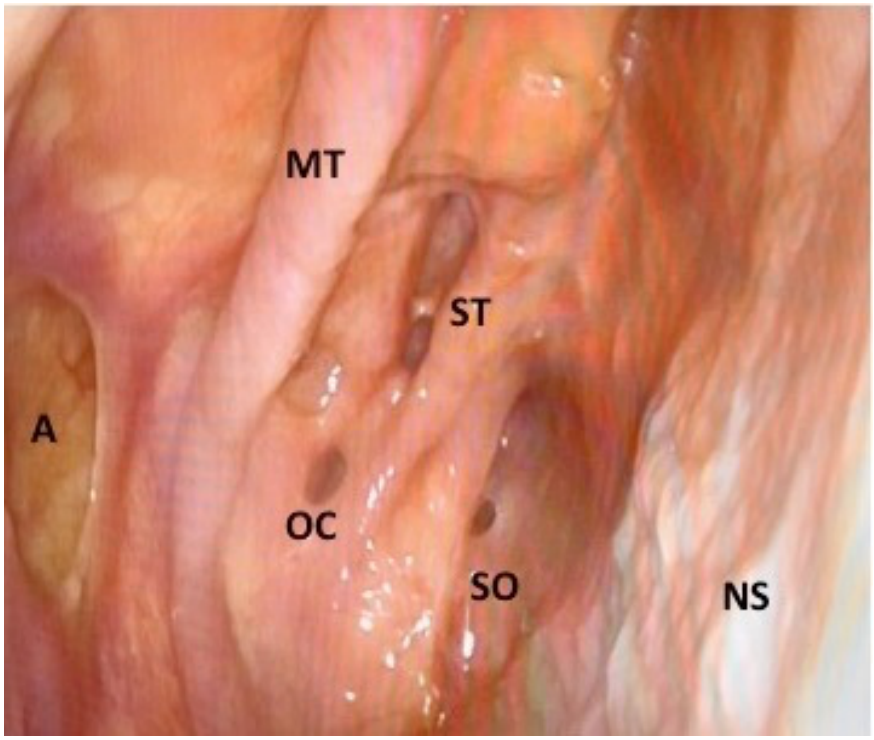

Fig.2 – Control after 2 years. Absence of recurrence in the nasal nostril. A, Antrostomia; MT, Middle Turbinate; OC, Onodi Cell; Superior Turbinate; SO, Sphenoid Ostium; NS, Nasal Septum.

The Elder Nose: a narrative review

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Abstract

Over the years senile rhinitis has become a common disease affecting the elder population with a mild yet very bothersome symptom- watery rhinorrhea. Other rhinitis symptoms such as congestion, sneezing, nasal/ocular pruritus, and postnasal drainage can significantly affect the quality of life for older adults. The pathophysiology of senile rhinitis is complex, there are multiple subsets of nociceptive, parasympathetic, and sympathetic nerves that innervate human nasal mucosa along with numerous morphologic alterations, with still much to be discovered about the aging effects these complex mechanisms. One of the objectives of this review is to gain insight to complex structural, physiological and morphological changes that lead to this pathology. The most essential factor in the diagnosis lies in detailed medical history and endoscopic ENT evaluation. Coexisting allergic rhinitis could complicate and delay diagnosis and treatment of senile rhinitis. Intranasal ipratropium bromide has been revealed as a treatment of choice, although this paper presents and explores other valid treatment options which could improve the quality of life of this fragile category of patients. This review aims to report the prevalence and etiopathology, diagnosis and treatment for senile rhinitis.

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Key Words: Senile rhinitis, Ipratropium Bromide, rhinorrhea

Abbreviations:

CRS- chronic rhino sinusitis
NAR- noninfectious rhinitis
AR- allergic rhinitis

IPB- Ipratropium Bromide
INCS- Intranasal corticosteroids
INAH- Intranasal antihistamines

