The role of FENO and spirometry in the evaluation of obstruction in pediatric asthma

Rolul FENO și al spirometriei în evaluarea de obstrucție în astmul pediatric

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Abstract

Introduction: *The fractional exhaled nitric oxide concentration (FENO) is used as a noninvasive biomarker for asthmatic inflammation. The role in establishing an asthma diagnosis is still under research. Spirometry remains the main investigation for the diagnosis and monitoring of asthma.*
Objectives: *The aim of the study was to assess the correlation between FENO level and the values of spirometric parameters defining airflow obstruction.*
Materials and methods: *The longitudinal study included a group of 89 children admitted to the Pediatric Clinic of "Grigore Alexandrescu" Emergency Clinical Hospital for Children. The inclusion criteria were: (i) a previously known diagnosis of asthma, (ii) age range between 5 and 18 years old. All patients underwent pulmonary function testing and measurement of FENO.*
Results: *Significant correlations were identified between FENO and a part of spirometric parameters: MEF75 (p=0.007), MEF50 (p=0.0.28), MEF25 (p=0.011) and PEF (p=0.020), but not with FEV1 (p=0.090), nor FVC (p=0.308). However, multiple linear regression analysis revealed that spirometric parameters poorly predict FENO variation (R=0.062, p=0.82). In adition, binary logistic regression analysis (LR) showed that FENO can not be used as a reliable biomarker (p=0.169) for the estimation of the risk for obstructive dysfunction.*
Conclusion: *The level of exhaled nitric oxide does not correlate with the values of spirometric values. More studies with a large number of patients are needed for establishing the role of FENO in the evaluation of airflow obstruction.*
Keywords *FENO, asthma, spirometric parameters*

Rezumat

Introducere: *Fracția de excreție a oxidului nitric (FENO) în aerul expirat este utilizată ca biomarker de inflamație a mucoasei bronșice. Pentru obiectivarea obstrucției căilor aeriene, spirometria este investigația principală de diagnostic și monitorizare a astmului bronșic.*
Scopuri și obiective: *evaluarea disfuncției obstructive în astmul bronșic prin determinarea FENO și spirometrie.*
Materiale și metode: *Acest studiu longitudinal a inclus 89 de copii internați în Spitalul Clinic de Urgență pentru Copii „Grigore Alexandrescu". Criteriile de includere au fost: (i) diagnostic cunoscut de astm, (ii) vârsta între 5 și 18 ani. Toți pacienții au fost evaluați prin spirometrie și măsurarea FENO.*
Rezultate: *FENO s-a corelat semnificativ cu o parte din parametrii spirometrici: MEF75 (p=0.007), MEF50 (p=0.028), MEF25 (p=0.011) si PEF (p=0.020). Totuși, analiza multiplă liniară de regresie a arătat că parametrii spirometrici au predicție slabă pentru variația FENO (R=0.062, p=0.82). În plus, analiza binară logistică de regresie (LR) a arătat ca FENO nu poate fi folosit ca un biomarker de încredere (p=0.169) pentru estimarea riscului de disfuncție obstructivă.*
Concluzii: *pentru stabilirea rolului FENO în definirea obstrucției, alături de probele funcționale ventilatorii sunt necesare mai multe studii, care să include un număr mai mare de pacienți.*
Cuvinte-cheie: *FENO, spirometrie,astm*

Introduction

Asthma is regarded as a chronic disease with an oscillating evolution influenced by a wide variety of factors: genetics, age, associated conditions (obesity, gastroesophageal reflux), compliance with, and responsiveness to, treatment[1].

For the diagnosis and monitoring of this disease, besides complete anamnesis, medical past history, physical examination, a spirometry is of paramount importance.

Obstruction is clinically defined through symptoms like spastic cough, thoracic constriction, wheezing, and expiratory dyspnea.

These clinical findings are the consequence of the limitation of airflow produced by the bronchial inflammation and bronchial hyperresponsiveness.

The obstruction can be evaluated through spirometry. International guidelines have established that the variation of the two parameters FEV1 >12% and PEF >15% between two periodical evaluations certify the bronchial obstruction[1]. Some researchers recommend the measurement of FENO before performing pulmonary function testing[2].

The measurement of the fractional concentration of exhaled nitric oxide (FENO) presents the most intriguing possibility on the quest to understanding asthma. It quantifies the severity of airflow obstruction. Recently, its role in the evaluation of airflow obstruction has started to be researched. In our study we have included all asthmatic children, with or without baseline treatment, and the performed investigations were not evaluated depending on the therapeutic step.

