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ORIGINAL PAPER

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Receiver operating characteristic analysis of the FeNO biomarker in the diagnosis of asthma

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Abstract

Introduction. The fraction of exhaled nitric oxide (FeNO) is used as a non-invasive biomarker that reflects inflammation in the airways. It is so versatile that it used to control asthma severity as well as to monitor response to treatment. However, the exact cut-off point of the nitric oxide level which allows one to make a precise diagnosis of asthma is unclear.

Aim. To examine the possibility of using advanced statistical methods such as receiver operating characteristic for the analysis of FeNO concentrations for improving the diagnosis of asthma.

Materials and methods. Receiver operating characteristic (ROC) was used for analyzing results to determine levels of nitric oxide which may be a prognostic indicator of asthma. The studied group consisted of 111 children including 69 asthmatic patients, and 42 age- and sex-matched healthy subjects. Measurement of exhaled nitric oxide was conducted in all subjects included in this study.

Results. FeNO level was higher in asthmatic patients. The analysis of results showed that the cut-off point for the FeNO concentration is 11.5 ppb. Sensitivity and specificity with the FeNO level allowed us to determine a value of the diagnostic variable of FeNO concentration of 14.0 ppb. A comparison of FeNO level and sex of the subjects showed there is no correlation between these parameters of patients.

Conclusions. Currently, the FeNO measurement provides complementary data in the care of a patient suffering from asthma, however analysis of more studies on a larger group of patients is needed.

Keywords. asthma, nitric oxide, ROC curve

Introduction

The problem of classifying data relevant to the diagnosis of diseases are often found in medicine. Establishing a correct diagnosis requires extensive medical knowledge and experience. Currently, it is helpful to use forecasting models based on advanced statistical analysis methods. Properly defined models show complex relationships and explain observed trends. Using advanced statistical

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analysis may be a basis for the diagnosis of many diseases including asthma based on non-invasive as well as invasive procedures. A series of tests are used to interpret the data and created models, adjusting their selection to the problems posed.[1] In human physiology, nitric oxide (fraction of exhaled nitric oxide, FeNO) was initially described as an endothelial diastolic agent which is able to modulate coronary artery tension.[2] In 1991, Gustafsson et al. discovered nitric oxide in exhaled air in humans, which aroused interest in its role in respiratory diseases.[3] Subsequently, it was confirmed that in persons suffering from asthma, the concentration of nitric oxide in exhaled air is higher than in control groups.[4] The results of the studies had also given the basis for the description of the effect of glucocorticosteroids on the reduction of FeNO, which confirmed the endogenous origin of nitric oxide and suggested its role in the underlying asthma and airway inflammation.[5,6] Currently, FeNO is used as a non-invasive biomarker for detection of inflammation if the airways. It is so versatile that it can be used successfully to evaluate asthma severity and assess response to treatment. The precise level of FeNO in the asthma management algorithm has not yet been established. However there is indisputable data on the usefulness of FeNO measurements in the diagnosis of asthma, in the assessment of its control and severity, and in the selection of inhaled glucocorticoid doses and the detection of the presence of inflammation in the airways.

The aim of the article is to examine the possibility of using advanced statistical methods for the analysis of FeNO concentrations which may be helpful in the diagnosis of asthma. In particular, the cut-off point was determined and the specificity and significance were calculated. In addition, we evaluate the influence of patient sex on the level of FeNO. The obtained results were compared with previous literature data.

Method of FeNO measurement and interpetation, factors affecting the concentration of FeNO

FeNO measurements were carried out using direct techniques using a real-time analyzer. This method is based on observations from 1997 where it was noted that the FeNO level is inversely proportional to the value of expiratory flow. This phenomenon is described as the dependence of concentration on flow. A high flow correlates with low FeNO levels and the low flow again is associated with a higher FeNO value. The dependence on flow is explained by the fact that nitric oxide appears in exhaled air as a result of diffusion occurring in the airways.[7,8]

Based on concentration-flow dependence and using standardized measurement techniques, the FeNO value can be used as a diagnostic tool. The position of the American Thoracic Society and the European Respiratory Society (ATS/ERS) standardizes FeNO measurements for a flow of 50 ml/s.[9] In addition to the flow rate, FeNO may be influenced by other factors, such as age height and gender.[10,11]

It should be mentioned that a low concentration of FeNO cannot exclude asthma in children.[9]

The American Society for Thoracic Diseases recommends, among others:

- use of the measurement of exhaled nitric oxide in the diagnosis of eosinophilic bronchitis
- use of FeNO in determining the likelihood of responding to glucocorticoid treatment in patients with chronic inflammatory symptoms of the respiratory tract
- use of FeNO in monitoring of airway inflammation in patients with asthma
- interpretation of FeNO concentration < 20 ppb in children as unlikely eosinophilic inflammation and poor response to glucocorticoid treatment
- interpretation of FeNO > 35 ppb in children as indicative of eosinophilic inflammation and a good response to treatment with glucocorticosteroids in symptomatic patients
- interpretation of FeNO concentrations between 20-35 ppb in children based on the clinical situation.[9,12]

Receiver characteristic operating curve

A receiver operating characteristic curve, (ROC curve), is a graphical illustration and fundamental tool used for the evolution and comparison of diagnostic systems that enable comparisons of sensitivity and specificity.

