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IMPROVED PREDICTION OF ASTHMA EXACERBATIONS BY MEASURING DISTAL AIRWAY INFLAMMATION

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TAKE HOME MESSAGE

Partitioning exhaled nitric oxide allows improved prediction of risk of an asthma attack in the subsequent four months. $C_{alv}NO > 7ppb$ was highly specific for a subsequent exacerbation, while $C_{alv}NO < 4$ ppb excluded risk of an attack with high specificity.

INTRODUCTION

The function of the distal airway generations has in the past been difficult to assess, and in particular spirometry is insensitive to small airway disease. Novel physiological tools such as the Forced Oscillation Technique (FOT) and multiple breath washout (MBW) have demonstrated that distal disease makes an important contribution to asthma severity. Distal obstruction may result from many different factors, including remodeling, inflammation, airway instability due to loss of alveolar tethering attachments and mucus plugging. Distal airway disease is known to independently contribute to the severity of airway hyper-responsiveness in asthma [1] but unsurprisingly there is a poor correlation between distal inflammation measured directly and physiological parameters. [2-4] Distal inflammation can be studied directly using transbronchial biopsy and has been implicated in the severity of asthma, [5] but this invasive technique is not suitable for routine monitoring and especially not in children.

Measurement of exhaled nitric oxide at an expiratory flow rate of 50 ml/sec ($FeNO_{50}$) has long been used as a marker of -mainly eosinophilic- inflammation, nitrosative stress, and altered nitrogen redox physiology of the airway in asthma [6]. Measurement of FeNO at multiple flow rates allows the contributions of distal (alveolar concentration of nitric oxide, $C_{alv}NO$) and proximal airway (bronchial flux

of nitric oxide, $J_{aw}NO$) to be determined [7]. Cohen et al demonstrated that a small particle inhaled corticosteroid (ICS), ciclesonide, which would be expected to be deposited distally, improved $C_{alv}NO$ and reduced gas trapping on Computed Tomography (CT) scanning. [8] A study of apparently steroid-refractory asthma, also in adults, demonstrated the presence of untreated distal inflammation, and control improved after treatment with ciclesonide. [9] Thus there is evidence that distal airway inflammation may be an independent contributor to poor adult asthma outcomes.

Measurement of future risk is an increasingly important part of the assessment of asthma, with the realization that good control does not exclude the possibility of a high risk of subsequent asthma exacerbations. For example, sputum eosinophilia and persistent elevation of $FeNO_{50}$ are markers of risk of asthma exacerbations [10,11] in apparently well-controlled asthmatics. We hypothesized that measurement of distal airway inflammation ($C_{alv}NO$) using variable flow measurements of FeNO would be a better marker of future risk than $FeNO_{50}$ or $J_{aw}NO$, which measure more proximal inflammation. We recruited asthmatic children to a prospective follow up study to determine which nitric oxide measurements were most closely associated with subsequent asthma exacerbations.

METHODS

Subjects

We recruited 68 asthmatic children (mean age ± standard deviation (SD) 9.0 ± 2.4 years, 45 males); all also had allergic rhinitis and were sensitized to aeroallergens (Table 1). None was a self-reported current or previous smoker. Inclusion criteria were as follows: (*1*) clinical diagnosis of asthma (a history of at least two of the following: cough, shortness of breath, recurrent wheeze, chest tightness) and 15% increase in first second forced expired volume (FEV_1) after administration of 400mcg short acting β-2 agonist; *2*) exclusion of other diseases mimicking asthma; (3) age more than 6 years to be able to perform FeNO measurements at incremental flows. All subjects were recruited from the asthma outpatient clinic of the Paediatric Respiratory Unit of the University Hospital of Alexandroupolis, Greece. The study was approved by the Ethics committee of the University Hospital of Alexandroupolis, consent was obtained from parents and age-appropriate assent from child participants.

Study Design

The study design is summarized in Figure 1. A detailed medical history including baseline medication and asthma control, physical examination, and specific IgE tests (RAST) for ten common aeroallergens were recorded. All subjects were stratified according GINA guidelines to controlled, partially controlled and uncontrolled at the enrollment (visit 1) and visits 2 (4 months after visit 1) and 3 (4 months after visit 2). We recorded Childhood Asthma Control Test (cACT), spirometry pre- and post-bronchodilator administration, and $FeNO_{50}$ and calculation of $J_{aw}NO$ and $C_{alv}NO$ was performed; the transfer factor and the airway wall concentration of nitric oxide ($D_{aw}NO$ and $C_{aw}NO$) were also computed. Any moderate or severe exacerbation in the

previous 4 months was recorded in all participants at visit 2 and 3. Moderate exacerbation was defined as including one or more of the following: deterioration in symptoms, deterioration in lung function, and increased rescue bronchodilator use, lasting at least two days, but not severe enough to warrant systemic corticosteroid prescription and/or hospitalization. Severe exacerbation was defined as one requiring any of high dose oral corticosteroids for at least three days, increase in maintenance oral corticosteroid dose, Emergency Department (ED) visit or hospitalization [12].