Table 1 The distribution of obstructive dysfunction in the subgroups of patients

Subgroup	No obstructive dysfunction (%)	With obstructive dysfunction (%)	Pearson χ2 *P*-value	OR (95% CI; *P* value)
Age **5-12 years** **13-18 years**	 26.4 47.1	 73.6 52.9	0.095	0.403 (0.136-1.196; 0.102)
Non allergic asthma **No** **Yes**	 35.7 28.8	 64.3 71.2	0.516	1.373 (0.527-3.572; 0.516)
Total	30.3 (n=27)	69.7 (n=62)	–	–

CrossTabs comparative analysis and statistic significance of Pearson χ^2 (Chi-square p-value) function regarding the distribution of allergic asthma cases. Risc score for allergic asthma expressed as Odds ratio (OR), confidence interval 95% (CI) and statistic significance (p-value). Threshold for statistic significance 5 % ($p < 0.05$).

Aims and objectives

The aim of the study was to assess the correlation between FENO level and the values of spirometric parameters defining airflow obstruction. Previous studies that have utilized FENO as a reference parameter for the evaluation of bronchial inflammation and investigated the correlation between FENO and other inflammatory markers reported positive results.

Materials and methods

Our study included a group of 89 children previously diagnosed with asthma with ages between 5 and 18. The study was performed in the Pediatric Clinic of "Grigore Alexandrescu" Emergency Clinical Hospital for Children from January 2013 to January 2014 with the approval of the Medical Ethical Commission of the hospital. Every legal guardian of each child included in the study signed the informed consent.

The inclusion criteria were: (i) a previously known and documented diagnosis of asthma, (ii) age range between 5 and 18 years old. The patients were divided into two subgroups according to their age. Therefore we evaluated one subgroup comprised of the children between the ages of 5-12 years old, and another one comprised of the children between the ages of 13-18 years old.

All patients underwent pulmonary function testing and measurement of FENO. Measurement of FENO was performed 15 minutes prior to spirometry because it has been demonstrated that the value of FENO can be lower when it is preceded by spirometry. The exclusion criteria were: (i) poor compliance and incorrect technique during pulmonary function testing and measurement of FENO; (ii) acute upper respiratory infection at the time of examination.

FENO measurement was performed with a NOBreath FENO monitor which measures the exhaled nitric oxide through the chemiluminescence method. The values obtained were interpreted according to reference tables established for age and sex. For pulmonary function testing we have used the Draeger and Pony FX spirometers. The results were calculated according to age and anthropometric parameters (weight, height).

Statistical analysis

Statistical analysis was performed using the statistical software SPSS for Windows 21.0 (IBM SPSS, IBM Ireland, Dublin, Ireland). The normal distributed quantitative variables (functional, biochemical and immunological) were expressed as means ± standard error of mean (SEM) and analyzed by using the Student T-test or Oneway Anova analysis with LSD Post-hoc test. Crosstabs distribution with chi-squared (χ2) analysis was performed to evaluate the differences between logistic data (age distribution, diagnosis). Parametric bivariate correlation analysis of FENO with spirometric parameters was performed using the Pearson's function and corresponding p-value. Multiple linear regression models of the FENO levels (as dependent variable) with spirometric functional parameters (as independent variables) were estimated and the significant adjusted R-squared estimates were used as a prediction score for the FENO variance (with checking for the model correction by using F-function score and Durbin-Watson analysis). Binary logistic regression (LR) analysis with the enter iteration method for the discrimination between obstructive dysfunction was done using the group without obstructive dysfunction considered as reference category and obstructive dysfunction group as risk category, while FENO (ppb), FEV1 (%), age group, gender, weight, height were introduced in the LR model as covariates. The threshold for statistical significance was set to 5% (p values lower than 0.05).

Results

The distribution of obstructive dysfunction among the 89 children does not statistically differ according to age group or the presence of allergic asthma ($p>0.05$, Table 1). However, a significant contribution has the distribution of obstructive dysfunction cases according to the age group. Therefore, 73.6% of the patients from the subgroup of 5-12 years old had obstructive dysfunction, while 52.9% from the other subgroup had obstructive dysfunction. This last distribution is not statistically significant, perhaps due to the relatively low number of cases.

