Electrochemical Detection of the Exhaled Nitric Oxide in Children
– A retrospective study

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Dosage of gaseous inflammatory markers is a promising method of evaluating various types of pediatric pathologies and not only. The fraction of exhaled nitric oxide (FeNO) can be quantified by electrochemical detection using a portable analyzer that delivers fast, reproducible, and widely available results. The objectives of our study were to evaluate the accessibility of the pediatric dosing method and the utility of FeNO for the diagnosis of atopic pathology in hospitalized children. We conducted a retrospective study that included children aged over 6 years admitted to the Pediatric Department of the Municipal Hospital Filantropia in Craiova who were evaluated according to the type of training performed for the determination of FeNO and according to the diagnosis of admission. Results. Using a human model was more effective in obtaining a valid result compared to the use of the demo provided by the manufacturer, especially at younger age (p = 0.05 and respectively, 0.04 - Student t test). Among the various allergic pathologies studied, children with asthma were the ones who recorded the highest levels of nitric oxide, even when compared to the rest of atopic children (p = 0.01 - t test). Moreover, the proportion of cases with positive values was increased in asthmatic children compared to the rest of the type of allergic pathology, but these values were at the limit of statistical significance (p = 0.08 - chi square and Mid-P exact test). The conclusion of our study was that it is possible to improve compliance with FeNO assessment especially at younger age, and the utility of the method is still limited, especially in asthma cases.

Keywords: electrochemical, children, nitric oxide, atopy

Nitric oxide (NO), a sensitive indicator of eosinophilic inflammation in the tracheobronchial tree [1] as well as in other apparatus and systems [2 - 4], is used both in diagnosis and in prediction and evaluation of the patient's response to anti-inflammatory therapy and compliance monitoring [5-7]. The difficulty in assessing the NO gas molecule results from the fact that the biological half-life varies from a few seconds to a few minutes, NO being extremely unstable.

The chemiluminescence method is the gold standard for NO analysis in the gas phase [1] because of its sensitivity and excellent response time, but has several disadvantages: the need for frequent calibration, the fairly significant dimensions of the required equipment and the prohibitive price that prevents the wide scale use of the method.

Electrochemical detection (EC) was the next step for the quantification of gaseous markers, in the case of NO the advantages of the method being the portability of the device, the relatively affordable price and the simple technique that allows large scale measurements without complicated maintenance or repeated calibrations [8, 9]. EC also has some drawbacks, most notably the instability of exhaled air samples, sensor instability, or the limited lifetime of the device requiring replacement after a certain period or after a specific number of determinations [10, 11].

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Experimental part

Our study is one step in a broader research effort that has been underway for around 10 years at the Pediatric Department of the Filantropia Municipal Hospital in Craiova, in connection with the dosing of nitric oxide in exhaled air in children. The objective of this phase was the evaluation of FeNO in exhaled air in pediatric patients according to several parameters: diagnosis on arrival, type of medication, type of training performed.

Material and method. We conducted a retrospective study with patients admitted to the Pediatric Clinic between 2016 and 2017, where functional ventilation samples were performed: spirometry, peakflowmetry and dosing of nitric oxide in exhaled air (FeNO). 106 consecutive pediatric patients over 6 years of age were included in the study, which were divided in two groups: children with atopic pathology (asthma, allergic rhinitis, atopic dermatitis, food allergies) and children without known atopy (control group). We evaluated the general demographics and compliance with the Niox Vero (Aerocrine, Sweden) analyzer, available at the clinic for the determination of nitric oxide in exhaled air. (Figure 1).

Fig. 1. The NioxVero FeNO portable analyzer

The statistical evaluation was done using MS OFFICE and OPENEPI (CDC, USA) with the Student t test and the chi square with relative risk (RR), the p values less than 0.05 being considered as significant results.

