RESEARCH PAPER

Fractional Exhaled Nitric Oxide in Children with Acute Exacerbation of Asthma

Dinesh Raj, Rakesh Lodha, Aparna Mukherjee, Tavpritesh Sethi, *Anurag Agrawal and Sushil Kumar Kabra

*From Division of Pediatric Pulmonology, Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029 and *Molecular Immunogenetics, CSIR-Institute of Genomics and Integrative Biology, New Delhi 110 007, India.*

Correspondence to: Dr Rakesh Lodha, Additional Professor, Department of Pediatrics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India. rlodha1661@gmail.com

Received: May 16, 2013; Initial review: July 03, 2013; Accepted: August 07, 2013.

Objective: To determine whether fractional exhaled nitric oxide (FENO) has a utility as a diagnostic or predictive maker in acute exacerbations of asthma in children.

Design: Analysis of data collected in a pediatric asthma cohort.

Setting: Pediatric Chest Clinic of a tertiary care hospital

Methods: A cohort of children with asthma was followed up every 3 months in addition to any acute exacerbation visits. Pulmonary function tests (PFT) and FENO were obtained at all visits. We compared the FENO values during acute exacerbations with those at baseline and those during the follow up.

Results: 243 asthmatic children were enrolled from August 2009 to December 2011 [mean (SD) follow up - 434 (227) days]. FENO during acute exacerbations was not different from FENO during follow up; however, FENO was significantly higher than personal best FENO during follow up ($P < 0.0001$). FENO during acute exacerbation did not correlate with the severity of acute exacerbation ($P=0.29$). The receiver operating characteristics curve for FENO as a marker for acute exacerbation had an area under the curve of 0.59. Cut-off of 20 ppb had a poor sensitivity (44%) and specificity (68.7%) for acute exacerbation.

Conclusions: FENO levels during acute exacerbation increase from their personal best levels. However, no particular cut off could be identified that could help in either diagnosing acute exacerbation or predicting its severity.

Keywords: *Acute exacerbation, Asthma, FENO, Nitric oxide.*

Published online: September 05, 2013. PII: S097475591300498

Nitric oxide (NO) was first measured in exhaled air in 1991 [1] and association with asthma was reported in 1993 [2]. Fractional exhaled nitric oxide (FENO) is a marker of asthma, and high levels correlate with ongoing eosinophilic inflammation [3]. FENO levels typically come down with inhaled corticosteroids (ICS) in a dose-dependent manner [4,5]. In case of loss of asthma control, FENO increases [6], and there are some data to suggest that serial monitoring of FENO can help in titrating corticosteroid doses [7] and predicting exacerbation [8].

American Thoracic Society guidelines recommend the use of exhaled NO in management of asthmatics, especially in asthma with eosinophilic inflammation and in predicting response to corticosteroids [9]. However, the role of FENO in asthma at present is limited to diagnosis of eosinophilic airway inflammation, monitoring of airway inflammation, and likelihood of steroid responsiveness. FENO has been studied in acute exacerbation, but has been limited to reproducibility of measurements, emergency department disposition, and response to corticosteroids [10-12]. Most of these studies have been done in Caucasian children and studies from the Indian subcontinent are lacking.

There is a need to evaluate the utility of FENO in acute exacerbations as it may reflect the extent of airway inflammation. We conducted this study to determine the utility of FENO measurements in acute exacerbations.

Accompanying Editorials: Pages 101-3

Methods

This study was conducted in the Pediatric Chest Clinic of the All India Institute of Medical Sciences (AIIMS), New Delhi, which is a tertiary care teaching hospital. We are following a cohort of pediatric asthma patients (up to 18 yrs age) since August 2009. Study protocol was approved by Ethics committees of AIIMS, New Delhi and CSIR-IGIB, New Delhi..

