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THE PRACTICAL UTILITY OF NITRIC OXIDE MEASUREMENT IN EXHALED AIR. EXPERIENCE OF THE PEDIATRICS DEPARTMENT OF THE FILANTROPIA MUNICIPAL HOSPITAL CRAIOVA

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ABSTRACT

Dosage of nitric oxide, a relatively new biomarker, is a non-invasive method, increasingly accessible to clinicians, used mainly in the field of pneumology, for the purpose of monitoring the respiratory tract inflammation. The goal of this paper is to present the experience of the Pediatric Department of the Filantropia Municipal Hospital in Craiova relating to nitric oxide dosing and asthma pathology, the result of the 10 years of experience with the portable exhaled air analyzer. We selected three of our most important researches, presented at the European Respiratory Society (ERS) Congresses

Keywords: nitric oxide, children, asthma

INTRODUCTION

Nitric oxide is a gaseous free radical with multiple roles in the body's homeostasis, the most important being neurotransmitter, vaso- and bronchodilator (1,2). Its endogenous production is the result of nitric oxide synthase enzyme activity (NOS) (3), and the easiest way to quantify is exhaled air dosage (FeNO), a non-invasive method that is becoming more accessible to clinicians.

Pneumology, including pediatric pneumology, was the first medical field to try to benefit from the dosing of this relatively new biomarker. In bronchial asthma, determinations made appear to correlate with the number of blood or sputum eosinophils (4) but its role in the diagnosis of chronic inflammatory pathology is still unclear (5).

The rapid accumulation of scientific evidence has explained this shortcoming through the multitude of factors that can alter the measured values. FeNO dosing may have low values in smokers during bronchoconstriction episodes (6,7) or if asthma has a neutrophilic phenotype (5) but may be increased in other atopic or chronic inflammatory conditions (eczema, allergic rhinitis, rheumatoid arthritis, chronic ENT pathology) (8,9).

MATERIAL AND METHOD

In 2010, in the Pediatric Clinic of Filantropia Craiova Hospital, was purchased the first portable nitric oxide dosing device in exhaled air for children – Niox Mino (Aerocrine, Sweden).

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The device is small, easy to use for both adults and children and requires a 10-second exhalation, the last 3 seconds being analyzed by a calibrated electrochemical sensor to obtain a definitive result in parts per billion (ppb). The device is pre-calibrated and designed to ensure operation without the need for service and calibration (10), but has a limited lifetime (3 years, or about 3,000 tests), after which a new investment is needed.

In 2015, Niox Vero was acquired – the latest generation of analyzer with an extended lifetime (5 years, or about 5,000 tests), and up to now over 800 pediatric determinations have been performed in our Department. Compared to the first generation, NioxVero solved a number of deficiencies, such as the use without a continuous connection to an external source of electrical power or an improved design of the mouthpiece, making it easier to use, especially by children. Perhaps the most important change is improved display visibility with an evolved graphics program so that visual and hearing aids ensure optimal compliance.

FIGURE 1. *Portable FeNO analyzers: NIOX Mino (left) and NIOX Vero (right)*

Our studies included children diagnosed with asthma aged 7 years and above, who were able to perform spirometry and dosing of nitric oxide in exhaled air. In particular situations, the control groups were composed of children close in age to asthmatic children, but without a history of atopic pathology.

The exclusion criteria in the studies were:

– Intense physical activity one hour before the beginning of the test
– Food consumption (especially foods rich in nitrites) one hour before the beginning of the test
– Extremely anxious children at the time of the measurements
– Adolescents who have admited or are legitimately suspected of smoking.

The statistical analysis was performed using chi square and t test with statistically significant values starting at $p = 0.05$, as used in medical literature. Microsoft Excell and EpiInfo (freeware distributed by the CDC – Center for Disease Control) were used.

RESULTS

In 2012, we conducted a study published at the European Respiratory Society Congress (11) that looked at the correlation between FeNO and other diagnostic tools in pediatric asthma. During the research, 149 asthma cases (89 boys) were compared with the non-atopic control group (100 cases – 55 boys). Average FeNO values per groups did not show any significant differences $p = 0.19$, RR = 0.91 (0.74-1.11). The second instrument used for asthma diagnostic was to determine specific serum IgE (with a mixed – food and respiratory – panel, collected in the ambulatory ward at the same medical unit). In this case the values had statistical significance, specific IgE being positive especially in the group diagnosed with asthma: $p = 0.01$; RR = 3.76 (0.99-14.25). There was no obvious correlation between nitric oxide levels with specific serum IgE values.

