Original Article
Fractional exhaled nitric oxide: a comparative study in patients with acute exacerbation of chronic obstructive pulmonary disease and bronchial asthma

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Abstract: Objective: The aim of this study was to investigate the value of fractional exhaled nitric oxide (FeNO) in the differentiation and diagnosis of patients with acute exacerbation (AE) of chronic obstructive pulmonary disease (COPD) and bronchial asthma (BA). Methods: A total of 46 AE-COPD and 48 AE-BA patients were selected during the same period. The levels of FeNO, serum C-reactive protein (CRP), peripheral blood eosinophil ratio (EOS%), and lung function in all patients were measured. Results: The levels of FeNO and EOS% in the AE-COPD patients were significantly lower than those in the AE-BA patients ($P < 0.01$) while the serum CRP level in the AE-COPD patients was significantly higher than that in the AE-BA patients ($P < 0.05$); the lung function indices in the AE-BA patients were superior to those in the AE-COPD patients ($P < 0.05$); the FeNO level in the AE-COPD patients showed no correlation with the lung function indices and EOS% ($P > 0.05$). The FeNO level in the AE-BA patients also showed no correlation with the lung function indices ($P > 0.05$) but did show correlation with EOS% ($r = 0.626$, $P < 0.01$). Receiver operating characteristic (ROC) analysis was used to investigate the diagnostic properties of various parameters. When combining the FeNO, EOS%, and CRP parameters to differentiate between AE-COPD and AE-BA, the area under the curve (AUC) value was 0.947 (95% CI: 0.882-1.000), with a corresponding sensitivity of 86.4% and a specificity of 88.9%, respectively. Conclusions: Assessment of FeNO, EOS%, serum CRP, and lung function indices may help clinicians distinguish between the acute exacerbation of two diseases.

Keywords: Fractional exhaled nitric oxide, chronic obstructive pulmonary disease, bronchial asthma

Introduction

Nitric oxide (NO) is an important gas molecule inside the airway and plays an important role in the relaxation of the blood vessels and bronchi which regulate inflammation and promote mucociliary movement in the airway [1, 2]. Evaluation of fractional exhaled nitric oxide (FeNO) is a technique used in recent years for clinical applications. It is a non-invasive technique characterized by convenience, safety, and good reproducibility. Therefore, FeNO assessments have been widely used for the auxiliary diagnosis of eosinophilic inflammation [3], evaluation of the status of asthma-control [4], determination of the response of asthma to glucocorticoid therapy [5], and prediction of the acute onset of asthma [6].

Chronic obstructive pulmonary disease (COPD) and bronchial asthma (BA) are both chronic obstructive airway diseases; however, they exhibit different pathogeneses and pathological features. Both COPD and BA manifest such symptoms as cough, expectoration, and wheezing in acute exacerbation, which may make identification difficult. FeNO assessments have been extensively studied in COPD and BA; however, the use of this technique is rarely reported in comparative studies of acute exacerbation in both diseases.

This study evaluated the FeNO levels in patients with acute exacerbation (AE) COPD and AE-BA. A comparative study was performed involving the assessment of serum C-reactive protein (CRP), peripheral blood eosinophil ratio (EOS%),

and lung function indices. The aim was to investigate the value of FeNO in the differentiation and diagnosis of AE-COPD and AE-BA.

Materials and methods

General information

A total of 46 AE-COPD and 48 AE-BA patients were selected. The patients were treated in the Department of Respiratory Medicine, the Second People's Hospital of Zhengzhou, from December 2013 to August 2015. The factors considered for inclusion criteria were as follows: COPD that met the diagnostic criteria of the Global Strategy for the diagnosis, management, and prevention of COPD [7] and BA that met the diagnostic criteria of the Global Initiative for Asthma [8]. The exclusion criteria included an association with other respiratory diseases, autoimmune disease, cancer, and severe heart, liver, or kidney dysfunction, the administration of systemic or inhaled corticosteroids within 2 weeks prior to treatment, or smoking within 1 week prior to admission. This study was approved by the Medical Ethics Committee of the Second People's Hospital of Zhengzhou, and all subjects signed the informed consent.

