

ROLE OF NASAL CYTOLOGY

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Nasal cytology represents a valid method in the differential diagnosis of allergic and non-allergic nasal diseases, as it is simple, safe, non-invasive, cost-effective, and easy to perform both in the medical and paediatric office. In particular, through cytological investigation it is possible to diagnose a group of “non-allergic infective rhinitis” that still today constitutes a vague aspect of the clinical-diagnostic-therapeutic approach to eosinophilic non-allergic rhinitis (NARES), non-allergic rhinitis mast cell (NARMA), neutrophilic non-allergic rhinitis (NARNA), and eosinophil-mast cell non-allergic rhinitis (NARESMA). Preventive treatment of nasal diseases, when guided by rhinocytograms, leads to a favorable clinical and time-dependent outcome. These advantages are reflected in a better quality of life and in a reduction in National Health Service costs, without chronic evolution of the disease to complications.

Nasal cytology covers an important area of research in rhino-sinus pathology, mainly concerning vasomotor rhinitis (VMR) and infective nasal disease, since it is a valid method in the differential diagnosis of allergic and non-allergic, bacterial and viral diseases. This is a well-known method, considering that Gollash in 1889 observed that the presence of eosinophils, in the nasal secretion of patient with bronchial asthma, was an important element in the pathogenesis of the disease (1-3).

Many factors have contributed to increase the interest about this diagnostic procedure, including the simplicity of nasal sampling, the scarce invasiveness of the techniques, the repeatability of the rhinological exams, which is often necessary in the follow-up visits for monitoring the efficacy of medical-surgical treatments (4). The simplicity of the methods, the non-invasive safety, the cost-effectiveness and the possibility of performing the exams in the clinical office setting, makes nasal cytology applicable also in paediatric subjects (5). The nasal mucosa is classically composed of four cell types (ciliated, muciparous, striated and basal), and some rare neutrophils. Therefore, finding other cell types is an index of probable disease.

Cytological techniques

The cytological method consists of the following:

- Sample collection (also known as sampling)
- Processing (which involves fixing and staining)
- Microscopic observation

Cytological sampling consists of harvesting the superficial cells of the nasal mucosa, which can be performed by using a sterile tampon (e.g. the same used for pharyngeal sampling), or a small plastic curette (Rhino-Probe®), or by scraping the nasal mucosa. In retrieving samples from the nasal mucosa the nasal tampon must be twisted with moderate pressure to remove a large quantity of mucosal cells, corresponding to the medial portion of the inferior turbinate, notably the area where there is an equal ratio of ciliated cells and goblet-muciparous cells (ratio of ¼ in favour of the ciliated cells). Usually, in the case of young patients, a nasal tampon is preferred instead of scraping the surface of the mucosa, since it is quicker with less inconvenience for the patient; furthermore an abundant quantity and quality of cell samples are collected. The sampling is always carried out with careful visual attention, with anterior rhinoscopy using a nasal speculum and good illumination. The method is not invasive, so it does not require any anaesthesia. After the samples are collected on the nasal tampon, they are spread on a microscope cover slide, they are fixed and stained with May Grunwald- Giemsa. This stain is usually used since it stains all the cytological components present in

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the nasal mucosa, including the inflammatory cells (such as neutrophils, eosinophils, lymphocytes and mast cells), bacteria and mycotic spores. The smear is observed under a common light microscope equipped with a 1000 x objective. For the rhinocytogram the procedure is to read as many fields as possible on the entire surface of the cover slide, in order to understand which cellular elements are involved for the diagnosis (neutrophils, eosinophils, lymphocytes and mast cells, bacteria and spores) and evaluating the percent of the cell types in at least 10 microscopic fields (6).

Cell Types in Nasal Disorders

Nasal disorders involve the more differentiated ciliated cells with a rearrangement of the respiratory mucosal epithelium favouring muciparous goblet cells (such as muciparous metaplasia). This observation has physiological and clinical implications: in fact, the reduction of the ciliated cell component and the proportional increase of muciparous cells, causes an abundant production of mucus which stagnates in the endonasal sinuses. In addition, the dynamics of mucociliary transport (TMC) is reduced, the stagnated mucus favours bacterial replication and triggers a vicious circle allowing recurrent inflammatory episodes [7]. As the normal turnover of ciliated cells is about three weeks, the recurrent inflammatory episodes impede the reconstruction of the normal ratio of the various cell types of the respiratory tract epithelium.

