Improved prediction of asthma exacerbations by measuring distal airway inflammation

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Shareable abstract (@ERSpublications)
Partitioning exhaled nitric oxide allows improved prediction of risk of an asthma attack in the subsequent 4 months. C_{alvNO}>7 ppb was highly specific for a subsequent exacerbation, while C_{alvNO} <4 ppb excluded risk of an attack with high specificity. https://bit.ly/3zWZWYp

Cite this article as: Paraskakis E, Sarikloglou E, Fouzas S, *et al*. Improved prediction of asthma exacerbations by measuring distal airway inflammation. *Eur Respir J* 2022; 60: 2101684 [DOI: 10.1183/13993003.01684-2021].

This article has an editorial commentary: https://doi.org/10.1183/13993003.00802-2022

Received: 14 June 2021
Accepted: 5 Jan 2022

Abstract

Introduction Partitioning parameters measured from exhaled nitric oxide, such as the alveolar concentration of nitric oxide (C_{alvNO}), may provide better predictors of future asthma exacerbation than exhaled nitric oxide fraction at an expiratory flow rate of 50 mL·s^{-1} ($F_{ENO_{50}}$). We aimed to determine whether any partitioned nitric oxide parameters were more closely associated than $F_{ENO_{50}}$ with subsequent asthma exacerbations.

Methods 68 asthmatic children (mean±SD age 9.0±2.4 years) were followed prospectively (134 visits) and exacerbations were recorded. Childhood Asthma Control Test (cACT), spirometry, $F_{ENO_{50}}$, C_{alvNO}, bronchial flux of nitric oxide (J_{awNO}), transfer factor of nitric oxide (D_{awNO}) and airway wall concentration of nitric oxide (C_{awNO}) were measured.

Results No exacerbation was recorded in 99 visits (Group 1) and an exacerbation was recorded in 35 visits (Group 2). The median (range) $F_{ENO_{50}}$, J_{awNO}, C_{alvNO}, D_{awNO} and C_{awNO} of Group 1 *versus* Group 2: 12.7 (4–209) *versus* 13.5 (3.8–149.9) ppb, 715 (10–12 799) *versus* 438 (40–7457) pL·s^{-1}, 3.4 (0.2–10.8) *versus* 5.2 (1.7–23.6) ppb, 38.3 (0.2–113.3) *versus* 38 (1.3–144.5) pL·s^{-1}·ppb^{-1} and 26.8 (4.1–2163) *versus* 29.9 (5.5–3054) ppb, respectively. Other than for C_{alvNO} ($p<0.001$), there was no difference between the two groups. C_{alvNO} >7 ppb predicted asthma exacerbation with specificity 90.9% and positive likelihood ratio (LR) 3.1. Conversely, C_{alvNO} <4 ppb excluded an exacerbation with sensitivity 71.4% and negative LR 0.48. An increase of C_{alvNO} by 0.5 ppb between visits could also predict an exacerbation with sensitivity 92%, specificity 92%, positive LR 11.8 and negative LR 0.08.

Conclusions Assessment of C_{alvNO} improved prediction of subsequent exacerbation, highlighting the importance of distal inflammation in asthma outcomes in children.