Detection of FeNO

FeNO was detected according to the standardized procedures and guidelines recommended by the American Thoracic Society and European Respiratory Society (ATS/ERS) [9]. An NO detector (NIOX MINO, Aerocrine, Sweden) was used and operated according to the operating instructions. FeNO was detected in all patients before the pulmonary function tests and the administration of medication to rule out the possibility of interfering factors. After performing two-cycle quiet breathing, the subject held the filter tightly in his/her mouth, deeply inspired to the forced vital capacity (FVC), and then exhaled the pulmonary gas with uniform airflow, maintaining the expiratory flow at 50 mL/s. The FeNO value was determined after 10 s of stabilization (unit: ppb, parts per billion).

Pulmonary function test

This test was performed according to the standardized operation procedures and guidelines recommended by the ATS and ERS [10]. A MasterScreen spirometer (JAEGER, Germany) was used for the measurement of the following

indices: forced expiratory volume in the first second (FEV_1), ratio of FEV_1 to the predicted volume ($FEV_{1\%}$ pred), FVC, ratio of FVC to the predicted volume (FVC% pred), FEV_1/FVC, and expiratory flow rate when reaching 25%, 50%, and 75% of FVC ($FEF_{25\%}$, $FEF_{50\%}$, $FEF_{75\%}$).

Detection of serum CRP and EOS%

A volume of 3 ml of peripheral blood was sampled and centrifuged at 3000 r/min for 10 min; the serum was then used for the determination of CRP levels (immune nephelometry); meanwhile, 3 ml of venous blood was also sampled for evaluation of EOS%.

Statistical methods

SPSS 19.0 statistical software was used. The normal distribution measurement data were expressed as mean ± standard deviation ($\overline{x}$±s); the intergroup comparison used the independent sample t-test; the non-normally distributed measurement data were expressed as the median (interquartile range), namely M (QR). The intergroup comparison used the independent sample Mann-Whitney U test. The data were compared with the x^2 test, and the correlation was analyzed with the Pearson correlation analysis. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic ability to differentiate between AE-COPD and AE-BA. The discriminative power of various parameters was determined by the mean of the area under the curve (AUC), sensitivity, specificity, and cut-off value. A *p* value < 0.05 was considered statistically significant.

Results

General data

The age of the AE-COPD patients was significantly higher than the AE-BA patients, and the difference was statistically significant (*P* < 0.01); general information such as body mass index (BMI), gender, and smoking status were factored into the ratio between the two groups and showed no statistically significant difference (*P* > 0.05, **Table 1**).

Comparison of FeNO level and pulmonary function

The FeNO level in the AE-COPD patients was significantly lower, and the difference was sta-

Table 1. General information between two groups

	COPD (n = 46)	BA (n = 48)	t/x^2	P
Age (years)	71.09±9.41	55.4±14.69	-3.939	0.001[a]
BMI (kg/m^2)	24.07±4.26	24.27±3.92	0.478	0.635[a]
Gender (M/F)	32/14	33/15	0.007	0.932[b]
Smoking constitute ratio (smoking/non-smoking)	38/8	40/8	0.009	0.926[b]

Note: [a]t test; [b]x^2 test. COPD: chronic obstructive pulmonary disease; BA: bronchial asthma.

Table 2. FeNO level and pulmonary functions between two groups

	COPD (n = 46)	BA (n = 48)	t	P
FeNO (ppb)	17.41±13.84	57.58±46.61	3.532	0.002
FVC (L)	1.19±0.49	2.05±0.95	3.461	0.002
FVC% pred	40.05±15.17	53.47±20.40	2.089	0.043
FEV$_1$ (L)	0.76±0.34	1.60±0.81	4.101	0.000
FEV$_{1\%}$ pred	35.23±15.21	52.65±22.88	2.573	0.016
FEV$_1$/FVC (%)	63.68±8.35	77.63±9.19	5.024	0.000
FEF$_{25\%}$ (L/sec)	1.13±0.68	2.55±1.77	3.218	0.004
FEF$_{50\%}$ (L/sec)	0.63±0.37	1.59±1.08	3.603	0.002
FEF$_{75\%}$ (L/sec)	0.35±0.18	0.81±0.46	4.038	0.001

FeNO: fractional exhaled nitric oxide; FEV$_1$: Forced expiratory volume in one second; FEV$_1$% pred: FEV$_1$/predicted value ratio; FVC: Forced vital capacity; FVC% pred: FVC/predicted value ratio; FEF$_{25\%}$, FEF$_{50\%}$, FEF$_{75\%}$: forced expiratory flow when 25%, 50%, 75% of the FVC has been expired.