Pulmonary Function Testing

A dry rolling seal spirometer was used for pulmonary function testing (MIR Spirodoc, Italy) based on ERS/ATS criteria [13].

Nitric oxide Testing

Exhaled nitric oxide was measured prior to spirometry using an analyzer (ECO-MEDICS, CLD88sp, Switzerland) [14] and according to ERS/ATS guidelines [15]. FeNO was initially measured at a flow rate of 50 ml/s ($FeNO_{50}$), followed by three measurements at 30, 100, and 300 ml/s; the latter were used to calculate $J_{aw}NO$, $C_{alv}NO$, $D_{aw}NO$, and $C_{aw}NO$ (Högman and Meriläinen algorithm) [16]. To ensure the higher possible success rate, a three-step approach was adopted: first, a specialized and experienced nurse demonstrates the procedure; second, two test measurements (at flows 50 and 300 ml/s) were performed to familiarize with the technique (lower and higher flows) and the device; third, the child performed the measurement at incremental flows. A single trial was performed per each flow rate; the trial was repeated only in the case of technical or quality issues (according to device quality-

control algorithms and to investigators' experience). The duration of the whole procedure ($FeNO_{50}$ and FeNO at incremental flow rates) was 10-15 minutes.

Statistics

We have previously reported a 40% higher $C_{alv}NO$ in children with poorly controlled asthma as compared with those with controlled disease [17]. Assuming 25% of asthmatic children would experience an exacerbation, 65 children examined twice (i.e. 130 visits) would be required to obtain a similar $C_{alv}NO$ difference at 5% alpha level with 85% power. Sample size estimation was performed using the G*Power software [18].

Continuous variables were compared with the Student's t test and categorical variables with the chi-square test. Linear mixed modeling with adjustment for repeated observations (i.e. study visits) was used to compare $FeNO_{50}$, $J_{aw}NO$, $C_{alv}NO$, $D_{aw}NO$, and $C_{aw}NO$ (log-transformed values) between visits followed and those not followed by an exacerbation. Linear Mixed Modeling was also applied to explore differences of log-transformed bronchial inflammation parameters in relation to the severity of exacerbations. Repeated measures (mixed effects) logistic regression was used to explore predictors of an asthma exacerbation. Spearman's correlation and Cox survival analysis was used to explore the relationship between $FeNO_{50}$, $J_{aw}NO$, $C_{alv}NO$, $D_{aw}NO$, and $C_{aw}NO$ z-score values and time to asthma exacerbation. ROC analysis was applied to calculate the overall predictive ability of bronchial inflammation markers; sensitivity, specificity, and positive and negative likelihood ratio (LR) were calculated for different $C_{alv}NO$ levels. As high-risk cut-off was considered the lower $C_{alv}NO$ value with positive LR >3. All analyses were performed using the IBM SPSS software version 25.0 (IBM Corp., Armonk, N.Y., USA).

RESULTS

The characteristics of the study population are presented in Table 1. Sixty-nine children were enrolled in the study (visit 1) of whom 21 reported at least one exacerbation prior to the study starting. At visit 2, 19/68 (one was lost to follow-up) reported that they had suffered an exacerbation, while 16/66 (two further children were lost to follow-up) reported an exacerbation at visit 3. In total, no exacerbation was reported in 99 visits (Group 1) and an exacerbation in 35 visits (Group 2); 10 (28.6%) of the exacerbations were severe. There were no differences at baseline in age, height, weight, treatment for allergic rhinitis, asthma control and cACT between the two groups (Table 2). Participants in the exacerbation group were more frequently treated with inhaled corticosteroids (ICS) or leukotriene receptor antagonists (LTRA). There were also no differences in spirometric parameters between the two groups, including post bronchodilation reversibility of FEV_1 (Table 2).

FeNO measurement at incremental flows was successful with the first attempt in 117 of the 134 visits (87.3%). A repeated measurement was required in 17 visits, mainly (15/17; 88.2%) due to expiratory flow instability at 300 ml/s that resulted in poor equation fitting [7] and even negative $C_{alv}NO$ values. In the participants who did not exacerbate the median (range) of $FeNO_{50}$ was 12.7 (0.2 - 10.8) ppb, of $J_{aw}NO$ 715 (10 - 12799) pl/s, of $D_{aw}NO$ 38.3 (0.2 - 113.3) pl/s·ppb, of $C_{aw}NO$ 26.8 (4.1 - 2163) ppb, and of $C_{alv}NO$ 3.4 (4.0 - 209) ppb (Figure 2). As compared to the previous group, those who experienced an exacerbation had significantly higher $C_{alv}NO$ [5.2 (3.8 - 149.9) ppb, $p < 0.001$], but similar $FeNO_{50}$ [13.5(1.7 - 23.6) ppb, $p=0.744$], $J_{aw}NO$ [438 (40 – 7457) pl/s, $p=0.708$], $D_{aw}NO$ [36 (0.2 - 144.5) pl/s·ppb, $p=0.431$], and $C_{aw}NO$ [29.9 (5.5 - 3054) ppb, $p=0.399$] (Figure 2) on the visit preceding the exacerbation. Participants who

experienced severe exacerbations had significantly higher $C_{alv}NO$, $FeNO_{50}$ and $C_{aw}NO$ on the preceding visit compared with controls (Figure 3).