Written informed consent was taken from the parents/ guardian. The diagnosis and treatment of asthma was based on the Global Initiative for Asthma (GINA) guidelines [13] by a pediatric pulmonologist. The patients

were followed up every 3 months, symptom diary was maintained, and control was assessed as per GINA guidelines. On each visit, lung function measurements (spirometry and impulse oscillometry) were performed and FENO levels were obtained. Blood was collected at enrollment for peripheral eosinophil counts. Therapy was modified, if required, on the basis of clinical features and spirometry.

In case of appearance of symptoms of acute exacerbation, they contacted the research team (led by a pediatrician) and a visit was scheduled. Children who were not able to perform spirometry or FENO were not included in the analysis.

The patients with acute exacerbation were evaluated and managed in the Pediatrics department by the study team. Acute exacerbation was defined as recent increase in asthma symptoms requiring hospital visit and treatment with salbutamol and/or steroids [14]. Initial evaluation included history and physical examination (including pulse oximetry). Severity of acute asthma was assessed using pulmonary score [15]. Child was initially evaluated in clinic, and subsequently managed in emergency room in case of moderate and severe acute exacerbation. Child underwent FENO measurement followed by spirometry. Patients were managed according to acute asthma guidelines [14]. Apart from asthma management, compliance and technique was checked and re-emphasized at each visit.

FENO measurement: FENO measurement was done using NIOX MINO (Aerocrine AB, Solna, Sweden) in accordance with ATS guidelines [9]. FENO was measured at the time of enrolment, on each follow up visit every 3 months and on each breakthrough visit that was assessed to be acute exacerbation. In mild to moderate exacerbations, FENO measurement was done in clinic before bronchodilator therapy. In case of severe exacerbation, child was managed in emergency room and FENO was done once child was stable. All measurements were performed on the same equipment and by similarly trained team-members, ensuring minimal technical variability.

Spirometry was performed after FENO measurement was done. Spirometry was done using portable spirometer (Superspiro MK2, Micro Medical Ltd, UK) in all children in accordance with ATS standards [16].

Pulmonary score is a validated measure of asthma severity for children with acute asthma exacerbation [15]. Each parameter is rated on a 0-3 scale, with a maximum total score of 9. Mild, moderate, and severe acute exacerbations were defined as pulmonary score of 0-3, 4-6, and 7-9, respectively.

Skin prick testing (SPT) was done using 12 aeroallergens during the follow up of the cohort. Saline was taken as negative control and histamine was used for positive control. Patients were not on antihistaminics for at least 48 hours preceding the test. The twelve allergens tested were rice grain dust, wheat threshing dust, housefly, female cockroach, dog dander, house dust mite (*Dermatophagoides farinae*), *Curvularia lunata*, *Aspergillus tamari*, *Alternaria tenius*, *Prosopis juliflora*, *Cynodon dactylon*, and *Holoptelea integrifolia*. Allergens were obtained from All Cure Pharma Pvt Ltd, Bahadurgarh, Haryana. Test was considered positive if wheal in any of the allergens was 3 mm or more than the negative control. Child was considered atopic if he demonstrated positive result to one or more allergen, and non-atopic if he had a negative SPT.

Statistical analysis: Data were entered using Microsoft Access. Statistical analysis was performed using Stata 9.0 statistical software (Stata Corp., College Station, TX, USA). We identified each patient's minimum FENO value during follow up (personal best). We determined the difference in FENO measured during acute exacerbation as compared to the follow up values and also as compared to the personal best for each patient. The distribution of FENO values was skewed to the left, so it was reported as median (IQR). The differences between FENO at various time points were analyzed using Wilcoxon signed-rank test. The difference in FENO across more than two groups was analyzed using Kruskal Wallis test. We constructed receiver operating characteristics (ROC) curves to assess the ability of FENO to predict acute exacerbation and we hypothesized FENO of 20 ppb as a cut off for acute exacerbation.

We divided patients on the basis of baseline FENO into three sub-groups (low, intermediate, and high) *i.e.*, <20 ppb, 20 to 35 ppb, and >35 ppb using ATS guidelines [9]. We then assessed the exacerbation rates and also various FENO values in these three categories. Correlation between FENO and pulmonary score was done using Spearman correlation test. A P value of <0.05 was considered significant.

