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Abstract

BACKGROUND:

Asthma is a chronic respiratory disease, characterised by airways inflammation, obstruction and hyperresponsiveness. Asthma control is the goal of asthma treatment, but many patients have sub-optimal control. Exhaled NO and exhaled breath condensate (EBC) NO metabolites (nitrites and nitrates) measurements are non-invasive tools to assess airways inflammation. Our aim was to investigate the relationships between asthma control and the above-named biomarkers of airways inflammation.

METHODS:

Thirty-nine non-smoking asthmatic patients (19 women) aged 50 (21-80) years performed measurements of exhaled NO (FENO), EBC nitrates, nitrites and pH, and answered Asthma Control Questionnaire (ACQ) and Asthma Control Test (ACT)-questionnaire.

RESULTS:

The ACT and ACQ score were strongly interrelated ($\rho = -0.84$, $p < 0.001$). No relationships between ACT or ACQ score and FENO were found ($p > 0.05$). EBC nitrates were negatively related to ACT score ($\rho = -0.34$, $p = 0.03$) and positively related to ACQ score ($\rho = 0.41$, $p = 0.001$) while no relation of EBC nitrites to either ACQ or ACT score was found ($p>0.05$).

CONCLUSION:

EBC nitrates were the only biomarker that was significantly related to asthma control. This suggests that nitrates, but not nitrites or FENO, reflect an aspect of airways inflammation that is closer related to asthma symptoms. Therefore there is a potential role for EBC nitrates in objective assessment of asthma control.

KEYWORDS

ASTHMA CONTROL; EXHALED BREATH CONDENSATE; NITRATE; NITRITE; EXHALED NO

Introduction

Asthma is a chronic respiratory disease characterised by reversible airway obstruction, airway inflammation and hyperresponsiveness. Asthma control is sub-optimal in many patients despite available effective therapies[1] and international guidelines for the diagnosis and management, based on clinical features and lung function tests.[2] The recent update of international GINA guidelines[2] suggests tailoring asthma treatment to the level of disease control rather than severity. Well-validated tools for assessing asthma control are available and two of the most widely used are Asthma Control Questionnaire (ACQ)[3] and Asthma Control Test (ACT).[4] These tools focus on the patient's reported symptoms and additionally, lung function, in the case of ACQ. Neither symptoms nor lung function alone seems to adequately reflect the underlying level of airway inflammation.

The analysis of exhaled breath is a non-invasive method to measure non-invasively airways inflammation in asthma and other respiratory diseases. Nitric oxide (NO) levels are increased in the air of asthmatic patients.[5] Fraction of NO in the exhaled air (FENO) is considered an indirect marker of steroid-sensitive, eosinophilic airway inflammation and it has been used to tailor inhaled steroid therapy in asthma.[6] However the level of evidence so far available could not recommend this approach for routine use.[7]

Exhaled breath condensate is another easy-to-use technique and several markers in exhaled breath condensate (EBC) have been related to airways' inflammation.[8] In the airway surface liquid, NO reacts with oxygen forming nitrite and nitrate, stable-end products of NO,[9] which can be measured in the EBC[8] and might reflect NO formation in the airways. EBC pH can be measured reliably[10] and the information regarding acidification of airways is regarded as relevant to asthma pathophysiology.[11]

Increased levels of FENO and EBC nitrate, nitrite and sum of nitrite and nitrate (NO_x) are reported in asthmatic subjects, when compared to healthy controls. 12 and 13 There are however fewer studies analysing the relation between these markers and asthma control. No relation between exhaled NO and asthma control is reported in cross-sectional studies.14, 15, 16 and 17 Peripheral airways inflammation, measured as increased alveolar NO, has been related to poor asthma control with contradictory results. 17, 18 and 19 The few studies that analysed the relation between nitrogen oxides in EBC and asthma control report contradictory results. 20, 21 and 22 No clear relationship has been established between EBC pH and asthma severity 23, 24 and 25 and no studies have reported a relationship between lower airways pH and poor asthma control.

