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Allergic and nonallergic rhinitis in children: The role of nasal cytology

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ABSTRACT

Nasal cytology is a diagnostic tool currently used in rhinology to study either allergic and vasomotor rhinological disorders or infectious and inflammatory rhinitis. Over the past few years nasal cytology has been rarely used in pediatrics, nevertheless its clinical and scientific applications seem to be very promising. The advantages of this technique are different: the ease of performance, the noninvasiveness allowing repetition and the low cost. We evaluated 100 children, from 2 to 15 years old, referred to our outpatient service for allergic children for suspected allergic rhinitis (AR). After skin prick test (SPT) or Radio Allergo Sorbent Test (RAST), 59/100 subjects were classified as affected by AR, while 8 children refused to be tested. According to ARIA guidelines, the 59 children with AR (4 - 15 years old) were divided in 56 with persistent AR and 3 with an intermittent form. Nine out of 59 children with AR had a significant number of neutrophils and eosinophils at the nasal cytology, documenting the presence of "minimal persistent inflammation". Eleven out of 59 AR patients showed a positive swab for bacteria. Children with nonallergic rhinitis (NAR) were 33/100 (2 - 15 years old). After nasal cytology, 17/33 children were classified as NARES (nonallergic rhinitis with eosinophils), including one X-linked agammaglobulinemia (XLA) child, 1/33 as NARESMA (nonallergic rhinitis with eosinophils and mast cell) and another 1/33 as NARMA (nonallergic rhinitis with mast cell). In conclusion, nasal cytology allowed us to correctly classify children with NAR and to better assess the condition of children with AR.

Keywords: Allergic Rhinitis; Children; Nasal Cytology; Nonallergic Rhinitis; X-Linked Agammaglobulinemia

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1. INTRODUCTION

Nasal cytology is a diagnostic tool currently used in rhinology to study either allergic and vasomotor rhinological disorders or infectious and inflammatory rhinitis [1-3].

The rationale of this method is based on the knowledge that the nasal mucosa of healthy individuals is constituted by four cytotypes (ciliata, mucipara, striata and basalis) and does not show other cells except, rarely, neutrophils and, very rarely, bacteria. So, the detection of eosinophils, mast cells, bacteria and fungal hyphae is a sign of a possible pathology [4].

Since it can detect cellular changes of epithelium exposed to physico-chemical inflammation [5,6], acute or chronic infections of different etiology (viral, bacterial, fungal or parasitic) [7], it has been a subject of clinical and scientific interest for the past decades [4,8]. In particular it provided an important contribution to the definition and understanding of the pathophysiologic mechanism of allergic and nonallergic rhinitis and to the identification of new pathological entities [9], such as the nonallergic rhinitis (NAR) with eosinophilia (nonallergic rhinitis with eosinophils, NARES), with mast cells (nonallergic rhinitis with mast cell, NARMA), neutrophilic forms (nonallergic rhinitis with neutrophils, NARNE) and, finally, the eosinophil-mast cells (nonallergic rhinitis with eosinophils and mast cell, NARESMA) [10,11].

There are still few reports on nasal cytology in pediatric population [7,12] and most of them are quite historical [3,4].

Samples can be obtained by biopsy but nasal biopsies are hardly feasible as a routine method and the caregivers may not agree [13].

Today the material can be collected without any traumatic intervention on the child and this technique (scraping and swab sampling) has opened a diagnostic path.

Considering an allergic child with seasonal or persis-

tent allergy we usually test him for the presence of specific IgE (skin prick test and/or Radio Allergo Sorbent Test), but this diagnosis of "allergic rhinitis" is an indirect one, inferred by the IgE positivity and by his medical history.

The microscopy examination of the inferior turbinate cells can directly document the allergic etiology (presence of eosinophils) and furthermore can show the presence of microbes and neutrophils.

These findings, which are not unexpected in allergic children that are prone to infections, allow us to tailor our treatment by adding antibiotics to the antiallergic drugs, usually nasal steroids. Local steroids, controlling the allergic inflammation, may favour infections due to their immunosuppressing effect and create a vicious circle.

The possibility to directly visualize what is going on can add information about the pathophysiology of the disturbance and it is therefore very important and helpful for an effective treatment.