Table 3. Serum CRP and EOS% between two groups

	COPD (n = 46)	BA (n = 48)	Z/t	P
EOS%	1.23±1.73	8.42±7.38	4.045	0.001[a]
CRP (mg/L)	9.94 (60.10)	2.49 (11.79)	-2.082	0.037[b]

Note: [a]t test; [b]Mann-Whitney U test. CRP: C-reactive protein; EOS%: Eosinophil ratio in peripheral blood.

Table 4. Correlation between FeNO level and pulmonary function indexes/EOS%

	COPD (n = 46) r	FeNO (ppb) P	BA (n = 48) r	FeNO (ppb) P
FVC (L)	-0.053	0.814	0.145	0.565
FVC% pred	0.218	0.329	0.040	0.878
FEV$_1$ (L)	-0.027	0.904	0.113	0.656
FEV$_{1\%}$ pred	0.236	0.290	0.007	0.980
FEV$_1$/FVC	0.022	0.924	-0.072	0.777
FEF$_{25\%}$ (L/sec)	0.120	0.595	0.062	0.808
FEF$_{50\%}$ (L/sec)	0.087	0.701	0.067	0.793
FEF$_{75\%}$ (L/sec)	-0.055	0.808	0.046	0.857
EOS%	0.107	0.636	0.626	0.005*

Note: *The FeNO level in the AE-BA patients was positively correlated with EOS% (r = 0.626, P < 0.01). FeNO: fractional exhaled nitric oxide; EOS%: Eosinophil ratio in peripheral blood.

tistically significant (P < 0.01), while the indices of lung function in the AE-BA patients (including FEV$_1$, FEV$_{1\%}$ pred, FVC, FVC% pred, FEV$_1$/FVC, FEF$_{25\%}$, FEF$_{50\%}$, and FEF$_{75\%}$) were significantly better than those in the AE-COPD patients, and the differences were statistically significant (P < 0.05, **Table 2**).

Comparison of serum CRP and EOS%

The serum CRP level in the AE-COPD patients was significantly higher than that in the AE-BA patients (P < 0.05); EOS% in the AE-COPD patients was significantly lower than that in the AE-BA patients (P < 0.01, **Table 3**).

Correlation analysis

The FeNO level in the AE-COPD patients showed no correlation with the lung function indices or EOS% (P > 0.05); the FeNO level in the AE-BA patients showed no correlation with the lung function indices (P > 0.05) but showed positive correlation with EOS% (r = 0.626, P < 0.01, **Table 4**; **Figure 1**).

Diagnostic value of parameters for the differentiation between AE-COPD and AE-BA

In the differentiation between AE-COPD and AE-BA using FeNO values, the AUC value was 0.889 (95% CI: 0.789-0.989), with a corresponding sensitivity of 77.8% and a specificity of 86.4%, respectively, based on the optimal cut-off value of 29.75 ppb (**Figure 2**).

In the differentiation between AE-COPD and AE-BA using EOS% values, the AUC value was 0.907 (95% CI: 0.819-0.994), with a corresponding sensitivity of 72.2% and a specificity of 81.8%, respectively,
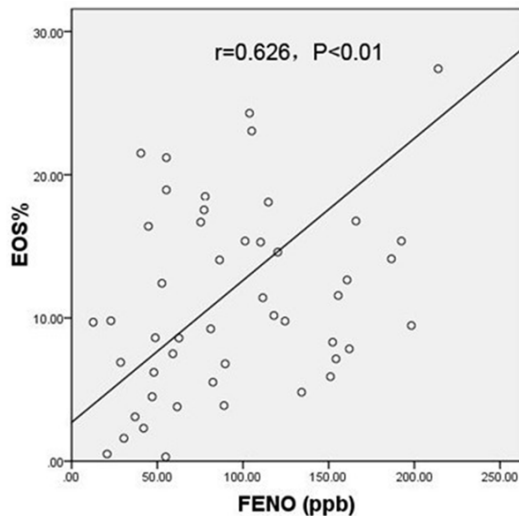
Figure 1. The FeNO level in the AE-BA patients was positively correlated with EOS%. FeNO: fractional exhaled nitric oxide. EOS%: Eosinophil ratio in peripheral blood.

based on the optimal cut-off value of 4.42 (**Figure 3**).

