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## The analysis of the exhaled nitric oxide in a group of patients with asthma, aged 3–18

### Analiza poziomu wydzielanego tlenu azotu w grupie pacjentów z astmą w wieku od 3 do 18 lat

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**Słowa kluczowe:** nadreaktywność dróg oddechowych, obturacja dróg oddechowych, tlenek azotu, młodzież

#### Abstract

The aim of this study was to analyse the results of exhaled nitric oxide examination in a group of patients with hyper-responsiveness. The study was performed on a group of 297 children who underwent spirometry, examination of exhaled nitric oxide, skin prick test and stress probe in years 2010–2012 in Pulmonology and Allergy Centre in Karpacz. Mean age of patients was 11.86. Patients were divided into four groups: with diagnosed asthma, with bronchial hyper-responsiveness (BHR), with described asthma without BHR and a group with BHR without diagnosed asthma. The results of the study confirmed the presence of higher amount of exhaled nitric oxide in patients with asthma and bronchial hyper-responsiveness. The elevation of NO concentration correlated with age; there was no significant difference between sexes.

#### Streszczenie

Celem pracy była analiza wyników badania ilości wydychanego tlenu azotu w grupie pacjentów z nadreaktywnością dróg oddechowych. Przeprowadzono grupę 297 dzieci, u których przeprowadzono badanie spirometryczne jak i badanie ilości wydychanego NO (Nitric Oxide – tlenek azotu), testy skórne oraz próbę wysiłkową. Badania przeprowadzono w Centrum Pulmonologii i Alergologii w Karpaczu, w latach 2010–2012. Średnia wieku pacjentów wyniosła 11,86. Pacjentów podzielono na 4 grupy: ze zdiagnozowaną astmą, z nadreaktywnością oskrzeli, z opisaną astmą bez nadreaktywności, z nadreaktywnością bez zdiagnozowanej astmy. Wyniki badania potwierdziły większą koncentrację NO u pacjentów ze zdiagnozowaną astmą i nadreaktywnością oskrzeli. Podwyższone stężenie NO korelowało z wiekiem, nie wykazano natomiast znaczącej statystycznie różnicy między płciami.

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## 1. INTRODUCTION

Asthma is a chronic illness of the airway tract, which affects all from the small to the big bronchi. It was believed that asthma symptoms were connected with pathological changes in the lobar and segmental bronchi. Recent studies indicate an importance of the changes in the small and smallest bronchioles, as well [van der Wiel & ten Hacken, 2013]. Main symptoms of an asthma such as spastic cough, dyspnoea and wheezes are an effect of the bronchial hyper-responsiveness (BHR), which can be described as an parallel syndrome [Rasmussen & Taylor, 2002]. It is important to differentiate these two entities to perform more effective and individualized treatment [Collins & Parson, 2011]. Widely available diagnostic methods such as the spirometry, the stress test and an allergy testing are not sufficient enough to fulfil the new expectations. New diagnostic and differential tests are looked for. Measurements of the exhaled nitric oxide [FeNO] seem to be encouraging.

## 2. OBJECTIVE

The aim of this study was the analysis of the results of exhaled nitric oxide [FeNO] levels in a group of patients with diagnosed asthma or BHR. Furthermore, the possible correlation between once sex, age, diagnosed ailments and the outcome of the FeNO test was assessed.

## 3. MATERIALS AND METHODS

### 3.1. Study Subjects

This study was performed on 297 kids: 144 girls and 153 boys, recruited from the outpatients of Pediatric Pulmonology Department from the "Pulmonology and Allergy Centre" in Karpacz, during years 2010–2012. All patients were inhabitants of the Lower Silesia area. These kids were directed in to diagnose or assess the severity state of asthma. Diagnosis and classification of

asthma were based on clinical history and currently guidelines. Among all the patients to research group, we chose only these kids, who went through examination of nitric oxide concentration in exhaled air, skin prick test to detect the allergy and confirmation origin of allergic asthma, and performed the stress test.

