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Review

Nasal Cytology and Clinical Rhinology Support a Translational Integrative Neuroscience Perspective

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Abstract

Nasal cytology is evolving into a promising tool for diagnosing neurological and psychiatric disorders, especially those such as Alzheimer's and Parkinson's diseases. Moreover, recent research has indicated that biomarkers differ greatly between samples taken before and after death. Nasal cytology might help to identify the early stages of cognitive decline. The association of olfactory disturbances with a host of these neurological disorders is remarkable. This means that the nose, something we probably take for granted, could well be the best means of establishing important biomarkers for earlier diagnoses in these conditions. The nose is a source of epithelial and neuroepithelial cells that can be used in *in vitro* cultured models and nasal cytology provides new avenues for translational, integrative neuroscientific research. The future incorporation of artificial intelligence into cytological analyses would facilitate the acceptance of nasal cytology as a screening platform for neurodegenerative and psychiatric conditions, facilitating early diagnosis and better management for patients.

Keywords: nasal cytology; biomarkers; neuroepithelium; chronic rhinosinusitis; neurodegenerative diseases

1. Introduction

In recent years, with the increase in non-invasive diagnostic techniques, nasal cytology (NC) has become a valid and reliable tool in the evaluation of many rhino-sinus pathologies, including allergic and non-allergic. These techniques are valid in both adults and children [1–3]. Nasal cytology is increasingly used in the study of rhinitis, due to the possibility of analyzing the type of cells that make up the nasal mucosa at the time of the examination. Therefore, we can define rhinitis from the detection of the cytological population, and study the pathological conditions that cause and the effect that allergenic, irritant and physicochemical stimuli can have on the nasal mucosa [4]. Finally, NC can allow patient follow-up, observing changes in the cells of the olfactory mucosa over time in patients undergoing nasal treatments for rhinitis [4,5]. This diagnostic procedure has considerable advantages because it is simple to apply in cell sampling, it is not an invasive technique, it is repeatable and it is very useful in the follow-up of patients of all ages [3]. Some studies have also used nasal cytology to assess histopathological changes in patients undergoing radiotherapy for tumors of the head-neck region. In these cases, notable findings include hyperplasia of the basal cell layer and mucosal cell metaplasia. Furthermore, a frequent observation in these patients is a reduction in the population of goblet cells. The overall goal is to improve the treatment of nasal disorders in such patients [6].

On the other hand, the olfactory mucosa was proposed as a valuable model for the study of neurological and neuropsychiatric illness [7]. Nasal cytology is a resource for investigating neuronal molecular markers of neuropsychiatric, neurological and neurodegenerative diseases, and nasal cavities exfoliation is a non-invasive, reproducible, and reliable method for the isolation, *in vitro* culture, and characterization of neuronal and glial cells from human olfactory epithelium [8]. As reported, the neuroepithelium might become a new translational research target to investigate alternative approaches for intranasal therapy and the treatment of brain disorders [8].