Introduction

Rhinitis is a symptomatic inflammation of the nasal mucosa, causing mild but irritating symptoms such as nasal obstruction, anterior or posterior continuous rhinorrhea, sneezing, or nasal itch. When these constant symptoms are present for at least 1 hour daily for a minimum of 12 weeks per year, the definition chronic rhinitis may be applied. Senile rhinitis affects both genders equally [1]. The pathology becomes (CRS) if the inflammation is extended to the sinonasal cavities and the presence of at least two or more symptoms such as nasal obstruction, facial pain, pressure or fullness, (thick and/or discolored) secretions, and/or decreased sense of smell. The prevalence of CRS is up to 10.9% of the Western population [2]. The severity of the disease may vary from mild to severe and several sub groups can be distinguished based on the etiology: infectious rhinitis, allergic rhinitis; non- allergic noninfectious rhinitis (NAR) and mixed rhinitis. In particular the subgroup of rhinitis without allergy or evident infectious processes will be discussed in this paper. Other subgroups of NAR are: drug-induced rhinitis, hormonal rhinitis including pregnancy-induced rhinitis, non- allergic occupational rhinitis, gustatory rhinitis, and idiopathic rhinitis [3]. Approximate prevalence of NAR worldwide is estimated to be more than 200 million people. Many patients with senile rhinitis may have a concomitant allergic disease that is the reason why the diagnosis of senile rhinitis most often refers to late-onset patients [4]. The manifestation is very variable: intermittent symptoms with perennial allergic rhinitis (AR), or persistent symptoms with seasonal AR The most frequent symptom are bilateral watery nasal secretions without endonasal mucosal and/or anatomic pathology.

Etiopathology

A neurogenic parasympathetic/sympathetic dysregulation is considered the main cause of the symptoms and it has been evidenced that inflammatory cells or type 2 pathways in the nasal mucosa have no influence in the pathogenesis of the senile rhinitis[5]. The neural regulation of the upper airways is quite complex and consists of sensory, parasympathetic, and sympathetic nerves that are in continuous interaction and regulate epithelial, vascular, and glandular processes in the nasal mucosa. The anatomically defined sensory, parasympathetic, and sympathetic neural systems contain heterogeneous populations of nerve fibers often carrying

unique combinations of neuropeptides [6]. Mucous secretion is mainly regulated by the parasympathetic nervous system and acetylcholine is the main parasympathetic neurotransmitter that regulates its secretion, thus rhinorrhea. Parasympathetic neurons may have two populations: larger diameter acetylcholine containing neurons and smaller diameter neurons that release vasoactive intestinal peptide (VIP) and nitric oxide [6].The sympathetic nervous system controls vascular tone with neurotransmitters norepinephrine (short acting) and neuropeptide Y (long acting) in the nasal mucosa and modulate secretions initiated by the parasympathetic system. Adenosine triphosphate is another transmitter in nasal sympathetic neurons. Sensory neuropeptides and nociceptive C-type fibers such as tachykinins, calcitonin gene-related peptide, neurokinin A, and gastrin-releasing peptide of the trigeminal nerve contribute to mast cell degranulation and itch/sneeze reflexes. Parasympathetic and sympathetic sensory neural systems contain heterogeneous populations of nerve fibers that often harbor unique combinations of neuropeptides. The sensory neurons are responsible for transmitting information about the conditions of inhaled air from the epithelium to the brain stem. Acetylcholine stimulates muscarinic M3 and possibly M1 receptors on glands to cause exocytosis so the response is given by efferent axons in the nasal mucosa and the immediate release of the neurotransmitters. In the elder population these complex mechanisms are altered creating an imbalance. Solitary chemosensory cells of the nasal cavity are specialized epithelial chemosensors that respond to irritants through the canonical taste transduction cascade stimulating peptidergic trigeminal nociceptive (or pain) nerve fibers. Activation of these nasal cells can trigger similar local inflammatory responses such as mast cell degranulation and plasma leakage, and this is only by cholinergic neurotransmission and neural activity and not by release of local inflammatory mediators as previously mentioned[7]. The physiological changes in the elder population are also due to anatomic alterations and mucosal atrophy. The body undergoes a gradual loss of water content, atrophy of collagen fibers and loss of elastic fibers in the dermis. These changes result in weakening of the lateral, superior, and inferior nasal cartilages, retraction of the nasal columella, and downward sagging of the nasal tip resulting in increased nasal airflow resistance [8]. On the other hand, mucosal gland degeneration, loss of lymphatic tissue, and decreased nasal blood flow contribute to atrophy and drying of the nasal mucosa and increased viscosity of the mucus.