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 and multiple breath washout have demonstrated that distal disease makes an important contribution to asthma severity. Distal obstruction may result from many different factors, including remodelling, 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 hyperresponsiveness 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 fraction at an expiratory flow rate of 50 mL·s^{-1} ($F_{ENO_{50}}$) has long been used as a marker of (mainly eosinophilic) inflammation, nitrosative stress and altered nitrogen redox physiology of the airways in asthma [6]. Measurement of F_{ENO} at multiple flow rates allows the contributions of distal airways (alveolar concentration of nitric oxide (C_{alvNO})) and proximal airways (bronchial flux of nitric oxide (J_{awNO})) to be determined [7]. COHEN *et al.* [8] demonstrated that a fine-particle inhaled corticosteroid (ICS), ciclesonide, which would be expected to be deposited distally, improved C_{alvNO} and reduced gas trapping on computed tomography scanning. A study of apparently steroid-refractory asthma, also in adults, demonstrated the presence of untreated distal inflammation and that asthma control improved after treatment with ciclesonide [9]. Thus, there is evidence that distal airways 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 realisation that good control does not exclude the possibility of a high risk of subsequent asthma exacerbations. For example, sputum eosinophilia and persistent elevation of $F_{ENO_{50}}$ are markers of risk of asthma exacerbations in apparently well-controlled asthmatic subjects [10, 11]. We hypothesised that measurement of distal airway inflammation (C_{alvNO}) using variable flow measurements of F_{ENO} would be a better marker of future risk than $F_{ENO_{50}}$ or J_{awNO}, 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±SD age 9.0±2.4 years; 45 males); all also had allergic rhinitis and were sensitised to aeroallergens (table 1). None was a self-reported current or ex-smoker. Inclusion criteria were: 1) clinical diagnosis of asthma (a history of at least two of cough, shortness of breath, recurrent wheeze and chest tightness) and 15% increase in forced expiratory volume in 1 s (FEV_1) after administration of 400 µg short-acting β_2-agonist; 2) exclusion of other diseases mimicking asthma; and 3) age >6 years to be able to perform F_{ENO} measurements at incremental flows. All subjects were recruited from the asthma outpatient clinic of the Paediatric Respiratory Unit of the University Hospital of Alexandroupolis (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 was obtained from child participants.

TABLE 1 Characteristics of the study population (n=68[#])

Male	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 sensitisation	
Seasonal (pollens)	38 (55.9)
Perennial	30 (44.1)
Dust mites	25 (36.8)
Moulds	14 (20.6)
Cat, dog	7 (10.3)
Seasonal and perennial	28 (41.2)
Multiple (⩾3 different allergens)	44 (64.7)
ICSs	36 (52.9)
LTRAs	10 (14.7)
ICSs and LTRAs	6 (8.8)
Nasal corticosteroids	10 (14.7)
Antihistamines	13 (19.1)

Data are presented as n (%) or mean±SD (range). BMI: body mass index; ICS: inhaled corticosteroid; LTRA: leukotriene receptor antagonist. [#]: children with at least one follow-up visit.

Study design

The study design is summarised in figure 1. A detailed medical history including baseline medication and asthma control, physical examination, and specific IgE tests (radioallergosorbent tests) for 10 common aeroallergens were recorded. All subjects were stratified according Global Initiative for Asthma guidelines to controlled, partially controlled and uncontrolled at enrolment (visit 1) and at 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 $F_{ENO_{50}}$, and calculation of J_{awNO} and C_{alvNO} was performed; the transfer factor of nitric oxide (D_{awNO}) and the airway wall concentration of nitric oxide (C_{awNO}) were also computed. Any moderate or severe exacerbation in the previous 4 months was recorded in all participants at visits 2 and 3. Moderate exacerbation was defined as including one or more of deterioration in symptoms, deterioration in lung function and increased rescue bronchodilator use, lasting at least 2 days, but not severe enough to warrant systemic corticosteroid prescription and/or hospitalisation. Severe exacerbation was defined as requiring any of high-dose oral corticosteroids for at least 3 days, increase in maintenance oral corticosteroid dose, emergency department visit or hospitalisation [12].

Pulmonary function testing

A dry rolling seal spirometer was used for pulmonary function testing (Spirodoc; MIR, Rome, Italy) based on European Respiratory Society/American Thoracic Society (ERS/ATS) criteria [13].

