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REVIEW

NASAL cytology: practical aspects and clinical relevance

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Summary

Nasal cytology is a simple and safe diagnostic procedure that allows to assess the normal and pathological aspects of the nasal mucosa, by identifying and counting the cell types and their morphology. It can be easily performed by a nasal scraping followed by May–Grunwald–Giemsa staining and optical microscopy reading. This procedure allows to identify the normal cells (ciliated and mucinous), the inflammatory cells (lymphocytes, neutrophils, eosinophils, mast cells), bacteria, or fungal hyphae/spores. Apart from the normal cell population, some specific cytological patterns can be of help in discriminating among various diseases. Viral infections, allergic rhinitis, vasomotor rhinitis and overlapping forms can be easily identified. According to the predominant cell type, various entities can be defined (named as NARES, NARESMA, NARMA). This implies a more detailed knowledge and assessment of the disease that can integrate the standard diagnostic procedures. Nasal cytology also represents a useful research tool for diagnosis and therapy.

Introduction and background

Nasal cytology (NC) represents a useful and easy-to-apply diagnostic tool to study rhinitis [1, 2], 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 also to evaluate the effects of various stimuli (allergens, infectious, irritants, physico-chemicals) or treatments.

At the end of the 1800s, Gollash and Von Mihalkovics [3, 4] 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 hayfever [5]. 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 non-standardized techniques, during the last decades [9, 10]. The technique of NC was better systematized and investigated in depth starting from 2006 [8, 9]. The NC approach subsequently provided relevant contributions to the knowledge of rhinitis from a pathophysiological point of view, allowing also to identify different phenotypes of non-allergic rhinitis: non-allergic rhinitis with eosinophils (NARES), with mast cell predominance (NARMA), neutrophilic (NARNE) or mixed (non-allergic rhinitis with eosinophils and mast cell, NARESMA) [11–13].

Nasal cytology: practical aspects

The nasal mucosa is a pseudo-stratified ciliated epithelium (Fig. 1a), containing also mucinous cells that are responsible for the continuous mucus secretion. The ciliated cell (Fig. 1b) 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 defence 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

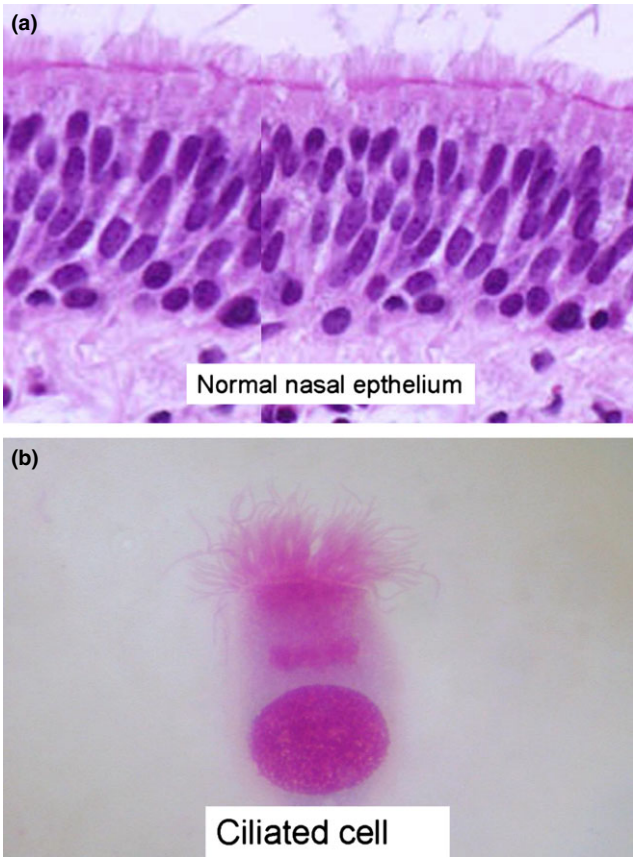

Fig. 1. Panel a: The normal nasal epithelium. Panel b: the normal ciliated cell.