This is particularly significant in preschool children where specific IgE are difficult to document as well as upper respiratory tract infections are very common.

2. MATERIALS AND METHODS

Referring to our outpatient service for allergic children for suspected allergic rhinitis (AR), 100 children (2 to 15 years old) were evaluated: 58 males and 42 females. Children with associated severe Asthma were excluded from the study.

We collect the medical history by a questionnaire (Appendix 1) in order to investigate presence of rhinitis, quality and recurrence of episodes. The child, with his parents' help, had to assign a score ranging from 0 to 10, depending on the intensity of each subjective symptom (sneezing, itching, eye symptoms, nasal obstruction, oral breathing, headache, nocturnal snoring, olfactory deficits and asthma).

Each child underwent to ear nose throat (ENT) evaluation, nasal cytology, skin prick test (SPT) or Radio Allergo Sorbent Test (RAST). We tested a panel of allergens: as pollens, velvet grass (Holcus lanatus), Bermuda grass (Cynodon dactylon), short ragweed (Ambrosia sp.), lichwort (Parietaria officinalis), olive tree (Olea europea), birch tree (Betula verrucosa), nut tree (Corylus avellana), depending on the allergen exposure of the area; we considered dust mites (Dermatophagoides pteronyssinus and Dermatophagoides farinae); as animals, dog (Canis familiaris) and cat (Felix domesticus); as mould Alternaria alternata; as food allergens, we tested α-lactalbumin, lactocasein, egg white and yolk, and peanuts (Arachis hypogaea).

According to the positivity or negativity of the SPT and/or RAST, individuals were divided into AR and NAR groups, respectively. AR group was further subdivided into "intermittent" and "persistent" disease according to ARIA guidelines [14]; we also correlated symptoms to perennial or seasonal allergens. NAR group was subdivided, according to the nasal smear cytologic result, into NARES, NARESMA, NARMA and idiopathic rhinitis.

Nasal cytology was performed being free of medications since at least 1 week, except for 10 children under sublingual immunotherapy (SLIT). Scrapings were collected from the inferior turbinate under careful vision in anterior rhinoscopy by means of a nasal speculum and good illumination. The material was transferred on a glass slide, air-dried and then stained by the May-Grunwald-Giemsa method. Observation was performed by an optical microscope at 1000× magnification. We divided the slide into 10 fields to detect neutrophils, eosinophils, mast cells and lymphocytes. The cell count was expressed, per each type, as percentage of total leucocytes.

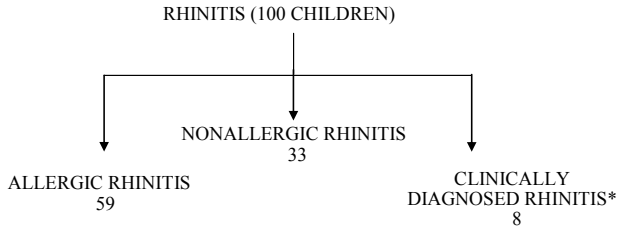
3. RESULTS

We evaluated 100 children, referred to our outpatient service for allergic diseases for suspected AR.

According to the correlation between medical history, physical examination and SPT and/or RAST positivity, 59/100 subjects were classified as affected by AR, while 33/100 children were SPT and/or RAST negative and were classified as NAR. We had one child affected by X-linked agammaglobulinemia (XLA), whose SPT were negative and who suffered of recurrent rhinitis. We performed nasal cytology to identify the nature of his nasal symptoms.

Eight out of 100 children could not be better defined, because their parents refused the SPT and/or RAST, so they had a clinical diagnosis of rhinitis (**Figure 1**).

According to ARIA guidelines, the 59 children with AR (age-range 4 - 15 years old) were divided in 56 with persistent AR and 3 with an intermittent form: 26/56 children had mostly seasonal symptoms associated to the prevailing allergy to grass pollens, while 30/56 children



*Parents refused allergic test *in vivo* or *in vitro* for their child.