RESULTS

The cohort of 243 children (76% males) was enrolled between August 2009 to December 2011. Forty-six children out of 243 were steroid naïve at baseline. Baseline characteristics of the cohort are given in ***Table* I**. Eosinophil count was available in 80 children. Absolute eosinophil count was 327/mm^3 (IQR: 216-609) and 264/mm^3 (IQR: 196-800) in non-atopic and atopic children, respectively (P=0.99). We had a total of 1183 FENO measurements from the children enrolled.

A flow chart of enrolled children depicting acute exacerbation and atopic status is given in ***Fig.* 1**. One hundred and seventy four exacerbations were diagnosed in 95 patients (39.1%) during the study period (***Table* II**). Forty seven patients (19.4%) had two or more exacerbations. FENO measurements were available in 143 exacerbations. The overall median number of FENO measure-ments in children who had an exacerbation was 8 (range 3 - 12).

The baseline and mean follow up FENO measurements are shown in ***Fig.* 2**. Median (IQR) FENO during follow up and baseline was 14.4 ppb (8.2-21.3) and 15 ppb (10-26), respectively. We looked at the difference in FENO at baseline and during acute exacerbation in children who suffered an exacerbation. Median (IQR) baseline FENO at enrolment was 15 ppb (9-26) while that during exacerbation was 17.7 ppb (12-25.3), the difference was not statistically significant (P=0.064). We identified each patient's minimum FENO value during follow up (personal best); the FENO during an acute exacerbation [17.7 ppb (12-25.3)] was significantly higher than the personal best FENO [8 ppb (5-12), P<0.0001]. The median difference in FENO values 3 months after the baseline measurement of FENO was 0.5 ppb in children who did not have an exacerbation in the first 3 months of follow up.

TABLE I BASELINE CHARACTERISTICS

Characteristics	
Number of patients	243
Mean (SD) age, mo	99 (41.7)
Males, *n* (%)	180 (76.1)
Duration of follow up, days; mean (SD)	434 (227)
Residence	
Rural, *n* (%)	50 (20.7)
Urban, *n* (%)	184 (76.35)
Urban slum, *n* (%)	7 (2.9)
Family history	
Asthma, *n* (%)	127 (52.7)
Asthma, nasal allergy, or eczema, *n* (%)	161 (66.8)
Exposure to tobacco smoke at home, *n* (%)	95 (39.6)
Baseline asthma severity	
Intermittent asthma, *n* (%)	25 (10.3)
Mild persistent asthma, *n* (%)	120 (49.4)
Moderate persistent asthma, *n* (%)	94 (38.7)
Severe persistent asthma, *n* (%)	4 (1.7)
Absolute eosinophil count, cells/μL (n=95)	655 (935)
Atopy (skin prick testing, n=180)	
Positive to at least one allergen, *n* (%)	100 (55.6)
Positive to more than one allergen, *n* (%)	68 (37.8)

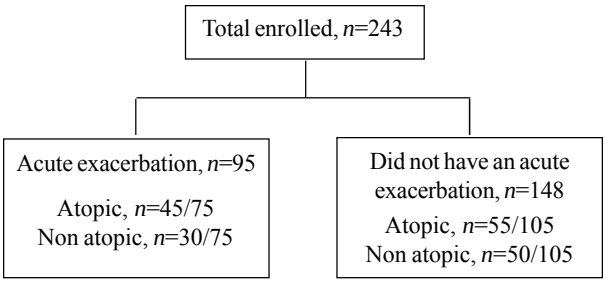

FIG. 1 *Flow chart depicting acute exacerbation during follow up and atopic status. (Atopic status was assessed using SPT in children above 5 years of age).*

FENO values increased above the personal best value for an individual patient in 114/143 episodes (79.7%). The median (IQR) personal best FENO (n=94) and FENO during acute exacerbation (n=84) were 7.5 (5-12) and 17.7 (12-25.3), respectively (P<0.001). The median change between personal best FENO and acute exacerbation FENO was 8.5 ppb (IQR: 4.3-13 ppb) (n=84). The median percentage increase in FENO (from personal best to acute exacerbation) was 121% (IQR: 46.4 - 200). The median absolute change in FENO (personal best to acute exacerbation FENO) was 5 ppb (IQR: 2-12), and 9 ppb (IQR: 5-13) in non-atopic and atopic children, respectively (P=0.10). ***Table* III** shows the FENO values at different time points in children according to the severity of asthma.