The main aim of this study was to investigate the relationships between EBC NO metabolites, nitrite and nitrate, EBC pH and asthma control. A secondary aim was to study the relationships between exhaled NO, and its estimated contributions from bronchial and alveolar compartment, and asthma control.

Material and methods

Study subjects

Thirty-nine non-smoking consecutive patients (19 women) aged 21–80 years (median age 50 years) with previously diagnosed asthma were included in the study. All patients were coming for regular follow-up in the outpatient clinic of Dept. of Allergology of Mauriziano Hospital, Turin, Italy between November 2008 and July 2009.

Exhaled NO measurements

Exhaled NO was measured at 50, 100 and 200 mL/s with a chemiluminescence analyser (NIOX, Aerocrine AB, Solna, Sweden), according to current recommendations.[26]Estimation of alveolar and bronchial contributions to exhaled NO was done by the slope–intercept model[27] using all the above mentioned flow-rates in 29 of the 39 subjects. Of those subjects, three had negative values of alveolar NO, leaving 26 subjects for further analyses. Alveolar NO was also calculated after adjustments for axial diffusion, according to Kerckx et al.[28]

Exhaled breath condensate

Exhaled breath condensate was collected with the R Tube EBC collection system (Respiratory Research, Inc, Charlottesville, Virginia, USA). EBC collections were obtained after thorough rinsing of mouth with water, at an initial

condenser temperature of −20 °C, for 10 min. Samples were store at −80 °C until assays, which were performed within 2 weeks of collection.

The pH of EBC was assayed immediately potentiometrically with a glass microelectrode before and after bubbling 200 µl of the sample with argon at 350 mL min^{-1} until pH reading was stable, as previously described.[11]
A modified anion chromatographic technique for nitrate (NO_2) and nitrite (NO_3) determinations, described in a previous publication, was used for measurements of $NO_2$and NO_3 in EBC.[29] Briefly, liquid chomatograph (Dionex, Sunnyvale, CA) equipped with a suppressed conductivity detector and an autosampling injector were used. The anion separator was an AG-4A-SC precolumn connected with an AS-4A column (Dionex). The eluent was an aqueous 1.28/1.60 mmol/L of sodium carbonate/bicarbonate solution flowing at 1.5 mL/min. Twenty-microlitre aliquots of EBC samples were injected into the column without pre-treatment.
The intra-assay variability of the method (for NO_2 and NO_3 detection), assessed by using the coefficient of variation, is 7 ± 1.2%.[29]

ACQ

ACQ[3] is a tool to assess asthma control which consists of 7 items: 6 questions, 5 related to symptoms during the last week and one related to the use of short-acting beta2 agonists, and the seventh item is an objective measure – a spirometric assessment (FEV1% predicted). Each item has 7 alternatives of answer, scored from 0 to 6. The ACQ score is the mean of all these individual answers and therefore, it ranges also from 0 to 6 with higher scores standing for poorer control of asthma. A score of ≤0.75 has been associated with "well-controlled" asthma while a score ≥1.5 has been associated with "not well-controlled" asthma.[30]

ACT

Asthma control test is a questionnaire developed to assess asthma control.[4] It consists of 5 questions, each with a 5 point scale from 1 (reporting all the time or very frequent the respective symptom) to 5 (never reporting the respective symptom). Therefore, the total ACT score is between 5 and 25, with a lower score standing for poorer controlled asthma. An ACT score ≥20 reflects well-controlled asthma.[4]

Lung function

Measurements of lung function were done with a water-sealed spirometer (Biomedin, Padua, Italy). The best of three measurements was automatically chosen by software. The parameters of interest were FEV_1, FEV_1/FVC-ratio, FEF25–75%.

