

A Multidisciplinary Approach In The Diagnosis Of Allergic And Non Allergic Respiratory Diseases: Nasal Cytology And Feno.

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Abstract:

Upper and lower airway disease is a common condition. Its prevalence is increasing in different areas of the world, but, at present, non-allergic rhinitis is underestimated. The aim of this study was to investigate FeNO (fractional exhaled nitric oxide) and nasal cytology in allergic and non-allergic patients, in order to reach a correct diagnosis.

This study was performed on 120 children with rhinitis and/or asthma, evaluated by an allergist and an otolaryngologist. Skin prick-test and nasal cytology were tested in all patients; FeNO only in the asthmatic ones.

The proportion of positive results in nasal cytology was higher in non-allergic than in allergic children: 22 out of 23 patients, vs 91 out of 97 patients. A significant correlation was found between FeNO levels and increase in nasal eosinophil counts.

There is compelling evidence of a close relationship between upper and lower airway in asthma and rhinitis. The presence of rhinitis should always be investigated in children with asthma; therefore, FeNO and nasal cytology have clinical benefit both in allergic and non-allergic children. Our finding also supports the use of nasal cytology to evaluate non-allergic rhinitis (NAR).

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Introduction :

Asthma and rhinitis are common throughout the world with a high burden of morbidity and cost. The quality of life of patients suffering from AR and allergic asthma is often severely impaired as is their social life, their career and their school performance,[1,2]. Despite the spread of respiratory allergies (AR: 113 million of patients; allergic asthma: 63 million of patients) the prevalence of non-allergic rhinitis is increasing and often largely underestimated (NAR:AR=1:4). NAR, a diverse syndrome including 4 main types of inflammatory disorders (NARES: NAR infiltrated by eosinophils; NARMA: NAR infiltrated by mast cells; NARESMA, NAR infiltrated by eosinophils and mast cells; NARNE, NAR infiltrated by neutrophils), is a risk factor for headache, sinusitis, nasal polyps and chronic nasal symptoms,[3].

Dysfunctions of the upper and lower airways frequently coexist: data from epidemiological studies indicate that rhinitis is experienced by as many as 80% of patients with allergic asthma and 75% of patients with non-allergic asthma; while asthma is experienced by as many as 34% of patients with AR and 25% of patients with NAR,[4,5].

Nasal cytology represents a valid method in the differential diagnosis of allergic and non-allergic nasal diseases as it is simple, safe, non-invasive (local anaesthesia is not required), easy to perform and able to detect both the cellular modification of the nasal epithelium (a ciliated pseudo-stratified epithelium) caused by either allergen exposure or irritative stimuli or inflammation. Such a consideration suggests the quality of a systemic use of nasal cytology in the diagnostic work-up of upper and lower airway disorders in children, in order to reach a proper defined diagnosis and to study the airway inflammation: the diagnosis of nasal disorders through nasal cytology is based on the consideration that, in healthy subjects, the nasal mucosa is composed of 4 normal subsets of cells, which commonly characterize the pseudo-stratified epithelium; besides neutrophils, no other cells are detected in healthy individuals. Therefore, on a rhinocytogram, the presence of eosinophils, mast cells, bacteria, spores and fungi has to be considered as a clear sign of nasal pathology.

FeNO is an easy to perform and non-invasive biomarker of airway inflammation and chronic airway inflammation is a characteristics key in the pathogenesis of asthma. Elevated levels of eNO are thought to result from increased expression and activity of inducible form of nitric oxide synthase in airway epithelial and inflammatory cells. These findings suggest that eNO provides information about the degree of eosinophilic airway inflammation and that the correlations between eNO and pulmonary function test characteristics are probably caused by the impact of eosinophils on pulmonary functions. Consistent with the observation that corticosteroids inhibit the expression of inducible NOS (iNOS) in epithelial cells, treatment of patients with nasal steroids leads to a fall in airway eosinophilia measured by nasal cytology and FeNO.

Therefore, the aim of this study was to combine measurements of upper and lower airway inflammation with FeNO and nasal cytology but also to study the clinical benefits of nasal cytology in children.

Materials AND Methods

A total of 120 patients, aged 3-17, coming from our outpatient service of allergic diseases were clinically examined from the 1st of November 2013 to the 31st of March 2014. All participants were interviewed about respiratory symptoms thanks to ACT (Asthma Control Test) or C-ACT (Childhood-Asthma Control Test) and SNOT (Sino-Nasal Outcome Test); lung function and airway inflammation were measured using HyPAIR FeNO and nasal cytology. Furthermore the allergic sensitization to common aeroallergens (birch, core, olive tree, grasses, ragweed, parietaria, dog, cat, house dust mite: DPP and DPF, mould: alternaria) and foods (cow lactalbumin, cow casein, egg white and yolk, peanuts) was evaluated by skin prick-test.

Nasal inflammation was assessed by nasal cytology in all patients free of treatment (systemic antihistamine and/or nasal steroids) for at least 10 days.

The following steps characterize the cytological technique:

- sampling and processing: the middle portion of the inferior turbinate was scraped using Rhino-probe; the sample was placed on a glass slide,

fixed by air drying and stained by May-Grünwald-Giemsa method.