The Diagnosis

Detailed medical history is essential because during this phase of interaction with the patient a probable diagnosis has to be made. The following tests have the scope of only confirming or denying the physicians suspicion. Therefore, the age of the patient, the duration and frequency of symptoms, the hormonal state, the occupational/environmental exposure to a list of triggers leading to nasal symptoms, and the systemic and nasal medication use should be investigated. Some of the prescription pharmaceuticals frequently and constantly assumed by the elder

population, such as alpha-1 adrenergic antagonists used for benign prostatic hyperplasia, ACE-inhibitors, beta adrenergic inhibitors and phosphodiesterase inhibitors can induce symptoms of rhinitis. The subsequent step includes anterior rhinoscopy checking up for any anatomical deformities, signs of infection or endonasal crust formation. The ENT specialist should proceed with fiber endoscopy and evaluate whole endonasal cavity including the ostiomeatal complex [9]. The nasal endoscopy is crucial in the diagnosis of prolonged courses of rhinitis, and it may reveal the presence of chronic rhino sinusitis with or without nnasal polyps[2]. Nasal cytology may help to distinguish between an inflammatory or neurogenic etiology of symptoms [10]. This diagnostic method represents a useful, inexpensive and easy-to-apply diagnostic procedure to better detail the phenotypic characteristics of rhinitis [11]. It also allows to detect and quantify the cell population within the nasal mucosa at a given time. In order to exclude allergic rhinitis, Prick test or allergen- specific IgE research in blood sample may be obtained, even though it is practically impossible to test against all possible allergens. Clinical relevance of detected sensitization may be confirmed by history and/or allergen provocation test [12]. Other diagnostic tools have been proposed but are not recommended by international consensus or position papers, such as: measurement of total IgE or allergen-specific IgE in nasal secretions, microbiological analysis of the nasal secretions (with the exception of suspect of symptomatic infection), measurement of nasal hyperactivity and allergy provocative testing. CT scan of the sinonasal cavities is not recommended in elder chronic rhinitis patients, especially without any suspect of polyp formation or surgical intention. And lastly, measurement of markers of cerebrospinal fluid leakage (b2- transferrin or b-trace) via a skull base defect are only indicated in unilateral watery rhinorrhea and should be excluded during the first phase of diagnosis- the medical history.

Chronic Rhino sinusitis with and without Nasal Polyps

There are significant differences in CRS prevalence between the elderly and young people. After endoscopic sinus surgery, nasal polyps recurred less often in the elderly, probably due to smaller eosinophilic infiltration which is known to increase the risk of recurrence[13]. Furthermore, this study investigated the segment of patients with allergy which was significantly higher in young people than in the older population, but found no difference in the prevalence of asthma in both populations. Another study confirmed these results, suggesting that the pathogenesis of the CRS in the elderly is different, less linked with allergy and eosinophilic infiltration, but more with nasal polyp formation [14].

Radiological alterations

There are only a few studies of radiological investigations of nasal symptoms. The estimated prevalence of sinus abnormalities on MRI ranges from 25 to 85%. This wide range may be explained with the simple fact that MRI is not an ideal technique for maxillary- facial imaging

because mucus excess seems bigger or more exaggerated leaving CT with coronal and sagittal sections a better technique for evaluation of nasal- paranasal district. An abnormality of the sinuses, which includes not only simple sinusitis but abnormalities such as tumor or fungal infection, was detected in 153 (47.1%) subjects[15]. In a Japanese study on the elder population, 654 (33.8%) subjects had paranasal sinusitis. Also this study found 17% Lund-Mackay (LM) >0 and 7.4% LM score ≥ 4 respectively[16]. Another study investigated the differences of the maxillary sinus in elderly dentate and edentulous patients with a computed tomography (CT). The most frequent alterations were mucosal thickenings and mucous cysts in dentate patients compared to edentate controls, but most of these abnormalities can be considered chronic [17]. Concluding, the majority of radiological findings regarding the nasal district in the elder population is incidental and asymptomatic.

Treatment

The decreased quality of life of untreated/undiagnosed senile rhinitis significantly increases the risk of other concomitant conditions such as obstructive apnea of sleep, fatigue, headache, general malaise, scarce appetite and weakness. Irritant avoidance and smoking stop should be advised to all the patients [18]. Pharmacological management of geriatric patients is never easy because of large assumption of many different pharmaceuticals and interactions are ought to be avoided. Luckily the therapy for CRS is usually local and does not compromise general status of the patient. The treatment of choice is ipratropium bromide that may be associated with intranasal corticosteroids and saline irrigations.