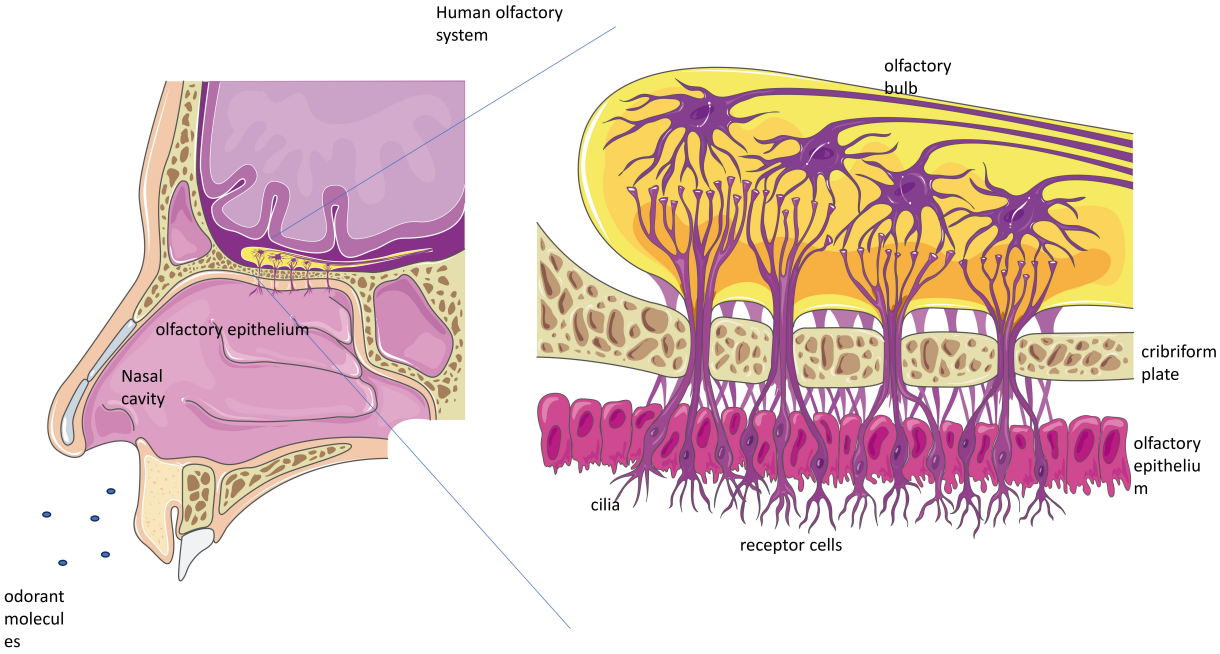

Fig. 1. Anatomical description of the human olfactory system. Odor molecules enter the nasal cavity and interact with the olfactory mucosa, which contains the olfactory epithelium and lamina propria. Olfactory sensory neurons in the olfactory epithelium detect odors and send signals to the olfactory bulb, where they connect with mitral cells. The monosynaptic mitral cell axons then project to brain regions such as the limbic system, (the pyriform cortex, amygdala, and entorhinal cortex) linking olfactory perception with memory and emotions. Supporting cells in the olfactory epithelium, like sustentacular cells, assist with metabolic and secretory functions, maintaining neuron health [8,9] (This schematic was created using Servier Medical Art templates, which are licensed under a Creative Commons Attribution 4.0 Unported License; https://smart.servier.com).

2. Olfactory Pathways

Odors, whether inhaled through the nose or present inside the oral cavity, reach the olfactory epithelium in the area of the hair cells and bind depending on their chemical nature and specific olfactory receptors present there, which are more than a thousand membrane proteins belonging to the G-protein-coupled receptor superfamily, resulting in the conversion of ATP to cyclic AMP (cAMP) by adenylate cyclase. The increase in ciliary cAMP opens a cyclic cation channel controlled by nucleotides, ultimately allowing the influx of sonic (Na^+) and calcium (Ca^{2+}) ions and the release of potassium (K^+). The binding with these receptors can be direct, or take place via proteins present in the secreted glycoprotein, called odorant binding proteins: these are extracellular proteins that bind to the hydrophobic portion of the odorous substances present in the mucus. The efflux of ions, which occurs at this juncture, can also explain certain mechanisms, called olfactory adaptation mechanisms, which depend precisely on the movement of the ions released. It is believed that the opening of channels in the ciliary membrane, which allows the entry of ions calcium, may play a modulating role. Intracellular Ca^{2+}, along with other factors, may, down-regulate the signal cascade leading to transduction [9].

The decreased perception of a specific odor occurs with prolonged exposure to that substance and can be attributed to peripheral mechanisms, such as this, or central (mechanism of olfactory adaptation). Signal transduction is a biochemical event that occurs at the end of each olfactory receptor dendrite. The resulting action potential is transmitted via the axons of the olfactory sensory neurons to the olfactory bulb in the ventral portion of the frontal lobe surface, passing through a thin porous bony covering called lamina cribrosa (Fig. 1, Ref. [8,9]). Here, the neurons organize to form glomeruli-like structures to glomeruli, where they collect with exceptional precision and convergence (approximately 5000–20,000 neurons per glomerulus) the axon fibers of neurons expressing the same receptor [9]. Thereafter, the signal is transmitted from the glomeruli to the second-order neuron, the so-called mitral cells, to the central areas: the olfactory area in the anterior part of the temporal lobe (anterior olfactory nucleus), the olfactory tubercle and the amygdala, also weaving relations with the limbic area. These anatomical interrelations explain how certain fibers in the hippocampus can generate mnestic and emotional responses in association with particular odor stimuli (Fig. 1). The system uses a combinatorial decoding mechanism to discriminate and identify inhaled molecules. Odor molecules can be detected even at low concentrations, normally dispersed in one part in a million. The multiple olfactory stimuli can be divided into: chemical patterns, divided by functional groups (e.g., example,

octanol is typical of citrusy odors such as orange), chain length and stereoselectivity. A single olfactory receptor is sensitive to different odors by binding to different structural portions, consequently, the same odour particle can activate several receptors and each molecule activates a unique combination of receptors, generating specific patterns [9]. In light of this, recognition of a substance results from the activation of patterns of neurons that converge on specific olfactory glomeruli (Fig. 1). Finally, the whole mechanism is affected by the modulation of more sophisticated cerebral areas of the cortex, undergoing the influence of factors such as age (perceptive acuity tends to decrease with advancing age perceptive acuity), gender (women have a more pronounced sensitivity for phylogenetic reasons related to motherhood), genetics, pathologies (viruses, chemical or mechanical damage) and finally cognitive-psychological cognitive-psychological mechanisms (given by the socio-economic, experiential and personal context) [10].

