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Exhaled nitric oxide in children with allergic rhinitis: a potential biomarker of asthma development.

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To the Editor,

Allergic rhinitis (AR) and asthma share a common pathogenic pathway, characterized by dys-regulation of immune response to allergen and airway inflammation. Allergic patients have an

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allergen-specific defect of T regulatory cells, promoting T helper (Th)-2 polarization. Expansion of Th2, stimulated by allergen exposure, induces increased production of Th2-dependent cytokines, including Interleukin (IL)-4, IL-5, and IL-13. Th2 cytokines are responsible of the two main immunologic features of allergic reaction: allergen-specific IgE synthesis and mucosal inflammation. Actually, allergen exposure causes mast cell activation followed by a cascade of inflammatory events that culminate in the typical phenomenon of tissue eosinophilia. Hence, airways eosinophilic inflammation is a common pathogenic pathway for both AR and allergic asthma. Really, it is possible to evaluate bronchial inflammation by measuring a surrogate biomarker, such as the fractional concentration of exhaled nitric oxide (FeNO) (1). There is evidence that the FeNO levels well correlate with peripheral or bronchial eosinophilia (2).

On the other hand, AR is considered to be a relevant risk factor for the development of asthma in children (3,4). In fact, it has been prospectively observed the existence of an allergic march starting from atopic dermatitis and progressively evolving towards AR and asthma (5).

However, a specific biomarker to precociously identify AR children at higher risk of developing asthma has not yet been identified. Therefore, the aim of this follow-up study was to investigate whether the levels of exhaled nitric oxide (FeNO) in children with AR alone could predict asthma onset 5 years later.

A prospective study was performed, enrolling one hundred nine paediatric patients (53 males, 56 females, mean age 8.4 years, age range 7-13) suffering from AR.

Inclusion criteria were: age range between 6 and 14 years, documented and validated diagnosis of AR, and written informed consent signed by parents. Exclusion criteria were, absence of sensitization, history of asthma symptoms, impaired lung function, previous or current specific immunotherapy, and chronic diseases.

The review board approved the study. Patients were treated with medications alone on demand.

All patients were evaluated at baseline and after 5 years of follow-up, performing the following assessments: skin prick test, spirometry, and FeNO measurement.

AR diagnosis was performed on the consistency between nasal symptoms history and sensitization.

Asthma diagnosis was performed on asthma symptoms history, impaired lung function, and functional testing (mainly bronchodilation).

Spirometry was performed by using a computer-assisted spirometer (Pulmolab 435-spiro 235, Morgan, England– predictive values ECCS 1993), with optoelectronic whirl flow meter. It was performed and interpreted as stated by the European Respiratory Society (ERS) guidelines (6).

FeNO was measured with a chemiluminescence analyzer (NiOX MINO®, Aerocrine, Solna, Sweden); the detection limit of the apparatus was 1–5 parts per billion (ppb), as required by American Thoracic Society/ERS guidelines (1), with a resolution of 1 ppb. The analyzer was calibrated daily, using a certified NO mixture. Children inhaled to total lung capacity from NO-free air and exhaled a single breath (without nose clip) through a mouthpiece at a mouth pressure of 45 cm water and at an expiratory flow of 50 ml/s. Mouth pressure was displayed on a computer screen to maintain a steady flow. The measurement was rejected if a stable flow was not maintained for at least 6 second of exhalation. NO was measured at the plateau and expressed in ppb. Normal values were considered if < 20 ppb; borderline ranging between 20 and 35 ppb; pathological values > 35 ppb.

The analysis was performed using parametric methods (ANOVA) and non-parametric ones (test U-Mann-Whitney and Wilcoxon). Contingency tables were evaluated by χ^2 test with Yates correction and by McNemar test. Logistic analysis assessed the risk of asthma onset considering FeNO values as independent variable, considering 10 ppb intervals.