Ipratropium bromide (IPB), an anticholinergic drug, is effective in reducing the severity and duration of the rhinorrhea in senile patients[19]. IPB is a quarternary ammonium derivative of atropine and is only minimally absorbed across biological membranes [20]. IPB was synthesized as a compound that would be less absorbed than atropine, thereby reducing the risk of systemic anticholinergic side-effects (mydriasis, xerosis, tachycardia), and still retaining its therapeutic action [21]. Its onset of action is 15 minutes and maximal effect is reached in 1 hour. IPB has been demonstrated to be highly effective for hypersecretion in idiopathic rhinitis [19], [22], but does not have any influence on other symptoms like nasal blockage or sneezing [21]. In a study that included 233 patients, this molecule, used three times a day has been shown to reduce rhinorrhea by thirty percent along with modest reduction of post nasal drip, sneezing and congestion [23]. It is the most widely experienced anticholinergic with the average follow-up of 4 weeks. Compared to the placebo, anticholinergic treatment significantly reduces the severity of rhinorrhea both in allergic and non -allergic patients [24]. The benefit was less considerable for nasal congestion, retro nasal drip and sneezing. Minor and infrequent episodes of epistaxis in 9.4% of patients and dryness of the nasal mucosa- 5% of patients- have been documented in literature, but these effects were never important enough to interrupt the treatment. No

alterations have been noticed in the nasal cytology after treatment [23]. Other adverse effects reported included irritation of the nasal mucosa, headache and pharyngitis, although the comparison with placebo has detected a significantly greater risk only for the epistaxis [21]. A practice parameter update on rhinitis, published in 2020, suggests that in patients with perennial allergic rhinitis and non-allergic rhinitis who have rhinorrhea as their main nasal symptom be offered intranasal ipratropium with low certainty of evidence for perennial allergic rhinitis and moderate for non- allergic rhinitis [25]. Combined IPB use with intranasal corticosteroid is more effective than the use of individual molecules.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active component of plants of the genus Capsicum such as chili peppers. It belongs to a group of chemicals identified as capsaicinoids. Capsaicin produces a burning sensation when a tissue comes into contact with it. This occurs via binding to transient receptor potential vanilloid 1 (TRPV1) receptor, an ion channel-type receptor, which can be stimulated by heat and physical abrasion [26]. The mechanism of action of capsaicin is quite unique. The initial neuronal excitation evoked by the irritant capsaicin is subsequently followed by a long-lasting refractory period, during which the previously excited neurons are no longer responsive to a broad range of stimuli [26]. It was established that capsaicin can reduce the density of the innervation of the nasal mucosa and the TRPV1-SP signaling pathway, without affecting the integrity and function of nasal epithelial cells or mast cells, and in this way is able to improve the symptoms in 80% of well-selected infectious rhinitis patients[27]. Unfortunately its effectiveness has not been demonstrated in any forms of allergic or non-allergic rhinitis.

Intranasal corticosteroids

Intranasal corticosteroids (INCS) remain the most effective monotherapy for allergic rhinitis meanwhile the association of intranasal ipratropium bromide and intranasal beclomethasone is demonstrated to be more effective than either active agent alone, in reducing the average severity and duration of rhinorrhea in allergic and non-allergic rhinitis [28, 29, 30].
The onset of action is 6-8 hours after the first dose although clinical improvement may not be apparent for a few days and maximal effect may not be apparent until after two weeks. The local side effects of intranasal corticosteroids include epistaxis (5% to 10%), nasal irritation (5% to 10%) dryness, burning and stinging, headache, crusting, nasal septal perforation (< 1%), candida infection of the nose and pharynx.

Nasal saline irrigations

Nasal lavage with saline solution has also been found to be a helpful alone or as an adjuvant therapy in patients with chronic rhinorrhea and rhino sinusitis [31]. It is best performed immediately prior to intranasal corticosteroids or IPB as it may improve mucus clearance;

remove antigen, inflammatory mediators, or biofilm; enhance ciliary beat; and protect the nasal mucosa. Lavages help to reduce postnasal drip, sneezing, and congestion. It has very minor (burning, irritation) –close to none side effects. Unfortunately, there is not an established consensus regarding method of delivery, volume to use, ratio of isotonic to hypertonic, or frequency.