3. The Nasal Mucosa

The nasal mucosa consists of a pseudostratified ciliated epithelium composed of columnar ciliated and not ciliated cells, muciparous goblet cells and basal epithelial cells. The ciliated cell is the most differentiated cellular element of the nasal mucosa. It, together with the muciparous cell, forms the first line of defense of the airway (mucociliary system). Into the intercellular spaces, even in normal conditions, it is possible to find few lymphocytes and neutrophils.

There are differences between nasal mucosa according to the studied anatomical region: the most anterior part of the nasal mucosa is called the "nasal vestibule" and it is characterized by a squamous, stratified and keratinized epithelium; moving posteriorly, nasal mucosa consists of pseudostratified nonciliated epithelium called "transitional epithelium", followed by a bathiprysmatic epithelium. Eventually, the remaining portion of nasal mucosa is made by ciliated columnar pseudostratified epithelium The finding in the rhinocytogram of eosinophils, mast cells, bacteria, spores and fungal hyphae will be a clear sign of nasal pathology. By collecting nasal secretions, it is possible to study the markers of inflammation expressed, through molecular biology techniques, and it is possible to profile the specific microbial flora of the patient by analyzing secreted microsomes. The most effective collection technique for the characterization of viruses, bacteria and fungi of the nasal microbiome is performed by a nasal swab, but it can also be performed by washing [11].

4. Nasal Cytology

The anatomy and morphology of the nasal mucosa were described microscopically for the first time at the end of the 19th century by Gollash and Von Mihalkovics [12,13].

Subsequently, in 1927, Eyermann [14] described the presence of eosinophils in nasal secretions by studying patients suffering from hay fever, a common pathology among farmers, highlighting that there was a relationship between a specific cell population and specific pathologies.

NC had great development as a tool for the evaluation and research of certain drugs and stimuli. The use of cultured human nasal epithelial cells was considered as a promising system enabling the prediction of nasal drug transport, metabolism and toxicity in humans, giving more direct clinical relevance. *In vitro* cultures of nasal epithelial cells showed in pharmacological and toxicological studies have several advantages: (i) reduction of mucosal factors, (ii) air-liquid interface more closely resembles evaluation of the potential permeability, metabolism and toxicity; (iii) *in vitro* exposure of human cells to solid, liquid and volatile compounds that could not be investigated directly in patients, revealing a more precise definition of drug transport, metabolism, and toxicity. Primary and cell lines of nasal epithelial cells have been used to investigate molecular and cellular mechanisms associated with nasal mucosal viral and bacterial infection, mucus secretion, ciliogenesis and ciliary movement, cystic fibrosis, and electrolyte transport [15]. Lastly, an *in vitro* human nasal cell culture system suitable for rhinobiome, chronic inflammation and metabolism studies is under validation in our lab. In addition,

NC was considered as a cellular resource to evaluate *in vitro* and *in vivo* models for Nose-to-Brain Drug Delivery Study by computational approaches [16]. An improvement of human nasal epithelial cells as an essential cell source to reconstruct a three-dimensional (3D) structure tissue models of the nasal epithelium was achieved by 3D bioprinting. One of the major purposes of bioprinting is to create functional tissue models that can be used for basic research, drug discovery, and potential therapeutic applications [17,18].

The birth of specific technical protocols for NC has been characterized since 2006 so that the technique could be standardized. Today, NC is a precious tool for the study of various forms of rhinitis, both allergic, such as that with eosinophils (NARES), with mast cell predominance (NARMA), neutrophilic (NARNE) or mixed (NARESMA), and non-allergic [4,5]. In addition to its application in otolaryngological disorders, it was suggested nasal cytology as a source of epithelial and neuroepithelial cells to *in vitro* cultured model aimed at biomarker assessment of neurological and psychiatric diseases. Scientists recently developed several procedures such as biopsies or noninvasive methods to obtain neuroepithelial cells as *in vitro* study models [7].

More recently, human olfactory neurosphere-derived cells were cultivated opening to an experimental approach to establish a study model for neurodegenerative and neuroinfectious disease research and the development of therapeutics [19,20].

Alternatively, in nasal secretions from Alzheimer's disease (AD) patients, the level of Aβ using interdigitated

Table 1. Techniques applied to obtain nasal cells.

