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Original Article

Fraction of Exhaled Nitric Oxide and Asthma Predictive Index in Infants Less Than Two Years-Old

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A B S T R A C T

Introduction: The fraction of exhaled nitric oxide (FE_{NO}) is considered an indirect marker of eosinophilic inflammation of the airway. In cooperative children, the usual method is a single breath. The impossibility of performing this in uncooperative children has led to the development of the online and offline tidal breathing technique. The objective of the study has been to analyse the relationship between the multiple-breath online FE_{NO} and the asthma predictive index (API) in children under the age of two.
Material and methods: An observational and cross-sectional study on a consecutive sample of boys and girls between 2 months and 2 years of age, over a period of 4 months. The post-prandial multiple breaths online FE_{NO} and flow spirometry between 40 and 60 ml/s, using a stationary chemiluminescence analyser (CLD 88 sp). The quantitative variables were: age, weight, IgE, eosinophilia, FE_{NO}, flow spirometry. The qualitative variables were: gender, atopic dermatitis, allergic rhinitis, food and medical allergies, family history of asthma and atopy, diagnosis and treatment. The relationship between API and FE_{NO} was analysed using the exact Fisher and Student t tests and the level of agreement between API and FE_{NO} using Cohen's Kappa. The relationship between eosinophilia, IgE, atopic dermatitis and FE_{NO} was also studied (exact Fisher and Student t tests).
Results: The cohort consisted of 38 patients. The determinations were successfully carried out on 32 (84.21) of the cases. The mean age was 10.9 ± 5.06 months. The cases with a positive API had significantly higher FE_{NO} values than those with a negative API, with a level of agreement between API and FE_{NO} of 0.71.
Conclusions: There is a significant relationship and a good level of agreement between the online tidal breathing FE_{NO} and the API.

Óxido nítrico exhalado e índice predictivo de asma en menores de dos años

R E S U M E N

Introducción: La fracción exhalada del óxido nítrico (FE_{NO}) se considera marcador indirecto de la inflamación eosinofílica de la vía aérea. En niños colaboradores la metodología habitual es mediante respiración única. La imposibilidad de realizarla en niños no colaboradores ha permitido desarrollar la técnica a respiración corriente *on-line* y *off-line*. El objetivo del estudio ha sido analizar la relación entre la FE_{NO} *on-line* a respiraciones múltiples y el índice predictivo de asma (IPA) en niños menores de dos años.
Material y métodos: Estudio observacional y transversal en una muestra consecutiva de niños y niñas entre dos meses y dos años de edad, durante un período de 4 meses. Se determinó la FE_{NO} postprandial *on-line* a respiración corriente con respiraciones múltiples y flujo espiratorio entre 40 y 60ml/s, mediante analizador de quimioluminiscencia estacionario (CLD 88 sp). Variables cuantitativas: edad, peso, IgE, eosinofilia, FE_{NO}, flujo espiratorio. Variables cualitativas: sexo, dermatitis atópica, rinitis alérgica, alergia alimentaria y medicamentosa, antecedentes familiares de asma y atopia, diagnóstico y tratamiento. Se ha analizado la asociación entre IPA y FE_{NO} mediante test exacto de Fisher y t de Student y el grado de acuerdo entre IPA y FE_{NO}

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mediante Kappa de Cohen. Se ha estudiado la relación entre eosinofilia, IgE, dermatitis atópica y FE_{NO} (test exacto de Fisher y *t* de Student).

Resultados: Cohorte constituida por 38 pacientes. Realizaron las determinaciones con éxito 32 (84,21%) casos. Edad media $10,9 \pm 5,06$ meses. Los casos con IPA positivo tenían valores de FE_{NO} significativamente superiores a los IPA negativos con grado de acuerdo entre IPA y FE_{NO} de 0,71.

Conclusiones: Existe asociación significativa y un buen grado de acuerdo entre la FE_{NO} a respiración corriente *on-line* y el IPA.

Introduction

In the last few years, several studies have established the utility of the fraction of exhaled nitric oxide (FE_{NO}) as a subrogated marker of eosinophilic inflammation of the airways.[1] A increase in FE_{NO} has been reported in asthmatic children as well as in close association with eosinophilic inflammation measured in the airway mucosa of these patients.[2] Given its high discriminative capacity, it has been used as a complement of lung function in the diagnosis of asthma.[3] Likewise, it had been used to monitor treatment with inhaled glucocorticoids due to its high sensitivity for detecting changes in response to the dosage received[4] and to detect a deterioration in the lung function even with the absence of respiratory symptoms.[5]

In cooperative children, it is a single-breath technique with maintained exhalation for 6 to 10 seconds. In uncooperative children, especially in those under the age of three, the measurement of FE_{NO} can be done without sedation, at tidal breathing, with multiple breaths (MB), using on-line and off-line systems analyses in accordance with the recommendations published by the *European Respiratory Society* (ERS) and the *American Thoracic Society* (ATS) in 2005.[6] This methodology is complemented with encouragement to achieve expiratory flows near 50 ml/s. If this is not possible, sedation can be used or the determination can be done during postprandial sleep.

Although it is not a rigorously standardized technique, some researchers[7,8] have documented the possibility of taking valid on-line measurements with MB and constant flow (40-60 ml/s), using continuous adjustment of the expiratory resistances during exhalation, through automatic flow restrictors. Other authors have used manual flow restriction.[9] Daniel et al[10,11] have determined FE_{NO} at MB using a face mask, both on-line as well as off-line, and have published normal values in children between the ages of 2 and 7 with said methodology. The data obtained with the on-line system in small children showed good correlation with those obtained with the on-line single-breath method in older children. Thus, it has been proposed to use FE_{NO} as a non-invasive parameter in the diagnosis, control of severity and activity of the disease in small children with episodes of recurrent wheezing and asthma.[12,13]

Currently, there are no sensitive and specific biological markers that allow us to catalogue infants with recurring wheezing as future atopic asthmatics. In order to identify this group of infants, Castro-Rodríguez et al[14,15] proposed the Asthma Predictive Index (API). Those infants with more than three episodes of wheezing or obstructive bronchitis a year for the first three years of their lives, who also met one major criterion or two minor criteria, were called positive API. Said infants have a sensitivity of 16%, specificity of 97%, positive predictive value of 77% and a negative predictive value of 68%, for developing asthma. In addition, the infants with positive API had seven times more risk for being asthmatics at school-age than those with a negative index (OR = 7.1; 95% CI: 3.5-14.1). Given the difficulty for diagnosing allergic rhinitis in small children, Guilbert et al[16] modified the API, adding the sensitization to one or more pneumoallergens as a major criterion, and food allergies to egg, milk or nuts as a minor criterion.

The objective of this study was to study the relationship between FE_{NO}, determined *on-line* with multiple breaths, and the Asthma Predictive Index by Guilbert.[16]

Patients and Methods

Our is an observational, cross-sectional study of a consecutive sample of patients between two months and two years of age who visited the Pediatric Pulmonology outpatient consultations for the first time from October 2008 to February 2009.

In all cases, postprandial determination of FE_{NO} was carried out with an on-line system and stationary chemiluminescence analyzer, CLD 88 sp (Eco Physics AG), at tidal breathing with MB. A facial mask was used to separate the airflow coming from the oral and nasal cavities (Hans Rudolph Inc, USA®), with an expiratory flow between 40 and 60 ml/s.[6] The facial mask was connected to a disposable antibacterial / antiviral filter. For each determination, mean FE_{NO} was obtained during the respiratory cycles (inspiration-expiration) performed by the patient for 60 seconds. FE_{NO} was measured at the end of the respiratory cycle, in the stable plateau phase when the expiration of 60-80% of the total volume had been produced. All the patients included performed three valid determinations with a variability ratio of less than 10% amongst them, and the mean was obtained from the three. The range of the determinations oscillated between 0.1-5,000 ppb (parts per billion). The determinations were carried out with NO-free room air (< 5 ppb) to avoid environmental contamination. Daily calibration of flow and volume verified the exactness of these measurements, as did an adjustment of NO to zero. Likewise, the NO gas was calibrated monthly. In accordance with published studies[7,10] normal values of FE_{NO} were considered to be between 2 and 8 ppb.

The diagnosis of recurrent wheezing, asthma and its severity were done in accordance with the criteria established by GINA.[17]

We defined:

1. Allergic rhinitis as compatible sign and symptom, specific IgE in blood (class III or higher) to one or more aeroallergens and or positive prick test;
2. Sensitization to one or more aeroallergens by specific IgE in blood and/or positive prick test;
3. Food allergies as signs and symptoms compatible with specific IgE in blood (class III or higher); and
4. Atopic dermatitis, compatible sign and symptom.[17,18]

The API modified by Guilbert et al[16] was used. Infants were considered API-positive if they had more than three episodes of wheezing or obstructive bronchitis a year during the first three years of life, while meeting a major criterion or two minor criteria. The major criteria were medical diagnosis of asthma in one of the parents, medical diagnosis of atopic dermatitis in the first three

Table 1
Descriptive characteristics of the study population

Mean FE_{NO} (ppb) (median ± SD)	11.11 ± 9.38
Median IgE (KU/L) (range)	7.09 (1-877)
Eosinophilia in peripheral blood (μL)	
Mean ± SD	377.06 ± 302.81
≥ 4% (N) (%)	11 (34.37)
Positive API (N) (%)	18 (56.25)
Atopic dermatitis (N) (%)	11 (34.37)
Food allergy (N) (%)	3 (9.37)
Family history of asthma (N) (%)	9 (28.12)
Family history of atopy (N) (%)	13 (40.62)
Diagnosis	
Recurrent wheezing (> 3 episodes) (N) (%)	13 (40.62)
Episodic asthma (N) (%)	3 (9.37)
Persistent asthma (N) (%)	7 (21.87)
Bronchopulmonary dysplasia (N) (%)	5 (15.62)
No evidence of pathology (N) (%)	4 (12.5%)

KU/L: kilounits per liter; ppb: parts per billion; μL: microliter.

years of life and/or allergic sensitization to one or more pneumoallergens. Likewise, we considered milk, egg or nut food allergy, wheezing unassociated with colds in the first three years of life and/or eosinophilia in peripheral blood ≥ 4% to be minor criteria.

The quantitative variables analyzed have been age, weight, IgE, eosinophilia, mean FE_{NO} value and expiratory flow. The qualitative variables studied have been sex, atopy (atopic dermatitis, allergic rhinitis, food allergy and/or allergy to medications), family history of asthma (medical diagnosis in one of the parents) and atopy (medical diagnosis in one of the parents of atopic dermatitis, food and/or medicine allergy) diagnosis and treatment. We studied the association between API and FE_{NO} by means of Fisher's exact test and Student's t test. The degree of agreement between API and FE_{NO} (categorized according to normal values) was analyzed using Cohen's Kappa (CK). We also analyzed the relationship between eosinophilia, IgE, atopic dermatitis and FE_{NO} by means of Fisher's exact test and Student's t test.

Given the limited validated and reliable information about the methodology and standardization of the technique for the determination of FE_{NO} in infants and its relationship with API that would provide us with a base to estimate the sample size necessary, we made a decision similar to other authors[8,10]: to obtain a sample large enough in statistical terms in order to describe hypothetical associations with a minimum of validity.

In all cases, an alpha level of 5% was established. The SYSTAT 9.0™ statistical package was used.

The study was approved by the hospital's Ethics and Research Committee. In all cases, we obtained the informed consent and the permission of both parents and/or guardians for the scientific exploitation of the data obtained on paper and on the Internet.

Results

Thirty-eight cases were studies, of which 32 (84.21%) successfully performed the determinations with an expiratory flow of 58.6 ± 6.9 ml/s. The cohort analyzed (n = 32) was distributed in 19 (59.37%) males and 13 (40.62%) females. Mean age of the patients included was 10.9 ± 5.06 months. The descriptive characteristics of the study population are reflected in table 1. None of the included subjects met criteria for allergic rhinitis or food allergy. Of those included (n = 32), 24 (75%) had received no treatment and 8 (25%) had received modifying treatment with anti-leukotrienes and/or inhaled

glucocorticoids on some occasion, having been withdrawn at least 4 weeks before the FE_{NO} determination.

There is a significant association between positive API, eosinophilia, IgE and FE_{NO} values higher than normal (p < 0.05). The patients with positive API had significantly higher FE_{NO} values than the API-negatives (16.31 ± 9.36 vs 4.43 ± 3.13) (mean ± SD) (fig. 1). There was substantial agreement between API and FE_{NO} (CK = 0.71). Likewise, the patients with FE_{NO} values higher than normal had total IgE values in peripheral blood significantly higher (75.9 ± 22.2 vs 6.24 ± 8.17) (p < 0.001). There was no significant association between atopic dermatitis and higher-than-normal FE_{NO} (p = 0.26), nor between eosinophilia in peripheral blood equal to or higher than 400 eosinophils/μL) and FE_{NO} (p = 0.19).

Discussion

The determination of FE_{NO} on-line at tidal breathing with multiple breaths was possible in our series in 84.21% of the cohort studied, with a variability coefficient lower than 10%. Likewise, with the methodology used it has been possible to obtain an expiratory flow between 40 and 60 ml/s during postprandial sleep. If we consider exhaled nitric oxide to be an indirect marker of inflammation, this technique offers the possibility to improve the diagnostic approximation in infants and toddlers with recurrent wheezing and for making comparisons between individuals or in the individual given his/her evolution, optimizing the control of the disease and its treatment.[12]

In our sample, there is a significant association and a good degree of agreement between FE_{NO} measured at tidal breathing with multiple breaths and the modified asthma predictive index.[16] In addition, as published by other authors,[12] in the API-positive group the FE_{NO} values obtained in our study were significantly greater than those obtained in the API-negative group.

Some authors have communicated FE_{NO} values higher in children under the age of 4 diagnosed with asthma and also in those with positive IgE for at least one aeroallergen. Nevertheless, they also did not find significant differences between FE_{NO} and eosinophilia in peripheral blood[19,20] as in our study. It is probable, as some publications point out, that in the two first years of life there is hardly any eosinophilic inflammation as it develops after the age of 3 and is more clearly established after the age of 5.[21] Gabriele et al[13] obtained
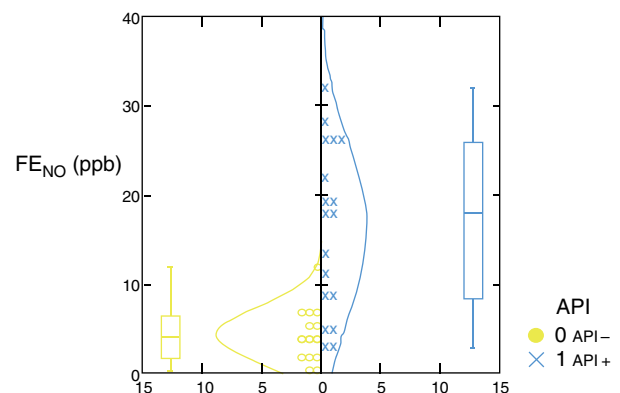

Figure 1. Measurement of the association between the asthma predictive index and the exhaled fraction of nitric oxide by means of Student's t test.
Significant association between positive API and higher-than-normal FE_{NO} (p < 0.05). The patients with positive API (blue) had significantly superior FE_{NO} values than the API-negative patients (yellow) (16.31 ± 9.36 vs 4.43 ± 3.13) (mean ± SD).
API: asthma predictive index; FE_{NO}: exhaled fraction of de nitric oxide; ppb: parts per billion.

higher FE$_{NO}$ values in atopic infants with recurrent wheezing compared with healthy infants or those affected with cystic fibrosis or bronchopulmonary dysplasia. Likewise, Moeller et al[12] found greater FE$_{NO}$ in the group of toddlers with a greater number of recurrent wheezing episodes, and they proposed FE$_{NO}$ as a method to reflect the activity of the disease and to differentiate patient subgroups with or without recurrent wheezing. However, they did not find a cut-point for FE$_{NO}$ that allowed calculating for sensitivity, specificity and predictive values to predict for the development of asthma at later ages in life.

Recently, de Mir et al[22] have reported in a group of children under the age of 4 a slight increase in FE$_{NO}$ in those with recurrent wheezing compared with healthy control subjects, although they found an extensive overlap of values in both groups. Likewise, they communicated a significant association between FE$_{NO}$, eosinophilia in peripheral blood and total IgE.

Regarding API, Goksor et al[23] communicated that the exposure to tobacco smoke, both pre- as well as post-natal, increased the risk for developing asthma between the ages of 17 and 20, with an OR of 3.5 (95% CI: 1.1-11.3) and 3.4 (95% CI: 1.2-10.1), respectively. Also, Piippo-Savalainen et al[24] observed that smoking in the family, and especially maternal smoking, behaved as a major criterion in the asthma predictive index in infants with recurrent wheezing. In our study, smoking in the family was not studied as a qualitative variable, which is why we have not provided this data. Another question is the difficulty to diagnose allergic rhinitis in the age group studied, a variable that is included as a minor criterion in the API by Castro Rodríguez et al.[14] This is the reason why we have used the modified API by Guilber et al,[16] which includes the sensitization to pneumoallergens as a major criterion and food allergies as a minor criterion.

One of the main limitations of our study is that there is currently no adequate standardization of the technique for the determination of FE$_{NO}$ on-line at tidal breathing with MB in non-sedated infants and toddlers, and therefore the values for normality and the results obtained by the different authors depend on the methodology used.[7-9,11,22,25] Another of the study limitations is that it is a limited sample, and therefore a greater number of population studies are needed to corroborate our results.

In our cohort, although the IgE and FE$_{NO}$ follow a practically normal distribution, in addition to Student's t test we carried out the statistical analysis with the Mann-Whitney U test for non-parametric variables, finding no differences in the results obtained.

Currently, there is no single parameter that can predict in infants or toddlers with respiratory symptoms the risk for developing asthma at later ages in growth and development.[26-28] Furthermore, the inflammatory phenotype that infants with recurrent wheezing present can remain, coexist or change throughout the evolution of the disease. Thus, the medical history, physical examination, lung function[29] and some subrogated tests of the inflammatory pattern that the patient presents at a certain moment, such as FE$_{NO}$ determination, can lead to a more accurate diagnosis and a more specific treatment of the recurring pathology of the small airway in infants.[30] The possibility to determine FE$_{NO}$ on-line quickly, innocuously and with guaranteed validity and reproducibility, significantly improves the standard clinical management of the patients with episodes of moderate or severe recurring wheezing in daily clinical practice. This results in more individualized treatment and better control and evaluation of the severity of the symptoms in different phases of the disease.

In summary, we can state that in our sample and with the methodology described, there is a significant association and a good degree of agreement between FE$_{NO}$ and the asthma predictive index.

Likewise, the determination of FE$_{NO}$ on-line has been possible in non-sedated small children with an expiratory flow between 40 and 60 ml/s during postprandial sleep.

Conflict of Interest

The authors declare having no conflict of interest.

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