

Alveolar nitric oxide and asthma control in mild untreated asthma

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Background: The role of the peripheral airways in asthma is increasingly being recognized as a potential target for the achievement of optimal control of the disease. We postulated that the inflammatory changes of the small airways are implicated in the lack of asthma control in mild asthma.

Objective: To test this hypothesis, we measured the alveolar fraction of exhaled NO ($C_{alv}NO$) in patients with mild asthma with different levels of control of symptoms.

Methods: Seventy-eight patients with asthma (35 men, age, 37 ± 15 years; FEV₁ percentage of predicted, $100\% \pm 9\%$) were studied. Asthma control was assessed by using the Asthma Control Test (ACT). Measurements of exhaled NO at multiple constant flows were performed.

Results: Bronchial NO concentrations were 27.1 ± 20 ppb, and $C_{alv}NO$ levels were 5.7 ± 3.4 ppb. The ACT score was 20 ± 4.2 . The level of asthma control was not associated with bronchial NO concentrations ($r_s = 0.16$, $P = .15$). However, a significant correlation was found between the ACT score and $C_{alv}NO$ ($r_s = 0.25$, $P = .03$). Moreover, $C_{alv}NO$ was significantly higher in patients with uncontrolled asthma than in patients with controlled/partially controlled asthma (6.7 ± 2.6 ppb vs 4.9 ± 2.6 ppb, respectively, $P = .02$). In the subgroup of patients with asthma who underwent extrafine inhaled corticosteroid treatment, the magnitude of the inhaled corticosteroid-induced improvement in asthma control positively correlated with baseline $C_{alv}NO$ at 1 month ($r_s = 0.39$, $P = .003$) and at 3 months ($r_s = 0.49$, $P < .0001$).

Conclusions: The alveolar component of exhaled NO is associated with the lack of asthma control in patients with mild, untreated asthma. This observation supports the notion that abnormalities of the peripheral airways are implicated in the mildest forms of asthma. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Asthma, biological markers, small airways

Asthma is a chronic respiratory disease characterized by inflammatory alterations that are distributed throughout the bronchial tree. In the pathogenesis of the disease, a major role

Abbreviations used

ACT: Asthma Control Test

CA: Controlled asthmatics

$C_{alv}NO$: Alveolar fraction of exhaled nitric oxide

FENO: Fraction of exhaled nitric oxide

FVC: Forced vital capacity

ICS: Inhaled corticosteroids

NCA: Uncontrolled asthmatics

NO: Nitric oxide

r_s : Spearman rank correlation coefficient

has been attributed to the pathologic changes that occur predominantly in the central airways. With the contribution of studies published in the last years, the involvement of the small airways (ie, peripheral membranous bronchioles <2 mm in diameter) in the pathophysiology and clinical appearance of asthma has become increasingly important, modifying the diagnostic and therapeutic approach to the disease.^{1,2}

The majority of asthmatic patients can achieve control of their disease with maintenance treatment. However, despite progresses in the knowledge of the pathophysiologic mechanisms underlying the disease and the development of novel therapeutic strategies, a not trivial proportion of asthmatic patients still present with uncontrolled symptoms, representing a real challenge for physicians. It is therefore crucial to develop strategies that may contribute to monitor the disease, improving the response to treatment. The assessment and monitoring of inflammatory-induced changes of the airways can be performed noninvasively by measuring biomarkers that are obtained from the lower respiratory tract; in this context, the evaluation of the nitric oxide (NO) concentration in the exhaled air has been proposed as a sensitive marker of allergic airway inflammation.³ An increase in the fraction of exhaled NO (FENO) has been demonstrated in asthma exacerbations and in the presence of recent asthmatic symptoms⁴; these observations allow us to hypothesize that exhaled NO could reflect or predict loss of asthma control. More recently, a method of monitoring the daily fluctuations in FENO values with quantification over a period of time has been established, providing information on asthma control and the risk of exacerbation.⁵ However, the role of exhaled NO to reflect the degree of unstable asthma or to predict loss of asthma control is controversial.⁶ Shaw et al⁷ did not find a significant reduction in exacerbation frequency with FENO-directed management in a large primary care setting. In a study conducted by Michils et al,⁸ FENO was found to be significantly related to asthma control over time; however, the overall ability of exhaled NO to reflect asthma control was largely influenced by the dose of inhaled corticosteroids (ICS).