The true-positive rate is also known as sensitivity, recall or probability of detection in machine learning. The false-positive rate is also known as the fall-out or probability of false alarm and can be calculated as (1 – specificity).[13] The ROC methodology was first used in medicine in the late 1960s after its development in 1950s. Currently ROC is widely used in medicine and psychology, radiology, biometrics, meteorology, and other scientific areas for many years.[14-17] The ROC is also known as a relative operating characteristic curve, because it is a comparison of two operating characteristics as the criterion changes.

Materials and methods

The research group consisted of children aged 6 to 14 years living in the Podkarpackie Province, which are covered by medical care in Hospital No. 2 in Rzeszów. The study group consisted of 111 children, 42 of including healthy subjects and 69 asthmatic patients, who were diagnosed according to standards. Generally 44 girls and 67 boys were examined, and their characteristics are presented in the Table 1. The level of nitric oxide was measured in all subjects included in this study by using Hyp'Air FeNO (MediSoft, Belgium) according to guidelines of ATS/ERS in September 2018.[9]

Table 1. Characteristics of the studied group (mean ± SD)

	n	Sex (F/M)	Age (years)	Height (cm)	Weight (kg)	BMI
healthy control	42	19/23	9.26 ± 3.18	139.32± 16.45	36.88 ± 11.57	18.72 ± 3.93
asthma	69	25/44	10.28 ± 3.74	144.34± 20.51	41.19 ± 17.08	18.91 ± 3.85

The study was approved by local Bioethical Commission.

In the statistical analysis, multivariate logistic regression analysis was performed to identify clinical features associated with asthma. In addition to the assessment of diagnostic accuracy, the sensitivity, specificity and predictive values were calculated, and the area under the curve (AUC) was calculated for the FeNO values. The statistical analysis of the data was performed using PQStat (version 1.6.6, PQStat Software, Poznań, Poland https://pqstat.pl/).

Results

The analysis showed that there is a significant correlation between the level of FeNO in the exhaled air and the diagnosed disease entity (p-value <0.001). The AUC is 0.879. Detailed data is given in Table 2.

Table 2. ROC Curve Analysis

ROC Curve Analysis	
Analysed variables	FeNO [bbp];disease entity
Number of unspecified	0
Number of missing data	0
Significance level	0.05
Size	111
Size STATE + (1)	69
Size STATE - (0)	42
Direction of diagnostic variable	stimulant
Prevalence	0.621622
-95% CI	0.524625
+95% CI	0.711957
DeLong's method	
AUC	0.879572
SE(AUC)	0.032277
-95% CI	0.816311
+95% CI	0.942833
Z statistic	6.688444
p-value	<0.000001
For cut-off	
Cost FN - wrong diagnosis	1
Cost FP - wrong diagnosis	1

The analysis allowed to determine the cut-off point for the concentration of 11.5 ppb FeNO yielding sensitivity and relevance, respectively, at the level of 0.928/0.643 as presented in Figure 1. Sensitivity and specificity in correlation with the FeNO concentration was used to determine a value of the diagnostic variable of 14.0 ppb FeNO concentration as shown in Figure 2.