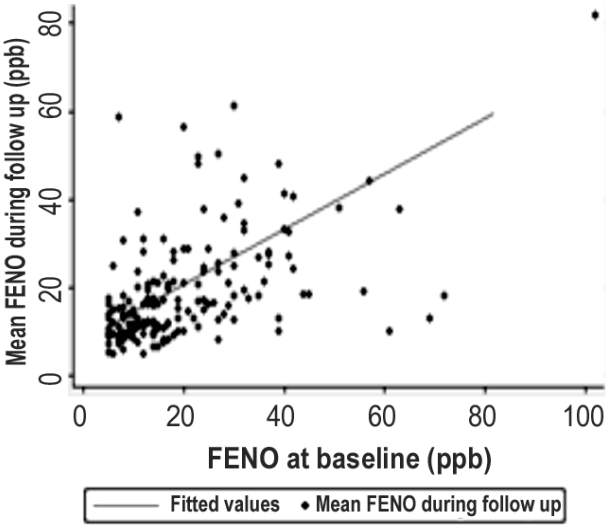

FIG.1 *FENO measurements of enrolled children at baseline and during follow up*

TABLE II CHARACTERISTICS OF STUDY SUBJECTS DURING FOLLOW-UP

Characteristics	
Total number of exacerbations, *n*	174
Exacerbation rate, per child per year	0.75
Children with at least 1 exacerbation, *n* (%)	95 (39.1)
Children with at least 2 exacerbations, *n* (%)	47 (19.4)
Severity of exacerbation (n=166)	
Mild (Pulmonary score 0-3)	139 (83.7%)
Moderate (Pulmonary score 4-6)	26 (15.7%)
Severe (Pulmonary score >6)	1 (0.6%)
FENO (ppb), median (IQR)	
Baseline (*n*=185)	15 (9-26)
Personal best (*n*=218)	8 (5-12)
During exacerbation (*n*=143)	17.7 (12-25.3)

We divided patients on the basis of baseline FENO into three sub groups (low, intermediate, and high) *i.e.*, <20, 20 to 35, and >35 ppb. The median FENO during follow up, personal best FENO, and FENO during acute exacerbation were higher in children with higher baseline FENO values (***Table* IV**). However, the exacerbation rates per child per year were similar in the 3 categories i.e. 0.81, 0.66, and 1.22 in the low, intermediate, and high FENO category, respectively (*P*=0.76).

As only one severe exacerbation was observed, so for the purpose of analysis, moderate and severe exacerbations were taken as one group. Median FENO during acute exacerbation was 18 ppb (IQR: 12-26) and 14 ppb (IQR: 10-25) in mild and moderate exacerbation respectively (*P*=0.39). Pulmonary score did not correlate with acute exacerbation FENO (r=0.1, Spearman correlation, *P*=0.29).

Sixty-three (75%) children had an FENO ≥20 ppb during exacerbation. Using ROC curve, a cut off of 20 ppb had a sensitivity of 44% and a specificity of 68.7%, with an area under curve (AUC) of 0.59. Cut off of 15 ppb had a sensitivity of 57.3% and a specificity of 53.5%. Cut off of 25 ppb had a sensitivity of 27.3% and a specificity of 78.7%.

The AUC was higher for children with a baseline FENO <20 ppb (AUC=0.64) as compared to those with baseline FENO ≥20 to <35 ppb (AUC=0.52) and FENO ≥35 ppb (AUC=0.36) [*P*=0.0009] suggesting better discriminatory value of FENO for exacerbation in children with a lower FENO at baseline.