Although the comparison of the 149 asthma cases with the non-atopic control group (100 cases) did not reveal significant differences $p = 0.19$, RR = 0.91 (0.74-1.11), increased values of nitric oxide in exhaled air were correlated with high levels (over 300 IU / l) of total serum IgE: $p = 0.008$, RR = 2.34 (1.15-4.72). Total dosage of IgE is a non-specific marker and its utility in the diagnosis of atopic pathology is limited – however, the investigation is technically and financially accessible, so it can still be used as a guideline.

In these first patient groups we could not identify a correlation between FeNO dosing and the degree of asthma control (assessed according to the Global Initiative for Asthma – GINA guide): $p = 0.45$.

The study group was then divided into two subgroups depending on the type of controller therapy (montelukast sodium or fluticasone propionate) given to children with asthma. The values obtained at the dosing of nitric oxide at that time were not statistically significant: $p = 0.27$.

In the second study, the research conducted at the clinic aimed to correlate the nitric oxide values with the therapeutic regimens in pediatric asthma.

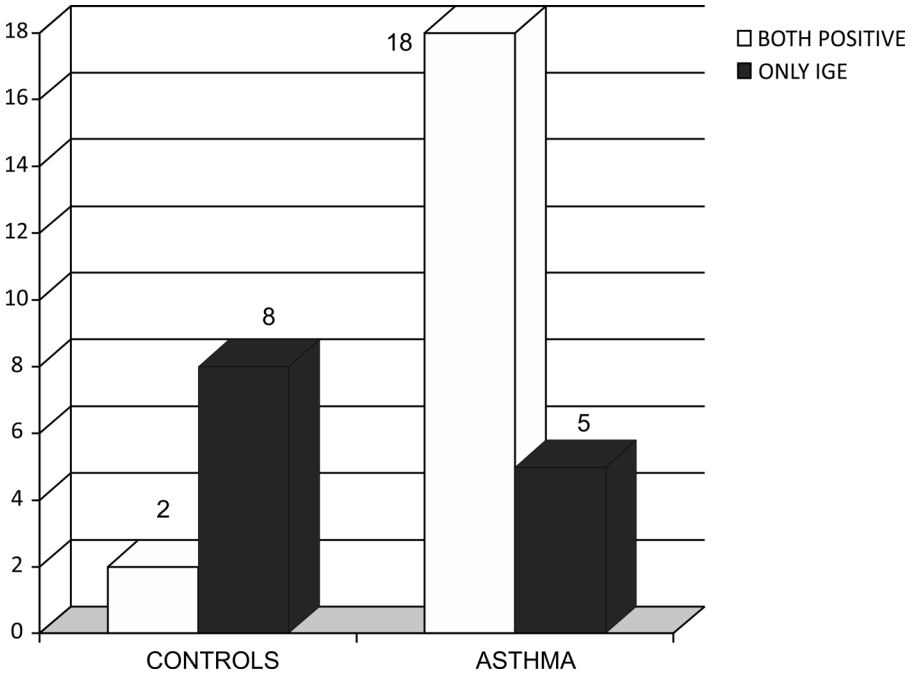

FIGURE 2. *Correlation between total IgE and FeNO*

The therapy used in our patients allowed us to evaluate children receiving inhaled corticosteroid therapy and/or leukotriene inhibitors (montelukast sodium). We did not include long-acting beta-agonists because the number of such cases admitted to our Department is rather low. In the study (12), 87 asthmatic children were included: 25 received inhaled corticosteroids (ICS), 54 Montelukast, 8 bi-therapy. FeNO was measured initially and after 3 months of treatment.

At the same time, children and caregivers were asked to fill out one of the standardized pediatric variants of the asthma control test (c-ACT).

After 3 months, 52 cases had better nitric oxide values in exhaled air, and 71 children reported better values in the asthma control assessment.