### 3.2. FeNO Measurement

FeNO measurement was performed with "Medisoft analyser FeNO" before and after stress test. This is a simple and noninvasive diagnostic test, which enables the precise determination of the condition of respiratory tracts. [Taylor & Palmay, 2010] This survey includes four steps: inspiration, exhaust, continuation of exhaust and results of research. In the first stage of research, patients take a deep breath. The beginning of exhalation takes place after 3 seconds, which is illustrated by appearing on the screen icon and audible signal. After the beep, patient starts to breath lasting about 12 s, during which he should keep the ball placed in the mouthpiece at the level indicated by the bar. The next audible signal announces the end of this study. Test results are shown on the screen immediately after the finish. The measured parameters are: the maximum value of the exhaust of nitric oxide (NO Exhale (ppb)) and averaged over speed of expiratory flow (VE)(NO Ex)(ml/sec).

### 3.3. Skin Prick Testing

The methods of skin prick test involve placing a substance, which are allergen in skin punctures. We perform a positive control with a solution of histamine and negative with physiological saline solution. The interpretation of results should be made after the end of 20 min, by measuring the bubble size and infiltration with a ruler. Number of surveyed allergens depends on the suspected aetiology.

### 3.4. The stress test

The study provocation of stress test was performed on a treadmill R5100 with a simultaneous monitoring heart rate by heart rate monitor. The effort was gradually increased during the 6–8 min, until the 80–90% of maximum heart rate (calculated according to the formula =  $220 - \text{age of patient}$ ). Value ventilation should reach 40–60% of the estimated maximum ventilation, defined as ( $\text{FEV1} \times 35$ ). This test is completed when it reaches the target ventilation or cardiac function, where it should be at least 4 min.

### 3.5. Statistical Analysis

The results were compared and analysed with GraphPad Prism 6. The normality of distribution was tested by Shapiro–Wilk test and Kolmogorov–Smirnov test. Next, all parameters with normal distribution were analysed with Student's *t*-test. These non-parametric methods by means of Mann–Whitney test or Wilcoxon test.

## 4. RESULTS

There were 297 children in age 3–18 years old, who had performed stress test and FeNO measurements during 2010–2012. There was  $n=279$  (93.94%) patients with diagnosed asthma, and  $n=213$  (71.72%) with bronchial hyperresponsiveness (Table 1.) Among the studied distinguished two another groups: with described asthma without BHR  $n=80$  (26.94%), and a group with BHR without diagnosed asthma  $n=14$  (4.71%). There was no significant difference between them ( $p>0.05$ ) (Fig. 1). The amount of FeNO and VE NO Ex in a group of patients with asthma and BHR was significantly higher than in a group with diagnosed asthma without BHR ( $p=0.0232$ ; Fig. 2). In this research group, there were 202 children with proved skin prick test allergy, and 95 kids with negative skin test. There was no statistically significant difference in the levels of FeNO and VE NO Ex between that groups.

Further, we have analysed the amount of exhaled NO and FEV1 before and after stress test, as a confounder. There was no significant difference in the level of FeNO. Results of FEV1 were meaningly lower after probe  $p<0.0001$  (Table 1).

Pearson's correlation test revealed a significant correlation between age and amount of exhaled nitric oxide either before or after the stress test (Table 2). There were no dependencies between once sex and FeNO results.

## 5. DISCUSSION

Asthma and bronchial hyperresponsiveness (BHR) are chronic diseases affecting respiratory system, and significantly may impact ones quality of life. According to Global Initiative for Asthma (GINA) guidelines, the aim of the treatment is to control the disease and improve once health-related quality of life (HR-QoL) [Scichilone & Braido, 2013]. To improve the HR-QoL medics have to meet the criteria:

**Table 1.** Characteristics of patients with diagnosed asthma and or bronchial hyperresponsiveness [BHR]. FEV1 – forced expiratory volume in 1 s, FeNO – fractional nitric oxide concentration in exhaled breath, VE NO Ex – averaged over speed of expiratory flow