A 2-compartment model of pulmonary NO production that can be used to differentiate the peripheral contribution to exhaled NO

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concentrations has been proposed.⁹ Moreover, the alveolar fraction of exhaled NO ($C_{alv}NO$) is related to features of allergic inflammation in the bronchial-alveolar lavage of asthmatic children¹⁰ and adults.¹¹ Unlike bronchial NO, $C_{alv}NO$ production is not reduced by inhaled fluticasone in individuals with asthma, suggesting that it may originate from a site not accessible to inhaled corticosteroid treatment.¹² In addition, $C_{alv}NO$ remains high in asthmatic patients who are oral steroid-dependent as opposed to patients with mild to moderate and severe asthma, suggesting that the most severe stages of asthma may be characterized by more peripheral airway disease. The elevated levels of $C_{alv}NO$ in steroid-resistant asthma¹³ would support the notion that refractory asthma is a disease of the distal airways with reduced distal drug deposition. In addition, elevated $C_{alv}NO$ in the presence of nocturnal symptoms¹⁴ would further suggest the involvement of the small airway in features of severe asthma. However, a vast proportion of patients may present with uncontrolled asthma and normal lung function. The physiopathologic mechanisms that underlie asthma control are not clearly understood, especially in patients with asymptomatic asthma who may experience periods of loss of control of their symptoms. Our driving hypothesis was that abnormalities of the periphery of the lung may already occur in mildest forms of the disease. We therefore investigated whether $C_{alv}NO$ is associated with the level of asthma control, as assessed by the Asthma Control Test (ACT), in individuals of various asthma control and FEV₁ value of more than 80% predicted. To comply with the objective of the study, we avoided any influence of corticosteroid treatment and smoking exposure on the explored relationships. As a secondary aim, we tested whether alveolar NO concentrations may predict changes in asthma control following proper treatment.

METHODS

Subjects

We aimed at recruiting 100 consecutive individuals attending the Allergy Outpatient Clinic of the Division of Respiratory Diseases of the University of Palermo (Palermo, Italy) who had received the diagnosis of asthma in accordance with the Global Initiative for Asthma guidelines.¹⁵ Patients were enrolled after consultation in our department, including the first visit during which clinical and functional assessments were performed. We aimed at selecting patients with mild asthma with FEV₁ value of more than 80% predicted, no regular treatment, and no recent exacerbations. To this objective, any regular asthma treatment, if used, was discontinued for 4 weeks, and the study day was scheduled at 1 month after enrollment. Patients who experienced acute exacerbations during this period were excluded. Short-acting β -agonists were withheld for at least 12 hours prior to the study evaluation. The study was also restricted to never smokers. None of the patients was receiving food supplements containing arginine or nitrates that might influence exhaled NO levels. Each subject underwent clinical, biological, and functional assessments on the same visit (visit 1). All measurements took place in the morning, and coffee or tea was not allowed prior to the evaluation. The subgroup of steroid-naïve patients was administered low doses of extrafine ICS (beclomethasone dipropionate-HFA 200 mcg/day) according to conventional asthma care and invited to repeat the clinical assessment at 1 (visit 2) and 3 (visit 3) months. The study was performed in accordance with the Good Clinical Practice guidelines recommended by the International Conference on Harmonization of Technical Requirements and was approved by the Ethics Committee of University Hospital of Palermo, and all participants gave written informed consent before inclusion.

Clinical assessment. The clinical assessment aimed at establishing the level of control of the disease. Asthma control was measured by using a self-administered questionnaire (ACT). The ACT is a validated 5-item

instrument, each item rated on a 5-point scale,¹⁶ providing a total score that allows one to discriminate among patients with “well-controlled” (total score, 25), “partially controlled” (total score, >20<25), and “not controlled” (total score, <20) asthma. The questions address symptoms and rescue medication use occurring within the 4 weeks preceding the evaluation.