In the differentiation between AE-COPD and AE-BA using CRP levels, the AUC value was 0.785 (95% CI: 0.644-0.926), with a corresponding sensitivity of 63.6% and a specificity of 77.8%, respectively, based on the optimal cut-off value of 11.5 mg/L (**Figure 4**).

Furthermore, when combining the FeNO, EOS%, and CRP parameters, the AUC value was 0.947 (95% CI: 0.882-1.000), with a higher sensitivity of 86.4% and a higher specificity of 88.9%, when compared with the above parameters alone (**Figure 5**).

Discussion

COPD and BA are both chronic airway inflammatory diseases, and the acute exacerbation of these diseases may have similar clinical symptoms, which often causes difficulties in diagnostic identification. FeNO has been widely used for the auxiliary diagnosis and disease management of asthma [3], but reports regarding the value of FeNO in the identification of acute exacerbation in both COPD and BA are rare.

This study found that the FeNO level in the COPD patients was significantly lower than that in the BA patients at the time of acute exacerbation, which is consistent with previous find-

ings. It has been observed that the FeNO level in AE-BA patients within 24 hours of admission was significantly higher than that in patients with AE-COPD and pneumonia [11]. In addition, the AE-COPD patients in this study had a significantly lower EOS% level than the AE-BA patients. While the EOS% of AE-COPD patients showed no correlation with the FeNO level, the AE-BA patients did show a positive correlation between the FeNO level and the EOS%. A previous study also showed that the FeNO level in BA patients showed correlation with EOS% [12]. It was observed that the FeNO and EOS% level were significantly different and showed different correlation characteristics in COPD and BA during acute exacerbation. This suggests that FeNO had a different pathological basis and mechanism in the two diseases; asthma showed airway inflammation characterized by eosinophilic infiltration. As a biomarker of eosinophilic inflammation, FeNO levels were significantly increased in cases of asthma [3]. COPD showed airway inflammation indicated by neutrophil infiltration; the activated neutrophils may potentially release superoxide anions, when combined with airway epithelial cells, nitric oxide (NO) may be produced, thereby reducing the FeNO level in patients with COPD [13]. Therefore, different levels and different correlation characteristics of FeNO and EOS% had important roles in the differentiation of acute exacerbation in COPD and BA.

CRP is a widely used clinical inflammation marker; previous comparative studies in patients with stable COPD and non-AE-BA revealed that the serum hypersensitive CRP level in the former was significantly higher than that in the latter, suggesting that varied levels of hypersensitive CRP may aid in the identification of the non-acute exacerbation status of COPD and BA [14]. The results of this study were similar to the previous study with regard to acute exacerbation; the serum CRP level in the COPD patients was significantly higher than that in the BA patients, indicating that there was a significant difference in the serum CRP level at the time of acute exacerbation in both diseases.