DISCUSSION

We evaluated the utility of FENO in children with acute exacerbation of asthma in this cohort study. This is the first study to have compared FENO levels during follow up to FENO levels during acute exacerbation. The personal best FENO was significantly lower than FENO during acute exacerbation.

Poor asthma control can lead to asthma exacerbation, so we expected increase in FENO during exacerbation from personal best levels. While in around 68% children, the FENO during acute exacerbation was at least 5 ppb higher than the personal best FENO, the difference was either less than 5 ppb or even negative (*i.e.*, best personal FENO less than acute exacerbation FENO) in the remaining one-third. Thus, FENO's utility in predicting the presence of acute exacerbation is limited.

FENO is influenced by a number of factors. Atopy [17], viral infections [18], allergen exposure [19, 20] and concomitant rhinitis [21] are known to increase FENO. FENO is known to decrease with smoke exposure [22], post-spirometry [23], and corticosteroid treatment [4,5]. In our study, almost all children were on anti-inflammatory treatment. There is some evidence that height correlates positively with FENO [24]. Smoke exposure and air pollution are particularly important to our patients in Delhi, India, where air quality is notoriously poor. It is likely that at one point in time there are multiple factors which play role in precipitating an acute exacerbation, and these factors have a complex effect on airway NO

TABLE III NIH ASTHMA SEVERITY AND FENO AT VARIOUS TIME POINTS

Asthma severity	*FENO at enrolment, ppb; median (IQR)*	*Minimum FENO, ppb;median (IQR)*	*Acute exacerbation FENO, ppb; median (IQR)*
Intermittent, *n*=4	13 (10-16)	5	11
Mild persistent, *n*=80	15 (10-23)	8 (5-11)	21 (13.5-26)
Moderate persistent, *n*=108	14 (9-25)	8 (5-11)	15 (12-22)
Severe persistent, *n*=24	20 (12-36)	12 (8- 20)	20 (14-29)
P value	0.46	0.0015	0.34

TABLE IV FENO AT VARIOUS TIME-POINTS IN DIFFERENT FENO SUBGROUPS

FENO category	*FENO at enrolment, ppb; median (IQR)*	*FENO during follow up, ppb; median (IQR)*	*Personal best FENO, ppb; median (IQR)*	*FENO during Acute exacerbation, ppb; median (IQR)*
FENO <20	11 (8-14), *n*=117	12 (9.3-17), *n*=105	7 (5-10), *n*=117	14 (11-21), *n*=45
FENO ≥20 to <35	26 (23-30), *n*=45	22.3 (16-32.9), *n*=42	12 (9-21), *n*=45	26.3 (18-35), *n*=19
FENO ≥35	42 (39-57), *n*=23	27.1 (18.3-38), *n*=23	13 (10-18), *n*=23	21.7 (19.5-23.5), *n*=7
P value	0.0001	0.0001	0.0001	0.0012

metabolism. Thus, in a pragmatic clinical setting, analogous to that seen by respiratory physicians in developing nations like India, FENO has probably only limited clinical utility such as predicting response to inhaled steroids.

The ROC curve for FENO as a marker for acute exacerbation had an AUC of 0.59. Cut of off 20 ppb had poor sensitivity and specificity for diagnosis of acute exacerbation. An FENO value of 16 ppb had a sensitivity of 56.6% and a specificity of 58.3%, which was the best combination of sensitivity and specificity. No study has so far evaluated FENO cut offs for acute exacerbation. It is evident from our study that FENO cut off values have poor sensitivity and specificity in predicting acute exacerbations.

Atopy is associated with high FENO, airway hyperresponsiveness, and deterioration with response to allergen exposure. We were interested in knowing whether atopic children demonstrate a higher increase in FENO during acute exacerbation than non-atopic children, which was not evident in the results (*P*=0.10). The possible explanation being that most children were on anti-inflammatory medications during acute exacerbations, and the complex interplay of NO metabolism affected by various factors.