Statistics

Statistical analyses were performed using STATA 10 software (Stata Corp., Texas, USA). Values are presented as median (range) and non-parametric statistics methods (Spearman's rank correlation test and Mann–Whitney *U*-test) have been used.
A *p*-value of <0.05 was considered statistically significant.

Ethics

Informed and written consent was obtained from all participating patients and the protocol was approved by Local Ethics Committee.

Results

The characteristics of the 39 included asthmatic subjects are described in Table 1.

Asthma control in the studied population

The ACT score (median (range)) was 22 (8, 25) and the ACQ score (median (range)) was 0.71 (0, 3.86). The ACT and ACQ score were strongly related ($\rho = -0.84$, $p < 0.001$) (Fig. 1). The relation was similar in subjects with steroid doses <400 μg budesonide ($\rho = -0.84$, $p < 0.001$) (open circles) and steroid doses ≥400 μg budesonide ($\rho = -0.84$, $p < 0.001$) (closed diamonds).

Asthma control and FENO

FENO levels at the exhalation flow-rate of 50 mL/s were ranging from 14 to 173 ppb, with a median value of 35 ppb. No relation between ACQ or ACT score and FENO at any of the measured flow-rates and alveolar and bronchial contributions to exhaled NO was observed (all p-values > 0.05). No differences were observed when adjusting the alveolar NO for trumpet shape of the airways and axial diffusion.

Asthma control and lung function

Significant correlations were observed between asthma control and spirometric parameters recorded in the study (Table 2).

Asthma control and EBC markers

EBC pH ranged between 6.55 and 7.33 with a median value of 7.07. No relation between ACQ score or ACT score and EBC pH was found (both p-values > 0.05).

EBC NO_3 ranged from 1.8 to 17 μM (median 6.5 μM) and EBC NO_2 ranged from 0.06 to 3.11 μM (median 0.68 μM). EBC NO_3 were negatively related to ACT score ($\rho = -0.34$, $p = 0.03$) and positively related to ACQ score ($\rho = 0.41$, $p = 0.001$) (Fig. 2A and B). No relation of EBC NO_2 to either ACT score ($p = 0.43$) or ACQ score ($p = 0.43$) was found (Fig. 2C and D). Stratifying the subjects after the doses of inhaled steroids used, we could observe that the relationships between EBC NO_3 and ACT or ACQ score were significant only in subjects on a dose of inhaled steroids of at least 400 μg budesonide ($\rho = -0.56$, $p = 0.004$ for ACT; and $\rho = 0.58$, $p = 0.003$ for ACQ).

Using the sum of nitrite and nitrate, nitrogen oxides (NO_x), significant relationships could be shown with the ACT score ($\rho = -0.32$, $p = 0.05$) and ACQ score ($\rho = 0.36$, $p = 0.02$). Similarly, this relation was found only in subjects on a dose of inhaled steroids of at least 400 μg budesonide ($\rho = -0.46$, $p = 0.02$ for ACT; and $\rho = 0.46$, $p = 0.02$ for ACQ score). The above reported results for nitrate were confirmed also when comparing the subjects with a good control of asthma vs those not controlled by means of ACT ($p = 0.008$) or ACQ ($p = 0.03$). This relation was stronger in subjects on doses of inhalation steroid ≥400 μg budesonide ($p = 0.002$ for ACT and $p = 0.004$ for ACQ score) (Fig. 3A and B). No

differences in EBC nitrite were found between subjects with well-controlled vs not well-controlled asthma, either for all subjects or after stratifying the subjects according to the dose of inhaled budesonide ($p > 0.30$ for all) (see Fig. 3C and D for subjects on high doses of inhalation steroids).