Characteristic	Total	Male (n = 153)	Female (n = 144)	P-value
Age (years)	11.85	11.84	11.85	
Asthma, n (%)	279 (93.94)	143 (48.15)	136 (45.79)	<0.0001
BHR, n (%)	213 (71.72)	108 (36.36)	105 (35.35)	<0.0001
Asthma without BHR, n (%)	80 (26.94)	41 (13.80)	39 (13.13)	<0.0001
Isolated BHR, n (%)	14 (4.71)	6 (2.02)	8 (2.69)	0.1884
FEV1 before stress test, mean (SD)	2.715 (1.008)	2.658 (1.01)	2.775 (1.02)	<0.0001
FEV1 after stress test, mean (SD)	2.573 (1.006)	2.542 (1.02)	2.607 (0.99)	<0.0001
FeNO before stress test, mean (SD)	57.13 (50.68)	56.84 (52.38)	57.44 (48.99)	<0.0001
FeNO after stress test, mean (SD)	57.32 (48.83)	56.99 (51.47)	57.68 (46.03)	<0.0001
VE NO Ex [ml/sec], before stress test, mean (SD)	48.04 (10.78)	48.48 (11.23)	47.57 (10.30)	<0.0001
VE NO Ex [ml/sec], after stress test, mean (SD)	48.63 (9.74)	48.68 (9.63)	48.57 (9.88)	<0.0001

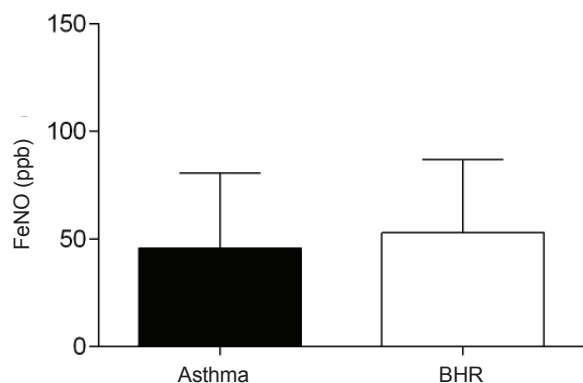


Fig. 1. Difference of amount of exhaled nitric oxide (FeNO) between a group of patients with isolated bronchial hyperresponsiveness (BHR) and a group of patients with asthma

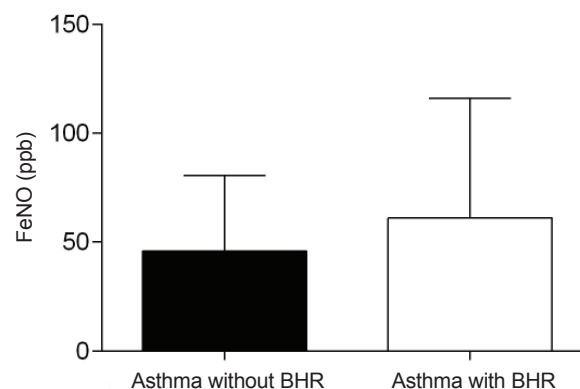


Fig. 2. Difference of amount of exhaled nitric oxide (FeNO) between a group of patients with isolated asthma and a group of patients with co-existing asthma and bronchial hyperresponsiveness (BHR)

- Eliminate occasional flare-ups.
- Reduce medicament doses to effective minimum.
- Eliminate side effects.

To achieve these goals, the therapeutic process should be personalized as much as possible [Haverkamp Dempsey, 2007]. To reach that point asthma should be different from isolated BHR. In most of the previous study, BHR was described as a cardinal feature of asthma. In fact, BHR may be another pathologic process, which requires another way of treatment [Colins & Parsons, 2011]. In the current research group, there were only  $n=80$  (26.94%) with asthma without BHR and  $n=14$  (4.71%) with diagnosed BHR without asthma. We expect that the real number of patients with isolated BHR is higher. Because of the retrospective character of this study, we could not check in detail the diagnostic process. To accurately differentiate these diseases, new methods are necessary.