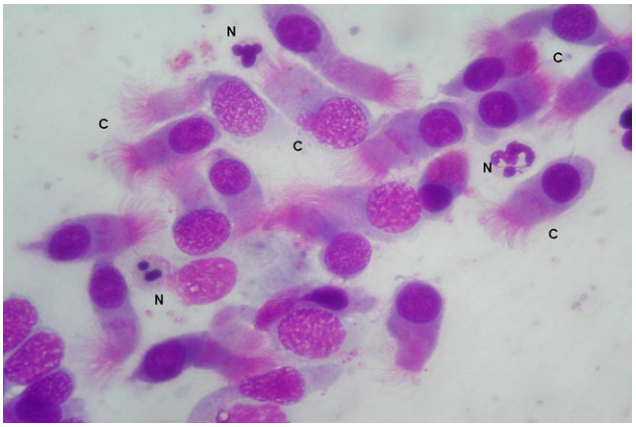

Fig. 2. Normal nasal cytology with ciliated cells (C) and sparse neutrophils (N).

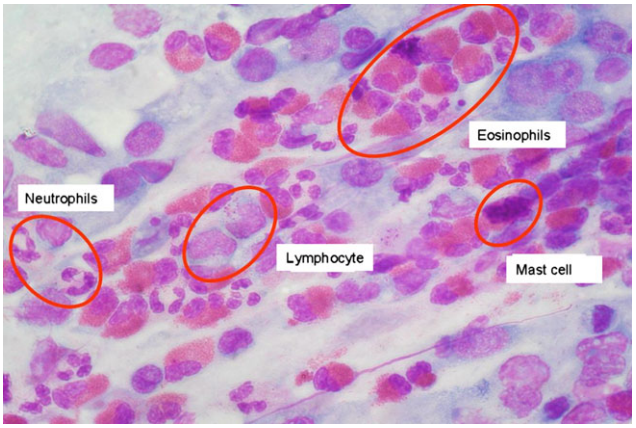

Fig. 3. Pathological findings at NC in allergic rhinitis: neutrophils, lymphocytes, eosinophils and mast cells.

cells, mucinous cells, basal cells/striated cells; only sparse neutrophils can be found occasionally (Fig. 2). The perinuclear halo or hyperchromatic supranuclear stria in ciliated cells is a hallmark of normal function [14]. 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 [15].

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 [16]. 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 anaesthetic 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) (Fig. 3), plus bacterial and fungal spore/hyphae. The traditional MGG staining procedure requires about 30 min, but pre-mixed compounds (e.g. MGG QUICK STAIN,

Table 1. Quantitative and descriptive grading for NC reporting

	Description	Quantitative	Grading*
Epithelial ciliated cells	Normal	–	N
	Abnormal	–	A (CCP/MN)
Mucinous cells	None	0	0
	Occasional	1–24%	1+
	Moderate number	25–49%	2+
	Large number	50–74%	3+
	Covering the entire field	75–100%	4+
Neutrophils and eosinophils	None	0	0
	Occasional	0.1–1%	½ +
	Few scattered cells, small clumps	1.1–5%	1+
	Moderate number, large clumps	5–15%	2+
	Large clumps not covering the field	15–20%	3+
	Clumps covering entire field	>20%	4+
Basophilic (mast cells)	None	0	0
	Occasional	0.1–0.3	½ +
	Few scattered cells, small clumps	0.4–1	1+
	Moderate number, large clumps	1.1–3	2+
	Large clumps not covering the field	3.1–6	3+
	Up to 25 per an X100 field	>6	4+
Eosinophil/mast cell degranulation	None observed	Present/absent	0
	Occasional granules		1+
	Moderate number of granules		2+
	Many granules easily seen		3+
	Massive degranulation, entire field		4+
Bacteria and spores	None observed	None standardized	0
	Occasional clumps		1+
	Moderate number		2+
	Many cells easily seen		3+
	Bacteria/spores over the entire field		4+

*CCP, ciliocytophthoria; MN, multinucleation.

Table 2. Examples of differential diagnoses at NC (Adapted from MELTZER 1988)

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

Bio-Optica®, Milan, Italy) are available and allow a satisfactory preparation in less than 30 s. 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 [17] (Table 1). 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 (Table 2).

Cytopathological aspects

NC should be always read and interpreted within the whole clinical context that includes symptoms, personal history, nasal examination and presence of IgE sensitization. The major aspects of differential diagnosis at NC in rhinitis can be at a glance subdivided in infectious rhinitis, allergic rhinitis, cellular vasomotor rhinitis, overlapping forms.

Infectious rhinitis

From a cytological viewpoint, any damage of the nasal mucosa firstly affects the ciliated cells, with an architectural rearrangement that favours mucinous cells (mucinous metaplasia). This phenomenon leads to an increased mucus secretion that cannot be efficiently cleared by the cilia and results into mucus deposition that can favour bacterial proliferation [18]. The normal turnover of ciliated cells is about 3 weeks; thus, recurrent/chronic inflammations impede the physiological cell replacement [19, 20]. Bacterial infectious rhinitis is usually characterized by the presence of a large number of neutrophils, with intra- and extracellular bacteria, that can be easily identified at optical microscopy. In addition, a proportional reduction of ciliated cells in favour of mucinous cells can be observed.