Effect of comorbidities and dose of inhaled steroids on asthma control, FENO and EBC markers

Chronic rhinosinusitis was associated with higher ACQ scores ($p = 0.05$) and increased EBC NO_3 levels ($p = 0.04$), with no effect on EBC pH, EBC NO_2, FE_{NO} levels or ACT scores (all p-values > 0.2).
Rhinitis was associated with higher ACQ scores ($p = 0.008$) and lower ACT scores ($p = 0.03$). No significant increase of EBC NO_3 ($p = 0.12$) or significant associations with EBC NO_2, EBC pH and FENO (all p-values > 0.2) and rhinitis were found.
The dose of inhaled steroids was not significantly associated with ACQ and ACT scores, FENO, EBC NO_3, EBC NO_2, EBC pH (all p-values > 0.30).

Discussion

The main finding of the present study was that exhaled breath condensate nitrates concentration was related to asthma control, assessed by means of two of the most common used instruments, ACT and ACQ, either using absolute scores, or validated cut-offs for well-controlled asthma. On the other hand, no significant relationships were found between asthma control and EBC pH, EBC nitrites and exhaled NO, including its contributions from peripheral and central airways.

The present study is one of the first studies looking separately at nitrates and nitrites in EBC when assessing their relation with asthma control. The NO metabolites in EBC have been previously reported to be increased in asthmatics compared to controls.12 and 13However few studies analysed separately nitrates,[12] while most of the studies have used a combination of nitrates and nitrites.13 and 20 It is still a matter of debate which NO metabolite, measured in EBC, nitrites or nitrates, is better related to airways inflammation. Recently, both experimental[31] and clinical studies,[32] demonstrated that salivary nitrites concentration may influence EBC nitrites, but not nitrates concentration, suggesting that EBC nitrates, but not nitrites originate in the lower airways. That nitrates and not nitrites concentrations are a more reliable marker of inflammation is also suggested by previous observations that BAL nitrates and not nitrites concentrations were related to airways inflammation induced by segmental allergen challenge.[33] Similarly, a recent study reported decrease of EBC nitrates, but not nitrites after treatment with a new anti-inflammatory drug.[34] Moreover, increased EBC nitrates and decreased EBC nitrites concentrations have been found to be related to asthma severity in a Dutch study of asthmatic children.[21] In our study no significant relationship between EBC nitrites and asthma control was found, in agreement with previous reports.20, 21 and 35
EBC pH too was not related to asthma control in our patients. It is well known that exhaled breath condensate pH reflects acidification of airways and this process has been demonstrated to occur in acute asthma.[11] In our study, we could not observe any differences in EBC pH between subjects with well-controlled and not well-controlled asthma, in agreement with a study in children, where EBC pH was not related neither to asthma severity nor to asthma control.[21] Therefore the acidic stress of the airways is probably less important in stable asthma, compared to acute

asthma. Moreover, the majority of our asthmatic patients had neutral–alkaline pH values, suggesting that no significant production of NO by nitrite reduction due to acidic environment took place in the airways of our asthmatic patients.[11] We did not find any significant relationship between exhaled NO and asthma control and this is in line with previous reports in adults and children,[14, 15, 16 and 17] underlining that symptoms and airway inflammation are two separate aspects of asthma disease.[36] However in longitudinal studies,[37 and 38] changes in FE_{NO} had been found to be related to changes in ACQ scores, both in smoking and non-smoking asthmatics. Alveolar NO has been reported in some small-scale studies to be associated with more symptoms in both adults and children[39 and 40] and two larger studies reported a significant association between poor asthma control and increased alveolar NO in children.[18 and 19] However these results could not be confirmed in a recent large-scale pilot study which enrolled both children and adults,[17] questioning the value of alveolar NO as a marker of asthma control, and our results agree with this study.