At baseline, all patients showed sensitization for aeroallergens: 62 (57.8%) for house dust mites (HDM), 38 (34.8%) for grass pollens, and 22 (20.2%) for tree pollens; 11 patients were polysensitized

(3 patients sensitized to HDM, tree and grass pollens, 2 patients to grass and tree pollens, 4 patients to HDMs and grass pollens, and 2 patients to tree pollens and HDM).

Lung function was normal in all patients according to the guidelines, with normal values of FVC (98 ± 4.7 % of predicted), FEV₁ (97.1 ± 5 % of predicted) and PEF (97.7 ± 5.1 % of predicted), as reported in Figure 1.

About FeNO measurement, 48 patients had normal values (8.6 ± 4.6), 34 had borderline values (28.1 ± 3.4), and 27 had high FeNO levels (74.9 ± 35.0). Thus, patients were subdivided in 3 sub-groups: normal FeNO, such as <20 ppb (Group 1), borderline FeNO, such as between 20 and 35 ppb (Group 2), and high FeNO, such as > 35 ppb (Group 3).

After 5 years, 21 children developed asthma: 16 intermittent and 5 mild persistent. All of them belonged to Group 3, whereas no patient of Group 1 or Group 2 developed asthma (Figure 2).

Sensitization re-evaluation showed the presence of 43 new sensitizations in 35 patients (20 for grass pollens, 2 for HDM, and 21 for tree pollens).

Lung function continued to be within normal ranges in all patients. However, the 3 sub-groups showed significant differences both concerning FEV₁ and FVC, as reported in Figure 1. In detail, FEV₁ significantly increased in Group 1 and 2 ($p<0.01$ for both), whereas significantly ($p<0.05$) diminished in Group 3. Similarly, FVC significantly increased in Group 1 and 2 ($p<0.01$ for both), whereas significantly ($p<0.05$) decreased in Group 3.

About FeNO measurement, FeNO values were 10.3 ± 7.6 in Group 1; 25.9 ± 4.4 in Group 2; $54.3 \pm$

5.7 in Group 3. There were no significant intra-group variations of FeNO values into the 3 sub-groups.

However, only children with high baseline FeNO values, such as Group 3, developed asthma, documented by asthma symptom occurrence (i.e. dyspnoea, wheezing, and cough after respiratory

infection and/or exercise and exposure to allergen) and lung function assessment, mainly concerning reversibility.

There was no significant relationship between FeNO values and FEV₁ ($r=0.17$); FVC ($r=0.11$); and Δ FEV₁ after bronchodilation test ($r=0.13$).

A logistic regression model was performed to evaluate the role of FeNO as independent variable in relationship with asthma onset. High FeNO values significantly and fairly predicted asthma onset (Odds Ratio: 2.49; C.I. 95%: 166.5 + 372; $p<0.01$).

Asthma and allergic rhinitis share airways eosinophilic inflammation (7). The FeNO measurement may be reasonably considered a surrogate biomarker of airway eosinophilic inflammation, mainly in allergic patients (2). Moreover, nasal inflammation is closely associated with bronchial airflow limitation, as well as it has been demonstrated that nasal eosinophil count was strongly related to eosinophil sputum (8). Moreover, FeNO measurement is a simple and available tool to assess bronchial inflammation.

On the other hand, AR frequently precedes asthma onset insomuch as the term asthma march has been fashioned to define the concept of progression from AR towards asthma (9). Therefore, this study aimed at investigating whether FeNO may predict asthma onset in children with allergic rhinitis alone.

Firstly, this study demonstrated that about 20% of AR children developed asthma within 5 years, confirming previous studies that underlined the role of AR as risk factor for asthma onset (5).