Nasal lavage:	Nasal washing is carried out by introducing liquid into the nasal cavity, waiting a few seconds and collecting the residual material. Used to better evaluate macromolecules such as proteins and cytokines, rather than cells, as there are abundant degenerated cells.
Nasal scraping:	Nasal scraping is performed by making two or three courrettages with a pen-shaped instrument, with a cup-shaped end, which is used to remove the mucosa of the inferior turbinates (Rhinoprobe®, Nasal scraping®).
Nasal brushing:	A nylon brush is rotated at the level of the middle meatus, then inserted and agitated in a polystyrene tube with 5 mL of PBS, finally brushing the bristles against the wall of the tube. The tubes are centrifuged at 400 g for 10 minutes. Used to living epithelial cells and ciliary ultrastructure studies.
Nasal biopsy:	It is an invasive technique not used in nasal cytology, however gold standard for the histological study of the nasal mucosa.
Microsucting aspiration:	It is used only in experimental contexts and is based on the aspiration of cellular material into a plastic tube of known weight. A known volume (1.0 mL) of PBS containing 10% Mesna is aspirated. Mucus aspiration can also be conducted with the endoscopic technique, when there is enough mucus, for a more targeted sample collection, safer for the patient and free from blood that can contaminate the field.

microelectrode biosensors was measured. This approach, conjugated with cellular biomarkers of nasal cells and clinical evaluation, maybe a possible predicting method for AD [21]. To date, it is evident that the nose-brain axis and the significance and mechanisms of nasal damage in the pathogenesis of brain diseases could represent a valuable route by improving nasal cytology.

5. Nasal Cytology Techniques

There are many nasal cytology techniques, the most standardized, practiced and listed in Table 1. Scientific journals will be listed below [3–5,16–24].

The nasal cytology is based on: sampling, processing (consists of fixing and coloring the samples) and microscopic observation.

Cytological sampling aims to collect a good quantity of nasal epithelial cells performed through an anterior rhinoscopy. The collected mucosa should include the region where hair cells and goblet mucous cells coexist, as in the middle portion of the inferior turbinate.

A variety of tools have been employed to collect human nasal epithelial (HNE) cells, but a universal consensus on the most effective device has yet to be established. This study examines two cytology brushes: Olympus (2 mm diameter) and Endoscan (8 mm diameter), both used for HNE cell collection via brushing the inferior turbinate. The findings revealed that the Endoscan brush significantly outperformed the Olympus brush in terms of total and viable cell collection, making it a more efficient choice. Additionally, the Endoscan brush offers better cost-effectiveness, being substantially cheaper than the Olympus brush while maintaining the quality of the collected samples [23].

This technique has the advantage of causing minimal discomfort to the patient since it does not require anesthesia. Furthermore, sampling can easily be repeated if necessary. The collected cells can be used for bacterial and viral diagnostic studies, as well as for biochemical and immunohistochemical analyses.

Microscopic Observation

A nasal cytogram is a diagnostic test that examines the cells present in nasal secretions, being useful to detect alterations or pathologies of the nasal mucosa. It is carried out by collecting mucus samples from the nasal cavity, which are then subjected to a staining process and observed under the microscope. The sample obtained after brushing the nasal mucosa is smeared on an uncharged glass microscope slide, and stained with the May-Grünwald-Giemsa (MGG) method or fixed in alcohol and stained with the Papanicolaou (PAP) method (Fig. 2). The cytological preparations are then examined under an optical microscope. The identification of cells on the cytological preparation allows quantifying the various components present: epithelial (cylindrical respiratory cells, goblet cells and squamous metaplasia cells) and inflammatory cells (mast cells, lymphocytes and polymorphonuclear neutrophils, eosinophils and basophils), to which can be added the bacterial analysis (rhinobiome), providing useful information to integrate the diagnostic algorithm for infectious, inflammatory, allergic or neoplastic diseases. All samples were analyzed independently by two pathologists, who were unaware clinical details of the patients. For each slide, ten fields were examined at high magnification (40×) and the present cells were counted and classified according to a five-grade scale (Table 2). Fig. 2 depicts several cellular components observed from nasal samples.

Table 2. The nasal cytogram analysis provides a quantitative and qualitative picture of the cellularity present in the nasal mucosa, providing the clinician with useful information for a correct diagnostic framework.

Nasal Cytogram					
Ciliated columnar cells	None	Occasional cells	Moderate number of cells	Many cells	Large clumps of cells
Cell counting (40×) magnification	0%	1–24%	25–49%	50–74%	75–100%
Muciparous cells	None	Occasional cells	Moderate number of cells	Many cells	Large clumps of cells
Cell counting (40×) magnification	0%	1–24%	25–49%	50–74%	75–100%
Metaplastic/squamous cells	None	Occasional cells	Moderate number of cells	Many cells	Large clumps of cells
Cell counting (40×) magnification	0%	1–24%	25–49%	50–74%	75–100%
Lymphocyte	None	Occasional cells	Moderate number of cells	Many cells	Large clumps of cells
Cell counting (40×) magnification	0%	1–5%	6–15%	16–20%	>20%
Polymorphonuclear neutrophils	None	Occasional cells	Moderate number of cells	Many cells	Large clumps of cells
Cell counting (40×) magnification	0%	1–5%	6–15%	16–20%	>20%
Polymorphonuclear Eosinophils	None	Occasional cells	Moderate number of cells	Many cells,	Large clumps of cells
Cell counting (40×) magnification	0%	1–5%	6–15%	16–20%	>20%
Mastcells/polymorphonuclear basophils	None	Occasional cells	Moderate number of cells	Many cells, easily seen	Large clumps of cells
Cell counting (40×) magnification	0%	1%	2–3%	4–6%	>6%
Bacteria	None	Occasional cells	Moderate number of cells	Many cells, easily seen	Large clumps of cells
Cell counting (40×) magnification	0%	1–24%	25–49%	50–74%	75–100%