Exhaled breath condensate nitrates and nitrites concentrations, EBC pH and exhaled nitric oxide were not significantly interrelated in our patients, as previously reported.[20 and 32] This lack of correlations between exhaled NO and its metabolites and airway pH is probably explained by the complex biochemistry of nitrogen oxides in the airways, and more specifically in the airway surface liquid (ASL). While FENO mainly reflects the NO produced by epithelial iNOS,[41] the nitrogen oxides reflect not only the oxidization of NO in the ASL, but they can also be generated through peroxidase-mediated reactions.[42] This might be an explanation for divergent findings of normal NO_x and low exhaled NO levels in cystic fibrosis patients. [43] Moreover a similar divergent pattern, with increased nitrate and decreased NO levels, has been observed immediately after allergen challenge.[44] Our findings reinforce the current view that EBC nitrates are not an alternative to exhaled NO, but their measurement should be considered complementary to the exhaled NO measurements.[45]

Almost all of our patients were receiving inhaled steroid therapy, which is well known to affect the levels of exhaled NO, leading to a prompt, dose-dependent decrease.[46] However, the effects of inhaled steroid treatment on EBC nitrites and nitrates are not so well studied. EBC NO_x appear to decrease following inhaled steroid therapy, but this effect is smaller than the effect on FENO and it was not dose-dependent. [46] NO_x are increased in the nasal lavage of patients with allergic rhinitis and these levels are not affected by intranasal steroids. [47] These observations might suggest that EBC NO_x concentration is poorly affected by inhaled corticosteroids therapy, yielding another explanation for the lack of correlation between FE_{NO} and EBC nitrates and nitrites concentrations. We observed in our material that the relation between EBC nitrate and asthma control was driven by the group with more severe disease, requiring higher steroid doses. This observation is intriguing and suggests that similar FENO concentrations may not necessarily indicate the same level of airway inflammation, but instead may reflect decreased NO bioavailability from increased NO oxidation.

Our asthmatics have a high prevalence of upper airway diseases, reflecting the interest of our clinic in this area.[48] Chronic rhinosinusitis was associated with poorer asthma control in our patients, in agreement with the literature.[49] No data exist on the levels of nitrates in EBC of patients with CRS, who have been reported to have lower levels of nitrate in nasal lavage.[50]

In conclusion, EBC nitrates concentration was the only biomarker among those measured in the present study to be significantly related to asthma control. This suggests that EBC nitrates, but not EBC nitrites or FENO, reflect an aspect of airways inflammation that is closer related to asthma symptoms. These results reinforce the current view that nitrogen oxides measurements in EBC provide information on airway inflammation that is different from that obtained from exhaled NO measurements. Particularly, our results suggest a potential role for measuring EBC nitrates in order to obtain an objective marker of asthma control in asthmatic patients out of exacerbations.

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References

1. Rabe KF, et al. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. J Allergy Clin Immunol 2004;114:40e7.

2. Bateman ED, et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008; 31:143e78.

3. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Respir J 1999;14:902e7.

4. Nathan RA, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol 2004;113:59e65.

5. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. Eur Respir J 1993;6: 1368e70.

6. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. N Engl J Med 2005;352:2163e73.

7. Petsky HL, et al. Tailored interventions based on exhaled nitric oxide versus clinical symptoms for asthma in children and adults. Cochrane Database Syst Rev; 2009:CD006340.

8. Montuschi P, Barnes PJ. Analysis of exhaled breath condensate for monitoring airway inflammation. Trends Pharmacol Sci 2002;23:232e7.

9. Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airways. Am J Respir Crit Care Med 1994; 149:538e51.

10. Kostikas K, Koutsokera A, Papiris S, Gourgoulianis KI, Loukides S. Exhaled breath condensate in patients with asthma: implications for application in clinical practice. Clin Exp Allergy 2008;38:557e65.

11. Hunt JF, et al. Endogenous airway acidification. Implications for asthma pathophysiology. Am J Respir Crit Care Med 2000; 161:694e9.

12. Corradi M, et al. Nitrate in exhaled breath condensate of patients with different airway diseases. Nitric Oxide 2003;8:26e30.

13. Ganas K, Loukides S, Papatheodorou G, Panagou P, Kalogeropoulos N. Total nitrite/nitrate in expired breath condensate of patients with asthma. Respir Med 2001;95: 649e54.