In addition, all the lung function indices in the BA patients were better than those in the COPD patients in this study. This may be related to the age differences between the two groups. Significant differences in the degree of airway obstruction between the two groups showed that the airway reversibility in the COPD pa-
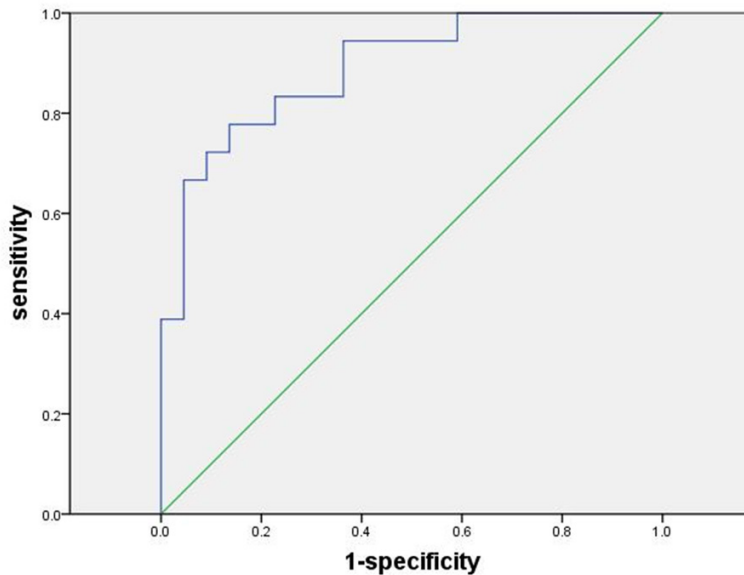
Figure 2. Receiver Operating Characteristic (ROC) curve of FeNO for distinguishing between AE-COPD and AE-BA, the AUC value was 0.889 (95% CI: 0.789-0.989), with a sensitivity of 77.8% and a specificity of 86.4%, respectively, based on the optimal cut-off value of 29.75 ppb.
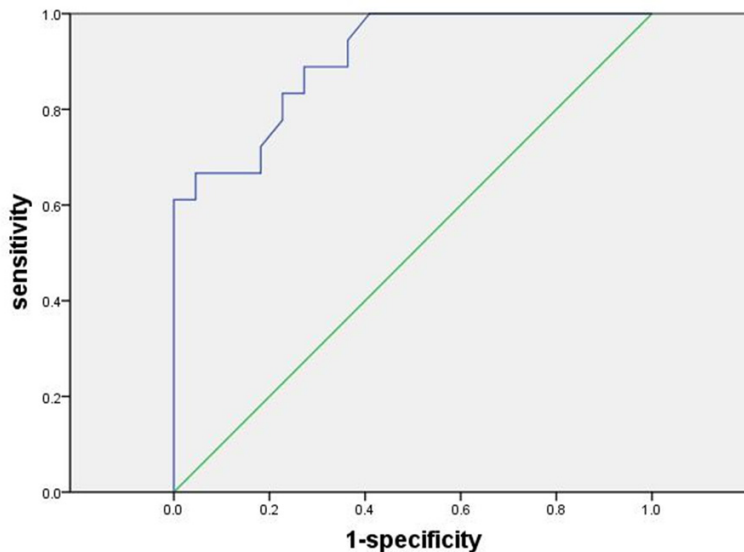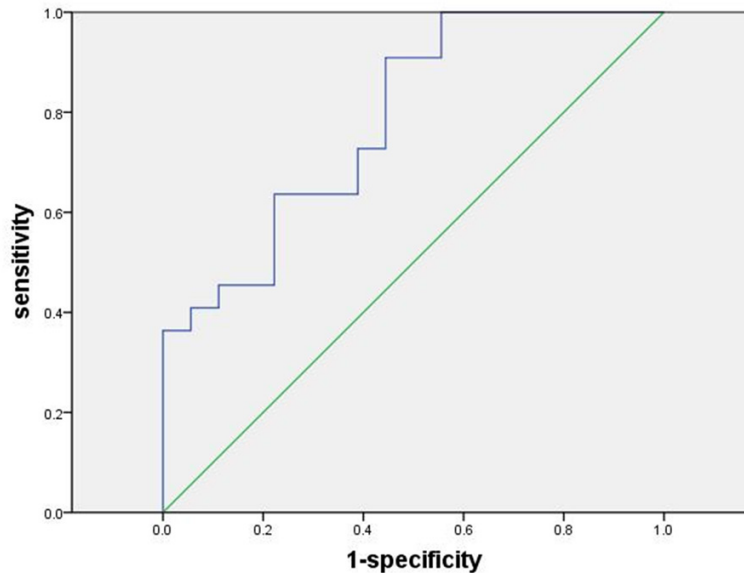

Figure 3. Receiver Operating Characteristic (ROC) curve of EOS% for distinguishing between AE-COPD and AE-BA. The AUC value was 0.907 (95% CI: 0.819-0.994), with a corresponding sensitivity of 72.2% and a specificity of 81.8%, respectively, based on the optimal cut-off value of 4.42.