Table 3. Non-allergic rhinitis can be sub-divided into several types as indicated.

(1) NARNA, Non-Allergic Rhinitis with Neutrophils: Microscopically characterized by a predominance of neutrophils, constituting more than 20% of the inflammatory cells.
(2) NARES, Non-Allergic Rhinitis with Eosinophilia: This is a non-IgE-mediated vasomotor rhinitis marked by a predominant eosinophilic infiltration of the nasal mucosa, typically accounting for 50–70% of the inflammatory cells.
(3) NARMA, Non-Allergic Rhinitis with Mast Cells: This type is frequently associated with nasal polyps, asthma, and rhinosinusitis.
(4) NARESMA, Non-Allergic Rhinitis with Eosinophils and Mast Cells: Similar to NARES, this type also exhibits eosinophilic and mast cell infiltration.

In addition, there are various cellular components that can be detected through nasal cytology, analyzing different preparations of nasal cell sampling slides (Fig. 2a–l).

6. Nasal Diseases

NC highlights mucosal alterations caused by chronic inflammation, represented by epithelial metaplasia, which can be proliferating, with an increase in muciparous cells (muciparous metaplasia) or atrophic, with an increase in squamous cells (platicellular metaplasia) [5].

Nasal diseases primarily affect ciliated cells, resulting in a remodeling of the mucosal epithelium in favor of mucipar calycephalic cells (muciparous metaplasia). This finding has both pathophysiologic and clinical implications; in fact, the proportional increase in mucipar cells results in increased mucus production, while the reduction in the ciliated cell component causes reduced mucociliary transport dynamics. All of this promotes the stagnation of catarrhal secretions within the naso-sinus cavities and predisposes to an increased risk of bacterial superimposition infection. Considering that the normal hair cell turnover is about three weeks, recurrent inflammation will prevent the restoration of the normal cytotype ratio, establishing a self-maintaining vicious cycle.

Nasal cytology analysis demonstrates increasing reliability in the diagnosis of the most frequent nasal pathologies, thanks to very precise techniques, such as those described in the following review. The most used techniques show various advantages, as they are economical, non-invasive and repeatable, even in children. Nasal cytology analysis requires qualified operators. It represents the diagnostic Gold Standard for rhinitis pathologies such as superimposed rhinitis, NARES, NARMA, Non-Allergic Rhinitis with Neutrophils (NARNA) and NARESMA (Table 3).

In infectious rhinitis when nasal cytology is conducted, a morphological change in the ciliated epithelium, indicating suffering cells, can be observed [4]. This phenomenon encompasses several features, including condensation of nuclear chromatin, nuclear margination, cytoplasmic vacuolization, and loosing of the apical portion of the ciliated cell resulting from the lateral confluence of cytoplasmic vacuoles.