Table 1
GROUP STRUCTURE

	Study group (n = 67)	*Control group (n = 39)*
Boys: girls, number	36:31	22:17
Average age(years); standard deviation	11,1; 2,91	10,4; 2,97
Environment: urban/rural, number	51/16	33/6
FeNO: average value; standard deviation	28,65; 24,41	24,07; 33,09

Most of the children tested came from an urban environment, this being the profile of the patients arriving in our clinic. There were no significant differences in nitric oxide according to the environment of origin (urban vs. rural) or gender. The average FeNO values recorded in the study group were slightly higher than those of children without atopic history, but without statistical significance (p = 0.41 - t test). However, the highest absolute mean values: 137 and 128 ppB (parts per billion) were recorded - surprisingly - in the group without proven allergies.

The next phase of our research tracked children's compliance with the measurement - that is, the number of determinations after which a valid record of nitric oxide was obtained in exhaled air. We used two different methods of training: the visual demo that came with the device and a "human model", that is, another child who could already perform the test correctly and who was used as an example for the one we wanted to train.

Our entire lot had an average of 2.5 determinations (1.58 standard deviation) required to obtain a correct measurement of the nitric oxide fraction in exhaled air. Stratified evaluation according to age and diagnosis is presented in Table 2.

Table 2
NUMBER OF TRIALS REQUIRED FOR A VALID RESULT: AVERAGE (STANDARD DEVIATION)

Age	6-12 years old	Over 12 years old	***T test***
Group - atopic	2.83 (1.67)	2.16 (1.15)	**0.05**
Group – non atopic	3.05 (2.34)	2.27 (1.12)	**0.04**
T test	0.7	0.46	

Pathological comparative analysis (atopic or non-atopic) did not identify significant differences in obtaining a valid result, children requiring approximately the same number of trials to make a correct determination.

As far as admission age is concerned, children under 12 years of age needed a greater effort to be able to achieve a correct determination in both the atopic and the atopic group, which is probably due to low compliance at the sampling technique, the younger children being harder to train. The values we recorded were at the limit of statistical significance, probably due to the relatively small sizes of the groups.

Regarding the teaching method, we also conducted a differentiated age assessment, the results are presented in Table 3.

Table 3
SAMPLE COMPLIANCE BY TYPE OF TRAINING: AVERAGE (STANDARD DEVIATION)

Age	***6-12 years old***	***Over 12 years old***	***T test***
Visual demo	3.48 (2.15)	2.16 (1.15)	0.11
Human demo	2.16 (1.1)	1.56 (0.8)	**0.02**
T test	**0.008**	1.09	

Age stratification shows lower average values in children over the age of 12 - as was expected, it was easier for them to identify the correct way to perform the test. The use of a human model as a "teacher" was more effective than the simulation provided by the device manufacturer, both in young children and after puberty. Under 12, however, the difference was statistically significant, that is, our method was obviously more effective at younger ages, where compliance with performing pulmonary function tests is more difficult to achieve.

In the last part of our study we focused on the group of children with atopic pathology, where we made an assessment of the nitric oxide fraction in exhaled air depending on the type of disease.

We divided the study group according to their type of pathology into asthmatic children (singular diagnosis or associated with other atopic manifestations) and allergic children without lower respiratory tract manifestations (allergic rhinitis, atopic dermatitis, food allergies). The mean values and the standard deviation of FeNO in the two sublots were 37.14 (31.07) and 22.75 (16.27) respectively, p = 0.01 (t test).

Comparative values for the type of pathology are shown in Figure 2.

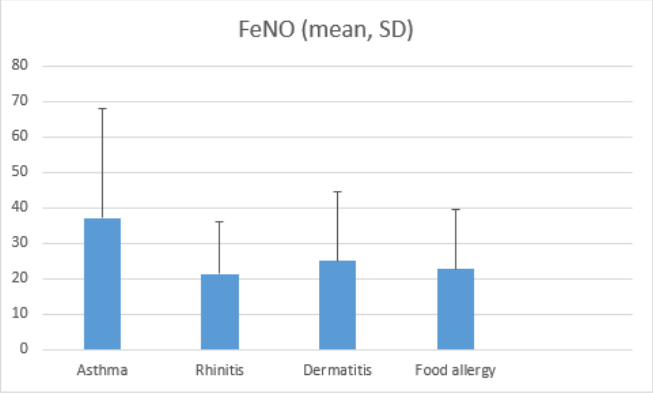

Fig. 2. FeNO (average, standard deviation) depending on the type of atopic pathology

The values recorded in children with asthma are clearly higher compared to other atopic pathologies.