In summary, our study showed that FeNO, EOS%, serum CRP, and lung function indices had significantly different detection levels and different correlation characteristics in the acute exacerbation of COPD and BA. Moreover, the ROC analysis also indicated that the combination of FeNO, EOS%, and CRP levels resulted in an AUC value of 0.947, with a higher sensitivity (86.4%) and a higher specificity (88.9%). Therefore, it is suggested that the combination of the above indices may have an important role in the differentiation of acute exacerbation in both diseases.

This study had some notable limitations. Based on previous studies, COPD showed certain heterogeneities, and regardless of the stable or acute exacerbation stage, COPD can be divided into two subgroups according to the different eosinophilic levels in sputum, namely the subgroups accompanied with or without eosinophilic inflammation. Subgroups that were characterized by eosinophilic inflammation had a higher level of FeNO, which improved the response to inhaled corticosteroid therapy [17, 18]. In addition, the patients with asthma-COPD overlap syndrome (ACOS) also had higher FeNO levels [19]. However, because this study did not find any COPD patient showing the typical characteristics of eosinophilic inflammation, the conclusions of this study may not be suitable for the identification of patients with COPD-eosinophilic inflammation or the acute exacerbation between ACOS and BA. However, the clinical rate of the subgroup accompanied by eosinophilic inflammation or ACOS was lower [20, 21]. This study had applicable values for the identification of acute

tients may be relatively poorer. However, in this study, no correlation was found between the FeNO level and pulmonary function parameters in both diseases, which is consistent with previous studies [15, 16], and the results suggest that the FeNO level was not entirely relevant to the degree of airway obstruction.

Figure 4. Receiver Operating Characteristic (ROC) curve of CRP for distinguishing between AE-COPD and AE-BA. The AUC value was 0.785 (95% CI: 0.644-0.926), with a sensitivity of 63.6% and a specificity of 77.8%, respectively, based on the optimal cut-off value of 11.5 mg/L.
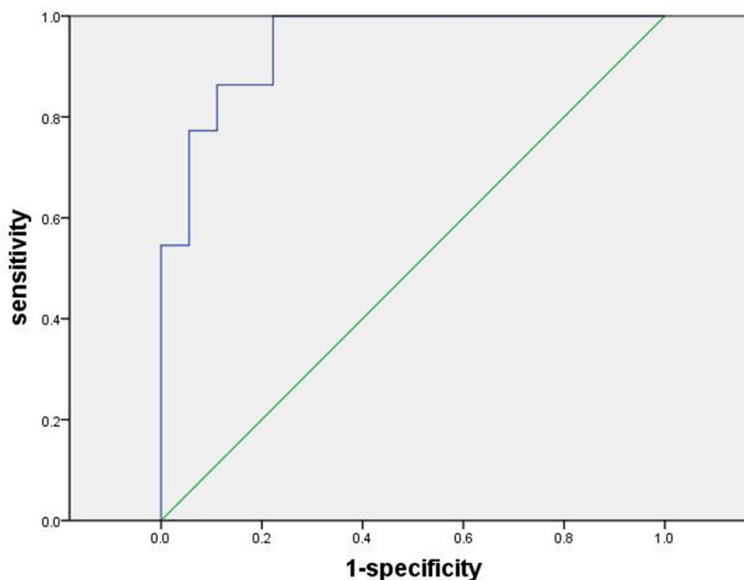
Figure 5. Receiver Operating Characteristic (ROC) curve of the combination of FeNO, EOS% and CRP parameters for distinguishing between AE-COPD and AE-BA. The AUC value was 0.947 (95% CI: 0.882-1.000), with a higher sensitivity of 86.4% and a higher specificity of 88.9%, when compared with the above parameters alone.

exacerbation between most non-eosinophilic inflammation present in both COPD patients and BA patients.

In conclusion, this study showed that the combination of FeNO, EOS%, serum CRP, and lung function indices may help to distinguish between acute exacerbation in COPD and BA. Furthermore, FeNO detection also had an important guiding significance and application value in the distinction of different subgroups of COPD that assisted the auxiliary diagnosis of ACOS and guided precise applications of glucocorticoids in the eosinophilic inflammation that accompanied COPD subgroups or ACOS and therefore, is worthy of further study.

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References

[1] Buga GM and Ignarro LJ. Electrical field stimulation causes endothelium-dependent and nitric oxide-mediated relaxation of pulmonary artery. Am J Physiol 1992; 262: H973-H979.