Antihistamines

Second generation oral antihistamines (cetirizine and levocetirizine, fexofenadine, loratadine and desloratadine) can be prescribed for the management of allergic rhinitis symptoms although they have not been adequately studied [25]. First generation antihistamines are not recommended because of the systemic side effects (sedation, performance impairment, dry mouth, constipation, urinary retention, delirium and ocular pressure changes.). Special caution should be taken into account using these agents in fragile elderly patients [32]. Patients who fail oral antihistamine treatment may be successfully treated with intranasal antihistamines (INAH) that have a more rapid onset of action compared to intranasal corticosteroids (INCS) and oral antihistamines, are more effective than oral antihistamines in the control of nasal congestion, and provide a favorable safety profile [25]. Two molecules- azelastine and olopatadine are approved by the FDA for the treatment of seasonal allergic rhinitis[33] [34]. Azelastine is also approved for the treatment of perennial allergic rhinitis and vasomotor rhinitis.

Discussion

Decreased water content of the body, degeneration of glands that secrete mucus and decreased blood flow in the nose require additional humidification of the nasal cavity [35]. Thus saline irrigations should be administrated before IP or ICS. Patient education is critical in managing senile rhinitis, as the elder are difficult to convince to abandon their habits of incorrect nasal irrigations, administrations or addiction to decongestants[36]. Considered the older age of the patients, at each visit, the physician should review all the current therapies to assess for any drug interactions, examine the technique of nasal instillation and provide clearly written treatment plans as memory may be an issue. Future considerations in the research area should involve aging alterations of microbiome and in local microbiome diversity of the patients with CRS and in patients with concomitant allergic disease [37, 38]. Research in the inflammatory pathways involving epithelial cells, including cytokine production or IgE involment should be another branch of consideration as the cellular mechanisms of aging nasal mucosa remain unknown.

Conclusions

Senile nose phenotype usually has very clear characteristics: watery rhinorrhea without other significant rhinitis symptoms. Through a combination of structural and physiological changes and impaired epithelial barrier function, the elderly are more susceptible to ulceration, atrophy and dried nasal mucosa, formation of intranasal crusts, and epistaxis. The elder individuals are eager to seek treatment because of the bothersome watery anterior drip that is often frequent and substantial. In addition to these physical and emotional impacts on patients, there is also an economic burden deriving from the diagnosis and missed/incomplete treatment of rhinitis. IPB is recommended for use in senile rhinitis and is considered a safe and quality-of-life-improving treatment that may be associated with INCS for even better results.

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Letter to the Editor

NEW INSIGHTS INTO THE IMPORTANCE OF ESTABLISHING HISTOMORPHOLOGICAL CRITERIA FOR CRSwNP

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To the Editor,

the advent of the newly developed monoclonal antibodies (mAbs) targeting type 2 (T2) inflammation has highlighted the importance of correctly typing patients with Chronic Rhinosinusitis with Nasal Polyposis (CRSwNP), as a function of both the endotype and phenotype, in order to identify the different clinical and biological markers associated with a poor response to conventional treatments and with an increased risk of relapse[1]. In this perspective, several biomarkers have been progressively identified and associated with poor prognosis in CRSwNP patients, including Charcot-Leyden crystals (CLSs), which are slender bipyramidal hexagonal crystals (~ 100 µm in maximum length) composed of Galectin-10[2]. As a matter of fact, CLCs are nowadays specifically considered as hallmarks of eosinophil involvement in many diseases, such as asthma, allergic rhinitis, CRSwNP, and atopic dermatitis[3]. However, it is still unclear how crystalline CLCs found in nasal biopsies can be used to predict CRSwNP recurrence (**Fig. 1**). Recent studies have proposed the use of histomorphology methods to define a possible cut-off of CLC in nasal polyps biopsies, such as to establish the actual risk of CRSwNP recurrence, which in turn would also be affected by the different forms of CLC[4]. Indeed, not only there is no unanimous histopathological criterion regarding a cut-off point to define eosinophilia in nasal polyps, but also the eosinophil count could be excessively time-costing, as even more than 500 eosinophils per high power field could be detected. On the contrary, counting CLCs might be more easy-counting.