Nitric oxide testing

Exhaled nitric oxide was measured prior to spirometry using an analyser (CLD 88 sp; Eco Medics, Dürnten, Switzerland) [14] and according to ERS/ATS guidelines [15]. F_{ENO} was initially measured at a flow rate of 50 mL·s^{-1} ($F_{ENO_{50}}$), followed by three measurements at 30, 100 and 300 mL·s^{-1}; the latter were used to calculate J_{awNO}, C_{alvNO}, D_{awNO} and C_{awNO} (Högman–Meriläinen algorithm) [14]. To ensure the highest possible success rate, a three-step approach was adopted: 1) a specialised and experienced nurse demonstrated the procedure; 2) two test measurements (at flow rates of 50 and 300 mL·s^{-1}) were performed to familiarise with the technique (lower and higher flows) and the device; and 3) 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 ($F_{ENO_{50}}$ and F_{ENO} at incremental flow rates) was 10–15 min.

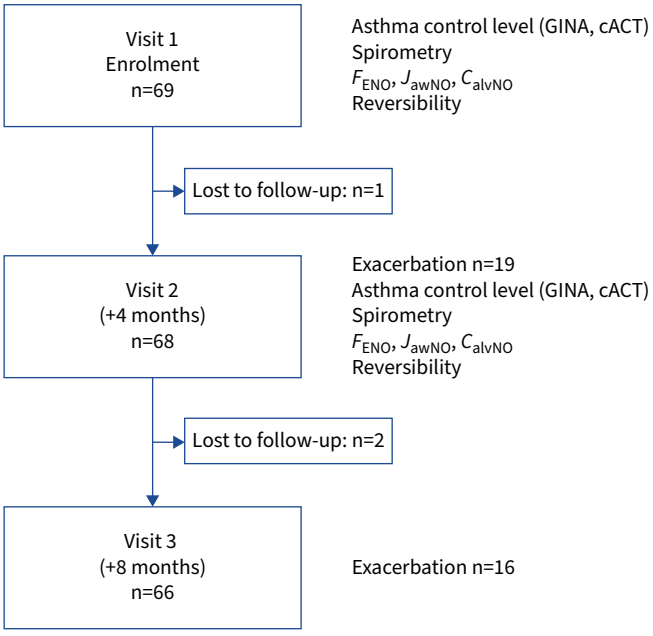

FIGURE 1 Study flowchart. GINA: Global Initiative for Asthma; cACT: Childhood Asthma Control Test; F_{ENO}: exhaled nitric oxide fraction; J_{awNO}: bronchial flux of nitric oxide; C_{alvNO}: alveolar concentration of nitric oxide.

Statistics

We have previously reported a 40% higher C_{alvNO} in children with poorly controlled asthma compared with those with controlled disease [16]. 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_{alvNO} difference at a 5% α-level with 85% power. Sample size estimation was performed using G*Power software [17].

Continuous variables were compared with the t-test and categorical variables were compared with the Chi-squared test. Linear mixed modelling (LMM) with adjustment for repeated observations (*i.e.* study visits) was used to compare $F_{ENO_{50}}$, J_{awNO}, C_{alvNO}, D_{awNO} and C_{awNO} (log-transformed values) between visits followed by an exacerbation and those not followed by an exacerbation. LMM 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 $F_{ENO_{50}}$, J_{awNO}, C_{alvNO}, D_{awNO} and C_{awNO} z-score values and time to asthma exacerbation. Receiver operating characteristic curve 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_{alvNO} levels. The lower C_{alvNO} value with positive LR >3 was considered as the high-risk cut-off. All analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA). $p<0.05$ was considered significant.

Results

The characteristics of the study population are presented in table 1. 69 children were enrolled in the study (visit 1), of whom 21 reported at least one exacerbation prior to the start of the study; at visit 2, 19 out of 68 children (one child was lost to follow-up) reported that they had suffered an exacerbation, while 16 out of 66 children (two further children were lost to follow-up) reported an exacerbation at visit 3 (figure 1). In total, no exacerbation was reported in 99 visits (Group 1) and an exacerbation was reported 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 ICSs or leukotriene receptor antagonists (LTRAs). There were also no differences in spirometric parameters between the two groups, including post-bronchodilation reversibility of FEV_1 (table 2).