14. Bernstein JA, et al. Is exhaled nitric oxide a useful adjunctive test for assessing asthma? J Asthma 2009;46:955e60.

15. 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;101:124e9.

16. Lopes C, et al. Assessing asthma control: questionnaires and exhaled nitric oxide provide complementary information. Eur Respir J 2008;32:1419e20.

17. Mahut B, et al. Multicentre trial evaluating alveolar NO fraction as a marker of asthma control and severity. Allergy 2010;65: 636e44.

18. Paraskakis E, et al. Measurement of bronchial and alveolar nitric oxide production in normal children and children with asthma. Am J Respir Crit Care Med 2006;174:260e7.

19. Puckett JL, et al. Clinical patterns in asthma based on proximal and distal airway nitric oxide categories. Respir Res 2010;11: 47.

20. Ratnawati, Morton J, Henry RL, Thomas PS. Exhaled breath condensate nitrite/nitrate and pH in relation to pediatric asthma control and exhaled nitric oxide. Pediatr Pulmonol 2006;41:929e36.

21. Robroeks CM, et al. Exhaled nitric oxide and biomarkers in exhaled breath condensate indicate the presence, severity and control of childhood asthma. Clin Exp Allergy 2007;37: 1303e11.

22. Sazlidere H, et al. The relation between nitric oxide levels in exhaled breath condensate and asthma control questionnaires in asthma patients. Tuberk Toraks 2010;58:5e15.

23. Kostikas K, et al. pH in expired breath condensate of patients with inflammatory airway diseases. Am J Respir Crit Care Med 2002;165:1364e70.

24. Tseliou E, et al. Exhaled nitric oxide and exhaled breath condensate pH in severe refractory asthma. Chest 2010;138:107e13.

25. Ueno T, et al. Inflammatory markers in exhaled breath condensate from patients with asthma. Respirology 2008;13:654e63.

26. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. Am J Respir Crit Care Med 2005;171:912e30.

27. Tsoukias NM, Tannous Z, Wilson AF, George SC. Single-exhalation profiles of NO and CO2 in humans: effect of dynamically changing flow rate. J Appl Physiol 1998;85:642e52.

28. Kerckx Y, Michils A, Van Muylem A. Airway contribution to alveolar nitric oxide in healthy subjects and stable asthma patients. J Appl Physiol 2008;104:918e24.

29. Rolla G, et al. Breath analysis in patients with end-stage renal disease: effect of haemodialysis. Eur J Clin Invest 2008;38: 728e33.

30. Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. Respir Med 2006;100:616e21.

31. Marteus H, Tornberg DC, Weitzberg E, Schedin U, Alving K. Origin of nitrite and nitrate in nasal and exhaled breath condensate and relation to nitric oxide formation. Thorax 2005;60:219e25.

32. Zetterquist W, et al. Oral bacteria e the missing link to ambiguous findings of exhaled nitrogen oxides in cystic fibrosis. Respir Med 2009;103:187e93.

33. Erpenbeck VJ, et al. Local nitric oxide levels reflect the degree of allergic airway inflammation after segmental allergen challenge in asthmatics. Nitric Oxide 2005;13:125e33.

34. Stefanska J, et al. Apocynin decreases hydrogen peroxide and nitrate concentrations in exhaled breath in healthy subjects. Pulm Pharmacol Ther 2010;23:48e54.

35. Vogelberg C, et al. Exhaled breath condensate nitrite e methodological problems of sample collection. Med Sci Monit 2008;14:CR416e22.

36. Leung TF, Wong GW, Ko FW, Lam CW, Fok TF. Clinical and atopic parameters and airway inflammatory markers in childhood asthma: a factor analysis. Thorax 2005;60:822e6.

37. Jones SL, et al. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 2001;164:738e43.

38. Michils A, Louis R, Peche R, Baldassarre S, Van Muylem A. Exhaled nitric oxide as a marker of asthma control in smoking patients. Eur Respir J 2009;33:1295e301.