Biological assessment. FENO was measured before spirometry at multiple constant flows (50, 150, 250, and 350 mL/s) in triplicate by using the electrochemical analyser (FeNO⁺; Medisoftware, Sorinnes, Belgium), which was calibrated daily. To determine whether subjects maintained a constant flow rate during exhalation, a continuous monitoring of expiratory flow rate by graphic display on the monitor screen was used. The technique was consistent with guidelines published by the American Thoracic Society/European Respiratory Society.¹⁷ The mean of 3 values obtained within 10% of each other was used to calculate bronchial NO and $C_{alv}NO$. The contributions of the bronchi (bronchial NO flux) and the alveoli (alveolar NO concentration) to FENO were derived from regression analysis, with NO output as the dependent and exhalation flow rate as the independent factor. The slope and intercept of the regression line are approximate values of alveolar NO concentration and bronchial NO flux, respectively.^{9,18}

Functional assessment. Functional assessment included conventional spirometry. FEV₁, forced vital capacity (FVC), and expiratory airflows at different lung volumes (forced expiratory flow [FEF] at 25% of FVC, FEF at 50% of FVC, FEF at 75% of FVC, and FEF at 25%-75% of FVC) were recorded for analysis. All spirometric measurements were obtained from a fully computerized water-sealed Stead-Wells spirometer (Baires System; Biomedin, Padua, Italy), which allowed compliance with criteria for acceptability and reproducibility online, following the European Respiratory Society/American Thoracic Society task force guidelines.¹⁹

Statistical analyses

The statistical package we used was StatView 5.0.1 (Abacus Concept, Berkeley, Calif). Data are presented as mean $\pm$ SD or median + range depending on whether they were normally distributed. The relationships between variables are expressed as Spearman rank correlation coefficient (r_s). Differences between groups were tested by using the Student *t* test or the Mann-Whitney test, as appropriate. Differences at *P* values less than .05 were considered to be statistically significant.

RESULTS

Out of the 100 individuals who met the inclusion criteria, 22 did not enter in the study protocol because they refused to provide the consent (3 subjects), or experienced an exacerbation in the 4 weeks preceding the study procedures (7 subjects), or did not adequately perform the study procedures (12 subjects). A total of 78 never smoker, mild asthmatic patients were enrolled in the present study. Demographic and functional characteristics of the recruited subjects are described in Table I. Fifty-five patients were newly diagnosed and had not received any specific treatment for asthma prior to inclusion. The remaining 23 patients were under corticosteroid treatment at the time of enrollment, which was discontinued by design. All subjects were skin test positive to at least 1 aeroallergen. No other comorbid conditions or nonrespiratory medications were present at the time of the study. In the entire group, bronchial NO concentrations were 27.1 ± 20 ppb and $C_{alv}NO$ levels were 5.7 ± 3.4 ppb. At baseline, no differences in terms of bronchial and alveolar NO were detected between subjects under corticosteroids and steroid-naïve subjects (bronchial NO, 27.7 ± 21 vs 25.8 ± 20 ppb, *P* = .70; $C_{alv}NO$, 5.7 ± 3.5 vs 5.8 ± 3.5 ppb, *P* = .95). The ACT score (mean $\pm$ SD) was 20 ± 4.2 (range, 5-25). In particular, 13 subjects (17% of the entire study population) obtained a score of 25 (well-controlled asthma), 30 subjects (38% of the total) presented with a score

TABLE I. Demographic and functional characteristics of the study subjects*

M/F	35/43
Age (y)	37 ± 15
FEV ₁ (% predicted)	100 ± 9
FVC (% predicted)	105 ± 15
FEV ₁ /VC	0.82 ± 0.07
FEF ₂₅ (% predicted)	79 ± 35
FEF ₅₀ (% predicted)	88 ± 33
FEF ₇₅ (% predicted)	90 ± 25
FEF ₂₅₋₇₅ (% predicted)	87 ± 27

F, Female; FEF, forced expiratory flow; FEF₂₅, FEF at 25% of FVC; FEF₅₀, FEF at 50% of FVC; FEF₇₅, FEF at 75% of FVC; FEF₂₅₋₇₅, FEF at 25% to 75% of FVC; M, male; VC, vital capacity.

*Data are expressed as mean ± SD.

between 20 and 25 (partially controlled asthma), and 35 subjects (45%) had a score below 20 (uncontrolled asthma).