Virus-induced rhinitis (e.g. common cold) is probably the most frequent infectious disease, and it can be easily diagnosed on a clinical basis. If NC is performed, a morphological change of the ciliated epithelium can be seen, known as 'ciliocytophthoria' [21], that includes : nuclear chromatin condensation, nuclear margination, appearance of an intranuclear halo with visualisation of the nucleolus, multiple cytoplasmic vacuoles, "decapitation" of the apical portion of the ciliated cell due to the lateral confluence of cytoplasmic vacuoles (Fig.S1).

Infectious rhinitis: the biofilm

Biofilms are surface-associated agglomerates of microorganisms (either bacteria or fungi) embedded in a self-produced extracellular polymeric matrix. Biofilms have been described in numerous diseases, including rhinosinusitis, otitis and nasal polyposis [22, 23]. The clinical importance of biofilm stands in the fact that the polysaccharide matrix may be responsible for an increased survival of microorganisms and for antibiotic resistance, thus leading to a difficult eradication or to a difficult-to-treat contamination of implanted medical devices. Consequently, identification of biofilm *in vivo* has both diagnostic and therapeutic implications. Due to their nature, biofilms have been always studied by complex and expensive techniques such as electron microscopy or confocal laser microscopy [23, 24], not feasible in the routine clinical practice. Recently, we showed that nasal cytology, performed by optical microscopy, is able to identify biofilms on nasal mucosal surfaces [25]. With this approach, biofilms appear as cyan-stained 'infectious spots', whose polysaccharide nature can be confirmed by the periodic acid–Schiff staining (Fig. S2)

Allergic rhinitis

The pathophysiological mechanisms of allergic rhinitis (AR) are currently quite well known. The triggering event is allergen–IgE–mast cell interaction that leads to the early-phase response (mainly mediated by histamine). If the allergenic stimulus is persistent over time, the allergen-triggered inflammation also becomes persistent, and other cellular components are involved, as well as adhesion molecules and cytokines. At NC, an intense infiltrate of eosinophils and mast cells (with lymphocytes and neutrophils) can be observed, strictly related to symptoms and exposure to allergens. When the exposure to the offending allergen is weak but persistent (typically in dust mite allergy), symptoms may be of low intensity, but a minimal persistent inflammation (predominantly neutrophils) is anyway present [26, 27]. Concerning pollen-induced AR, within the pollen season, the typical symptoms are present, and NC identifies neutrophils, eosinophils and degranulated mast cell (Fig. 3). In such case, the concordance among pollen exposure, symptoms and skin test results are usually sufficient for a correct diagnosis.

Non-allergic ('Cellular') Vasomotor Rhinitis

In the setting of chronic rhinitis, the category of non-allergic ('cellular') rhinitis still remains an unclear entity, lacking an unambiguous clinical, diagnostic and therapeutic approach. The term 'non-allergic' obviously implies that a specific IgE sensitization is clearly excluded (negative skin prick test or serum IgE assay). These forms of rhinitis are often underdiagnosed and/or labelled as 'non-specific' vasomotor rhinitis or as local allergic rhinitis [28]. Failure to identify them is solely due to the fact that nasal cytology is not included among the routine investigation They account for around 15% of all nasal diseases, which is quite a considerable proportion, and are usually accompanied by intense pseudo-allergic symptoms (nasal congestion, itching, bouts of sneezing, burning in the nose or rhinorrhea) that often leads them to be confused with IgE-mediated rhinitis.

Overall, patients with cellular vasomotor rhinitis also display a non-specific nasal reactivity that causes the onset of symptoms in the presence of non-specific stimuli (cold air, humidity, strong odours, cigarette smoke, nasal irrigation, topical drugs). This aspect is well known in swimmers who are constantly in contact with the chlorinated swimming pool water and develop an irritative neutrophilic rhinitis with persistent obstruction [29]. In such cases, NC provides a robust diagnostic tool.