- observation through microscopy (a light microscopy able to magnify up to 1000x)

For the rhinocytogram analysis, the slide, divided into 10 microscopic fields, has to be read in order to detect eosinophils, mast cells, neutrophils, bacteria, spores and calculate their percentages comparing to the number of total leukocytes.

FeNO was measured by using the online single breath method with HyPAIR FeNO according to ERS/ATS guidelines.

The subject, seated comfortably and with the nose clip, inserted the mouthpiece and inhaled NO-free air to total lung capacity over a period of 2 to 3 seconds through the mouthpiece of the instrument. The subject then started exhalation with a flow rate of 50ml/sec. Children younger than 10 years performed a 6-second exhalation, and FeNO was calculated during the last 2 seconds of the exhalation. Children ≥ 10 years performed an exhalation of 10 seconds, and FeNO was calculated during the last 3 seconds of the exhalation. FeNO values was recorded if the reliability index was higher than 60%. Each subject performed no more than a total of 6 exhalations. The interval between exhalations was at least 30 seconds. FeNO was calculated as the mean of 3 correct exhalations. All measurements were performed in parts per billion (ppb).

Results :

Participants

We evaluated 120 children from our outpatient service for allergic diseases, 75 males and 45 females, aged 3-17, (46% aged 6-10, 35% aged 11-15, 13% aged 3-5, 6% older than 15). 74% children were affected by rhinitis, 63% of children by rhinitis and concomitant asthma or conjunctivitis. 25% of children only asthma.

According to prick test positivity patients were classified in two groups: allergic (97) and non-allergic (23). Of allergic children 55% were polyallergic, 9% were polysensitized but only for perennial allergens. The most common sensitizing allergens were grass pollen (70%) and DPP (69%), followed by DPF (64%), birch (45%), cat (44%).

Nasal Cytology

Eosinophils were found in 80 allergic patients. 6 patients had normal nasal cytology but two of them were sensitized only for grass pollen and the exam was performed out of the pollen season; 2 were on therapy (Fluticasone nasal spray and antihistamine) which could have invalidated the results; 2 were polyallergic. According to the correlation between symptoms and prick test 6 patients, diagnosed only with allergic rhinitis also had overlapped rhinitis (Table 1a)

The nasal cytogram of the 23 non allergic children showed the presence of eosinophils and mastcells; Of these children 12 had rhinitis (4 associated with asthma), 9 were affected by asthma (they reported to have also blocked nose and rhinorrhea but they didn't have a physician-diagnosed rhinitis), 1 child reported to only suffer from persistent cough. According to the symptoms NARES was diagnosed in 20 and NARESMA in 2 out of 23 patients; in one case the child had only current wheezing so a follow up was suggested in order to monitor the clinical situation. The child with persistent cough had normal nasal cytology. (Table 1b)

FeNO and Nasal Cytology

FeNO was evaluated in 72 asthmatic patients. FeNO was higher in allergic children (medium FeNO =21,76ppb) than in non-allergic children (FeNO =9,39ppb) and in subjects affected by both asthma and rhinitis (FeNO =26,06ppb allergic; 21,30ppb non allergic) than in subjects only with asthma (FeNO =20,21ppb allergic; 6,97ppb non allergic). Moreover FeNO was increased in patients with high level of nasal eosinophilia (Fig.1).

Discussion :

Rhinitis and asthma have a huge impact on the quality of life of children and their families, social life, career and school performances are often severely impaired; moreover costs for these diseases amount to several millions of Euros,[1].

Patients with both of these respiratory diseases often need emergency treatments and hospitalization with lower physical activity, productivity loss and higher costs for the Health Medical Care.

Rhinitis causes not only physical problems but also social problems, especially in interpersonal relationships. The need to constantly blow their nose, cough and sneeze

make children sad, frustrated and irritable. They feel unsuitable and this leads to a kind of “social exclusion”.

Nasal symptoms are one of the most frequent causes of school absenteeism: 23% of children interviewed reported the loss of 1-5 school days, 11% 6-10 days and 7% more than 10 days.

As pointed out by the Global Initiative for Asthma (GINA), it is necessary to find new non-invasive tools for the diagnosis and the management of respiratory inflammation,[8].

Nasal cytology and FeNO are suitable to assess the cellular modification of the nasal epithelium and the fraction of exhaled nitric oxide and so we allow us to study the situation of the upper and lower respiratory airways and the role played by rhinitis on their deterioration,[7]. Nasal cytology is now part of the diagnostic algorithm of rhinitis and it is included in the latest Italian guidelines (ARIA 2010 and 2014), although it is still used by few medical centers,[6].

Thanks to Nasal cytology it is possible to understand the conditions of allergic and non-allergic patients whose symptoms do not correspond to the first diagnosis. In allergic subjects nasal cytology is extremely useful to recognize and treat overlapped rhinitis. Some children who present a sensitization to seasonal allergens may experience a perennial symptomatology along with a

positive cytology, even outside the pollen season. Our study detected 6 overlapped rhinitis: a clinical follow up was suggested for these children since there was a higher possibility of nasal polyposis.