Secondly, FeNO can be considered a reliable predictor for asthma onset as only children with high FeNO values developed asthma. Particularly, the risk of having asthma in a close future is rather high. In fact, every 10ppb increase (over the cut-off of 35) corresponds to have a more than doubled risk (OR 2.4) of developing asthma. This finding has a clinical relevance as underlines the role of bronchial eosinophilic inflammation as a possible marker of bronchial involvement in AR patients. In addition, This article is protected by copyright. All rights reserved.

the present study confirms a previous trial that demonstrated FeNO as reliable predictor of bronchial reversibility in children with allergic rhinitis alone (9,10). In fact, it has been reported that the presence of > 34 ppb FeNO values represented a relevant risk (ORAdj 1.9) for having a positive response to bronchodilation testing. In fact, reversibility may be considered the pathognomonic functional determinant for asthma diagnosis.

All these data underline the close link among allergic inflammation, AR, and asthma. Thus, this study might suggest that a FeNO value > 34 ppb, such as pathological, may predict future asthma onset with good reliability in AR children.

Therefore, all these previous studies were consistent with the present report, confirming the role of FeNO as marker of bronchial impairment. On the contrary, lung function assessment was not able to predict asthma onset in all children, also those with high FeNO values. This fact highlights the possible capability of FeNO as potential candidate of predictive marker for asthma onset in AR patients.

However, this study has a main limitation, such as the restricted number of children, who were enrolled.

In conclusion, this study provides a preliminary evidence that FeNO may predict with fair capability the risk of asthma onset in children with allergic rhinitis alone. Therefore, a simple FeNO measurement could suggest relevant information about possible bronchial involvement in AR.

Signature

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References

- 1) Dweik RA, Boggs PB, Erzurum SC, et al. American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. *Am J Respir Crit Care Med*. 2011;184:602-15.
- 2) Roadway GW, Chou JY, Hoffman LA, Sethi JM. Exhaled nitric oxide in the diagnosis and management of asthma: clinical implications. *Chron Respir Dis* 2009; 6:19-29
- 3) Esteban CA, Klein RB, Kopel SJ, McQuaid EL, Fritz GK, Seifer R, et al. Underdiagnosed and Undertreated Allergic Rhinitis in Urban School-Aged Children with Asthma. *Pediatr Allergy Immunol Pulmonol*. 2014;27:75-81
- 4) Deliu M, Belgrave D, Simpson A, Murray CS, Kerry G, Custovic A. Impact of rhinitis on asthma severity in school-age children. *Allergy*. 2014 Jun 24. doi: 10.1111/all.12467
- 5) Shaker M. New insights into the allergic march. *Curr Opin Pediatr*. 2014 May 30. [Epub ahead of print]
- 6) Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. *Eur Respir J* 2005;26:948-68
- 7) Amorim MM, Araruna A, Caetano LB, Cruz AC, Santoro LL, Fernandes LG. Nasal eosinophilia: an indicator of eosinophilic inflammation in asthma. *Clin Exp Allergy* 2010; 40:867-74.
- 8) Ciprandi G, Signori A, Tosca MA, Cirillo I. Spirometric abnormalities in patients with allergic rhinitis: indicator of an asthma march? *Amer J Rhinol Allergy* 2011;32:22-8
- 9) Ciprandi G, Tosca MA, Capasso M. High exhaled nitric oxide levels may predict bronchial reversibility in allergic children with asthma or rhinitis. *J Asthma* 2013;50:33-8
- 10) Colon-Semidey AJ, Marshik P, Crowley M, Katz R, Kelly HW. Correlation between reversibility of airway obstruction and exhaled nitric oxide levels in children with stable bronchial asthma. *Pediatr Pulmonol*. 2000;30:385-92.

Figures Legends

Figure 1: Upper Panel= FEV₁ values in the sub-groups of baseline FeNO levels, at baseline and at the 5-year follow-up); Lower Panel= FVC values in the sub-groups of baseline FeNO levels, at baseline and at the 5-year follow-up). * p<0.05; ** p<0.01.

Figure 2: Number of patients with asthma within 5 years in the 3 sub-groups.