39. Lehtimaki L, Kankaanranta H, Saarelainen S, Turjanmaa V, Moilanen E. Increased alveolar nitric oxide concentration in asthmatic patients with nocturnal symptoms. Eur Respir J 2002;20:841e5.

40. Mahut B, et al. Increase in alveolar nitric oxide in the presence of symptoms in childhood asthma. Chest 2004;125: 1012e8.

41. Lane C, et al. Epithelial inducible nitric oxide synthase activity is the major determinant of nitric oxide concentration in exhaled breath. Thorax 2004;59:757e60.

42. Brennan ML, et al. A tale of two controversies: defining both the role of peroxidases in nitrotyrosine formation in vivo using eosinophil peroxidase and myeloperoxidase-deficient mice, and the nature of peroxidase-generated reactive nitrogen species. J Biol Chem 2002;277:17415e27.

43. Chapman AL, et al. Myeloperoxidase-dependent oxidative metabolism of nitric oxide in the cystic fibrosis airway. J Cyst Fibros 2010;9:84e92.

44. Dweik RA, et al. NO chemical events in the human airway during the immediate and late antigen-induced asthmatic response. Proc Natl Acad Sci USA 2001;98:2622e7.

45. Silkoff PE, et al. ATS workshop proceedings: exhaled nitric oxide and nitric oxide oxidative metabolism in exhaled breath condensate. Proc Am Thorac Soc 2006;3:131e45.

46. Kharitonov SA, et al. Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. Thorax 2002;57:889e96.

47. Garrelds IM, van Amsterdam JG, de Graaf-in't Veld C, Gerth van Wijk R, Zijlstra FJ. Nitric oxide metabolites in nasal lavage fluid of patients with house dust mite allergy. Thorax 1995;50: 275e9.

48. Guida G, et al. Determinants of exhaled nitric oxide in chronic rhinosinusitis. Chest 2010;137:658e64.

49. Boulet LP. Influence of comorbid conditions on asthma. Eur Respir J 2009;33:897e906.

50. Deroee AF, Naraghi M, Sontou AF, Ebrahimkhani MR, Dehpour AR. Nitric oxide metabolites as biomarkers for followup after chronic rhinosinusitis surgery. Am J Rhinol Allergy 2009;23:159e61.

Table 1. Patients' characteristics.

Age (median (range))	50 (21–80)
Females (*N* (%))	19 (49%)
Atopy (*N* (%))	30 (77%)
Inhaled steroid therapy (*N* (%))	
No therapy	2 (5%)
<400 µg[a]	15
400–800 µg[a]	9
800–1200 µg[a]	9
>1200 µg[a]	4 (10%)
Oral steroids (*N* (%))	2 (5%)
Rhinitis (*N* (%))	32 (82%)
Chronic rhinosinusitis (*N* (%))	22 (56%)
Nasal polyps (*N* (%))	14 (36%)

a Equivalent budesonide dose.

Figure 1.
Correlation between ACQ and ACT scores. Closed diamonds represent subjects on steroid doses <400 µg budesonide while open circles represent subjects on steroid doses ≥400 µg budesonide.