The rhinological cytogram of a child with acute or chronic non-allergic rhino-sinusitis presents a higher percentage of inflammatory cells (neutrophils, macrophages and lymphocytes), respect to normal subjects, and an inversion of the ratio of ciliated and muciparous cells, in favour of the latter. The characteristic of the infective rhino-sinusitis is the presence of numerous bacterial elements, both in the extracellular space and inside neutrophils (such as phagocytosis).

An interesting observation, recently obtained from a randomized study on 800 children, is the condition of "asymptomatic carrier" with high endonasal bacterial count, seen in children of 3-4 years (8). These young children are at higher risk, not only for nasal-sinus diseases, but also for infective diseases of the local regions (otitis, pharyngitis, tonsillitis), and more distally, laryngeal-tracheal-bronchitis, bronchopneumonia and rhino-bronchial syndrome. In these cases, it is important to follow the disease during the medical treatment with periodic cytological controls, as only significant reduction of inflammatory cells and disappearance of bacterial elements, in the cytological samples, will confirm the recovery.

A low intensity, but temporally persistent, exposure

to an allergen, typical of perennial rhinitis (i.e., *Dermatophagoides*, *Parietaria* pollen) leads to a condition called "Minimal persistent inflammation" (9), characterized by an infiltrate mostly made by neutrophils and, in minimal part, by eosinophils, and rarely mast cells. Instead, the "pollen forms" are characterized by eosinophils and mast cells, which are mostly degranulated (10).

Eosinophils are found in all types of allergic diseases and at all ages, while the presence of bacteria in the intra- and extra-cellular spaces, associated with numerous neutrophils in allergic patients, is a sign of superimposed bacterial infection. Other inflammatory causes of rhinitis, besides the ones mentioned, are known as non-infective, non-allergic rhinitis (vasomotor rhinitis) that give rise to forms of rhinitis that are considered serious, very difficult to treat and require advanced testing. Although these forms are described in the literature as adult forms, our current data also include pediatric cases. In particular, through cytological investigation it is possible to diagnose a group of non-allergic infective rhinitis" that still today constitutes a vague aspect of the clinical-diagnostic-therapeutic approach to eosinophilic non-allergic rhinitis (NARES) (Figure 1A), non-allergic rhinitis with mast cell (NARMA) (Figure 1B), neutrophilic non-allergic rhinitis (NARNA) (Figure 1C) and eosinophil-mast cell non-allergic rhinitis (NARESMA) (Figure 1D) [11]. These forms are usually termed "aspecific" and they express, in fact, the scarce knowledge of etiological factors that cause these particular diseases, which have determined diagnostic and therapeutic failure. The pseudo-allergic symptoms of these diseases are usually accompanied by nasal obstruction, pruritus, sneezing, burning sensation of the nasal mucosa, rhinorrhea, etc. These symptoms are often confused with IgE-mediated rhinitis. They also present with a non-specific reactivity, which causes symptoms deriving from a change in posture, temperature changes, and exposure to: cold air, intense odours, and tobacco smoke. These symptoms are often misdiagnosed as vasomotor rhinitis. The intense and persistent nasal symptoms, together with the tendency to associate these symptoms with more serious diseases, such as, bronchial asthma, aspirin intolerance, rhino-bronchial syndrome, nasal polyps, and sinusitis, is a negative aspect of these rhinological diseases which should not be taken lightly in terms of health care costs and the quality of life of these patients.(12-14). Since these are cellular disorders, it is indispensable to perform a microscopic diagnosis of the nasal mucosa in order to determine the prevalent cellular type to arrive at a precise diagnosis.

Another frequent finding recorded during history is the surgical treatments of the turbinates to resolve the "obstructed nasal passages". These surgical procedures

are however insufficient and can sometimes damage the patient, as cause scarring (turbinate-septal synechia), crusting rhinitis, and atrophy of the mucosa. Typical is the chronic use of nasal decongestants containing nafazoline, or similar compounds, to reduce nasal congestion. It must be kept in mind that chronic use of nasal decongestants can cause a serious form of rhinitis (pharmacological rhinitis) that can be superimposed on the pre-existing condition.

