RELATIONSHIP BETWEEN FRACTION OF EXHALED NITRIC OXIDE AND SPIROMETRY PARAMETERS IN ASTHMA

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RELATIONSHIP BETWEEN FRACTION OF EXHALED NITRIC OXIDE AND SPIROMETRY PARAMETERS IN ASTHMA (Abstract): **Aim:** Asthma is a chronic airway inflammatory disease with significant social and economic impacts, being associated with a high annual rate of premature deaths. Asthma severity and response to inhaled corticosteroids therapy can be assessed by spirometry parameters and fractional exhaled nitric oxide (FeNO) evaluation. The aim of this study was to evaluate the relationship between spirometry parameters and FeNO values in a group of asthmatic patients. **Materials and methods:** We performed a retrospective analysis on 124 patients with history of asthma of different degrees of severity. All patients have been classified according to the last Global Initiative for Asthma (GINA) criteria and had a complete spirometry and a FeNO measurement. **Results:** The analyzed cases showed a female predominance (62.9%) and a mean patient age of 50.6±16.0 years. A significant association between Forced Expiratory Volume in one second (FEV1) values ($F = 231.167$, $p < 0.001$) or FEV1/FCV ratio ($F = 52.630$, $p < 0.001$) and the degree of asthma control was observed in all patients, regardless of inhaled corticosteroids administration. An inverse correlation between FEV1 and FeNO values ($r = -0.52$, $p < 0.01$) was registered. **Conclusions:** Spirometry parameters and FeNO represent valuable tools in the management of asthma, with higher FeNO values being associated with impaired lung function. **Keywords:** ASTHMA, FEV1, FCV, FeNO.

INTRODUCTION

Asthma is a chronic respiratory disease characterized by wheezing, chest tightness, shortness of breath and cough, with a variable incidence in the population, between 1% and 29%, in different geographical areas (1). According to the latest Global Asthma Report, asthma affects 9.1% of children and 6.6% of the adult population worldwide (2). Due to its continuous increasing incidence in recent decades, asthma is considered a global health problem with impact on the quality of life and is responsible for over 450,000 deaths each year (3). Epidemiological studies on asthma estimate an increase in its incidence in the population worldwide, affecting approximately 520 cases per 100,000 people by 2050 (4).

Mainly produced by epithelial cells, endothelial cells, and neurons in the lung, NO promotes ciliary motility and surfactant secretion, acting also as an airway bronchodilator factor (5-7). However, NO may also be generated by inducible nitric oxide synthase (iNOS) in lung, supporting Th2 lymphocyte differentiation and inhibiting Th1 and Th17 Ly activity in the bronchial mucosa, reflecting its chronic inflammatory status that lead to bronchial epithelial lesions, goblet cell hyperplasia, increased vascular permeability and eosinophilia (6, 7). Thus, NO plays a dual role, as it can also act as a potent pro-inflammatory factor that supports the airway hyper-responsiveness in T2 asthma (6, 8).

Although the role of NO is still controversial, the evaluation of the fractional exhaled nitric oxide (FeNO) may be an adjunct to diagnostic tools used to assess the type 2 airway inflammation in asthma. Moreover, the addition of FeNO to blood eosinophil counts has been considered as a valuable marker for the assessment eosinophilic bronchial mucosa inflammation in the asthmatic patients (7). Last but not least, FeNO measurement is a non-invasive test with increased compliance in adult and pediatric population, its increased values being correlated with lower lung function, limited response to inhaled corticosteroids, and higher risk for asthma exacerbations (4).

In this context, our study aims to evaluate the association between FeNO values and spirometry parameters in a group of asthmatic patients.