Patients with cellular rhinitis frequently have a family history of asthma and/or nasal polyposis or a history of turbinate surgery (often resulting in septum–turbinate–synechiae, crusting, mucosal atrophy). Another typical finding is the overuse of nasal decongestants (over-the-counter drugs) that lead to 'rhinitis medicamentosa'. These conditions display severe and persistent nasal symptoms and often occur with other diseases (bronchial asthma, acetylsalicylic acid sensitivity, polyposis, chronic rhinosinusitis), with a relevant significant detrimental effect on the quality of life. According to the NC aspects, the 'cellular' forms of rhinitis can be subdivided into non-allergic rhinitis with neutrophils (NARNE), non-allergic rhinitis with eosinophilia syndrome (NARES), non-allergic rhinitis with mast cells (NARMA) and non-allergic rhinitis with eosinophils and mast cells (NARESMA).

Non-allergic Rhinitis with Neutrophils (NARNE)

NARNE is characterized at the microscopic examination by a predominant infiltration of neutrophils (> 20%) (Fig. 4a). Different from infectious rhinitis, neutrophils are not accompanied by the presence of bacterial or spores/fungal hyphae. The increasing prevalence of this disease, especially in recent years, is probably linked to physical and chemical irritants, because the majority of subjects are industrial and craft workers, people living in industrialized areas or smokers [29]. NARNE can be often found in patients with gastroesophageal reflux disease, where the inhalation of hydrochloric acid derivatives can easily explain the recruitment of inflammatory cells. The prolonged presence and continuous release of chemical mediators (in particular neutrophil elastase) are the main cause of free radical formation and consequent impairment of the mucosal epithelium, which is translated clinically into 'vasomotor' symptoms (seromucous rhinorrhea, sneezing bouts, burning sensation and nasal congestion). At variance with other forms of cellular rhinitis [14], symptoms are usually less intense and resolve once the pathogenic cause is identified and removed.

Non-allergic Rhinitis with Eosinophilia Syndrome (NARES)

NARES is a non-IgE-mediated vasomotor rhinitis, characterized by a predominant eosinophilic infiltration of the nasal mucosa, usually up to 50–70% of inflammatory cells (Fig. 4b). Like NARMA and NARESMA, it often co-occurs with nasal polyposis, and/or asthma, and/or sensitivity to acetylsalicylic acid. In a small proportion of patients, nasal eosinophilia can be accompanied by peripheral hypereosinophilia. Sometimes, these forms of rhinopathy can recruit, for reasons that are still unknown, mast cells, thereby turning into eosinophilic mast cell forms (NARESMA), in which the symptoms become more intense and continuous.

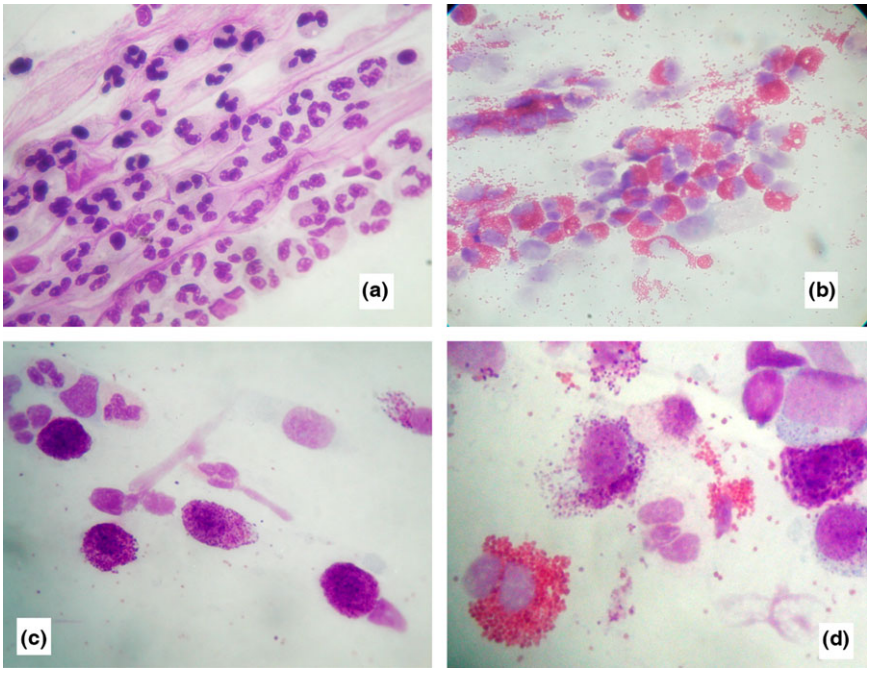

Fig. 4. (a) Non-allergic rhinitis with neutrophils (NARNE), (b) non-allergic rhinitis with eosinophils (NARES), (c) non-allergic rhinitis with mast cell (NARMA) and (d) non-allergic rhinitis with eosinophil–mast cell (NARESMA).