We did not record significant differences between patients diagnosed with rhinitis, dermatitis or food allergies, or related to the age group.

According to the American Thoracic Society guidelines, the FeNO level indicating a possible eosinophilic inflammation is 25 ppB (parts per billion) in children. Depending on this threshold, we have made an assessment of the FeNO positive cases compared to the types of atopic pathology included in our research. Values are shown in Table 4.

Table 4

FeNO values	Pozitive (over 25 ppB)	Negative (bellow 25 ppB)	TOTAL number of cases	p (chi square) vs. asthma	RR (95% confidence intervals)
Asthma	15	11	26		
Allergic rhinitis	6	13	19	0.08	1,8 (0.87-3.82)
Atopic dermatitis	5	8	13	0.24	1.5 (0,69-3,21)
Food Allergies	2	7	9	0.08 (Mid-P exact test)	2.59 (0.73-9.02)

As we can see, cases with asthma have had positive levels of nitric oxide in the exhaled air to a greater extent than the rest of children diagnosed with allergic diseases. However, compared to the rest of atopic diseases, the values are not statistically different, approximating the significance limit for allergic rhinitis and food allergies.

Discussion

Determination of nitric oxide in exhaled air is a method increasingly used in assessing inflammatory respiratory pathology. However, its applicability is much broader because a series of difficult-to-diagnose illnesses could benefit from FeNO sampling [12, 13, 14]. Moreover, chronic inflammatory processes with various localizations (cutaneous, articular, upper respiratory, etc.) could be the beneficiaries of this mode of assessment of inflammation [15, 16, 17]. At present, most structured data are related to pneumology where the nitric oxide fraction is used in the diagnosis and management of asthma [18, 19]. Its utility seems to be particularly confirmed in the pediatric field, where it can be used as a tool to guide treatment or to prevent the exacerbation rate, the data for adults not being as encouraging [20, 21].

As far as the field of pediatrics is concerned, compliance is one of the major impediments to using sampling at younger ages. Portable analyzers, like those used in the Clinic of Pediatrics at the Filantropia Municipal Hospital in Craiova, have made this determination more accessible, but - as with classic pulmonary function tests - the age of 5-6 years seems to be a threshold below which it is difficult to get valid results [22]. There are methods to allow on-line assessment of nitric oxide in exhaled air for smaller children, but they are only available in specialized centers and no sufficiently large amount of data has been collected to be able to include them in current practice. [23, 1].

A series of investigations have been directed towards the validation of new types of electrochemical portable analyzers compared to the chemiluminescence standard, or between different types of portable analyzers [24, 25, 26]. In general, the results supported both methods, and portable and accessible equipment for a large number of researchers was accepted by the medical community.

Conclusions

Evaluation of inflammatory markers with the help of an electrochemical analyzer is an opportunity for the management of pediatric respiratory pathology. The improved design of the latest generation of devices has allowed more patients, especially younger children, to access the nitric oxide fraction of exhaled air.

In our clinic we tried to improve adherence to the technique by using a "human model" - that is, another child already trained in making the sample, and this method gave better results than the program used by the manufacturing company, especially in younger children. Patients over the age of 12 had, as expected, better outcomes in terms of compliance.

On the whole, the values obtained in children with atopic diseases were somewhat higher than in non-atopic patients, but the limit of statistical significance was not reached. In those allergic, children with asthma diagnosis had higher values of FeNO ($p = 0.01$ – t test). The proportion of positive values were also increased in asthma subgroup near the statistically significant limit ($p = 0.08$ – chi square and Mid-P exact tests).

The results of this study are thus in line with the trend of our previous research in the last decade [27- 30], in which nitric oxide in exhaled air had a limited utility in evaluating the inflammatory-allergic pathology [31-36] in children.

The lack of another biochemical marker capable of quantifying the inflammatory syndrome [40-44] on a large scale, as well as the still high preoccupation of medical literature for exhaled nitric oxide, are the main reasons for our support towards further research in this field.

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