Nitric oxide measurements seem to be promising. NO is recognized as a multifunctional molecule in human and animal organisms. This highly reactive compound plays a role inter alia in: neurotransmission, vasodilatation, bronchodilatation, and inflammation process [Nathan & Xie, 1994]. In pathophysiology of asthma, the last two functions are most important. The exact role is not clear. On the one hand, it may act as a proinflammatory mediator predisposing to the development of bronchial hyperresponsiveness (BHR). On the other, NO is a weak smooth muscle relaxant. In the early 1990s, NO measurements were introduced for the first time [Gustafsson & Leone, 1991]. About 20 years after introduction, FeNO measurements are still an experimental diagnostic test. Mostly FeNO results are used as an additional,

less valuable markers. American Thoracic Society suggests that FeNO assessment may be used inter alia to:

- Diagnose eosinophilic airway inflammation (strong recommendation, moderate quality of evidence).
- Support the diagnosis of asthma in situations in which objective evidence is needed (weak recommendation, moderate quality of evidence).
- Monitor airway inflammation in patients with asthma (strong recommendation, low quality of evidence).
- Determine the likelihood of steroid responsiveness in individuals with chronic respiratory symptoms possibly due to airway inflammation (strong recommendation, low quality of evidence).

Still is underlined that in spite of advance technology and standardization before FeNO will be useful as a clinical tool, more work is still needed [Jongste & Carraro, 2011; Moon & Choi 2013]. In our study, the amount of FeNO and VE NO Ex in a group of patients with asthma and BHR was significantly higher than in a group with diagnosed asthma without BHR  $p = 0.0232$  (Fig. 2).

There are more and more metaanalysis concerning the usefulness of exhaled NO analysis. Jartti and Wendelin-Saarenhovi (2012) questioned the helpfulness of this tool. In their opinion, there are too many factors affecting FeNO values. They find a correlation between once height, age, sex, cooperation, nasal inflammation, food, smoking or environmental exposures, to levels of exhaled NO. Our results do not confirm the relationship between once sex, but there is a strong correlation between patients age. In our opinion, nasal inflammation or coexisting infections may be a strong factor affecting the FeNO result. We did not

Table 2. Results of the Pearson's correlation test between age and stress test's parameters

	Age vs. distance	Age vs. HR	Age vs. FeNO before stress test	Age vs. FeNO after stress test
<i>R</i>	0.2837	0.03499	0.1715	0.2067
<i>R</i> <sup>2</sup>	0.0805	0.001225	0.02941	0.04272
<i>P</i> -value	<0.0001	0.5535	0.003	0.0003
Significant (alpha = 0.05)	Yes	No	Yes	Yes

analyse this part because of the lack in medical documentation. We suspect that FeNO measurements may be helpful in diagnosis of diseases, which imitate asthma like Gastro Esophageal Reflux Disease [Pirogowicz & Patyk, 2013].

A step forward was made by Montuschi et al. [2013], who designed the "Electronic Nose" to analyse the exhaled air. With the gas chromatography and mass spectrometry, they found several volatile organic compounds (VOCs). This method was useful especially in combination with FeNO measurements to discriminate asthmatics and healthy people. This tool may be helpful in future to diagnose another lung disorders like COPD, cancer [Montuschi & Santonico, et. al, 2013].

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## 6. CONCLUSION

Nitric oxide measurements may be helpful in assessment of asthma control. The results of the study confirmed the presence of the higher amount of exhaled nitric oxide in patients with bronchial hyperresponsiveness than in patients with diagnosed asthma. There was significant correlation between age and FeNO results (in Pearson's correlation test  $r=0.2067$ , and  $p<0.001$ ). The FeNO measurement may be used as a complementary method in asthma diagnosis. There was no statistically significant difference between groups of girls and boys. Present methods require standardization.