Table 2 Mean values of measured parameteres in the two subgroups of patients studied

Parameter	Obstructive dysfunction	Mean ± SEM	p-value
FENO (ppb)	NO	30,00 ± 8,11	0,240
	YES	19,24 ± 3,90	
	Total (n=87)	22,33 ± 3,63	–
VEMS (FEV1) (%)	NO	100,23 ± 3,418,11	< 0,001***
	YES	68,39 ± 3,07	
	Total (n=88)	77,89 ± 2,85	–
FVC (%)	NO	94,34 ± 3,40	< 0,001***
	YES	56,18 ± 3,10	
	Total (n=83)	68,14 ± 3,07	–
MEF75 (%)	NO	99,14 ± 4,81	< 0,001***
	YES	53,23 ± 2,13	
	Total (n=88)	66,79 ± 3,04	–
MEF50 (%)	NO	97,10 ± 5,68	< 0,001***
	YES	50,98 ± 2,13	
	Total (n=87)	64,61 ± 3,17	–
MEF25 (%)	NO	104,51 ± 8,29	< 0,001***
	YES	52,13 ± 2,44	
	Total (n=88)	67,61 ± 3,92	–
PEF (%)	NO	99,07 ± 4,14	< 0,001***
	YES	53,22 ± 2,21	
	Total (n=87)	66,40 ± 2,97	–

Mean values and mean standard error deviation(SEM) of analyzed parameters, the evaluation of statistical difference with Student T-test function (threshold $p<0.05$), after analysis of variances equalities (Levene test). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

The mean values of FENO does not differ depending on the diagnosis obstructive dysfunction, nor on the distribution of group age, probably because of the large dispersion of values, a consequence of the heterogeneity of subjects or of other functional factors ($p>0.05$, Table 2). It should be noted that the 6 spirometric parameters statistically differ in the age subgroups, taking into account the diagnosis of obstructive dysfunction ($p<0.001$, *data not shown*). Therefore an advanced multiparametric analysis of FENO in correlation with spirometric should be adjusted according to age.

FENO values statistically significant correlates only with a part of spirometric parameters: MEF75, MEF50, MEF25 and PEF ($p<0.05$, Table 3) and not with FEV1, nor FVC. In addition, FENO positively correlates (or directly proportional) and is statistically significant ($r=0.203$, p-0.042, see Table 3).

The analysis of correlations according to age subgroups revealed that statistically significant FENO correlates with the same spirometric parameters MEF75, MEF50, MEF25 and PEF only in the 13-18 years old subgroup (Table 3) and not with the age, FEV1 and FVC, probably because of a more precise appreciation of this parameter in teenagers, in comparison with children aged between 5 and 12 years old.

The use as an independent biomarker of FENO for obstructive dysfunction can be estimated on the basis of the variation of other associated parameters used for diagnosis. Multiple linear regression analysis (multiparametric) estimates, according to the R-squared variable, that a variation of 6.2% of FENO is predicted by a corresponding variation of spirometric parameters ($p=0.0082$, Table 4). If the age and sex of the patients is taken into account (model 2 in Table 4), then a statistically significant prediction of the FENO variation is estimated with 9.2% ($p=0.047$). As a result, FENO variation is scarcely estimated by the spirometric values, even though it correlates with some of them.

The risk for obstructive dysfunction can be statistically estimated with the binary logistic regression analysis which takes into account parameters with normal distribution (Gaussian) and binary variables (discrete such as sex, age group or other secondary diagnostics). This analysis reveals that FENO is not a significant

Table 3 Statistical correlations estimated between FENO (ppb) and spirometric parameters measured in the subgroups of patients included

Parameter	R	p-value
Age (years)	**0.203***	0.042
Age subgroup		
5-12 years	0.106	0.342
13-18 years	-0.175	0.486
FEV1 (%)	0.177	0.090
Age subgroup		
5-12 years	-0.001	0.992
13-18 years	0.456	0.088
FVC (%)	0.110	0.308
Age subgroup		
5-12 years	-0.095	0.425
13-18 years	0.290	0.294
MEF75 (%)	**0.278****	0.007
Age subgroup		
5-12 years	0.003	0.982
13-18 years	0.621*	0.014
MEF50 (%)	**0.227***	0.028
Age subgroup		
5-12 years	-0.037	0.747
13-18 years	0.619*	0.014
MEF25 (%)	**0.262***	0.011
Age subgroup		
5-12 years	-0.043	0.705
13-18 years	0.668**	0.006
PEF (%)	**0.242***	0.020
Age subgroups		
5-12 years	-0.007	0.953
13-18 years	0.550*	0.034

Parametric correlations Pearson (R, 2-tailed) and statistical significance (p-value, threshold 0.05). * $p<0.05$, ** $p<0.01$.