Therapeutic strategies of allergic and non-allergic vasomotor nasal diseases

Nasal cytology is considered more than just a simple diagnostic tool, since it can monitor the medical treatment of various forms of nasal diseases. The literature of the last decade has emphasized this aspect many times, highlighting the reduction of immune-inflammatory cells, degranulation phenomena, and the reduction of bacteria, etc. due to modern drug therapies which include: corticosteroids, local and systemic antihistamines, antibiotics, vasoconstrictors, etc. (15,16).

The possibility to reveal allergic rhinitis in a preclinical phase and to follow its evolution in the post-clinical stage depends on cytological staging. These phases are marked by absence of symptoms, even if there are immune-inflammatory processes present in the mucosa, as evidenced by cytological alterations known as persistent minimal inflammation, complicated at times by infection due to superimposed bacteria, and always considered serious since the disease tends towards a chronic state, or worst, towards complications. In fact, during the acute phase there are evident clinical manifestations that lead to medical treatment intervention, which at times can be aggressive or exaggerated (overtreatment), which controls the pathological event on the clinical side, but the minimal persistent inflammation remains, escaping any form of therapy. On the contrary, the minimal persistent inflammation continues to aggravate the tissue damage liberating pro-inflammatory cytokines and causing a continuous flow of immunological-inflammatory mediators, perpetuating the vicious circle.

It is therefore possible to trace a therapeutic strategy based on cytological examination (Figure 2), as this diagnostic method is able to control the natural history of allergic rhinitis, in particular when inflammation persists in the latent or asymptomatic form. It is also important to underline that the preventive treatment of pollinosis, if guided by a rhinocytogram, promotes a more favourable clinical picture and duration of treatment, and produces advantages that reflect on the quality of life and reduced medical costs, avoiding the evolution of the disease towards a chronic and complicated state. Instead, in the asymptomatic phase of nasal allergy, which is rarely diagnosed with common clinical methods, it is more

difficult to program a treatment schedule. The only exam capable of unveiling the latent disease is nasal mucosa cytology, which highlights the cellular elements and the pathogenesis. In absence of any signs and symptoms and a negative cytological exam, there is no need for any therapeutic treatment. Local corticosteroids (budesonide, fluticasone furoate, mometasone furoate, etc.) are recommended (18) for treating moderate symptoms, possibly associated with local (azelastine) (19) or systemic antihistamines (desloratadine, levocetirizine, ebastine, etc) (20-22). Systemic corticosteroids (such as deflazacort, prednisone, etc.) should be used only when eosinophils with degranulated mast cells are abundant, and there are severe symptoms (23). This treatment protocol should be continued until the symptoms start to subside and, above all, when the cytology of the nasal mucosa indicates a partial or total remission of the acute state. Therefore, it becomes necessary to return to topical corticosteroid treatments and systemic antihistamine treatment.

Concerning the medical therapy of chronic nasal congestion, the point of no return exists where either systemic or topical therapies does not have any effect on the symptoms, therefore surgical intervention of the turbinates appears to be justified with a return to medical therapy prescribing topical or systemic corticosteroid, antihistamines, antileukotrienes, etc. (24-25). The correct therapeutic orientation is very often "personalized" temporally, depending on the disease severity, in the hope of not intervening surgically once again or developing complications. Another important aspect of these cellular nasal disorders is the frequent association with polyps. Preliminary data show a greater incidence of cellular forms in comparison with allergic rhinitis (polyps: 11.5% vs 1.7%; asthma 12% vs 6.7%).

Since they are chronic diseases, chronic therapy is necessary and personalized follow-up aimed at controlling the symptoms and at preventing complications (rhinosinusitis, polyposis, asthma, etc.).

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Legend to figures:

Fig. 1 A,B,C,D: Nasal scraping with different cellular predominance. A eosinophilic, B mast cell predominance, C neutrophilic, D eosinophilic-mast-cell.

Fig.2 Cytologic evaluation aimed at instituting a more rational treatment strategy in allergic rhinitis.

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