MATERIALS AND METODS

The study group consisted of 124 asthmatic patients undergoing inhaled corticosteroid therapy, diagnosed in the Specialty Ambulatory of the Pneumology Clinic Hospital, Iasi, Romania. Clinical data was anonymously collected from the medical records without active patient participation, in accordance with the principles of the Declaration of Helsinki. Participants were included in the study group based on informed consent and a set of clearly defined inclusion criteria, including age over 18 years, non-smokers (total non-smoker or former occasional smoker), absence of other chronic or acute lung diseases, and the capacity to adequately and correctly perform a spirometry test. The exclusion criteria in the study group were smokers, inability to adequately and correctly perform spirometry tests, and patients with other chronic or acute lung diseases. The FeNO level assessment was performed using the NIOX® device, in accordance with the manufacturer's recommendations. A FeNO value $\geq$ 25 ppb in patients with a medium dose of inhaled corticosteroid therapy and a FeNO value > 20 ppb in patients with a high dose of inhaled corticosteroid therapy were considered pathological according to current guidelines (3). Spirometry test has been performed after FeNO level assessment, by using Vitalograph Alpha Touch after a minimum six-hour withdrawal from a short-acting inhaled beta-agonist, in agreement with the American Thoracic Society recommendations (9). The test was repeated three times and the best spirometry indices values were recorded (9). Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and FEV1/FVC ratio were registered for each patient. A gender, age and height-wise comparison of spirometry indices was done, being automatically calculated by the device. Airway obstruction was defined as an FEV_1/FVC ratio below the lower normal limit (LLN; Z-score < −1.64).

Statistical analysis has been performed

using *SPSS version 25* (IBM, Armonk, NY, USA) and *Microsoft Excel* 2016. Means and standard deviations were computed for continuous variables. Differences in FeNO levels between patient subgroups were analyzed using ANOVA and Pearson correlation tests. A p-value <0.05 was considered as statistically significant.

RESULTS

The predominant female gender was registered in our study group, with 78 cases (62.9%), compared to 46 male cases (37.1%), with a women-to-men ratio of 1.69:1. The most patients were from an urban area (81 of cases; 65.32%) and only 43 patients (34.68%) from the rural area. The mean patients' age was 50.6±16.0 years, showing relatively similar gender ranges, with 51.5±16.4 years in men and 50.1±15.9 years in women. The youngest patient included in the study was 19 years old, while the oldest was 83 years old, and the highest number of cases was observed in the 50-69 age group (28.2%) followed by 60-70 age group (20.2%) (fig. 1).

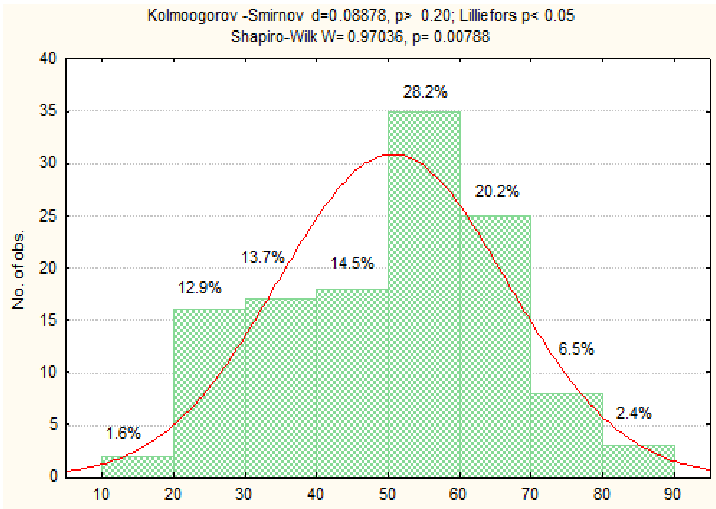

Fig. 1. Age group distribution of the asthmatic patients

Following clinical examination and respiratory exploratory tests, the patients have been included into the following stages: 62 cases (50%) of usual well-controlled (intermittent/mild) asthma, 36 cases (29.03%) of partially controlled (moderate) asthma, and 26 cases (20.97%) of uncontrolled (severe) asthma.