FeNO levels improved significantly in the bi-therapy group compared to patients with a single type of controller treatment: p = 0.04, OR = 0.18 (0.007-1.29) versus the Montelukast group and p = 0.09, OR = 0.22 (0.008-1.76) vs. ICS group.

The criteria used to confirm the positive evolution were:

- Reduction by at least 20% at initial values above 50 ppB (parts per billion)
- Reduction by at least 10 ppB (nominal value) at initial values below 50 ppB.

The secondary objective of the study was the correlation of FeNO with the asthma control level quantified by c-ACT (Asthma Control Test). In this case the results did not show an obvious correlation, with 53% of the children enrolled in the study having a dissonant evolution of the two monitored indicators.

TABLE 1. *Correlation between therapeutic regimens FeNO and ACT*

		FeNO improvement	ACT improvement	TOTAL
ICS group	***Males***	**9**	**12**	**21**
	Females	**6**	**7**	**13**
M group	***Males***	**19**	**29**	**48**
	Females	**11**	**20**	**31**
ICS + M group	***Males***	**4**	**2**	**6**
	Females	**3**	**1**	**4**
TOTAL		**52**	**71**	

A third study (13) conducted in our clinic focused on a special category of pediatric patients (children practicing professional sports) and was based on collaboration with the Sports Medicine Department in Craiova. 87 children (49 boys) aged 12-17 practicing professional sports were included, versus 75 children (39 boys) in the control group.

TABLE 2. *Children practicing professional sports*

		TRACK AND FIELD	FOOTBALL	FENCING	JUDO
STUDY GROUP	male	13	19	10	7
	female	14	7	12	5
CONTROL GROUP	male	39			
	female	36			

The prevalence of asthma was similar between the two groups, 13 athletes and 11 in the control

group, p = 0.96, RR = 1.01 (0.48-2.13) and – in the group of athletes – between outdoor and indoor sports. FeNO dosing was relatively similar between groups with an average of 15.66 ppB (in athletes) versus 14.6 ppB (control group): p = 0.54 (t test). However, when the indoor athletes were compared with the control group, the results had a significant difference because the mean FeNO in this sublot was 20.21 ppB: p = 0.007 (t test). Practically, athletes practicing an indoor sport had elevated levels of nitric oxide in exhaled air.

DISCUSSIONS

As with spirometry, large-scale determination of FeNO is related to patient compliance, and in pediatrics – although data for younger ages is available (14) – it is usually impossible to obtain reliable values under 5-6 years, even if the new analyzers in current use have an improved design (15,16,17). However, there are researches that report successful percentages of over 90% in children around the age of 4 (18,19). Yet, the role of the investigator is much less important than in spirometry because the device is calibrated so that valid results are automatically displayed only after the subject has complied with the current technical recommendations (20).

A series of research studies over the past decade has attempted to establish equivalence between the most commonly used nitric oxide determination devices in exhaled air, and the results are encouraging, which has allowed the establishment of widely applicable standards (21,22).

Although there are shortcomings in the method, dosing of nitric oxide in exhaled air is still a subject of great interest in asthma management, especially pediatric asthma, the last GINA (Global Initiative for Asthma) guide recognizing its predictive role for asthma in younger children (23), but also its usefulness in monitoring treatment in children (24).

CONCLUSIONS

Dosage of nitric oxide in exhaled air was initially perceived as a huge leap in the monitoring of inflammation in pediatric chronic respiratory diseases, especially asthma. That is why our research has been directed from the beginning in the evaluation of its effectiveness in the diagnosis and the therapeutic management of this kind of pathology.

If we were to systematize the conclusions of our research, the most important are:

The correlation of FeNO values with other pediatric asthma monitoring tools is still unlikely. The link we have established with total serum IgE is not very useful, as long as these too are quite unspecific.

Corroboration of FeNO values with therapeutic regimens in asthma is also quite relative, the positive values being recorded mostly in severe cases, with multiple controller therapy.

The sustained physical effort typical to athletes did not significantly influence the data obtained, except for indoor sports, where the environment in which the athletes were performing likely had a decisive role.

As a final conclusion, in our opinion the dosing of nitric oxide in exhaled air is a promising but still adjuvant method in the monitoring of the asthmatic chronic inflammatory pathology in children.

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