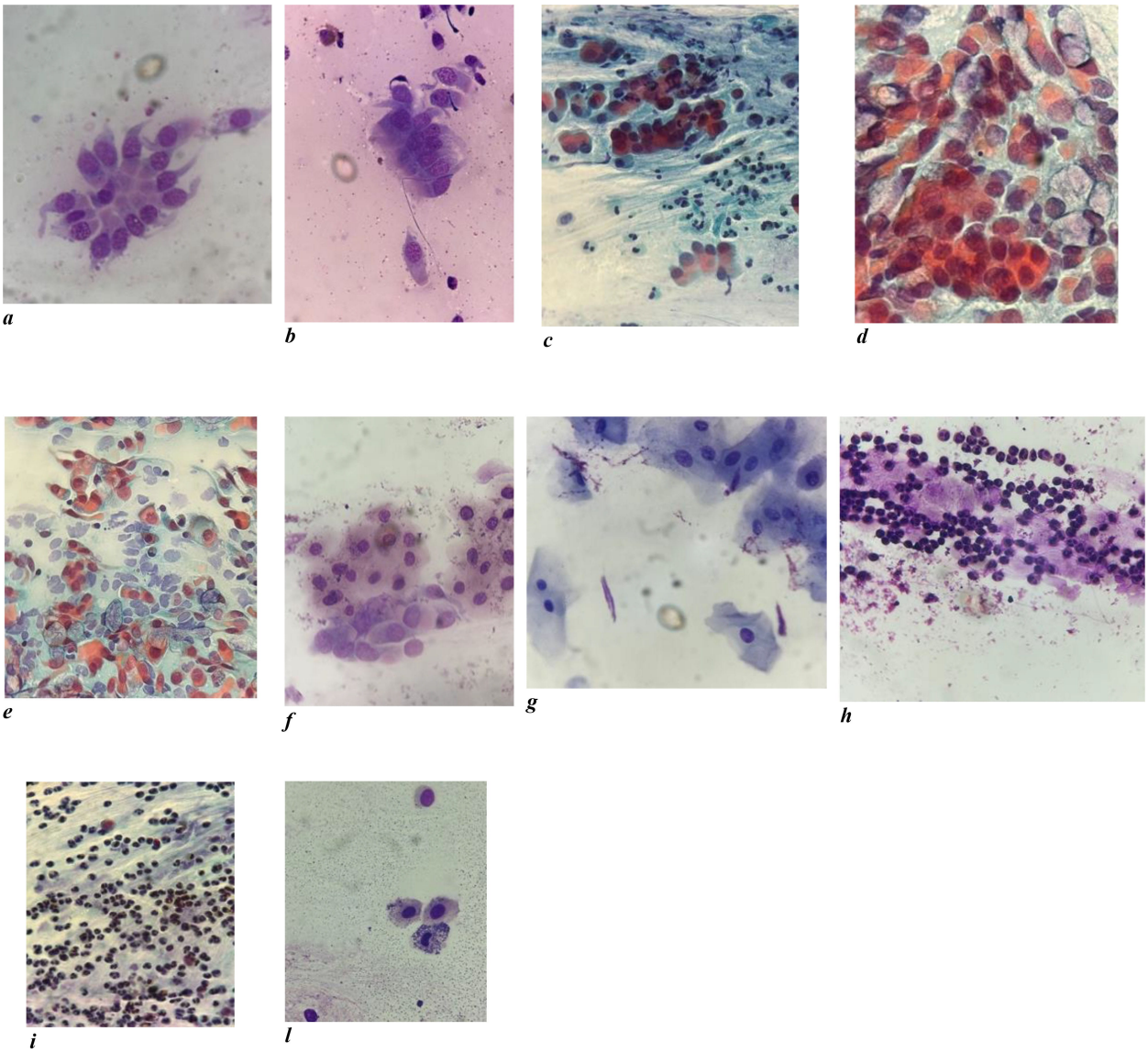

Fig. 2. Several cellular components detected through nasal cytology. (a) Ciliated cylindrical cells, MGG, 63×. (b) Ciliated cylindrical cells and polymorphonuclear eosinophils MGG, 63×. (c) Columnar cells and Polymorphonuclear neutrophils PAP, 40×. (d) Ciliated columnar cells, muciparous cells PAP, 63×. (e) Ciliated columnar cells, muciparous cells along with a few small-sized lymphocyte with basophilic mucus, PAP 40×. (f) Metaplastic cells and Ciliated cylindrical cells MGG, 63×. (g) Metaplastic cells, MGG, 63×. (h) Columnar cells and polymorphonuclear neutrophils MGG, 63×. (i) Polymorphonuclear neutrophils, PAP, 40×. (l) Mast cells, metaplastic cells ciliated cylindrical cells and lymphocytes MGG, 63×; (from Complex Operative Unit (UOC) Anatomic Pathology, San Camillo De Lellis Hospital, Rieti, Italy). MGG, May-Grünwald-Giemsa; PAP, Papanicolaou.

Nasal cytology of allergic rhinitis, shows a prominent infiltration of eosinophils and mast cells, alongside lymphocytes and neutrophils. This infiltration is closely related to the symptoms experienced and the exposure to allergens [24]. Non-Allergic Vasomotor Rhinitis, termed cellular, is characterized by the absence of IgE in the pathophysiological mechanism [25].

The cellular forms of non-allergic rhinitis can be subcategorized into several types, as reported in Table 3 or following other recent classifications [25]. Chronic nonallergic rhinitis syndromes encompass several forms, among these the most common the vasomotor rhinitis also referred to as nonallergic rhinopathy, nasal hyperreactivity, neurogenic rhinitis, or idiopathic rhinitis. Nonallergic rhinitis is characterized by clinical symptoms triggered by chemical irritants and climatic changes through chemosensors, mechanosensors, thermosensors, and osmosensors activated through different transient receptor potential calcium ion channels [24,25]. Several co-morbidities can increase the clinical diagnosis and as a consequence a more precise therapy is mandatory. The reactivity of the sympathetic nervous system is very important to understand

the pathophysiological mechanisms involved in nonallergic rhinopathy [24,25]. Studies on neurogenic pathways represent an important research route where ear, nose, throat (ENT) and neurologists could be allied.