Nasal cytology in non-allergic children allow us to highlight the existence of non IgE-mediated rhinopathies: non-allergic rhinitis such as NARES, NARNE, NARESMA, NARMA, which, without this exam, would have remained non-diagnosed. 19 NARES and 2 NARESMA were diagnosed in our study.

In our study we find a correlation between the nasal level of inflammation and clinics: generally children with a high level of eosinophils and mast-cells at nasal cytology have worse symptoms than others with lower levels.

Nasal cytology is a useful tool in children with asthma as well. Epidemiological studies have shown that asthma and rhinitis often co-exist in the same patient. However upper airways symptoms may be underestimated because of the presence of lower airways symptoms. This fact could be dangerous because nasal inflammation may not be treated, leading to unsatisfactory asthma control (“United Airway”, [9])

Using both nasal cytology and FeNO on the same patients it can be observed that FeNO is higher in

Table 1a: Nasal cytology in allergic children

No	No	CYTOLOGY	CYTOLOGY	No	
97 ALLERGIC CHILDREN	80	EOSINOPHILS	Only eosinophils	67	
			Eosinophils+ mastcells	4	
			Eosinophils + fungi	1	
			Eosinophils+ bacteria	2	
				Only eosinophils but with overlapped rhinitis	6
				Mastcells + neutrophils	1
				Fungi	1
	11	NO EOSINOPHILS	Neutrophils + bacteria	1	
			Mastcells	2	
			Neutrophils	6	
	6	NORMAL			

Table 1b: Nasal cytology in non-allergic children

No	CYTOLOGY	No	DIAGNOSIS
23 NON ALLERGIC CHILDREN	Only eosinophils	20	NARES
	Eosinophils+ mastcells	2	NARESMA
	Normal	1	

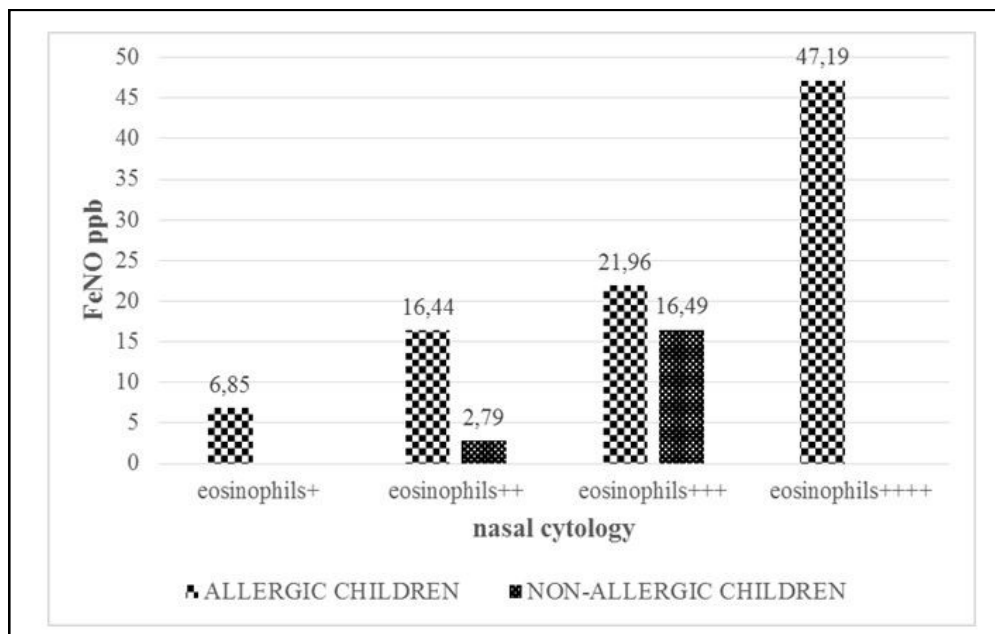


Figure 1 FeNO in patients with nasal eosinophilia:

Eosinophils +++++: FeNO 47,19ppb allergic children; eosinophils +++: FeNO 21,96ppb allergic and 16,49ppb non allergic; eosinophils ++: FeNO 16,44ppb allergic, 2,79ppb non allergic; eosinophils +: FeNO 6,85ppb allergic children.

subjects only with eosinophilia or mastcells than in patients with other kinds of NAR.

Our research confirmed the role played by eosiniphilia which is able to induce the NO- synthase and the direct correlation between pulmonary and nasal inflammation, [10]: when nasal eosinophils grow, the fraction of exhaled nitric increases as well (Fig. 1).

Conclusion :

According to our experience and to other studies, nasal cytology is a useful, simple and safe technique. Thanks to its non-invasiveness allowing repetition it can be used especially among children, and it is also well accepted by parents who more and more often ask for specific diagnosis and rational approaches.

It can provided an important contribution to identification of new pathological entities such as non-allergic rhinitis (NAR 17%), which are underdiagnosed.

Its association with FeNO is useful in the prevention and management of all allergic and non-allergic respiratory diseases in order to improve patients' quality of life.

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