Figure 1. Diagnosis of rhinitis based on clinic and skin prick test or RAST.

were allergic to housedust mites and molds and showed perennial symptoms. Seventeen out of 59 children were monosensitized to perennial allergens, 12/59 were sensitized to grass pollens and 2/59 children were allergic to Betula V. and Corylus A. The remaining 28/59 children were polyallergic patients. Ten out of 59 children with AR were under SLIT: 5 for perennial allergens and 5 for grass pollens.

Children with NAR were 33/100 (from 2 to 15 years old).

The results of the nasal smear of the 59 allergic children are reported in **Table 1**.

Eosinophils were found in 38/59 patients with AR; 17 patients out of these 38 had also at the rhinoscopy hypertrophic and pale inferior turbinates, pathognomonic sign of AR. Nasal cytology showed neutrophils and eosinophils in 11/59 children with AR; 9/11 were allergic to perennial allergens, although they were asymptomatic at time of evaluation. This result is coherent to the "minimal persistent inflammation" theory [7]. In 6/38 allergic children we found also bacteria at the rhinocytogram exam, so we could add antibiotic treatment. In 21 smears of AR patients eosinophils were not present: 16 children had a normal cytology, because we did the exam out of the allergic season, whereas 4 of them had few neutrophils, but still in the normal range. Five subjects had normal cytology but bacteria were present in the smear suggesting an associated infection.

The nasal cytogram of the children under SLIT showed eosinophilia in the 5 housedust mites allergic patients, while 4 smears of grass pollens allergic children were normal, although the exam was done during the spring season (April and June).

The swab results of the 33 non allergic children are shown in **Table 2**.

The nasal cytogram of 17/33 children with NAR showed eosinophilia with a persistent disease, so we could make diagnosis of NARES. One of these patients was the XLA child. Six of these 17 children at ENT examination showed hypertrophic and pale inferior turbinates. NARESMA was diagnosed in 1/33 patients and in another 1/33 NARMA was documented.

The **Table 3** shows the results of the 8 SPT/RAST not tested patients.

One child had several neutrophils in the smear, indicating an infectious rhinitis. Seven with eosinophils could not be classified as AR or NARES because they were not tested for type 1 allergy. Nevertheless one child had a good response to anthistaminic.

The summary of the final results in our patients after SPT and/or RAST and nasal cytology is reported in **Figure 2**.

Table 1. Nasal cytology in allergic rhinitis.

No.		No.		No.	CYTOLOGY
59	AR	38	Eosinophils	21	Only eosinophils
				11	Eosinophils + neutrophils
				6	Eosinophils + neutrophils + bacteria
		21	No eosinophils	16	Normal cytology
				5	Normal cytology + bacteria

4. DISCUSSION

While nasal cytology has proved to be very effective in adult with rhinosinusitis [5-7], it is rarely used in children. It is thus difficult to compare our results with scientific references because of the lack of previous studies. The sample group analyzed is one of the most numerous groups among those reported in literature [7,12,15].

We must stress that this technique should not be used routinely, but it is very helpful and informative when treating children in which allergic tests and/or history are not concordant. One of this is the XLA child, who is prone to bacterial infections in the ENT district as first reported by Bruton in 1952 [16]. The swab showed an unexpected prevalence of eosinophils.

XLA is a primary immunodeficiency characterized by the lack of immunoglobulin, B cells, and plasma cells, secondary to mutation in Bruton's tyrosine kinase (Btk) gene. We expected to find an infectious rhinitis, but the

Table 2. Nasal cytology in nonallergic rhinitis.

No.		No.	CYTOLOGY	DIAGNOSIS
33	NAR	17	Eosinophils	NARES
		1	Eosinophils + mastcells	NARESMA
		1	Neutrophils + mastcells	NARMA
		14	Normal cytology	IDIOPATHIC

Table 3. Nasal cytology in not tested rhinitis.