Finally, it is also of fundamental assistance in the follow-up of patients suffering from nasal pathologies, since NC allows us to observe the changes induced by pharmacological treatments on the nasal epithelium over time by studying its cytology.

7. Nasal Mucosa, Neurological and Psychiatric Diseases

One potential application of nasal cytology is the evaluation of various biomarkers associated with mucosal diseases that can indicate disease severity or predict recurrence. Usually, these biomarkers are identified through histopathological methods, which tend to be more invasive and expensive than nasal cytology. Patients with rhinopaties and indications for septoplasty and turbinectomy in case of turbinate hypertrophy are directed to mucosal histopathological analysis to determine the extent of the epithelial lesion and degree of basement membrane thickening. However, it is also possible to detect biomarkers in cytological samples using techniques such as immunofluorescence and confocal laser microscopy [26].

Neural stem/progenitor cells derived from the olfactory neuroepithelium, known as olfactory neural stem/progenitor cells, are gaining attention as a potential tool for investigating psychiatric disorders [7]. Among the studies aimed at identifying potential biomarkers of schizophrenia, which have investigated the alterations of the olfactory neuroepithelium, a recent pilot study on a small sample of patients, by nasal brushing and culture in appropriate media, has highlighted a reduction in the proliferation of olfactory neuroepithelium cells in patients with schizophrenia compared to healthy controls [27]. In schizophrenia, overall cognitive function was inversely associated with cell proliferation evaluated by BrdU incorporation, Cyclin-D1 and p21 protein level in cultivation passage 3 compared to passage 9, and measured by cognitive tests, suggesting a potential the nasal cell culture as a useful tool to support the individuation of early alterations in psychosis [27].

It was suggested that nasal brushing may enhance our understanding of the mechanisms underlying the pathophysiology of schizophrenia. In this direction, starting from the observation that the olfactory epithelium has received increased interest as a model to study brain and psychiatric diseases, several protocols were assessed [28–30].

The olfactory neuroepithelium samples were obtained invasively by biopsies after nasal surgery [31] or postmortem at autopsies [32], or by non-invasive method [33, 34]. A methodological step-forward was performed by an easy and non-invasive exfoliation [31] which opened new perspective to brain investigations.

However, all these studies *in vitro* on the evaluation of the olfactory neuroepithelium and epithelium cells, from healthy or neurological/psychiatric patients, represent an important development on the potential employment of nasal cytology [35].

To date, a pathophysiological hypothesis underlying childhood epilepsy is associated with focal brain inflammation, which contributes to epileptogenesis [36]. The degranulation of mast cells, triggered by certain factors, leads to the release of inflammatory and neurotoxic molecules. There is a growing need to elucidate the inflammatory pathways involved. In this regard, a cohort study has established a link between allergic rhinitis and an increased risk of childhood epilepsy [37], and could be interesting to analyze the nasal cytology with the intent to establish a potential expression of clinical epilepsy alterations [38]. In this regard, nasal cells are thought to be useful for identifying biomarkers that could facilitate the early diagnosis of neurodegenerative diseases, such as Parkinson's disease (PD). These conditions are often associated with olfactory dysfunction that manifests in the early stages. A previous study described how the genes *OR10A4*, *OR9A2*, and *IFIT1B* were found to be significantly altered in cells isolated from the nasal fluid of patients with PD, confirming their relevance as biomarkers for diagnosis [39].

Nasal cytology has shown a connection between mild nasal symptoms and cellular damage. One study found that all patients analyzed displayed few neutrophils and a decrease in the "hyperchromatic supranuclear stripe of the Golgi apparatus" which is a key marker for the anatomical and functional integrity of ciliated cells. The reduced presence of this marker indicates cellular distress [25].

Olfactory neuroepithelium as a cellular model to evaluate and identify biomarkers of AD, was recently reviewed [40]. A main difference is between studies using postmortem olfactory neuroepithelium from subjects with AD antemortem nasal endoscopy [40], and nasal exfoliation of living AD patients [41].