In addiction to CLCs count, we believe that histomorphology methods should aim at establishing a cut-off point for mast cells in nasal polyps' biopsies. Indeed, the great attention paid over the years

to the undoubtedly crucial role that eosinophils play in the pathogenesis of CRSwNP has led to underestimating the importance of other cells of immunophlogosis, such as mast cells, which are equally involved in the inflammatory mechanisms underlying the CRSwNP[5]. As a matter of fact, mast cells have been shown to be directly involved in the pathogenesis of CRSwNP, orchestrating type-2 cytokines and chemokines. Moreover, although CLCs have been traditionally considered a marker of eosinophilic inflammation, they have been shown to colocalize with both eosinophils and mast cells, thus being a marker of both cytotypes[6].

Interestingly, our preliminary studies have demonstrated that mast cells infiltrate the lamina propria of almost all nasal polyps and the epithelial layer of the most difficult to treat forms. Since the intraepithelial localization of mast cells is properly related to the severity of CRSwNP, a cut-off of intraepithelial mast cells could be established to identify the most severe endotypes of CRSwNP[7]. This would overcome a disadvantage of CLCs counting: hematoxylin-eosin staining is complicated and requires an optimal eosin pH value to visualize the smallest CLCs. As proof of this, CLCs are rarely detected during common histological investigations. On the contrary, intraepithelial mast cell counts could be performed using May-Grunwald Giemsa staining, which is already used for nasal cytology. This staining, unlike hematoxylin-eosin, would allow to visualize all the inflammatory cells that infiltrate the nasal polyps, including mast cells, without the risk of underestimating the count (**Fig. 2**).

In light of these evidence, establish standardized histomorphological criteria that define the different forms of CRSwNP is mandatory. We believe that the keystone could be represented by intraepithelial mast cells count. For this purpose, further studies are needed to define the cut-off of infiltrating intraepithelial cells associated with increased severity of CRSwNP.

Conflict of Interest Statement:

The authors have no conflicts of interest to declare.

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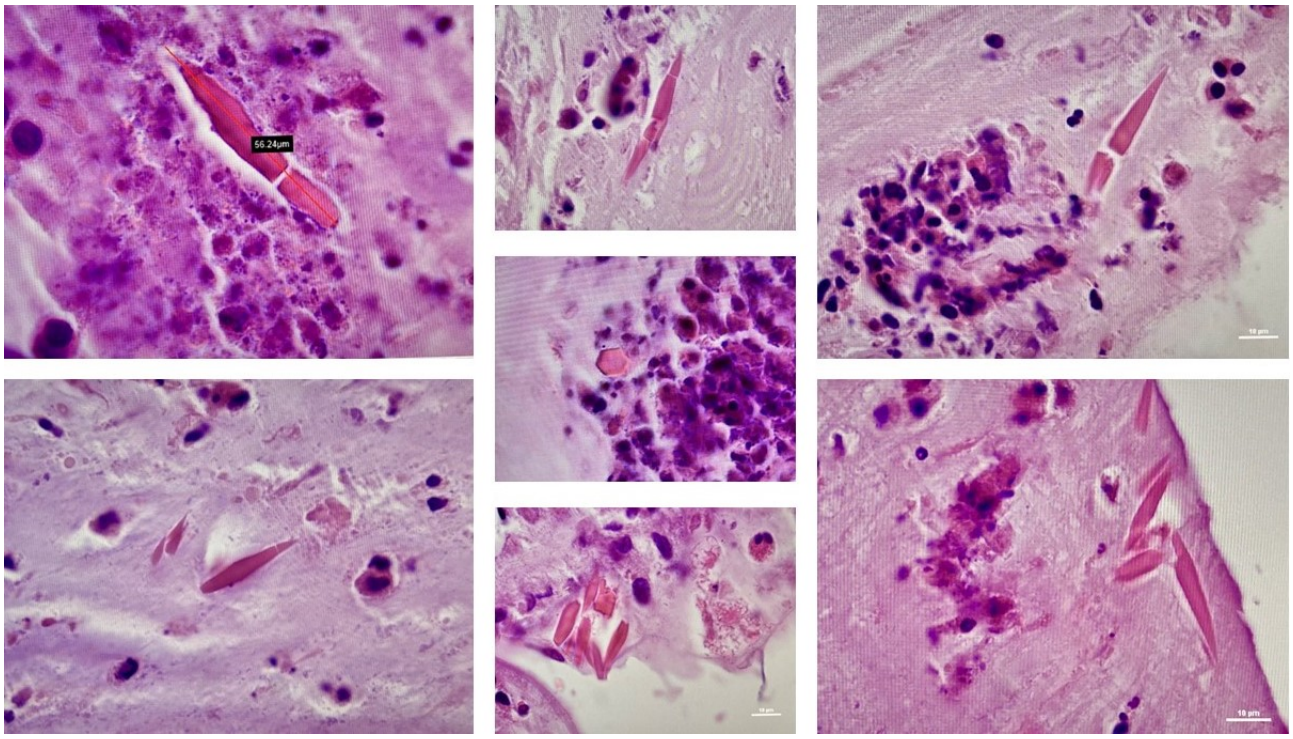