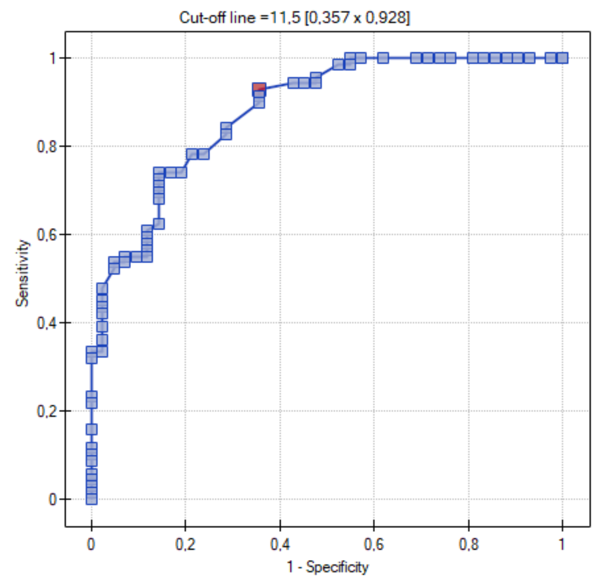

Fig. 1. ROC curve – determination of cut-off point

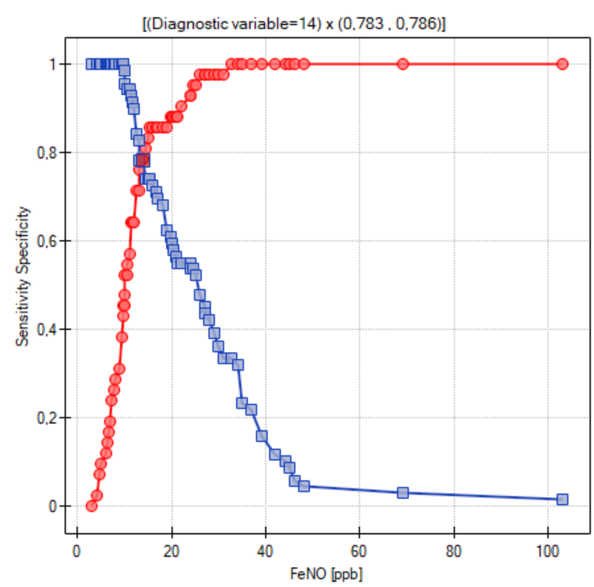

Fig. 2. ROC curve – determination of diagnostic variable

A comparison of the age of the subjects with disease showed that occurrence of asthma does not correlate with age of patients. The distribution curve presents typically a random character. Detailed data is presented in Table 3 and Figure 3.

Table 3. Dependent ROC curves – comparison of age and occurrence of the asthma

Dependent ROC curves - comparison	
Analyzed variables	FeNO [bbp];age;disease entity
Number of unspecified	0
Number of missing data	0
Significance level	0.05
Grouping variable	disease entity
Size	111
Size STATE + (1)	69
Size STATE - (0)	42
Variable age	
Direction of diagnostic variable	stimulant
AUC	0.584886
SE(AUC)	0.055132
-95% CI	0.476829
+95% CI	0.692943

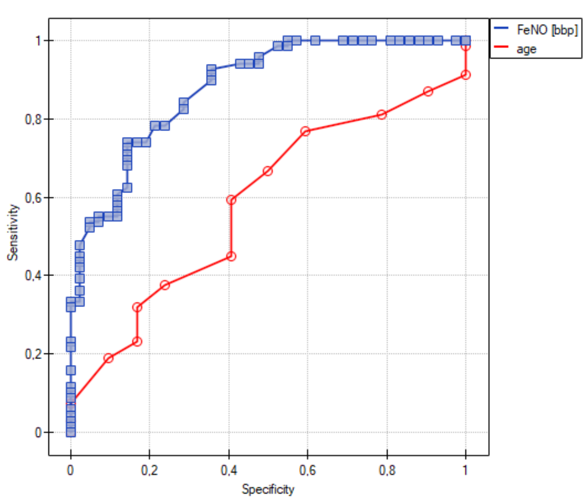

Fig. 3. Dependent ROC curves – comparison of age and occurrence of the asthma

The gender of patients does not have a significant impact on the measurement results. The analysis showed that there is no significant correlation between FeNO concentration in exhaled air and gender (p-value 0.076). It is worth noting that the results of the boy's measurements were lower by an average of 11%. Detailed data is provided in Table 4 and Figure 4.

The FeNO cut-off value is 11.5 ppb (AUC 0.879, p <0.001) and this showed the best combination of sensitivity and specificity for the positive reaction for the whole group. The FeNO cut-off value of 32.6 ppb showed a specificity of 100%. The negative prognostic level with high sensitivity and negative predictive value was not determined. With the use of logistic regression, no significant correlation between the results obtained by boys and girls was confirmed. Currently, the FeNO measurement provides complementary data in the care of a patient suffering from asthma.