All subjects took part in visit 1. The ACT score was weakly associated with the degree of airway obstruction, as expressed by the FEV₁ percentage of predicted ($r_s = 0.23$, $P = .04$) and FVC percentage of predicted ($r_s = 0.22$, $P = .05$). The ACT score did not correlate with the bronchial NO concentrations ($r_s = 0.16$, $P = .15$) (Fig 1, A). However, a statistically significant relationship was found between the ACT score and C_{alv}NO ($r_s = 0.25$, $P = .03$) (Fig 1, B). To further investigate the association between the level of asthma control and markers of distal airway inflammation, we pooled the study subjects into 2 groups: uncontrolled asthmatics (NCA, 35 subjects) and well or partially controlled asthmatics (CA, 43 subjects). The 2 groups did not differ in terms of age (40 ± 15 years vs 34 ± 14 years, respectively, $P = .08$) and lung function (NCA vs CA: FEV₁ percentage of predicted, $96\% \pm 17\%$ vs $102\% \pm 17\%$, $P = .12$; FVC percentage of predicted, $101\% \pm 13\%$ vs $107\% \pm 16\%$, $P = .09$). The bronchial NO did not discriminate between NCA and CA (28 ± 20 ppb vs 26 ± 22 ppb, respectively, $P = .71$). However, C_{alv}NO was significantly higher in NCA (6.7 ± 4.1 ppb) than in CA (4.9 ± 2.6 ppb) ($P = .02$) (Fig 2).

At visit 2, the group of 55 asthmatic patients who underwent extrafine ICS treatment showed a significant improvement in the ACT score (21 ± 4.2 vs 19 ± 4.2 , post vs pre, $P = .0006$), which was confirmed at visit 3 (22 ± 5.3 , $P = .0004$). We analyzed the relationship between the baseline C_{alv}NO and the change in the ACT score. This analysis yielded significant results both at visit 2 ($r_s = 0.39$, $P = .003$) and at visit 3 ($r_s = 0.49$, $P < .0001$) (Fig 3, A and B). However, bronchial NO did not predict the response to ICS (at visit 2: $r_s = 0.26$, $P = .07$; at visit 3: $r_s = 0.19$, $P = .10$).

DISCUSSION

The findings of the present study demonstrate that in patients with mild, untreated asthma, the level of asthma control is associated with inflammatory changes of the peripheral airways. In addition, the alveolar NO concentrations appear to predict the response to extrafine ICS treatment in terms of asthma control. Taken together, these observations suggest that abnormalities of the peripheral airways are implicated in clinical presentations of mildest forms of asthma.

The use of markers of airway inflammation, such as NO concentrations in the exhaled air, instead of direct measurements

of inflammation, that is, the eosinophils in the sputum, could represent a limitation of the study. However, elevated alveolar NO has been demonstrated to reflect eosinophilic inflammation in the distal lung.¹¹ Measurements of alveolar NO concentrations in the exhaled air are increasingly used to indirectly estimate the degree of inflammation at the level of distal airways. Paraskakis et al²⁰ demonstrated significant relationships between alveolar NO concentrations and spirometric parameters of small airway abnormalities in children. Similarly, Battaglia et al²¹ showed that in patients with mild asthma, exhaled NO concentrations were positively correlated with nonhomogeneous emptying of lung units, as reflected by a steeper slope of phase III at the single breath nitrogen test (functional evidence of small airway involvement) but were not correlated with spirometric measurements of bronchial obstruction.

The current observations suggest that increasing levels of markers of distal airway inflammation significantly correlate with the lack of asthma control in a cohort of patients with mild asthma. In a study conducted in consecutive asthmatic children,²² increased levels of C_{alv}NO were related to poor asthma control independent of baseline lung function, atopic status, or use of inhaled corticosteroids. The finding that C_{alv}NO increases in patients with uncontrolled asthma supports the observations that the site of inflammation might change with the disease severity, as demonstrated by other studies,^{23,24} becoming more prevalent and clinically important in the periphery of the airways. However, data are conflicting: a recent study performed in a population of children and adults with asthma²⁵ failed to demonstrate that alveolar NO concentrations are predictive of asthma control in individuals who were under treatment with ICS. Perhaps, the discrepancy between these studies could be attributed to different characteristics of study populations, such as the use of anti-inflammatory treatment or smoking exposure.

Several mechanisms linking exhaled NO rising from distal airways and asthma control can be proposed. If the pathophysiologic changes of the peripheral airways are considered as defining a phenotype of asthma, it is logical to assume that the distal airway involvement is the primary pathogenetic mechanism, characterizing a condition of excessive bronchoconstriction and, perhaps, unstable clinical conditions. A large body of evidence supports this hypothesis. In cases of fatal asthma, the outer area of the small airways was found to be the major site of extracellular matrix remodeling²⁶; even in less severe patients, a higher grade of inflammatory processes has been demonstrated in the small airways compared with the large airways.²⁷ Recently, Farah et al²⁸ demonstrated that abnormal small airway function contributes to the expression of asthma symptoms. In Farah's study, ventilation heterogeneity was severely impaired in individuals with poorly controlled asthma, and changes in ventilation heterogeneity following a period of ICS treatment correlated with improvements in symptom control independently of all other measured physiologic variables. These mechanisms are consistent with observations that airway closure²⁹ and air trapping³⁰ as physiologic manifestations of small airways disease are associated with recurrent asthma exacerbations and unstable asthma. Moreover, Lehtimäki et al¹⁴ found that nocturnal symptoms in asthmatic patients are related to a higher alveolar NO concentration.