Fig. 1 Charcot-Leyden Crystals (CLCs) in nasal polyps samples stained with hematoxylin-eosin.

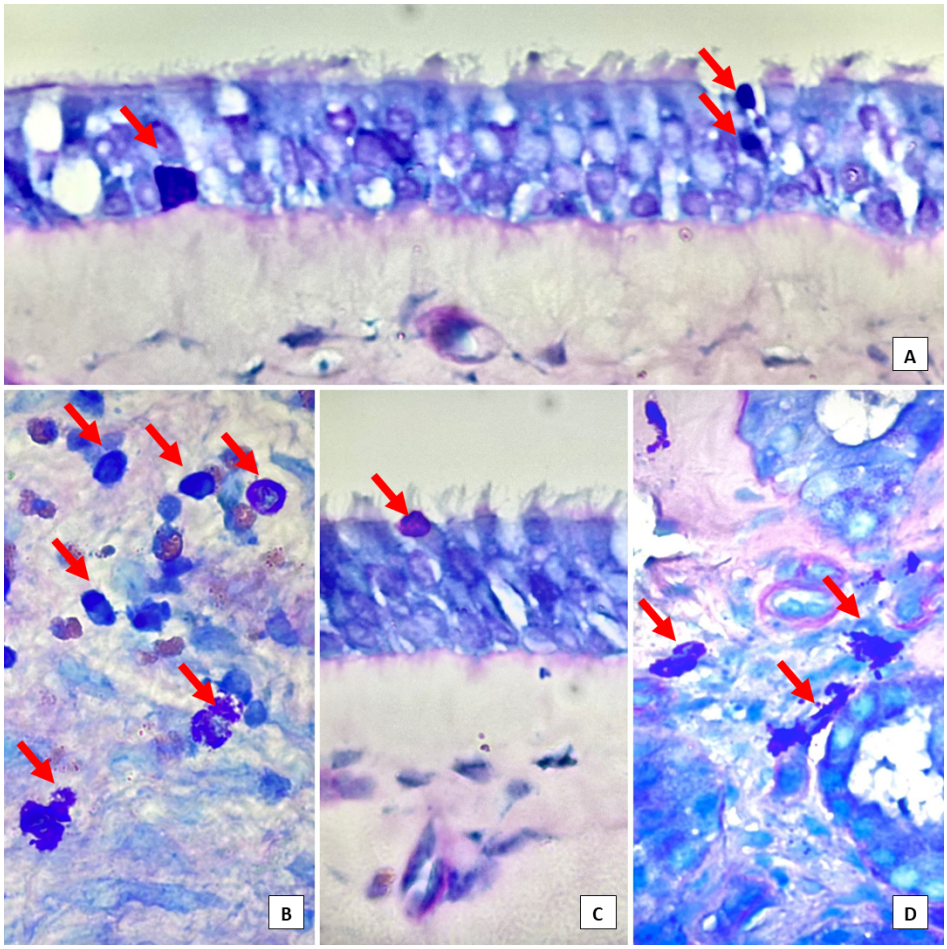

Fig. 2 Nasal polyp biopsies stained with May-Grunwald Giemsa (MGG). The arrows indicate mast cells, located in the epithelial layer (**A-C**) and in the lamina propria (**B-D**) of nasal polyps.

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