Predictors and time to exacerbations

Repeated measures logistic regression revealed that the $C_{alv}NO$ was the only bronchial inflammation parameter that was associated with increased risk of asthma exacerbation within the following four months (Table 3). The effect of $C_{alv}NO$ was independent of the spirometric parameters (including reversibility to bronchodilation), $FeNO_{50}$ levels, gender and use of controller therapy (ICS, LTRA) (Table 3). With the exception of $D_{aw}NO$, all other bronchial inflammation indices were negatively correlated with the time to asthma exacerbation. The strongest correlation was observed for $C_{alv}NO$ and the weakest for $J_{aw}NO$. Cox survival analysis corroborated these results (Figure 4).

Predictive ability of bronchial inflammation parameters

The AUC of $FeNO_{50}$ was 0.507 (95% CI 0.390-0.623), of $J_{aw}NO$ 0.516 (95% CI 0.407-0.641), of $D_{aw}NO$ 0.521 (95% CI 0.420-0.652), and of $C_{aw}NO$ 0.565 (0.448-0.681), reflecting the low ability of the above parameters to identify children at risk. The AUC of $C_{alv}NO$ was 0.69 (95% CI 0.585-0.794), indicating moderate overall ability to differentiate children at risk for asthma exacerbation. The predictive characteristics of different $C_{alv}NO$ levels are shown in Figure 5. A $C_{alv}NO$ >7 ppb predicted asthma exacerbation with high accuracy (specificity 90.9%, LR + 3 .1), while a $C_{alv}NO$ >10 ppb had a specificity of 99% and a positive likelihood ratio of 19.8, but a sensitivity of 20% and a negative likelihood ratio 0.8. Conversely $C_{alv}NO$ levels less than 4 ppb for example, had lower ability to exclude an exacerbation within the next four months (sensitivity 71.4% likelihood ratio -0. 48).

Children with $C_{alv}NO$ levels higher than 7 ppb had a high probability of experiencing an exacerbation within the next four months (Table 4); they also had more exacerbations (50% vs 21.9%, p<0.001), more severe exacerbations (90% vs 0.9%, p<0.001) and shorter time to exacerbation (4.2±2.9 vs 12.5±2.1 weeks, p<0.001). The corresponding differences in cACT, $FeNO_{50}$ and FEV_1 were not significant, but participants with $C_{alv}NO$ levels higher than 7 ppb presented lower forced mid-expiratory flows (Table 4).

The predictive characteristics of a change in $C_{alv}NO$ between visits was assessed in children with an attack after visit 2 but without an attack between visits 1 and 2 since treatment with systemic corticosteroids could have affected NO measurements. The median (range) $C_{alv}NO$ change was –0.1 (–3.6 to 5.8) ppb or –5.4 (–56.3 to 150) % in those who did not exacerbate *versus* 1.2 (0.2 to 4) ppb or 60 (3.6 to 480) % in those who experienced an asthma exacerbation (P<0.001) (Figure 6). $C_{alv}NO$ rise was a better risk predictor of future exacerbation (AUC 0.939; 0.832-0.987) than a single $C_{alv}NO$ measurement (AUC 0.650; 0.507-0.776; P<0.001). An increase of $C_{alv}NO$ of 0.5 ppb from visit 1 to visit 2 had 92% sensitivity, 92% specificity, 11.8 positive LR and 0.08 negative LR for the identification of an exacerbation within the next four months. Similarly, the AUC for the relative $C_{alv}NO$ change between the two visits was also informative (0.931; 0.817-0.984); a 10% increase of $C_{alv}NO$ from the previous visit had 92.3% sensitivity, 88.2% specificity, 7.9 positive LR and 0.09 negative LR in predicting a future exacerbation. Given the small numbers, we did not subdivide further to explore whether the predictive power was greater within one month of the measurement.

DISCUSSION

In this prospective study which recruited children with a wide range of asthma severities, we have shown that partitioning exhaled nitric oxide allows improved prediction of risk of an asthma exacerbation in the subsequent four months. Specifically, a marker of distal inflammation, $C_{alv}NO$, was the best predictor of risk of all the parameters measured. We found decreased forced mid-flows in children with high $C_{alv}NO$ levels (i.e. >7 ppb), which seems to support the value of $C_{alv}NO$ as a marker of small airway disfunction in asthma [7]. More important, a $C_{alv}NO$ level of greater than 7 ppb was highly specific but not very sensitive for a subsequent exacerbation, and a level of less than 4 ppb excluded risk of an attack also with high specificity but low sensitivity. Even more sensitive, in a small subgroup, was a rise in $C_{alv}NO$ of >0.5 ppb between visits four months apart. As expected, neither symptoms (cACT) nor FEV1 or airway reversibility test were useful in the assessment of risk.