The mean value of FEV1 (% of predicted) for the study group was 78.2%, ranging between 24.1% and 147%, the lowest mean values being registered in the uncontrolled (severe) asthma cases (45.9±9.4), with a minim 24.1% and maximum values of 55.9%. FEV1/FVC ratio had the lowest mean values in uncontrolled asthma patients (55.9±8.0) and the highest values in well-controlled asthma cases (77.6±7.5) (tab. 1). Statistical analysis showed a statistically significant association between asthma severity levels and FEV1 ($F = 231.167$, $p < 0.001$) or between asthma severity and FEV1/FCV ratio ($F = 52.630$, $p < 0.001$).

TABLE I.
Patients' distribution according to FEV1 values (% of predicted) and FEV1/FVC ratio (%) in the study group

Bronchial asthma	FEV1		FEV1/FVC ratio	
	mean ±SD	minimum; maximum	mean ±SD	minimum; maximum
Well-controlled (intermittent/mild)	99.75±10.7	80.3; 147.0	77.6±7.5	59.4; 91.5
Partially controlled (moderate)	68.8±6.3	59.2; 79.8	66.4±7.0	51.2; 78.0
Uncontrolled (severe)	45.9±9.4	24.1; 55.9	55.9±8.0	39.6; 69.2

FEV1 - forced expiratory volume in one second; FVC - forced vital capacity; SD - standard deviation

Asthma duration ranged from 1 to 40 years among the study group patients (Figure 2). The mean asthma duration was 13.1±9.6 years in uncontrolled (severe) asthma, 12.9±11.1 years in partially controlled (moderate) asthma, and 8.25±8.6 years in usual well-controlled (intermittent/mild) asthma.

Most of the asthmatic patients (79 of cases; 63.7%) displayed FeNO values ranging between 20-60 ppb, while 41 patients (33.06%) had FeNO values between 20-40 ppb. A significant FeNO increased value (> 60ppb) has been registered in 31 asthmatic patients (25%) (tab. II).

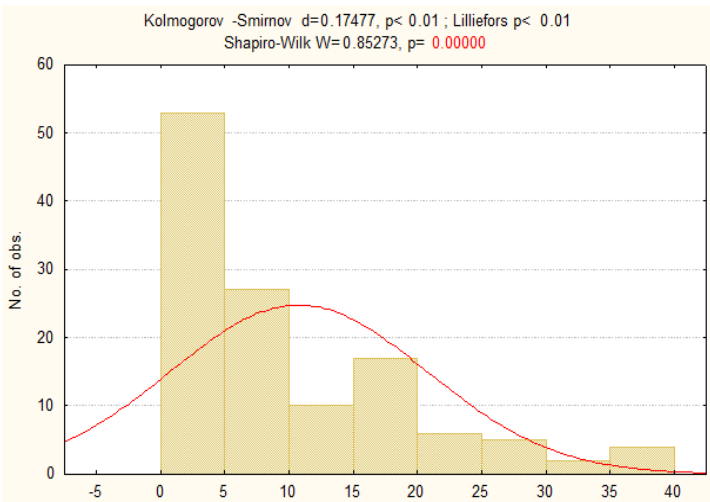

Fig. 2. Distribution of the asthma duration (in years) in the study group

The highest FeNO value in patients with uncontrolled asthma was 126 ppb, while it was only 75 ppb in patients with partially controlled asthma. The lowest FeNO value of 6 ppb was observed in a patient with well-controlled asthma. The mean FeNO value increased progressively with disease severity, being 33.75±17.2 in well-controlled asthma, 43.1±14.9 in partially controlled asthma, and 86.1±22.7 in uncontrolled asthma. Statistical analysis showed a statistically significant association between FeNO levels and asthma severity ($F = 43.905$, $p < 0.001$).

TABLE II.
FeNO values distribution in the study group

	Number of cases	%
0<FENO<=20	14	11.29%
20< FENO <=40	41	33.06%
40< FENO <=60	38	30.65%
60< FENO <=80	11	8.87%
80< FENO <=100	12	9.68%
100< FENO <=120	4	3.23%
120< FENO <=140	4	3.23%
Total	124	

Pearson correlation analysis demonstrated a moderate inverse correlation between FEV1 and FeNO values (r = −0.52, $p < 0.01$, 95% CI) (fig. 3).