TABLE 2 Participant characteristics on study visits[#]

	Group 1: visits not followed by exacerbation (n=99)	Group 2: visits followed by exacerbation (n=35)	p-value
Male	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
ICSs	54 (54.5)	26 (74.3)	0.041
LTRAs	15 (15.6)	11 (32.4)	0.036
Nasal corticosteroids	15 (15.2)	6 (17.1)	0.781
Antihistamines	19 (19.2)	9 (25.7)	0.415
FEV_1, % pred	101.8±8.9 (82–126)	101.6±9.7 (83–119)	0.911
FVC, % pred	95.7±8.4 (82–120)	97.4±7.9 (84–113)	0.298
$FEF_{25–75\%}$, % pred	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, % pred	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 presented as n (%) or mean±SD (range). BMI: body mass index; ICS: inhaled corticosteroid; LTRA: leukotriene receptor antagonist; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC; PEF: peak expiratory flow; ΔFEV_1: % change in FEV_1 after administration of 400 µg salbutamol inhaler. [#]: visit 1 of 68 children who attended visit 2 and visit 2 of 66 children who attended visit 3 (n=134 visits in total). Comparisons were performed by the Chi-squared test or the t-test.

F_{ENO} measurement at incremental flows was successful at the first attempt in 117 of the 134 visits (87.3%). A repeated measurement was required in 17 visits, mainly (15 out of 17 (88.2%)) due to expiratory flow instability at 300 mL·s^{-1} that resulted in poor equation fitting [7] and even negative C_{alvNO} values. In the participants who did not exacerbate (Group 1), the median (range) values were: $F_{ENO_{50}}$ 12.7 (4–209) ppb, J_{awNO} 715 (10–12 799) pL·s^{-1}, D_{awNO} 38.3 (0.2–113.3) pL·s^{-1}·ppb^{-1}, C_{awNO} 26.8 (4.1–2163) ppb and C_{alvNO} 3.4 (0.2–10.8) ppb (figure 2). Compared with Group 1, those who experienced an exacerbation (Group 2) had significantly higher median (range) C_{alvNO} (5.2 (1.7–23.6) ppb; p<0.001), but similar $F_{ENO_{50}}$ (13.5 (3.8–149.9) ppb; p=0.744), J_{awNO} (438 (40–7457) pL·s^{-1}; p=0.708), D_{awNO} (38 (1.3–144.5) pL·s^{-1}·ppb^{-1}; p=0.431) and C_{awNO} (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_{alvNO}, $F_{ENO_{50}}$ and C_{awNO} on the preceding visit compared with controls (figure 3).

Predictors and time to exacerbations

Repeated measures logistic regression revealed that C_{alvNO} was the only bronchial inflammation parameter that was associated with increased risk of asthma exacerbation within the following 4 months (table 3). The effect of C_{alvNO} was independent of the spirometric parameters (including reversibility to bronchodilation), $F_{ENO_{50}}$ levels, gender and use of controller therapy (ICSs and LTRAs) (table 3). With the exception of D_{awNO}, all other bronchial inflammation indices were negatively correlated with time to asthma exacerbation. The strongest correlation was observed for C_{alvNO} and the weakest for J_{awNO}. Cox survival analysis corroborated these results (figure 4).

Predictive ability of bronchial inflammation parameters

The area under the curve (AUC) values of $F_{ENO_{50}}$ 0.507 (95% CI 0.390–0.623), J_{awNO} 0.516 (95% CI 0.407–0.641), D_{awNO} 0.521 (95% CI 0.420–0.652) and C_{awNO} 0.565 (0.448–0.681) reflect the low ability of the these parameters to identify children at risk. The AUC of C_{alvNO} was 0.690 (95% CI 0.585–0.794), indicating moderate overall ability to differentiate children at risk for asthma exacerbation. The predictive characteristics of different C_{alvNO} levels are shown in figure 5. C_{alvNO} >7 ppb predicted asthma exacerbation with high accuracy (specificity 90.9%, positive LR 3.1), while C_{alvNO}>10 ppb had specificity