This is the first study prospectively evaluating $C_{alv}NO$ as a marker of risk in children. The strengths of the study include its prospective design and the fact that were assessed children with varying levels of asthma severity, although all participants had well-controlled asthma at recruitment. The use of the improved EcoMedics device for measuring exhaled nitric oxide enabled us to measure $C_{alv}NO$ and $J_{aw}NO$ in all our subjects, unlike our previous experience [17]. However, inevitably there are some weaknesses. There were some potential markers of risk that we did not include. Well-known risk factors for an asthma exacerbation are especially a previous bad exacerbation, overuse of short-acting β-2 agonists and under-use of ICS. [19] We did not have any objective assessments of inhaler use. We also did not measure other markers of risk such as induced sputum and peripheral blood eosinophil count, and we

did not attempt to see if small particle ICS reduced risk of exacerbations in those with a high $C_{alv}NO$. We had no data on lung diffusion capacity, and we could not assess the effect of other factors, such as cardiac output, hemoglobin concentration, and airway lining fluid pH on $C_{alv}NO$ levels [6]. Finally, our findings need to be ideally validated in a second cohort.

This study has mechanistic and clinical implications for children with asthma. The risk of an asthma exacerbation associated with small airway inflammation, as measured by $C_{alv}NO$, underscores the importance of distal as well as proximal airways disease in the pathophysiology of asthma. As a clinical test, the partitioning nitric oxide is likely only to be useful in specialist settings. The equipment is expensive and the test is time-consuming. There is also considerable overlap between children who did and did not relapse. However, those children who have a really high $C_{alv}NO$ on the present data form a subgroup who are at high risk and need a focused reassessment of risk factors. Those children with a rise in $C_{alv}NO$ between visits also merit this approach, although the usefulness of a change in $C_{alv}NO$ is limited by the need to make measurements at two time points. Conversely, those with a very low $C_{alv}NO$ and no change over time would not be expected to be having exacerbations, and if exacerbations are reported, the paediatrician might consider whether symptoms are being over-reported [20].

Ultimately, whether partitioning nitric oxide has clinical value depends on whether taking action on the results improves outcomes; there is little point in making measurements clinically if no useful action results. The obvious next study is to determine whether fine particle ICS improves outcomes when added to the treatment regime of those suffering acute exacerbations and have an elevated or rising $C_{alv}NO$.

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Table 1. Characteristics of the study population (N=68*)

Male sex	45 (66.2)
Age, years	9.0 ± 2.4 (6 - 15)
Height, cm	139 ± 13 (116 - 180)
Weight, kg	38.8 ± 15 (21 - 71)
BMI, kg/m^2	19.5 ± 4.4 (12.8 - 35.6)
Allergic sensitization, n (%)	
Seasonal (pollens)	38 (55.9)
Perennial	30 (44.1)
Dust mites	25 (36.8)
Molds	14 (20.6)
Cat, dog	7 (10.3)
Seasonal and perennial	28 (41.2)
Multiple (≥ 3 different allergens)	44 (64.7)
ICS	36 (52.9)
LTRA	10 (14.7)
ICS and LTRA	6 (8.8)
Nasal CS	10 (14.7)
Anti-H_1	13 (19.1)

Data are number of cases (%)

* children with at least one follow-up visit

BMI: body mass index, ICS: inhaled corticosteroids, LTRA: leukotriene receptor antagonists, CS: corticosteroids, anti-H_1: antihistamines

Table 2. Participant's characteristics on study visits*

	Visits not followed by exacerbation Group 1 (N=99)	Visits followed by exacerbation Group 2 (N=35)	P
Male sex	68 (68.7)	19 (54.3)	0.125
Age, years	9.0 ± 2.4 (6 - 15)	8.9 ± 1.8 (6 - 15)	0.760
Height, cm	139 ± 13 (116 - 180)	138 ± 11 (122 - 160)	0.808
Weight, kg	38.8 ± 15 (21 - 71)	36.9 ± 15.5 (22 - 80)	0.585
BMI, kg/m^2	19.5 ± 4.4 (12.8 - 35.6)	18.7 ± 4.9 (13.2 - 35.6)	0.421
ICS	54 (54,5)	26 (74.3)	0.041
LTRA	15 (15.6)	11 (32.4)	0.036
Nasal CS	15 (15.2)	6 (17.1)	0.781
Anti-H_1	19 (19.2)	9 (25.7)	0.415
FEV_1, % predicted	101.8 ± 8.9 (82 - 126)	101.6 ± 9.7 (83 - 119)	0.911
FVC, % predicted	95.7 ± 8.4 (82 - 120)	97.4 ± 7.9 (84 - 113)	0.298
FEF_{25-75}, % predicted	106.5 ± 14.8 (78 - 139)	105.2 ± 18.7 (74 - 140)	0.678
FEV_1/FVC, %	89.1 ± 3.6 (83 - 97)	88.2 ± 3.8 (81 - 93)	0.212
PEF, % predicted	94.3 ± 9.6 (82 - 118)	93.3 ± 9.2 (82 - 111)	0.593
ΔFEV_1, %	4.1 ± 3.0 (1 - 11)	4.9 ± 3.4 (2 - 16)	0.193

Data are number of cases (%) and mean ± SD (range). Comparisons were performed by chi-square and student's t test.

* visit #1 of 68 children who attended visit #2 and visit #2 of 66 children who attended visit #3 (N=136 visits in total)

† as assessed on the visit prior to exacerbation; ‡ time elapsed from the last visit until asthma exacerbation; ¶ need of oral or intravenous corticosteroids

BMI: body mass index, ICS: inhaled corticosteroids, LTRA: leukotriene receptor antagonists, CS: corticosteroids, anti-H_1: antihistamines; GINA: global lung initiative, cACT: childhood asthma control test; ΔFEV_1: % change in FEV1 after administration of 400 mcg salbutamol inhaler; NA: not applicable

Table 3 Probability of exacerbation

	Exploratory models		Multivariable model	
	Odd ratios (95% CI)	P	Odd ratios (95% CI)	P
cACT	0.70 (0.34 - 7.60)	0.350	-	
FEV1	0.22 (0.02 - 9.44)	0.529	-	
FEV_1/FVC	0.15 (0.02 - 8.68)	0.302	-	
ΔFEV_1	1.16 (0.59 - 2.25)	0.672	1.02 (0.53 - 1.97)	0.945
FeNO	1.18 (0.80 - 1.88)	0.406	1.13 (0.57 - 2.25)	0.720
$J_{aw}NO$	0.99 (0.80 - 1.24)	0.950	-	
$C_{alv}NO$	**1.46 (1.13 - 1.88)**	**0.004**	**1.65 (1.07 - 2.53)**	**0.023**
$C_{aw}NO$	0.73 (0.39 - 1.37)	0.326	-	
$D_{aw}NO$	1.03 (0.79 - 1.33)	0.847	-	

Repeated measures (mixed effects) logistic regression with adjustment for sex and controller therapy (ICS, LTRA). In exploratory models the effect of each factor was assessed separately. The multivariable model presents the combined effect of $C_{alv}NO$, FeNO and ΔFEV_1.

cACT: childhood asthma control test, ΔFEV_1: % change in FEV_1 after administration of 400 mcg salbutamol inhaler, FeNO: fractional expired concentration of nitric oxide, $J_{aw}NO$: bronchial flux of nitric oxide, $C_{alv}NO$: alveolar concentration of nitric oxide, $C_{aw}NO$: airway wall concentration of nitric oxide, $D_{aw}NO$: transfer factor of nitric oxide

Table 4. Characteristics of children with increased $C_{alv}NO$

Parameter *	$C_{alv}NO$ < 7 ppb (N=114)	$C_{alv}NO \geq 7$ ppb (N=20)	P
ACT	25.1 ± 3.1 (25; 14 - 27)	25.0 ± 4.1 (27; 15 - 27)	0.434
FEV_1, % predicted	102.0 ± 9.5 (103; 82 - 126)	99.9 ± 7.0 (98; 91 - 115)	0.307
FEV_1/FVC, %	88.8 ± 3.7 (88; 81 - 97)	89.3 ± 3.7 (89; 83 - 94)	0.537
FEF_{25-75}, % predicted	106.2 ± 14.5 (78 - 139)	94.8 ± 14.6 (74 - 114)	0.021
ΔFEV_1, %	4.3 ± 2.9 (3; 1 - 11)	4.5 ± 3.9 (4; 1 - 16)	0.813
FeNO, ppb	21.9 ± 30 (11.4; 3.8 - 209)	39.6 ± 42 (24.4; 8.5 - 149.9)	0.005
Exacerbation	25 (21.9)	10 (50.0)	<0.001
Severe exacerbation	1 (0.9)	9 (90)	<0.001
Time to exacerbation, weeks	12.5 ± 2.1 (13; 8 - 16)	4.2 ± 2.9 (4; 1 - 8)	<0.001

Data are mean ± SD (median; range) or number of cases (%). Comparisons were performed using Mann-Whitney U or chi-square test, as appropriate.

$C_{alv}NO$: alveolar concentration of nitric oxide, ACT: asthma control test, ΔFEV_1: % change in FEV_1 after administration of 400 mcg salbutamol inhaler, FeNO: fractional expired concentration of nitric oxide

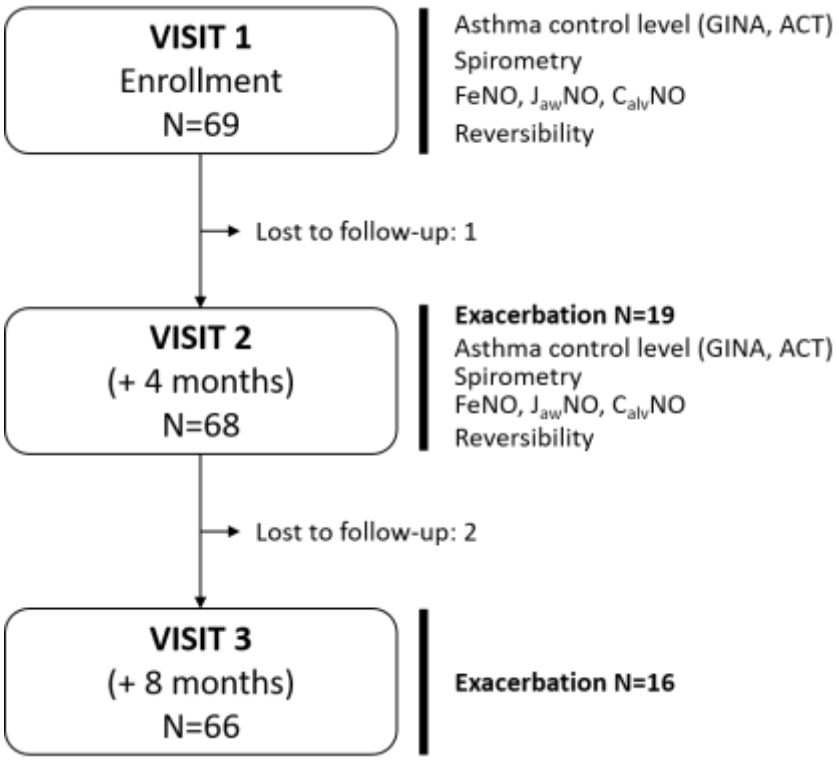

Figure 1.

Study flow

GINA: global lung initiative, cACT: childhood asthma control test, FeNO: fractional expired concentration of nitric oxide, J_{aw}NO: bronchial flux of nitric oxide, C_{alv}NO: alveolar concentration of nitric oxide

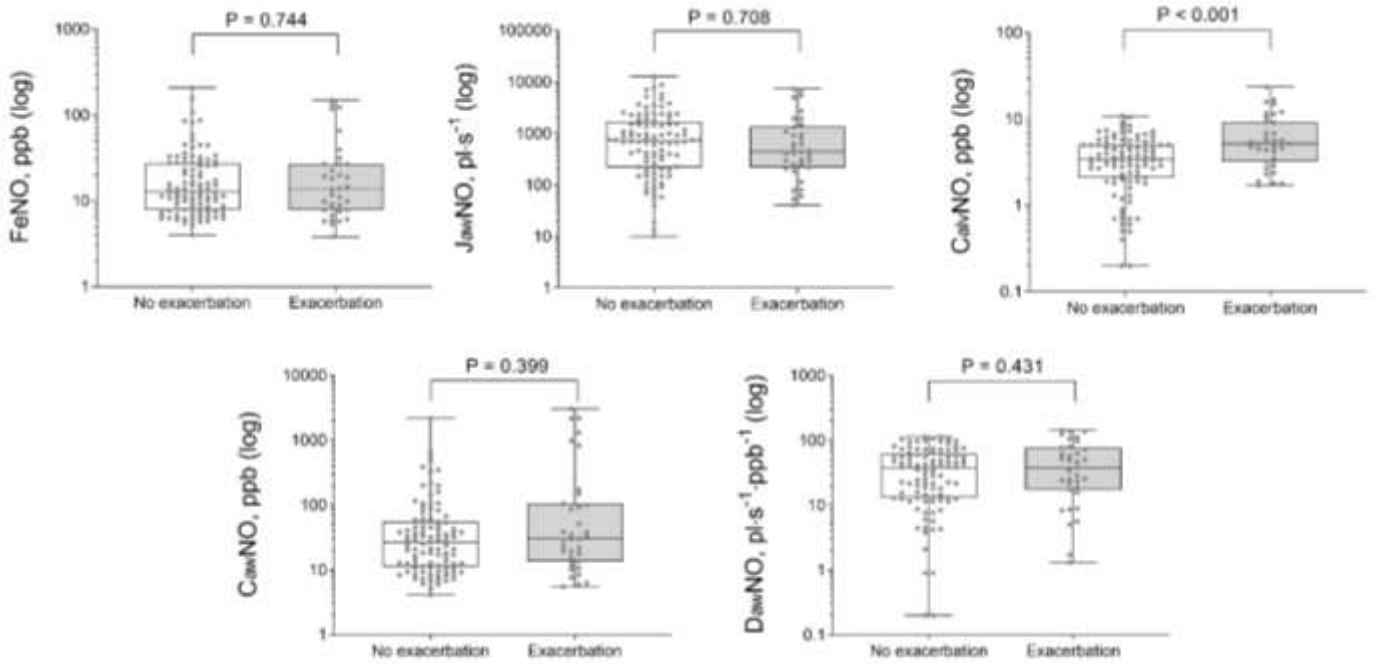

Figure 2.

Bronchial inflammation parameters (log-transformed) in visits not followed by an exacerbation and in those followed by an exacerbation. P-values were calculated with Linear Mixed Modeling with adjustment for repeated observations.

FeNO: fractional expired concentration of nitric oxide, $J_{aw}NO$: bronchial flux of nitric oxide, $C_{alv}NO$: alveolar concentration of nitric oxide, $C_{aw}NO$: airway wall concentration of nitric oxide, $D_{aw}NO$: transfer factor of nitric oxide

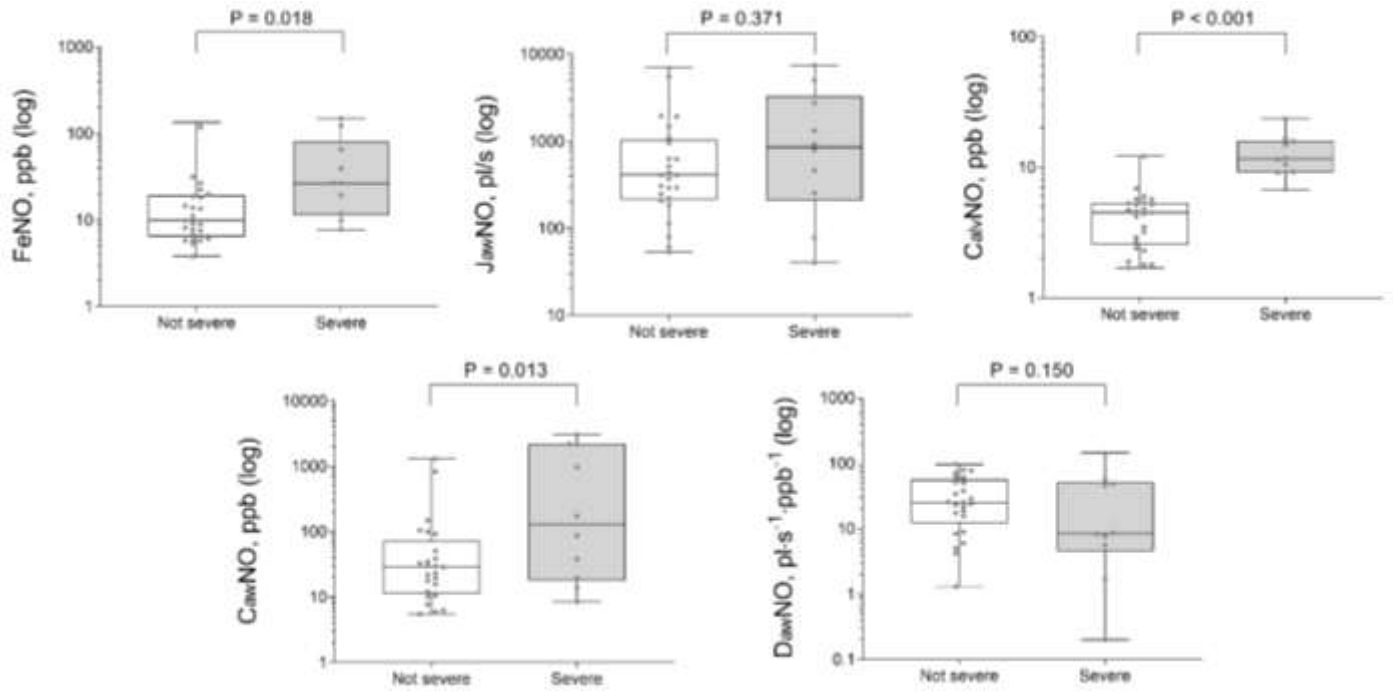

Figure 3.

Bronchial inflammation parameters (log-transformed) according to severity of asthma exacerbation (N=35). P-values were calculated with Linear Mixed Modeling.

FeNO: fractional expired concentration of nitric oxide, $J_{aw}NO$: bronchial flux of nitric oxide, $C_{alv}NO$: alveolar concentration of nitric oxide, $C_{aw}NO$: airway wall concentration of nitric oxide, $D_{aw}NO$: transfer factor of nitric oxide

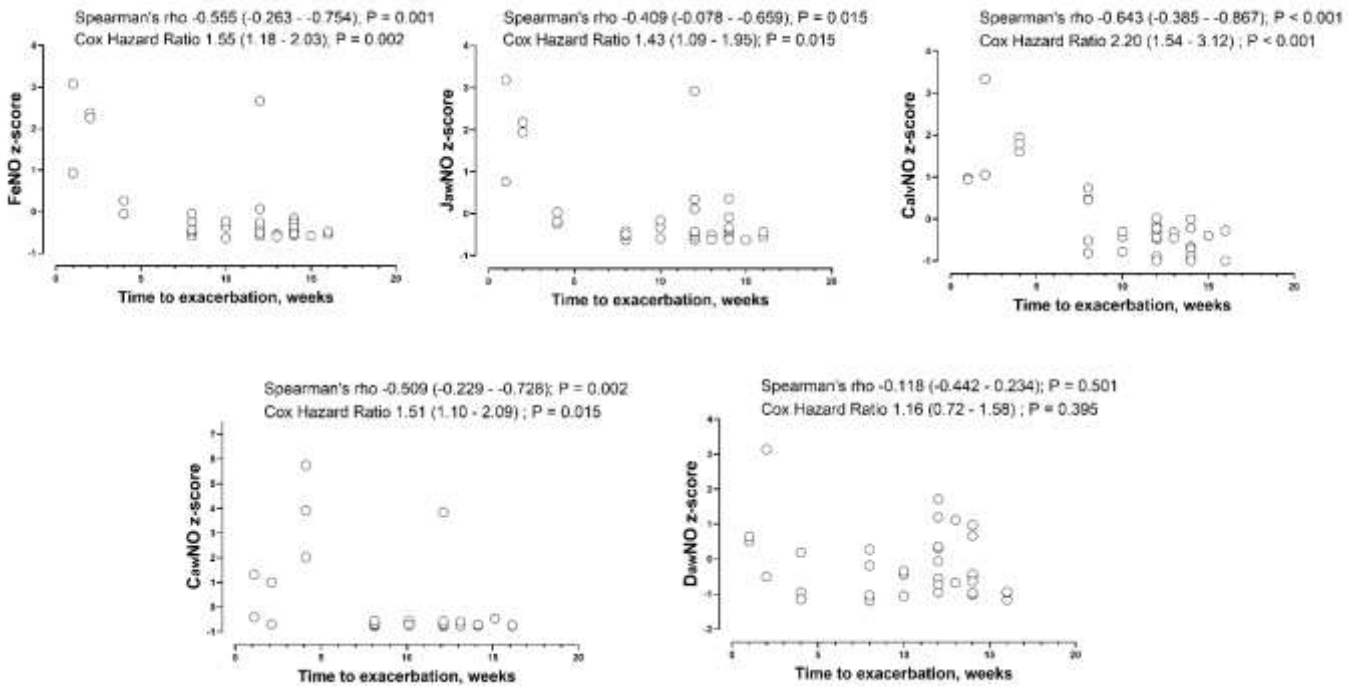

Figure 4.

Bronchial inflammation indices (z-scores) and time to exacerbation.

FeNO: fractional expired concentration of nitric oxide, $J_{aw}NO$: bronchial flux of nitric oxide, $C_{alv}NO$: alveolar concentration of nitric oxide, $C_{aw}NO$: airway wall concentration of nitric oxide, $D_{aw}NO$: transfer factor of nitric oxide

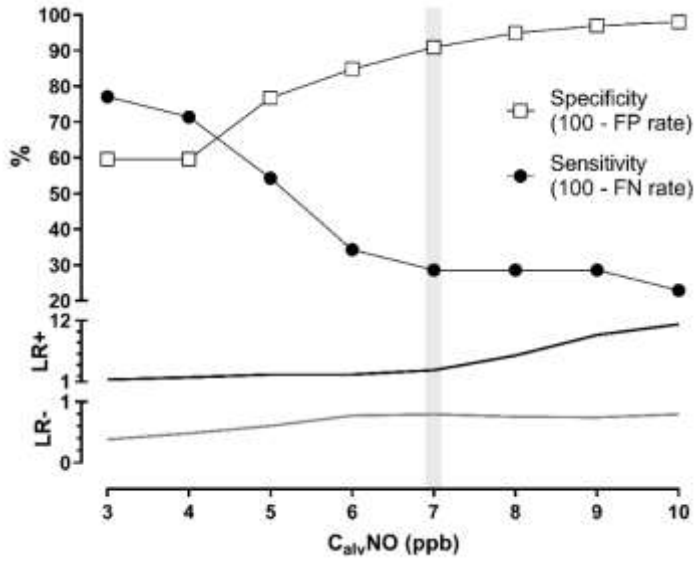

Figure 5.

Predictive characteristics of different $C_{alv}NO$ levels. The lower $C_{alv}NO$ value with LR+ >3 (high-risk cut-off) is marked.

$C_{alv}NO$: alveolar concentration of nitric oxide, FP: false positive rate, FN: false negative rate, LR+: positive likelihood ratio, LR-: negative likelihood ratio

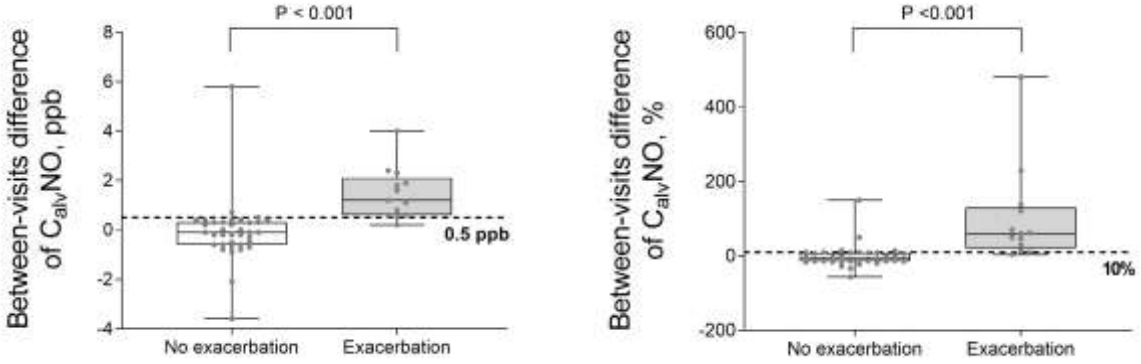

Figure 6.

Comparison of absolute and relative $C_{alv}NO$ difference between visits 1 and 2 in relation to exacerbation occurrence after visit 2.

The dotted line marks the cut-off value with the best sensitivity-specificity combination in predicting an exacerbation (ROC analysis).

$C_{alv}NO$: alveolar concentration of nitric oxide