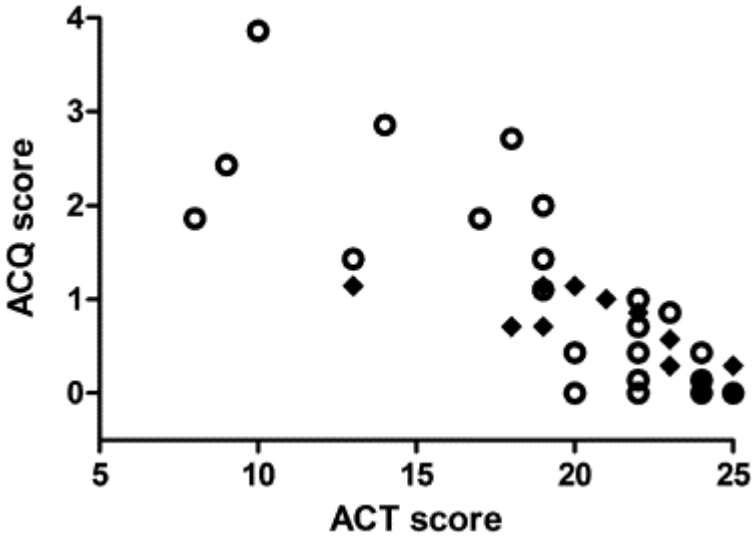

Table 2.
Relationships between asthma control (measured as either ACQ or ACT score) and lung function parameters. Results are presented as Spearman ρ (p-value).

	ACQ score	ACT score
FEV_1 (% pred)	−0.61 ($p < 0.001$)	0.47 ($p = 0.003$)
Tiffeneau index	−0.42 ($p = 0.008$)	0.40 ($p = 0.01$)
FEF25–75 (% pred)	−0.49 ($p = 0.001$)	0.41 ($p = 0.01$)

Figure 2.
Correlation between EBC nitrate and ACT score (Panel A) and ACQ score (Panel B), respectively, as well as correlations between EBC nitrite and ACT score (Panel C) and ACQ score (Panel D). Closed diamonds represent subjects on steroid doses <400 µg budesonide while open circles represent subjects on steroid doses ≥400 µg budesonide.

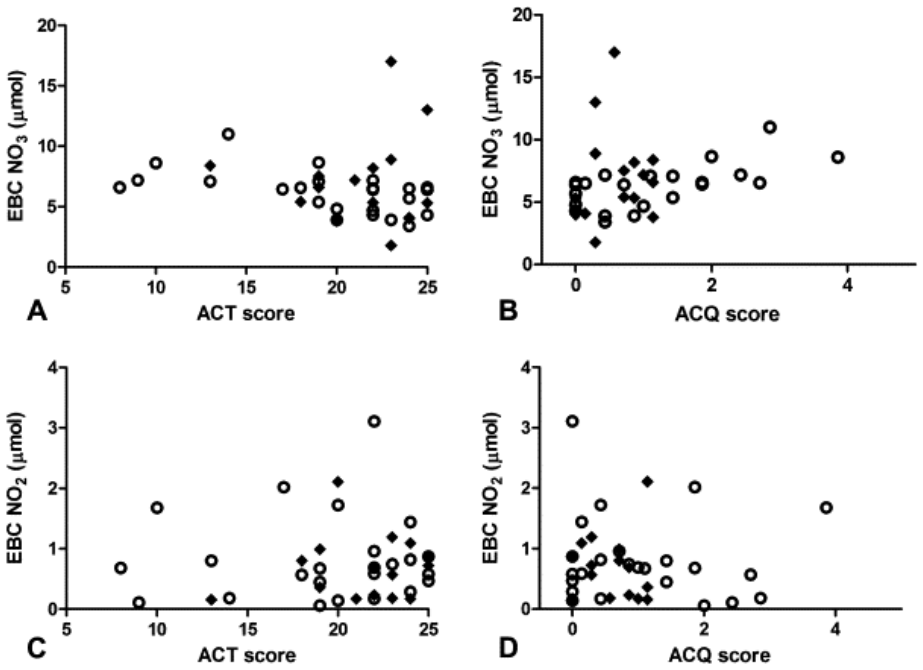

Figure 3.
Box-plot of EBC nitrate (Panels A and B) and EBC nitrite (Panels C and D) in subjects on steroid doses ≥400 µg budesonide with well-controlled and not well-controlled asthma, according to ACT scores (Panels A and C) or ACQ scores (Panels B and D). Box-plot shows median (line), interquartile range (IQR) (box) and whiskers extend to 1.5 IQR. Values outside 1.5 IQR are considered outliers (dots).