We investigated the association between severity of acute exacerbation and FENO concentrations. We used pulmonary score [14] to assess the severity of acute exacerbation. Majority of the acute exacerbations were of mild-moderate severity. Only one severe exacerbation was seen in our cohort. The reason behind this could be rigorous follow up, telephonic interactions, and home visits to ensure adequate adherence to the controller treatment regimen which may have prevented severe exacerbations.

We did not find any association between FENO and severity of acute exacerbation. Kwok et al measured FENO during acute exacerbation in children aged 2-18 years of age, and reported no difference in their median FENO concentration, regardless of their severity of acute asthma [10].

It is logical to think that the more severe the exacerbation, the more inflammation should be evident. Airway caliber also affects FENO concentrations, and decrease in airway caliber has been shown to decrease FENO [25, 26]. This is one of the reasons which possibly could negate the increase in FENO in moderate-severe exacerbations, apart from the other confounders like, age, height, viral infections, allergen exposure etc.

Not much work has been done on evaluation of FENO in the setting of acute exacerbation. Kwok et al found measurement of FENO difficult for a large proportion of children with acute asthma. In their study FENO measurement could be obtained in only 68% children [10]. Gill, *et al*. found poor reproducibility of FENO measurements obtained in emergency department patients with acute asthma exacerbations [11]. However, Baptist, *et al*. showed acceptable intraclass correlation coefficient and coefficient of variation values (0.98 and 9.42%, respectively) for reproducibility [12]. With the advent of portable hand held NO analyzer, it is possible to measure FENO not only in clinic setting but also in the emergency department, but the utility of FENO in acute exacerbation setting is probably limited.

Asthma is now increasingly being recognized to be a heterogeneous disease constituting of several inflammatory phenotypes. FENO is a surrogate marker of eosinophilic inflammation in asthmatic children. Other phenotypes like neutrophilic and pauci-granulocytic are unlikely have high FENO. Our study enrolled heterogeneous children with asthma and assessed the utility of FENO in acute exacerbations. In our study, it is possible that children with non-eosinophilic inflammation had near normal FENO even during an inflammation. The heterogeneity of patients (steroid naïve, intermittent, mild persistent, moderate persistent, and severe persistent) could be one of the reasons of inability of FENO to predict exacerbation. We did not evaluate viral infection as a cause for acute exacerbation as it is known to increase

WHAT IS ALREADY KNOWN?

- FENO, a marker of eosinophilic inflammation is recommended for monitoring of airway inflammation.
- The role of FENO in the setting of acute exacerbations has not been established.

WHAT THIS STUDY ADDS?

- FENO measurements add little to the diagnosis of acute exacerbation of asthma in children.

FENO. The personal best FENO value was taken as minimum of the follow up values. The observed personal best FENO value may not have been true for children with fewer visits.

To conclude, FENO levels during acute exacerbation increase from their minimum follow up levels. However, no appropriate cut off could be identified which could help diagnosing acute exacerbation. The FENO values did not correlate with the severity of acute exacerbation. It appears that FENO measurement may add little to the diagnosis of acute exacerbation of asthma in children. Diagnosis of acute exacerbation should be based on history, clinical examination, and spirometry and rising FENO (from personal best to acute exacerbation) may be taken as supportive evidence.

Contributors: RL, SKK, and AA: were involved in conception and design of the study; DR, AM, and TS: were involved in data generation; DR, RL, AM, TS, SKK, and AA; were involved in analysis and interpretation of data; DR, RL, AM, TS, SKK, and AA: were involved in preparation and critical revision of the manuscript. All authors approved the final version of the manuscript.

Funding: The study was funded by a grant from CSIR-IGIB; *Competing interests*: None stated.