AD, the leading cause of dementia, affects millions of people worldwide, and its diagnosis is primarily based on clinical criteria. Unfortunately, the diagnosis is often made late, by which time neurodegenerative damage is already widespread. A study focuses on the potential of identifying biomarkers, mainly in the cerebrospinal fluid (CSF) and blood; however, the olfactory neuroepithelium could also play a crucial role. It is well established that olfactory dysfunction is a characteristic that is often altered many years before the clinical diagnosis, even in the early stages of AD (present in 85% of patients with early-stage disease) [8]. The reason is still being investigated, it is thought that there may be an accumulation of pathological plaques at the olfactory circuits [42]. The olfactory neuroepithelium was investigated to see how markers of AD, such as amyloid-β and paired helical filament-tau (PHF)-tau were more present in olfactory neuroepithelium obtained by nasal en-

doscopy biopsy. Amyloid-β was evident in 71% of AD cases compared to 22% of normal cases and 14% of cases with other diseases. PHF-tau was present in 55% of cases with AD, in 34% with normal brains and 39% with other neurodegenerative diseases [42]. In addition, they obtained olfactory neuronal precursors (ONPs) from olfactory neuroepithelium (ONE) by nasal exfoliation, a nasal cytology technique described above using middle turbinate cell harvesting from living individuals. They found increased levels of t-tau and p-tau in olfactory neuroepithelium precursor cultures from patients with AD compared to control patients without AD. Furthermore, t-tau was distributed in a punctuated pattern in a greater number of AD olfactory neuronal precursors. The ease of obtaining these samples by accessing the nasal cavities could also be used for disease monitoring. Briefly, contrasting results were evidenced in post-mortem nasal tissues, with respect the brain area associated with neurodegeneration, in terms of expression levels and density staining of biomarkers studied in AD, protein tau and amyloid-beta peptide. Conversely, olfactory neuronal precursors obtained by nasal exfoliation showed a greater number of total tau-labeled neuronal cells about age, AD and control. Olfactory neuroepithelium cultured *ex vivo* from patients and healthy individuals represents a new and valuable model for studying the cellular and molecular mechanisms involved in neurodegenerative diseases such as AD, PD and mental illness.

8. Conclusions

At present, various procedures are available for analyzing the nasal epithelium field very rich in information useful for the diagnosis of the most common nasal diseases. Thus, based on the patient's clinical condition, the physician should select the relevant technique, making the sample analysis easier and facilitating obtaining any particular information required. Nasal cytology nicely complements clinical evaluation, especially for rheumatological conditions, both allergic and non-allergic and infectious forms. Interesting implications regarding the study of nasal neoplasm or diseases like anosmia due to neurodegenerative conditions would be apparent. Lastly, cytological follow-ups on patients can yield useful information on the effectiveness of medications administered and the progress made by the underlying disease.

In non-allergic rhinitis and chronic rhinosinusitis, nasal cytology offers insight into using various biomarkers relevant for assessing both surgical and biological therapies. Moreover, it ought to be investigated for its use in follow-up evaluations when predicting a recurrence of nasal polyps' prognostic index.

Additionally, nasal cytology could form the basis for a standardized pre-treatment assessment of patients with head and neck tumors, followed by post-treatment cytological assessment to explain any ongoing inflammatory and epithelial changes in the nasal cavity. This approach may have important risks associated with infection and airway colonization. Furthermore, nasal cytology can help a physician choose the best treatment for each patient's case.

It has been suggested that nasal cytology may be of help in diagnosing neurological and psychiatric illnesses particularly chronic neurological diseases such as AD and PD.

The purpose of this direction is to underline that many differences compared to the expression of well-known biomarkers can provide sufficient guidance in the selection of nasal cytology as a valid diagnostic support for the categories of patients suspected to have either neurological or psychiatric diseases. The relationship between olfactory dysfunction and the initiation or progression of neurological diseases may suggest some diagnostic markers.

In this respect, hopes are set toward the possibility of standardizing the analysis and procedures about the study of nasal cytology. The introduction of AI in the field of they're quickly revealing a concrete simplification of some experimental methods, thus also applying to nasal cytology.

A breakdown of this use in medicine has become rhinocytology, which allows better traditional rhinocytological analyses as the Rhino-Cytology Interface [43].

Research regarding AI-enhanced rhinocytological analysis is a must do not only for otolaryngologist, but also neurologists and psychiatrists [43].

Moreover, we think that nasal cytology is winning recognition as an essential investigatory technique as several other device manufacturers with AI-powered technology are trying to automate cell counting, obviating time and reducing human error during microscopic observation [43]. This happening with Rhino-Cyt is moving toward quantitatively an understanding that is more precise and scientifically valid for standardization, to catalog cellular elements and gives a more accurate diagnosis as fast as possible. Such advancements could enable nasal cytology-a test yet to gain acceptance into diagnosis gain further application into wider use among newer pairs of physicians.

Author Contributions

WAR, MR, GC, and CL, designed the research study, performed the research, and wrote the draft manuscript. DR and GC assisted with data collection and analyzed the data and AS, CP, support on literature research. FL and SV assisted with data collection and analyzed. VC, CC visualization and formal analysis. AM provided clinical interpretation. VC, CC and CB provided help and advice on project management. CB and AM contributed to original draft; manuscript revision; project supervision, supervised manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Dr. Christian Barbato, given his role as Guest Editor, had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Zhi Dong Zhou and Bettina Platt.

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