We sought to extend these observations to patients with mild asthma. The driving hypothesis of the current study was that abnormalities of the periphery of the lung may already occur in mildest forms of the disease. Patients with mild asthma do not

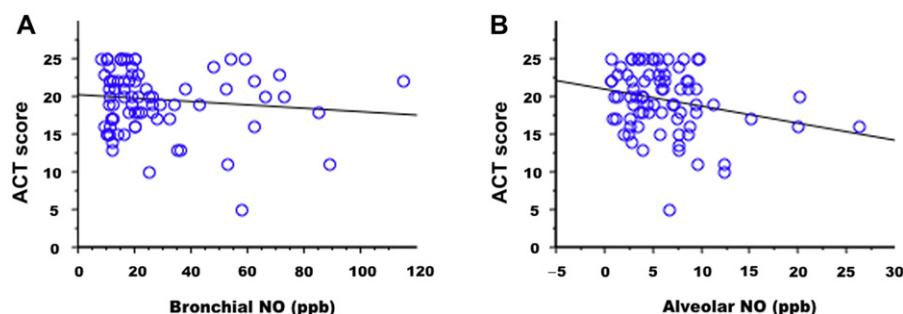


FIG 1. Relationships between the level of asthma control and the concentrations of bronchial (A) and alveolar (B) exhaled NO.

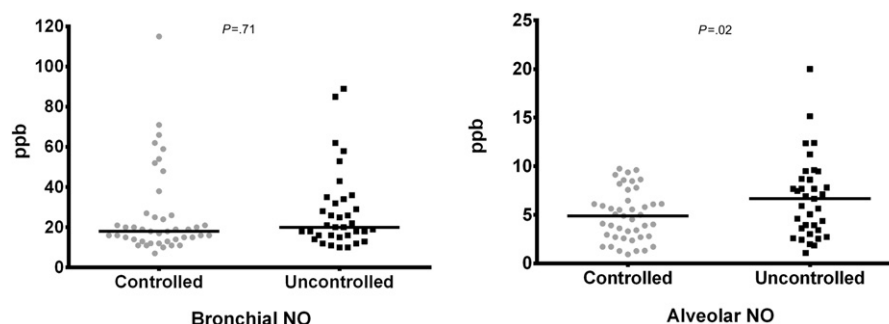


FIG 2. Histograms showing the levels of bronchial and alveolar exhaled NO concentrations in patients with well/partially controlled asthma and in patients with uncontrolled asthma.

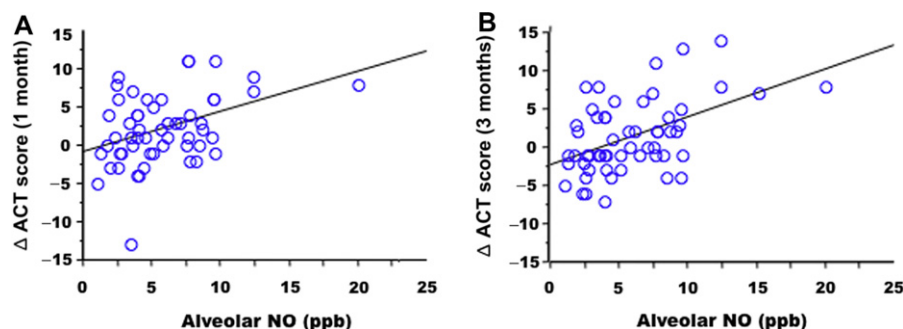


FIG 3. Relationships between the concentrations of alveolar exhaled NO and changes in the ACT score at 1 month (A) and 3 months (B) of treatment.

represent a challenge for physicians, and only rarely experience near-fatal or fatal asthma attacks; however, the impact of loss of asthma control on quality of life and asthma-related costs may be relevant. Despite it, the mechanisms underlying the lack of asthma control in this population are largely unknown. In particular, the role of small airways in predicting the loss of asthma control in patients with mild asthma has not been specifically explored. Our findings seem to confirm that inflammatory changes of the small airways are already present in patients with mild uncontrolled asthma. Whether this represents the primary step for further worsening to more severe stages of asthma could be an interesting topic of research. Similarly, the detection of small airway abnormalities could identify patients at major risk of asthma attacks, or predicts those likely to benefit from a specific intervention. This speculation requires specifically designed studies.