[2] Jain B, Rubinstein I, Robbins RA, Leise KL and Sisson JH. Modulation of airway epithelial cell ciliary beat frequency by nitric oxide. Biochem Biophys Res Commun 1993; 191: 83-88.

[3] Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL and Taylor DR. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide

levels (FENO) for clinical applications. Am J Respir Crit Care Med 2011; 184: 602-615.

[4] Yang S, Park J, Lee YK, Kim H and Hahn YS. Association of longitudinal fractional exhaled nitric oxide measurements with asthma control in atopic children. Respir Med 2015; 109: 572-579.

[5] Zeiger RS, Szefler SJ, Phillips BR, Schatz M, Martinez FD, Chinchilli VM, Lemanske RF Jr, Strunk RC, Larsen G, Spahn JD, Bacharier LB, Bloomberg GR, Guilbert TW, Heldt G, Morgan WJ, Moss MH, Sorkness CA and Taussig LM. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. J Allergy Clin Immunol 2006; 117: 45-52.

[6] Gelb AF, Flynn Taylor C, Shinar CM, Gutierrez C and Zamel N. Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics. Chest 2006; 129: 1492-1499.

[7] Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, Stockley RA, Sin DD and Rodriguez-Roisin R. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013; 187: 347-365.

[8] Global Initiative for Asthma (GINA). The global strategy for asthma management and prevention [EB/OL]. Updated 2012. Available from: http://www.ginasthma.org.

[9] American Thoracic Society and European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005; 171: 912-930.

[10] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G and Wanger J. Standardisation of spirometry. Eur Respir J 2005; 26: 319-338.

[11] Al-Ali MK and Howarth PH. Exhaled nitric oxide levels in exacerbations of asthma, chronic obstructive pulmonary disease and pneumonia. Saudi Med J 2001; 22: 249-253.

[12] Strunk RC, Szefler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, Hodgdon K, Morgan W, Sorkness CA and Lemanske RF Jr. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. J Allergy Clin Immunol 2003; 112: 883-892.

[13] Jones KL, Bryan TW, Jinkins PA, Simpson KL, Grisham MB, Owens MW, Milligan SA, Markewitz BA and Robbins RA. Superoxide released from neutrophils causes a reduction in nitric oxide gas. Am J Physiol 1998; 275: L1120-L1126.

[14] Tilemann L, Gindner L, Meyer F, Szecsenyi J and Schneider A. Differences in local and systemic inflammatory markers in patients with obstructive airways disease. Prim Care Respir J 2011; 20: 407-414.

[15] Schafroth Torok S and Leuppi JD. Bronchial hyper-responsiveness and exhaled nitric oxide in chronic obstructive pulmonary disease. Swiss Med Wkly 2007; 137: 385-391.

[16] Gemicioglu B, Musellim B, Dogan I and Guven K. Fractional exhaled nitric oxide (FeNo) in different asthma phenotypes. Allergy Rhinol (Providence) 2014; 5: 157-161.

[17] Papi A, Romagnoli M, Baraldo S, Braccioni F, Guzzinati I, Saetta M, Ciaccia A and Fabbri LM. Partial reversibility of airflow limitation and increased exhaled NO and sputum eosinophilia in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000; 162: 1773-1777.

[18] Soter S, Barta I and Antus B. Predicting sputum eosinophilia in exacerbations of COPD using exhaled nitric oxide. Inflammation 2013; 36: 1178-1185.

[19] Donohue JF, Herje N, Crater G and Rickard K. Characterization of airway inflammation in patients with COPD using fractional exhaled nitric oxide levels: a pilot study. Int J Chron Obstruct Pulmon Dis 2014; 9: 745-751.

[20] Brightling CE, Monteiro W, Ward R, Parker D, Morgan MD, Wardlaw AJ and Pavord ID. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. Lancet 2000; 356: 1480-1485.

[21] Miravitlles M, Soler-Cataluña JJ, Calle M, Molina J, Almagro P, Quintano JA, Trigueros JA, Piñera P, Simón A, Riesco JA, Ancochea J and Soriano JB. A new approach to grading and treating COPD based on clinical phenotypes: summary of the Spanish COPD guidelines (GesEPOC). Prim Care Respir J 2013; 22: 117-121.