No.		No.	CYTOLOGY	DIAGNOSIS
8	NOT TESTED	7	Eosinophils	AR or NARES
		1	Neutrophils	INFECTIOUS RHINITIS

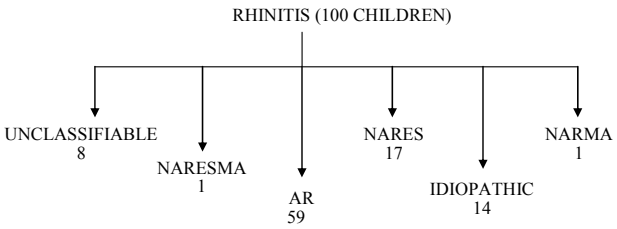


Figure 2. Rhinitis classification after nasal cytology.

nasal smear showed an eosinophilic infiltration allowing us the diagnosis of NARES. This indicates that the treatment with systemic antibiotic was not able to control the recurrent rhinosinusitis because of these allergic cells [11] and nasal steroids improved the situation.

This observation is in favor of the specificity of the NARES diagnosis, which should not be considered as due to allergy towards an unknown allergen, but a true novel entity.

The nasal cytogram helped us in the diagnosis of AR.

In 9/59 asymptomatic children with AR to perennial allergens we expected to see a normal exam, but we found neutrophils and eosinophils, documenting the presence of "minimal persistent inflammation" [7].

The nasal cytogram of 38/59 children with AR (symptoms and positivity to SPT and/or RAST) showed eosinophilia, confirming the isolated allergic form in these patients. In 21/59 allergic patients the nasal smear was normal. This confirmed the effectiveness of the treatment, in particular in those under SLIT. On the other hand, a significant proportion of allergic children (11/59) showed also bacteria. In the absence of this result an ineffectiveness of the antiallergic therapy would have been suspected and it would not have been added the correct therapy with antibiotic.

Using nasal cytology we could identify cellular rhinitis (17 NARES, 1 NARESMA and 1 NARMA) in our group of patients, who without this exam would have remained with no specific diagnosis and treatment.

Despite our intent was to design nasal cytology just for allergic patients, it allowed us a specific diagnosis even in nonallergic ones.

5. CONCLUSIONS

Nasal cytology is useful both from the pathophysiological and clinical point of view to better understand the disease and to follow especially children in which allergic test and/or history are not concordant.

The advantages of nasal cytology are different: the ease of performance, the noninvasiveness allowing repetition and the low cost.

It is useful to follow the disease during medical treatment by periodic cytologic controls, showing, for example, a significant reduction of inflammatory cells or the disappearance of bacteria.

When considering an allergic child with SPT positivity, the etiology of an existent rhinitis could not be assumed to be certainly allergic: only the nasal cytology can directly confirm such etiology by showing the presence of an eosinophilic infiltrate. On the other side, the allergic child is prone to long lasting bacterial infection and even in this case the nasal cytology can show the existence of a secondary bacterial infection (neutrophilic infiltrate ± bacteria).

The effectiveness of the SLIT could be documented by this technique allowing us to show the disappearance of the eosinophilic infiltrate. The compliance to the SLIT could also be assessed by nasal cytology.

It has provided an important contributution to identification of new pathological entities, such as the nonallergic eosinophilic rhinitis (NARES) or mast cell mediated nasal inflammation (NARESMA).

Despite the proven usefulness of nasal cytology, we suggest to use this technique not routinely, but mainly for selected patients or for scientific survey.

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APPENDIX 1

NASAL CYTOLOGY

PATIENT CARD N:
DATE:
SURNAME:
NAME:

Family and Medical History:
Physiological anamnesis/breastfeeding?
Familiarity:
Previous surgery ENT:
Medical therapy:
Associated diseases:
Current medical history (predominant symptom/onset/ recurrence/circadian pattern/triggers/asthenia? irritability?)

Symptoms:

Rhinorrhea:	NO	ES	serous mucous mucopurulent intensity: 1→10	intermittent persistent
Sneezing		NO	YES	(……)
Itching		NO	YES	(……)
Eye symptoms		NO	YES	(……)
Nasal obstruction		NO	YES	(……)
Oral breathing		NO	YES	(……)
Headache		NO	YES	(……)
Nocturnal snoring		NO	YES	(……)
Olfactory deficits		NO	YES	(……)
Asthma		NO	YES	(……)

Clinic Examination:
Oropharynx:
Inferior turbinates: (hypertrophic?/pale?)
Tympanic membranes:

Exams:
Skin prick test:
RAST:
Spirometry:

Nasal Cytology:

Other tests

DIAGNOSIS:

THERAPY:

