

THE ROLE OF EXHALED NITRIC OXIDE ASSESSMENT IN CHILDREN WITH BRONCHIAL ASTHMA

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Abstract

Background: Exhaled nitric oxide (eNO) assessment in children with bronchial asthma (BA) is an easy and non-invasive test that provides informations on the presence of bronchial inflammation.

Methods: The study included 110 children aged between 5–18 years with a diagnosis of BA. The patients were assessed clinically (objective examination, questionnaire regarding the disease control) and by medical tests (exhaled breath analysis for nitric oxide, induced sputum, spirometry).

Results: Of the 33 patients with non-atopic BA, 9 (27.27%) presented normal eNO values, and 24 (72.72%) had higher than normal levels. There were 26 patients with controlled BA, 59 with partially controlled BA and 25 with uncontrolled BA, the levels of eNO demonstrating the existence of the statistically significant differences between the mean values of the variables obtained in the studied groups ($p=0.003$). Increased individual values were more frequently noted in patients with an exacerbation of asthma in the last month (70% vs 56.6%), but the differences were not statistically significant. Of the 77 patients with atopic BA, 35 (45.45%) had normal eNO, while 42 (54.54%) had increased values. The normal values of the eNO were most frequently noted in patients undergoing treatment with inhaled glucocorticoids (IGC). Patients with low percent of eosinophils in sputum had increased eNO values ($p=0.03$).

Conclusions: The investigation of the airways by measuring the eNO levels is a useful method of assessing bronchial inflammation in children with bronchial asthma, in order to establish the disease responsiveness and therapy.

Keywords: bronchial asthma, child, inflammation, exhaled nitric oxide

Aims

Chronic bronchial inflammation is the most important pathogenetic link in bronchial asthma (BA). The presence and intensity of bronchial inflammation are greatly correlated with the asthma symptomatology.

Documented evidence of the airways inflammation in BA may be obtained by invasive methods (broncho-alveolar lavage and bronchial mucosa biopsy). In practice non-invasive methods are used in order to evidence bronchial inflammation, such as exhaled nitric oxide (eNO). The studies on the eNO changes in BA have shown that this indicator increases mainly in asthma with an eosinophilic infiltrate at the level of the airways. This finding may have therapeutic implications, as it was noted that the forms with eosinophilic infiltrate responded well to IGC. Therefore higher levels of eNO in a patient with naïve BA may be

predictive for the favourable response to IGC. On the other hand, it was found that during the IGC therapy the eNO decreases in asthmatic patients [1-6].

Our main objective was to study the correlation between asthma symptomatology and eNO. Other objectives included the investigation of the eNO level changes in relation to the sputum cell counts (eosinophilic/non-eosinophilic) and the presence or absence of atopy.

Patients and methods

The study included 110 patients with BA from the 3rd Pediatric Clinical Hospital of Cluj-Napoca, Romania, in the period July 2008–March 2012. The study was performed after obtaining informed consent of the parents, according to the regulations of the Ethics Committee of the Emergency Hospital for Children.

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Inclusion criteria:

- documented diagnosis of BA
- age between 5 – 18 years.

The diagnosis of BA was established based on the classical symptoms and at least one of the following criteria:

- positive bronchial reversibility test (increase of the forced expiratory volume in 1 second (FEV₁) by over 12% after administration of Salbutamol)
- previous hospital admissions for repeated bronchial exacerbations events
- at least two calls to the emergency unit due to asthma attacks.

The children who were in the course of a severe bronchial exacerbation and thus unable to perform the eNO measurement technique properly, were excluded from this study.

In order to measure the NO level in the exhaled breath, a Niox-MiNo Sweden device was used. The patient was asked to take the deepest breath they can and then exhaled a tidal volume during 10 seconds. In the patients unable to maintain expiration for 10 seconds, or those smaller than 135 cm, a special kit for 6 seconds expiration was used. Results were expressed as p.p.b. (parts per billion). For the children under IGC treatment increased eNO levels were considered >20 p.p.b. in those under 12 years old and >25 p.p.b. in those who were 12 or older. For the patients without IGC treatment, the increased values were considered >5 p.p.b.

The presence and intensity of bronchial obstruction were evidenced by functional respiratory tests. We considered bronchial obstruction evidenced by spirometry, the case in which FEV₁ was <80%. The bronchial reversibility test was considered positive if FEV₁ was increased by over 12% after the administration of 200-400 µg Salbutamol.

Patients with atopic BA were those who presented one or more of the following characteristics: association with allergic rhinitis, positive skin tests for one or several allergens, high immunoglobulin E (IgE) titer. For allergy skin tests the prick method was used and the total IgE were determined using the ELISA kit.

In order to obtain the sputum for cellularity analysis, we used an ultrasound device to nebulize sodium chloride solutions in increasing concentrations – 3%, 4%, 5%. We changed the nebulized solutions every 5 minutes. Both before and after saline nebulization, the patients underwent a spirometry and were given 200 µg Salbutamol. Sputum was analyzed microscopically, no longer than 2 hours after harvesting, using Giemsa stain. The eosinophils, neutrophils, macrophages, lymphocytes and bronchial epithelial cells counts were expressed as percentages of the total count of non-squamous cells. An eosinophilic infiltrate of the airways was considered at ≥ 2.5% eosinophils in the induced sputum, while an eosinophil level <2.5% was interpreted as non-eosinophilic inflammation of the airways.

The control level of BA was established based on the Global Initiative for Asthma [7].

Statistical analysis

The data obtained were statistically processed using the SPSS v.15 and Microsoft Excel. We calculated the statistically significant differences between the mean values of the variables between the groups studied. When 3 groups were formed, the Kruskal-Wallis test was used, while for 2 groups we applied the Mann-Whitney test. The statistical significance was established at a p value ≤ 0.05.

Results

The characteristic features of the 110 patients with BA are presented in Table 1.

Table 1. Characteristic features of the patients included in the study

Age (Mean±SD)	10.07±3
Male (%)	52.7
Positive allergic skin tests (%)	38.1
Allergic rhinitis (%)	50
Inhaled glucocorticoid therapy (no.)	63
Doses (budesonid equivalent)	
<200 µg (%)	58.3
200-400 µ (%)	31.6
>400 µg (%)	10
Without IGC (no.)	47
Level of disease control (GINA)	
Controlled BA (%)	23.6
Partially controlled BA (%)	53.6
Uncontrolled BA (%)	22.7

The analysis of the eNO values in relation to the level of control of the disease evidenced statistically significant differences between the mean values of the variables obtained in the study groups. The analysis of the distribution of the normal and increased eNO levels evidenced a higher frequency of increased values in patients with uncontrolled asthma as compared to normal values (84% vs. 16%). A higher frequency of increased levels was also found in uncontrolled asthma patients as compared to those with controlled or partially controlled disease (84% vs. 73% and 57.6% respectively).

The analysis of eNO in the patients with BA who had an exacerbation of the disease in the past month in comparison with the patients free of obstructive events in the same period is presented in Table 3. Increased individual values were more frequently noted in those with bronchial obstructive events in the past month (70% vs 56.6%), but the differences were not statistically significant.

Table 2. eNO according to the level of control of the disease

eNO (p.p.b.)	Controlled asthma (26 patients)	Partially controlled asthma (59 patients)	Uncontrolled asthma (25 patients)	p
Mean±SD (median [min- max])	29.6±36 (16.5 [5-162])	29.5±38.7 (19 [2-256])	55.8±37.1 (42 [0-124])	0.003
Increased values	19/26 (73%)	34/59 (57.6%)	21/25 (84%)	
Normal values	7/26 (26.9%)	25/59 (42.3%)	4/25 (16%)	

Table 3. eNO in patients with BA who had a bronchial exacerbation in the past month in comparison with patients free of obstructive events in the same period

eNO (p.p.b.)	> 1 month since the last bronchial exacerbation	< 1 month since the last bronchial exacerbation	p
Increased values			
Mean±SD	54.6±53.6 34/60 (56.6%)	54.5±109 14/20 (70%)	0.4
Normal values			
Mean±SD	10±17 26/60 (43.3%)	14.5±12 6/20 (30%)	0.1

According to FEV₁ at the time of eNO determination, the patients were divided into 2 groups: patients with bronchial obstruction and patients with normal respiratory function. The comparative values of eNO in the 2 groups are presented in Table 4. No statistically significant differences were found between the eNO values in the patients with bronchial obstruction and those with normal values of FEV₁.

Table 4. eNO in relation to FEV₁

Noe (p.p.b.)	FEV ₁		p
	≤80%	>80%	
Increased values			
Mean±SD	46±46 3/6 (50%)	38.16±256 31/84 (36.9%)	0.7
Normal values			
Mean±SD	13±33 3/6 (50%)	12±20 53/84 (63.09%)	0.5

According to the presence of atopy, the patients included in the study were divided into 2 groups: atopic BA and non-atopic BA. Our study did not find statistically significant differences of the eNO levels in relation to atopy.

In 39 patients we performed induced sputum in order to analyze the cellularity of the sputum. The sputum sample obtained could be interpreted in 24 patients. According to the sputum cellularity we formed 2 groups: BA in which eosinophils were predominant (eosinophils ≥ 2.5%) – 8 patients, and BA with non-eosinophilic sputum (eosinophils <2.5%) – 16 patients. The eNO levels in the two groups are presented in Table 6. All the 8 patients with eosinophilic sputum had increased eNO levels, though the arithmetic means indicated higher values in those with non-eosinophilic sputum.

Table 5. eNO in relation to atopy

Noe (p.p.b.)	Atopic BA	Non-atopic BA	p
Increased values			
Mean±SD	54.8±49.4 44/77 (57.1%)	33.5±90 24/33 (72.7%)	0.3
Normal values			
Mean±SD	12±24 33/77 (42.8%)	11±20 9/33 (27.2%)	0.1

Table 6. eNO in relation to sputum cellularity

Noe (p.p.b.)	Eosinophilic sputum (eosinophils ≥ 2.5%)	Non- eosinophilic sputum (eosinophils < 2.5%)	p
Increased values			
Mean±SD	22±27 8/8 (100%)	37±89 12/16 (75%)	0.03
Normal values			
Mean±SD	no cases no cases	8.5±19 4/16 (25%)	

Statistically, higher eNO values in relation to sputum cellularity were found in the cases of non-eosinophilic sputum, though increased individual values were 100% increased in patients with eosinophilic sputum.

Discussions

In our study we analyzed the concentration of eNO in children with BA subdivided according to the disease control. Higher individual eNO levels were found more frequently in patients with uncontrolled BA as compared to those in which the disease was fully or partially controlled. Finding some increased values of eNO in patients with controlled BA and normal values in uncontrolled BA is in accordance with certain reports in literature that show a weak correlation between eNO levels and symptoms, especially in those under IGC therapy [2].

Patients fulfilling the clinical criteria of BA diagnosis may present normal levels of eNO, especially in non-atopic BA. Therefore normal eNO values do not exclude the diagnosis of asthma. Asthma is a heterogeneous disease and eNO measurement provides information only on one aspect of the asthmatic syndrome [3,4].

Exhaled NO measurement is valuable in the investigation of patients with chronic respiratory symptoms in which the diagnosis has not been established, among which: eosinophilic bronchitis, asthmatic cough, postviral bronchial hyperactivity, gastro-esophageal reflux, vocal chords dysfunction, recurrent post-bronchiolitic wheezing, cystic fibrosis, congenital anomalies of the respiratory tract, ciliary dyskinesia syndrome. In these cases the increased eNO values indicate the existence of the eosinophilic infiltrate, with a good prediction of corticotherapy effectiveness [5,6,8].

Exhaled NO measurement may be also performed in

preschool children, in which spirometry and induced sputum tests are not possible. Increased eNO values represent a marker of responsiveness to glucocorticoids, especially at the initial assessment, before the IGC therapy is initiated.

In our study we recorded more frequently increased individual values in patients without IGC treatment as compared to those under such treatment. These results are in accordance with other studies in literature and may be explained by the strong anti-inflammatory effect of IGC. The IGC effects may influence the relation between the clinical picture, the investigations and the asthma expressivity. Patients with severe forms or poor disease control are more frequently treated with IGC, often with high doses. In these patients lower eNO levels will be found as compared to less severe forms of the disease [9].

The randomized control trials that assessed prospectively the role of eNO measurement in guiding anti-inflammatory management in mild/moderate forms of asthma have reported ambiguous results. However, these results do not deny the role of eNO parameter in the therapeutic management of asthma. eNO is a useful indicator, correlated with other tests, especially in the "difficult to treat" asthma. Normal eNO values are suggestive of a non-eosinophilic infiltrate, which prompts for other therapeutic choices [9-11].

Increased values of eNO in BA are recorded especially during the active period of the disease [12]. Warke *et al.* found a correlation between eNO and recent symptoms [13]. In our study we recorded high individual values more frequently in patients with a bronchial exacerbation in the past month (70% vs. 56.6%), but the differences were not significant statistically.

There are studies that did not evidence differences between patients with active asthma and those in remission for at least 12 months. In the study by Cano-Garcinuño *et al.*, eNO did not correlate with the asthma control, unplanned visits to the emergency room, or limitation of daily activity. In this study only cough in the past 4 weeks was associated with high eNO levels [3].

In our study we did not find statistically significant differences between eNO values in patients with bronchial obstruction (evidenced by FEV₁) and those with normal bronchial caliber (normal spirometric tests). There is a consensus in literature regarding the absence of correlation between baseline FEV₁ values and inflammation, both in children and adults, both in those treated with IGC and those without the treatment [3,6,14,15].

Epidemiological studies have confirmed the existence of increased eNO values in patients with atopic BA, with our without respiratory symptoms. The high value in asymptomatic patients were explained by the existence of minimal bronchial inflammation responsible for the increased eNO [16-18]. In our group we did not find statistically significant differences between the eNO levels in relation to the atopic background.

Eosinophils, mastocytes and T lymphocytes are involved in the development of airway inflammation in BA. Various studies have shown a correlation between eNO and sputum eosinophilia, in the bronchial biopsy or lavage samples [19-22]. Eosinophilic sputum is relevant for bronchial inflammation especially during symptomatic period of the disease. In our study, all the 8 patients with eosinophilic sputum had high eNO levels, though the arithmetic means indicated higher values in patients with non-eosinophilic sputum.

There are studies that have evidenced in some asthma patients a non-eosinophilic infiltrate, especially with neutrophils. Turner *et al.*, in a group of 34 patients with moderate uncontrolled asthma, found no eosinophils in the sputum in about half of them (16 of the 34) [23]. In a study on 45 patients with moderate asthma under IGC therapy, Basyigit *et al.* found eosinophils in 33 patients and a non-eosinophilic infiltrate in 12 patients [24]. A non-eosinophilic infiltrate was noted in patients with a longer term disease and atopy. Uribe Echevarría *et al.* found eosinophils in 23 of 41 steroid naive patients with moderate to severe asthma, the other patients having neutrophils in the sputum [25]. Patients with non-eosinophilic sputum had less bronchial frailty and responded better to anti-leukotriene treatment.

The pathogenesis of neutrophil recruitment in the airways is not completely elucidated. It is not clear whether neutrophils are the predominant cells of the primary inflammatory process or whether this is the consequence of glucocorticoid therapy, knowing that this medication inhibits neutrophil apoptosis and increases their survival in the respiratory tree [26]. Other interpretation of the non-eosinophilic sputum invokes the heterogeneity of asthma, with a diversity of inflammatory patterns, such as the involvement of viral infection, which is associated with predominantly neutrophilic infiltrate [27]. According to Pavord *et al.* the presence of non-eosinophilic sputum in a patient with BA is correlated with a poorer response to IGC [28].

One of the limits of our study has been the relatively small number of patients, taking into account that the analysis of the results was performed in correlation with the level of the disease control and also the fact that induced sputum test was performed in only 67 patients.

In conclusion, there is a weak correlation between the intensity of airways inflammation as evidenced by eNO and the clinical expression of asthma. Though respiratory functional tests are extremely valuable in the evaluation of asthma, eNO represents an additional examination for evidencing inflammation.

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