Table 4 Multiple linear regression for the prediction of FENO variation depending on the variation of spirometric parameters measured in the subgroups

Multiple linear regression[1]	F score	Adjusted R squared	p-value
Model 1[a]	1.953	0.062	0.082
Model 2[b]	2.091	0.092	0.047*

1. Dependent variable: FENO (ppb) (n=87). * $p<0.05$; **a.** Independent variables (predictors): FEV1, FVC, MEF75, MEF50, MEF25, PEF;
b. Independent variables (predictors): FEV1, FVC, MEF75, MEF50, MEF25, PEF, sex, age

biomarker ($p>0.05$) for the risk of obstructive dysfunction, even though its variation can correctly predict more than 72% of cases, a good, but not a strong score (model no 1, Table no 5). Therefore, FENO can be used as a significant predictive biomarker only together with one spirometric parameter FEV1 (with which it does not correlate individually but independently) in almost 80% of the cases ($p<0.001$, model 2 in Table 5). Taking into account other parameters like age, sex, weight, height improves the prediction up to more than 82%. Among these covariables only FEV1 is an independent significant predictor ($p=0.001$), the other parameters not having a significant contribution to the estimation of obstructive dysfunction risk (Table 5).

Table 5 Binary logistic regression for the diagnosis of obstructive dysfunction based on FENO variation and spirometric parameters in the two subgroups of patients

Binary logistic regression[1]	Prediction obstructive dysfunction (%)	Chi-square step	p-value step	Chi-square model	p-value model
Model 1[a]	72.1	–	–	1.895	0.169
Model 2[b]	79.1	32.491	< 0.001***	34.387	< 0.001***
Model 3[c]	82.5	4.137	0.368	33.503	< 0.001***

1. Dependent variable: obstructive dysfunction. Chi-square test for parameters coefficients (covariate) in model (B). * p<0.05, ** p<0.01, *** p<0.001.

a. Covariable (predictors): FENO (ppb); **b.** Covariable (predictors): FENO (ppb), FEV1 (%); **c.** Covariable (predictors): FENO (ppb), FEV1 (%), age group, sex, weight, height.

Discussions

The role of FENO in the pathology of pediatric asthma is intensely researched. Even though the number of studies conducted up until now are insufficient, researchers from numerous academic centers have outlined the benefits of FENO measurement in the diagnosis and monitoring of airway inflammation.

N.Gupta et al. have performed a study on a group of patients between the ages of 6 and 38 years old in which the authors investigated FENO variation depending on FEV1 and FVC. They have revealed that there is no correlation between the value of exhaled nitric oxide and the values of the spirometric parameters[3]. Our results are consistent with these findings.

FENO values can vary according to a wide variety of factors, including spirometry. A number of studies recommend that measurement of FENO should be performed before spirometry[4]. Other researchers recommend the measurement of exhaled nitric oxide before and after the spirometry examination, when obstruction is improved after bronchodilator administration.

FENO could be used for the diagnosis of asthma when the diagnosis is not confirmed[5]. Even though pulmonary function testing represents the gold standard for objective and precise evalution and monitoring of the disease, FENO could significantly improve the guideline for monitoring. In addition it could be regarded as an additional tool which could safely be included in the clinical guidelines, thus improving the quality of life of asthmatic patients[6]. The same study recommends FENO as diagnostic and monitoring tool for pediatric asthma[6].

The correlations outlined in our study between FENO and spirometric values (FENO and MEF 25-75) are consistent with del Guidice et al.[7] findings. The significant correlations were obtained between FENO and FEV1 ($p<0.0059$, $r0=0.468$). Currently, the scientific community does not explain why FENO correlates only with the spirometric parameters which describe the airflow obstruction present in the small airways. Therefore, further studies are critical for the elucidation of these hypotheses.

Conclusions

1. Along with functional respiratory testing, FENO measurement can be used for establishing the diagnosis for asthma.

2. In our study, even though FENO does not correlate with FEV1, nor FVC, it statistically correlates very well with MEF 25 and PEF.

3. FENO can successfully predict the risk for obstructive dysfunction only with a single spirometric parameter FEV1 (with which it correlates only independently) in almost 80% of cases ($p<0.001$)

4. The results published in the scientific literature convey the necessity for peforming more studies in the pediatric population, concerning the evaluation of these parameters. ■

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