The absence of regular inhaled treatment in our population does not allow us to compare our findings with those from other studies. This could represent a limitation of the study. The withdrawal of ICS was a pre-requisite for inclusion in the study because we wanted to explore whether the degree of asthma control is associated with inflammatory markers of distal inflammation of the airways, without any inference of anti-inflammatory factors. It has been demonstrated that exhaled NO values recover in few days after the suspension of ICS therapy and there is no carryover effect.³¹ The suspension of ICS treatment could be envisaged as a weakness of the study, in that the definition of control requires treatment. However, asthma guidelines suggest to assess the level of asthma control even at the initial consultation, and if the disease is uncontrolled, to start treatment at step 3. For the same reason, we recruited only never smokers and subjects with no recent exacerbations. Thus, we believe that our sample is truly

representative of patients with mild, untreated asthma with various levels of asthma control.

Although the study did not specifically evaluate the role of exhaled NO in predicting the risk of loss of asthma control, the findings of the current study appear to suggest that $C_{\text{alv}}\text{NO}$ could be a useful noninvasive marker to anticipate the loss of symptom control. The concept of “future risk” is gaining increasing importance in the management of the disease, as emphasized by recent guidelines.¹⁵ Whether the detection of small airway abnormalities indicates patients at major risk of asthma attacks or more severe disease, or predicts those likely to benefit from a specific intervention cannot be inferred from our findings. Moreover, the relationship between alveolar NO concentration and the level of asthma control, as assessed by the ACT score (see Fig 1), does not allow us to draw definite conclusions as to whether an isolated measure can predict the magnitude of control of the disease.

In conclusion, the results of the current study showed that $C_{\text{alv}}\text{NO}$ is associated with lack of asthma control in patients with mild asthma. These findings support the role of the peripheral airways in the clinical presentations of mildest forms of asthma. Assessment of alveolar NO could contribute to the definition of future risk and the optimal choice of treatment in this asthmatic population.

Clinical implications: In patients with mild untreated asthma, the alveolar NO was associated with lack of asthma control, supporting the role of the peripheral airways in the clinical presentations of the mildest forms of asthma.