REFERENCES

1. Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem Biophys Res Commun. 1991; 181:852-7.
2. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J. 1993; 6:1368-70.
3. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. Thorax. 1998;53:91-5.
4. Kharitonov SA, Donnelly LE, Montuschi P, Corradi M, Collins JV, Barnes PJ. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. Thorax. 2002;57:889-96.
5. Jones SL, Herbison P, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, *et al*. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. Eur Respir J. 2002;20:601-8.
6. Jones SL, Kittelson J, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med. 2001;164:738-43.
7. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. Am J Respir Crit Care Med. 2005;172:831-6.
8. Gelb AF, Flynn Taylor C, Shinar CM, Gutierrez C, Zamel N. Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics. Chest. 2006; 129:1492-9.
9. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, *et al*. American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184:602-15.
10. Kwok MY, Walsh-Kelly CM, Gorelick MH. The role of exhaled nitric oxide in evaluation of acute asthma in a pediatric emergency department. Acad Emerg Med. 2009;16:21-8.
11. Gill M, Walker S, Khan A, Green SM, Kim L, Gray S, *et al*. Exhaled nitric oxide levels during acute asthma exacerbation. Acad Emerg Med. 2005;12:579-86.
12. Baptist AP, Sengupta R, Pranathiageswaran S, Wang Y, Ager J. Evaluation of exhaled nitric oxide measurements in the emergency department for patients with acute asthma. Ann Allergy Asthma Immunol. 2008;100:415-9.
13. Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2011. Available from: http://www.ginasthma.org/.
14. Saharan S, Lodha R, Kabra SK. Management of status asthmaticus in children. Indian J Pediatr. 2010;77:1417-23.
15. Smith SR, Baty JD, Hodge D 3rd. Validation of the pulmonary score: an asthma severity score for children. Acad Emerg Med. 2002;9:99-104.
16. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, *et al*. Standardisation of spirometry. ATS/ ERS Task Force. Eur Respir J. 2005;26:319-38.
17. Silvestri M, Sabatini F, Spallarossa D, Fregonese L, Battistini E, Biraghi MG, *et al*. Exhaled nitric oxide levels in non-allergic and allergic mono- or polysensitised children with asthma. Thorax. 2001;56:857-62.

18. Kharitonov SA, Yates D, Barnes PJ. Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. Eur Respir J. 1995;8:295-7.
19. Pedrosa M, Barranco P, López-Carrasco V, Quirce S. Changes in exhaled nitric oxide levels after bronchial allergen challenge. Lung. 2012;190:209-14.
20. Bodini A, Peroni D, Loiacono A, Costella S, Pigozzi R, Baraldi E, *et al*. Exhaled nitric oxide daily evaluation is effective in monitoring exposure to relevant allergens in asthmatic children. Chest. 2007;132:1520-5.
21. Linhares D, Jacinto T, Pereira AM, Fonseca JA. Effects of atopy and rhinitis on exhaled nitric oxide values - a systematic review. Clin Transl Allergy. 2011;1:8.
22. de la Riva-Velasco E, Krishnan S, Dozor AJ. Relationship between exhaled nitric oxide and exposure to low-level environmental tobacco smoke in children with asthma on inhaled corticosteroids. J Asthma. 2012;49:673-8.
23. Silkoff PE, Wakita S, Chatkin J, Ansarin K, Gutierrez C, Caramori M, *et al*. Exhaled nitric oxide after beta2-agonist inhalation and spirometry in asthma. Am J Respir Crit Care Med. 1999;159:940-4.
24. Franklin PJ, Turner SW, Le Souëf PN, Stick SM. Exhaled nitric oxide and asthma: complex interactions between atopy, airway responsiveness, and symptoms in a community population of children. Thorax. 2003;58:1048-52.
25. García-Río F, Ramírez M, Mediano O, Lores V, Rojo B, Villasante C, *et al*. Exhaled nitric oxide and airway caliber during exercise-induced bronchoconstriction. Int J Sports Med. 2006;27:905-10.
26. de Gouw HW, Hendriks J, Woltman AM, Twiss IM, Sterk PJ. Exhaled nitric oxide (NO) is reduced shortly after bronchoconstriction to direct and indirect stimuli in asthma. Am J Respir Crit Care Med. 1998;158:315-9.