Table 4. Independent ROC curves – comparison of patients' sex and FeNO level

Independent ROC curves - comparison	
Analyzed variables	FeNO [bbp];disease entity
Number of unspecified	0
Number of missing data	0
Significance level	0.05
Grouping variable	sex
Direction of diagnostic variable	stimulant
Method	DeLong
Group name	F
Size	44
Size STATE + (1)	25
Size STATE - (0)	19
AUC	0.944211
SE(AUC)	0.031646
-95% CI	0.882185
+95% CI	1
Group name	M
Size	67
Size STATE + (1)	44
Size STATE - (0)	23
AUC	0.837451
SE(AUC)	0.051184
-95% CI	0.737132
+95% CI	0.937769
AUC1-AUC2	0.10676
SE(AUC1-AUC2)	0.060177
Z statistic	1.774102
p-value	0.076046

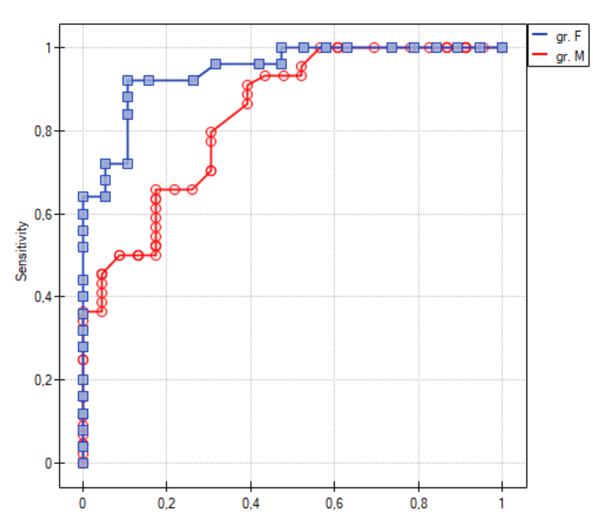

Fig. 4. Independent ROC curves – comparison of patients' sex and FeNO level

Discussion

Measurements of nitric oxide in exhaled air is considered as a parameter which provides complementary data in the care of patients suffering from asthma.

Currently, there is no significant value of FeNO which is a precise indicator of asthma. The analysis of the usefulness of nitric oxide concentration in patients with asthma as a marker of inflammation that helps in making clinical decisions has been studied and discussed in many studies. The conclusions are ambiguous; some studies have shown a close correlation between FeNO and the degree of bronchial hyperresponsiveness and others have presented only a partial correlation with no statistical significance. On the other hand, some researches have questioned the usefulness of this method in making clinical decisions. Paraskakis et al. determined FeNO levels at different expiratory flow rates in 132 children. Based on the assessment of follicular nitric oxide using a two-compartment model, they confirmed the presence of inflammatory process in small airways.[18]

In the present study, the relationship between FeNO level in children with asthma and results in healthy children was demonstrated. We have found that FeNO level was significantly higher in asthmatic patients as compared to healthy subjects.

Similar results were revealed by Silvestri et al. In their study, although the FeNO concentration in the group of children with asthma (mean 30 ppb) was higher than in the control group (mean 4.1 ppb), FeNO did not correlate with spirometric parameters.[19] Moreover, Olin et al. determined the average value of FeNO in healthy people as well as asthmatic patients. The average level of FeNO was 15.8 ppb (range between 11.4 – 22.4) in control group, while in patients with asthma FeNO level was 22.5 (range between 16 – 31.2).[20] Afterwards, Buchvald et al. studied healthy children aged 4-17. The geometric mean for FeNO from measurements made in 405 children was 9.7 ppb.[21]

Moreover, a meta-analysis of 4,691 patients with asthma conducted by Li et al. demonstrated that measurement of FeNO showed a diagnostic sensitivity of 0.78.[22] Wang et al. demonstrated that the optimal cut-off value of FeNO was 19.5 ppb for the diagnosis of typical bronchial asthma, while our results have indicated that the cut-off point is 11.5 ppb.[23] A higher cut-off point of 35.5 ppb was determined by Gao & Wu in 75 adult uncontrolled asthmatic patients and was considered as a predictor of sputum eosinophilia.[24]

Buchvald et al. also showed that the FeNO level was higher with age of tested subjects.[21] Literature data indicate that in adults there is no consistent relationship between FeNO and age, but there are reports that in children the FeNO concentration increases with age.[25,26] Negative correlation between FeNO and age as well as BMI in patients with asthma was also found by Rawy & Mansour.[27] Our research has not confirmed a correlation between the FeNO level and sex of the examined children. Obtained results and their analysis have showed random distribution of results. It seems that the FeNO values should be higher in boys than girls, but there are conflicting results on this subject.[28] Nevertheless, some researchers notice that measurements of nitric oxide cannot be routinely recommended for all children with asthma.[29]

In summary, studies indicate that measurements of nitric oxide in the lower respiratory tract may be helpful in the diagnosis of respiratory diseases, including asthma. However, more studies of large group of patients are required to set precise points of FeNO which will be indicator of various respiratory diseases. Currently, measurements of nitric oxide may be only useful in combination with clinical outcomes and traditional respiratory tests.

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