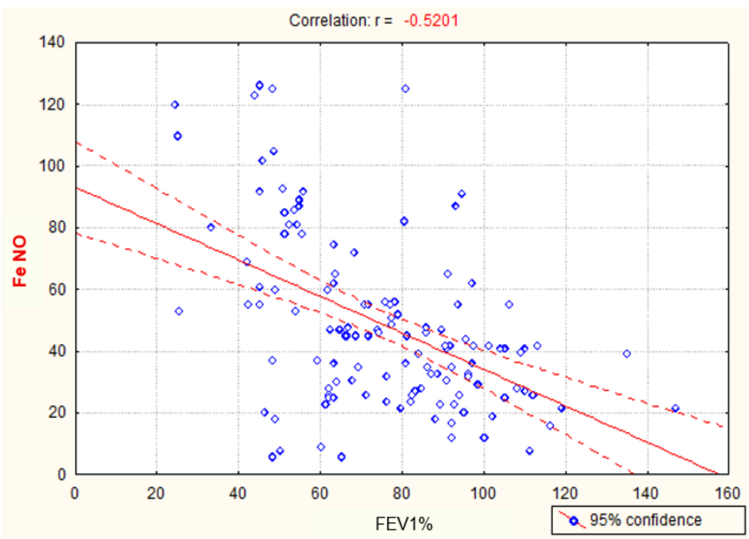

Fig. 3. Scatter plot with regression line showing the correlation between FEV1 and FeNO

DISCUSSION

Although substantial advances have been achieved in the management of bronchial asthma, a significant improvement in patient's quality of life has not been yet demonstrated, despite the implementation of recent standardizations and clinical guideline and the availability of effective therapies (9).

This study was conducted on a group of asthmatic patients diagnosed under background treatment. The study group was predominantly female, a finding that aligns with global epidemiological data, which suggest a higher incidence of asthma in women than in men (9-11). This shift is linked to potentially stronger immune responses in women, smaller airways, and hormonal changes (estrogen and progesterone) affecting airways, leading to more severe symptoms and poorer asthma control in adult women (11). Moreover, estrogens and progesterone were associated with an enhancement of type 2 (T2) airway inflammation by supporting the antigen presentation, Th2 lymphocyte polarization, eosinophils infiltration, and mast cell degranulation (12, 13). Most patients were from urban areas, which may reflect an easier access to medical services and the potential role of the environmental factors (e.g. air pollution), along with unhealthy diet and sedentary life style (11, 14).

GINA emphasizes that asthmatic patients may have a decreased lung ventilation function, expressed by a reduction in FEV1 and FEV1/FVC values. Therefore, spirometry is an important diagnostic tool for asthma management, helping the assessment of the functional status of the patient and guiding the therapy (3). A decrease in FVC and FEV1 values compared to the predicted value was observed in our study, which was significant in patients with partially controlled or uncontrolled asthma. These data are consistent with similar studies that also reported reduced FEV1 and FEV1/FCV values in most asthmatic cases (9, 15). However, low values of these functional parameters could be partially influenced by various factors, such as incorrect inhalation technique, low adherence to treatment, and irregular follow-up visits to medical centers. As a result, additional biomarkers, such as FeNO assessment, may offer supplementary data regarding both diagnosis and disease progression, providing information about the local inflammatory status of the bronchial mucosa (6, 7).

Chronic inflammatory status is a one of the hallmarks of asthma, as in other chronic inflammatory diseases (16-20). According to this criterion, asthma is currently divided into type-2 high, type-2 low, and mixed endotypes (6, 21). Type-2 high asthma is associated with increased eosinophilic airway inflammation and elevated values of inflammatory cytokines (e.g., IL-2, IL-4, IL-5, and IL-13), whereas Type-2 low asthma is characterized by a predominantly neutrophilic inflammation of the bronchial mucosa (6, 22). The mixed asthma includes features of both endotypes (6).