Non-allergic Rhinitis with Mast Cells (NARMA)

Microscopically, this disease is characterized by the presence of mast cells in the nasal mucosa, partially degranulated (Fig. 4c). The clinical presentation is usually severe (nasal congestion, rhinorrhea, sneezing bouts, nasal itching) and it is often associated with the presence of asthma and/or nasal sinus polyposis. Like NARES, NARMA can be considered a transitional form leading to NARESMA.

Non-allergic Rhinitis with Eosinophils and Mast Cells (NARESMA)

NARESMA was identified as a cytological entity only in recent years. It is characterized by the presence of eosinophils and mast cells, in variable proportions, and with a relevant degranulation (Fig. 4d). The most important aspect of NARESMA is that, at variance with other forms described above, it is more frequently associated with nasal polyposis, asthma and rhinosinusitis. When associated with nasal polyposis, NARESMA represents an unfavourable prognostic factor, associated with frequent relapses. NARESMA responds well to corticosteroid therapy, both topical and systemic and, like all the other forms of vasomotor rhinitis requires a regular clinical and cytological assessment.

The 'Overlapping' forms

In the field of rhinology, one of the most important contributions provided by NC in recent years is the concept of 'overlapping rhinitis'. By means of NC examination, it is possible to identify patients who are affected by multiple diagnostic entities (e.g. allergic rhinitis associated with NARES or with NARESMA). From a clinical point of view, these patients, despite testing positive for one or more 'seasonal' allergens, have 'persistent' nasal symptoms, together with a rhinocytogram showing the presence of eosinophils and/or mast cells also outside the pollen season. In such cases, nasal cytology may be an additional useful criterion, because it can unmask this inflammatory basis of the clinical condition [30, 31]. The diagnosis of these forms of rhinitis is crucially important, especially in the field of allergy, where therapeutic strategies range from pharmacologic approaches (antihistamines, corticosteroids, leukotriene modifiers, decongestants, etc.) to allergen immunotherapy (AIT). In this regard, it should be remembered that most patients with overlapping rhinitis and treated by AIT may experience less benefit than expected. This can be attributed to the fact that AIT has no effect on the concomitant 'non-IgE-mediated' component of rhinitis. In these cases, it will always be necessary to combine AIT with an appropriate pharmacologic treatment to control symptoms. Therefore, a detailed rhinological and allergological diagnostic work up, to identify the presence of clinical and cytological signs that might raise the suspicion of 'overlapping' rhinopathies, is essential to plan a targeted therapeutic strategy.

Conclusion

The increasing importance of NC as an adjunct diagnostic tool in nasal diseases has progressively been recognized in the last decades. The modern methods of sampling, staining and interpretation have been sufficiently standardized, so that NC now represents an easy to do procedure, even in routine practice. The use of NC, in addition to the diagnosis of allergic or non-allergic rhinitis, is currently providing a useful instrument for research purposes, such as the investigation of conditions less common than allergic rhinitis [32]. It is true that some costs have to be afforded (microscope and staining preparations), but it is also true that in the case of a high prevalence condition, such as rhinitis is, the cost-to-benefit ratio remains favourable. It should be also considered that more expensive and complex diagnostic approaches are currently used in other diseases (e.g. thyroid, lung or breast nodule biopsies).

In the case of nasal cytology, the procedure is non-invasive, repeatable, easy to apply in all conditions and age range. Currently, NC allows to detect and discriminate various inflammatory aspects of nasal mucosa [33–37]. If allergic rhinitis (when symptoms, skin test and CAP results are in accordance) is easy to be diagnosed, other diseases (cellular or vasomotor rhinitis) could benefit from a diagnostic NC procedure [34] to detect the non-IgE-mediated component. As already applied to the lower respiratory airways by means of fiberoptic bronchoscopy, NC could represent an attractive investigational tool to detect, at mucosal level, more refined aspects of rhinitis. In addition to the simple staining herein described, the use of 'omic' techniques could be envisaged.

Conflict of interest

The authors declare no conflict of interest.

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Authorship

All the signing authors equally contributed in the literature search, drafting and writing the MS and approved the final version.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. The progressive degeneration of the ciliated cells in the case of virus-induced rhinitis.
Figure S2. The microscopical appearance of the nasal 'spot' (biofilm), clearly cyan stained at MGG.