REFERENCES

- Contoli M, Bousquet J, Fabbri LM, Magnussen H, Rabe KF, Siafakas NM, et al. The small airways and distal lung compartment in asthma and COPD: a time for reappraisal. *Allergy* 2010;65:141-51.
- Scichilone N, Battaglia S, Olivieri D, Bellia V. The role of small airways in monitoring the response to asthma treatment: what is beyond FEV1? *Allergy* 2009;64:1563-9.
- Barnes PJ, Dweik RA, Gelb AF, Gibson PG, George SC, Grasemann H, et al. Exhaled nitric oxide in pulmonary diseases: a comprehensive review. *Chest* 2010;138:682-92.
- Mahut B, Delacourt C, Zerah-Lancner F, De Blic J, Harf A, Delclaux C. Increase in alveolar nitric oxide in the presence of symptoms in childhood asthma. *Chest* 2004;125:1012-8.
- Stern G, de Jongste J, van der Valk R, Baraldi E, Carraro S, Thamrin C, et al. Fluctuation phenotyping based on daily fraction of exhaled nitric oxide values in asthmatic children. *J Allergy Clin Immunol* 2011;128:293-300.
- Khalili B, Boggs PB, Shi R, Bahna SL. Discrepancy between clinical asthma control assessment tools and fractional exhaled nitric oxide. *Ann Allergy Asthma Immunol* 2008;101:124-9.
- Shaw DE, Berry MA, Thomas M, Green RH, Brightling CE, Wardlaw AJ, et al. The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial. *Am J Respir Crit Care Med* 2007;176:231-7.
- Michils A, Baldassarre S, Van Muylen A. Exhaled nitric oxide and asthma control: a longitudinal study in unselected patients. *Eur Respir J* 2008;31:539-46.
- Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol* 1998;85:653-66.
- Mahut B, Delclaux C, Tillie-Leblond I, Gosset P, Delacourt C, Zerah-Lancner F, et al. Both inflammation and remodeling influence nitric oxide output in children with refractory asthma. *J Allergy Clin Immunol* 2004;113:252-6.
- Berry M, Hargadon B, Morgan A, Shelley M, Richter J, Shaw D, et al. Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma. *Eur Respir J* 2005;25:986-91.
- Lehtimäki L, Kankaanranta H, Saarelainen S, Turjanmaa V, Moilanen E. Inhaled fluticasone decreases bronchial but not alveolar nitric oxide output in asthma. *Eur Respir J* 2001;18:635-9.
- Gelb AF, Taylor CF, Nussbaum E, Gutierrez C, Schein A, Shinar CM, et al. Alveolar and airway sites of nitric oxide inflammation in treated asthma. *Am J Respir Crit Care Med* 2004;170:737-41.
- Lehtimäki L, Kankaanranta H, Saarelainen S, Turjanmaa V, Moilanen E. Increased alveolar nitric oxide concentration in asthmatic patients with nocturnal symptoms. *Eur Respir J* 2002;20:841-5.
- Global Initiative for Asthma. GINA Guidelines. Global strategy for asthma management and prevention. 2011. Available from: <http://www.ginasthma.org/>. Accessed August 2012
- Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;113:59-65.
- ATS/ERS. 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;171:912-30.
- George SC, Hogman M, Permutt S, Silkoff PE. Modeling pulmonary nitric oxide exchange. *J Appl Physiol* 2004;96:831-9.
- Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. *Eur Respir J* 2005;26:948-68.
- Paraskakis E, Brindicci C, Fleming L, Krol R, Kharitonov SA, Wilson NM, et al. Measurement of bronchial and alveolar nitric oxide production in normal children and children with asthma. *Am J Respir Crit Care Med* 2006;174:260-7.
- Battaglia S, den Hertog H, Timmers MC, Lazeroms SPG, Vignola AM, Rabe KF, et al. Small airways function and molecular markers in exhaled air in mild asthma. *Thorax* 2005;60:639-44.
- Puckett JL, Taylor RW, Leu SY, Guijon OL, Aledia AS, Galant SP, et al. Clinical patterns in asthma based on proximal and distal airway nitric oxide categories. *Respir Res* 2010;11:47.
- Brindicci C, Ito K, Barnes PJ, Kharitonov SA. Differential flow analysis of exhaled nitric oxide in patients with asthma of differing severity. *Chest* 2007;131:1353-62.
- van Veen IH, Sterk PJ, Schot R, Gauw SA, Rabe KF, Bel EH. Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma. *Eur Respir J* 2006;27:951-6.
- Mahut B, Trinquart L, Le Bourgeois M, Becquemin MH, Beydon N, Aubourg F, et al. Multicentre trial evaluating alveolar NO fraction as a marker of asthma control and severity. *Allergy* 2010;65:636-44.
- Dolnikoff M, da Silva LF, de Araujo BB, Gomes HA, Fernexlian S, Mulder A, et al. The outer wall of small airways is a major site of remodeling in fatal asthma. *J Allergy Clin Immunol* 2009;123:1090-7.e1091.
- Hamid Q, Song Y, Kotsimbos TC, Minshall E, Bai TR, Hegele RG, et al. Inflammation of small airways in asthma. *J Allergy Clin Immunol* 1997;100:44-51.
- Farah CS, King GG, Brown NJ, Downie SR, Kermod JA, Hardaker KM, et al. The role of the small airways in the clinical expression of asthma in adults. *J Allergy Clin Immunol* 2012;129:381-7.e381.
- in 't Veen JC, Beekman AJ, Bel EH, Sterk PJ. Recurrent exacerbations in severe asthma are associated with enhanced airway closure during stable episodes. *Am J Respir Crit Care Med* 2000;161:1902-6.
- Sorkness RL, Bleecker ER, Busse WW, Calhoun WJ, Castro M, Chung KF, et al. Lung function in adults with stable but severe asthma: air trapping and incomplete reversal of obstruction with bronchodilation. *J Appl Physiol* 2008;104:394-403.
- Nicolini G, Chetta A, Simonazzi A, Tzani P, Aiello M, Olivieri D. Both bronchial and alveolar exhaled nitric oxide are reduced with extrafine beclomethasone dipropionate in asthma. *Allergy Asthma Proc* 2010;31:85-90.