NO secretion in the airway tract may occur *by* enzymatic and non-enzymatic pathways, high FeNO level being correlated with the T2-high (Type 2) asthma (6, 22). The NO enzymatic synthesis is controlled by nitric oxide synthases 1-3 (NOS1-3), while NOS-1 and NOS-3 isoforms are constitutively expressed (6). NOS-2 overexpression is induced by pro-inflammatory cytokines (e.g. TNF-α, IL-4, and IL-13), which are intensely produced in Type 2 asthma (6). Increased NO levels in asthmatic patients may induce bronchial mucosae hyperemia and edema, bronchial hyper-reactivity, decreased apoptosis of inflammatory cells, and mucus hypersecretion in the bronchial epithelium (7). Therefore, FeNO is considered an adjunct biomarker, reflecting underlying airway inflammation. In this context, an increased concentration of FeNO was detected in asthma patients, including well-controlled ones, compared to normal individuals in different studies (23, 24). These data are consistent with our results, when a mean FeNO value of 33.75±17.2 was identified even in mild forms of asthma, its mean values increasing with asthma severity. Additionally, a meta-analysis performed on a large cohort of asthmatic patients showed that FeNO exhibits higher specificity than sensitivity, which supports its role in the diagnosis of asthma (25). Moreover, the combination of spirometry test results, value of symptom intensity scales, and FeNO measurement increased the asthma diagnostic accuracy with area under curve of 0.76 (95% confidence interval 0.66-0.84) in a group of untreated patients with symptoms suggestive of asthma (26).

However, FeNO evaluation remains an additional tool for asthma diagnosis, high FeNO values increasing the probability of asthma diagnosis, while a negative result does not exclude asthma (3). This is attributable to the fact that FeNO level may be influenced by numerous factors, including time of the day, age, gender or allergen exposure. In this regard, FeNO has a higher value in men than in women, in the afternoon than in the early morning or in patients with other allergic diseases (allergic rhinitis or atopic dermatitis), respectively a lower value in patients who smoke, take inhaled corticosteroids or have a reduced lung function (3, 6). Even so, a FeNO > 20 ppb, added to blood and sputum eosinophils is considered the cut-off for Type 2 inflammation of severe asthma, according to GINA criteria, and it is used to assess this asthma phenotype (3, 6). In addition, a FeNO value > 50 ppb in adults is associated with eosinophilic inflammation in bronchial mucosa, feature that is used to predict the anti-inflammatory therapy response. Furthermore, FeNO value added to lung function parameters is a useful tool to predict the future risk of symptoms exacerbation and loss of asthma control in practice (3, 7, 27). Not least, glucocorticoids are used to reduce the eosinophilic inflammation in asthmatic patients, therefore FeNO evaluation has a significant role to predict the corticotherapy response of these patients (28). Regarding FeNO and FEV1, a moderate inverse correlation between these two variables has been identified in our study, which is partially consistent with the literature. Thus, a strong correlation between FeNO and FEV1 have been observed in different asthmatic patients (29-32), while other studies have not reported such an association (33, 34). These contradictory results are most likely due to the heterogeneity of the asthma patients' groups studied so far. Despite its limitations, a refractory Type 2 inflammation in asthma is associated with a FeNO > 20 ppb value in patients with high-dose inhaled corticosteroids and itself has been demonstrated to be the strongest predictor of asthma exacerbation in the same patients, compared to periostin and peripheral blood eosinophils count (7, 35). Thus, alongside spirometry, FeNO is a non-invasive method to assess airway inflammation and a significant tool for the asthma therapy management.

CONCLUSIONS

FeNO evaluation is a cost-effective method that is used to detect Type 2 airway inflammation in asthma.

The highest FeNO levels were observed in patients with uncontrolled asthma compared to partially controlled or well-controlled asthma, and these were negatively correlated with FEV1 and FCV values.

Although its value may be influenced by different factors, FeNO may serve as a bio marker for asthma diagnosis, prediction for lung function decline, evaluation of poor asthma control, future symptoms exacerbation risk, and assessment of response to the inhaled corticosteroid therapy in practice.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no funding regarding this research.

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