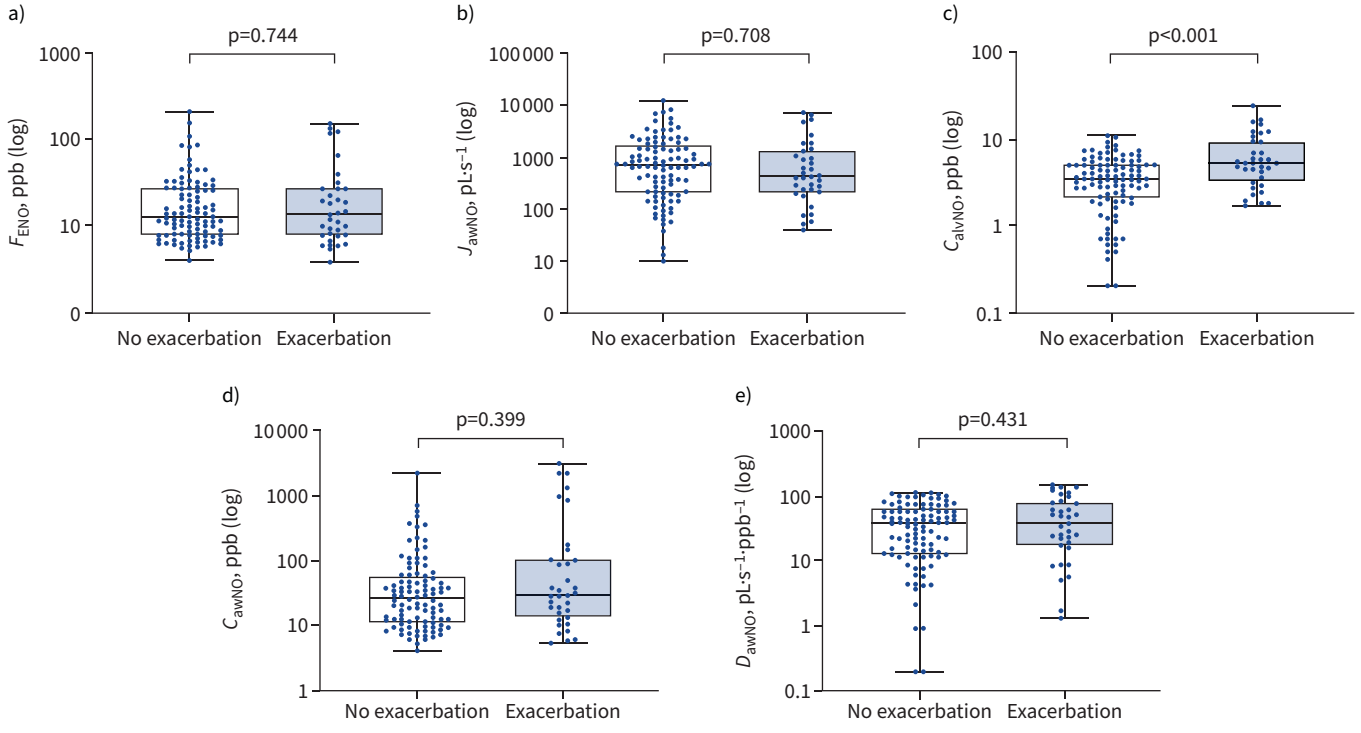

FIGURE 2 Bronchial inflammation parameters (log-transformed) in visits not followed by an exacerbation and in visits followed by an exacerbation: a) exhaled nitric oxide fraction (F_{ENO}), b) bronchial flux of nitric oxide (J_{awNO}), c) alveolar concentration of nitric oxide (C_{alvNO}), d) airway wall concentration of nitric oxide (C_{awNO}) and e) transfer factor of nitric oxide (D_{awNO}). p-values were calculated with linear mixed modelling with adjustment for repeated observations.

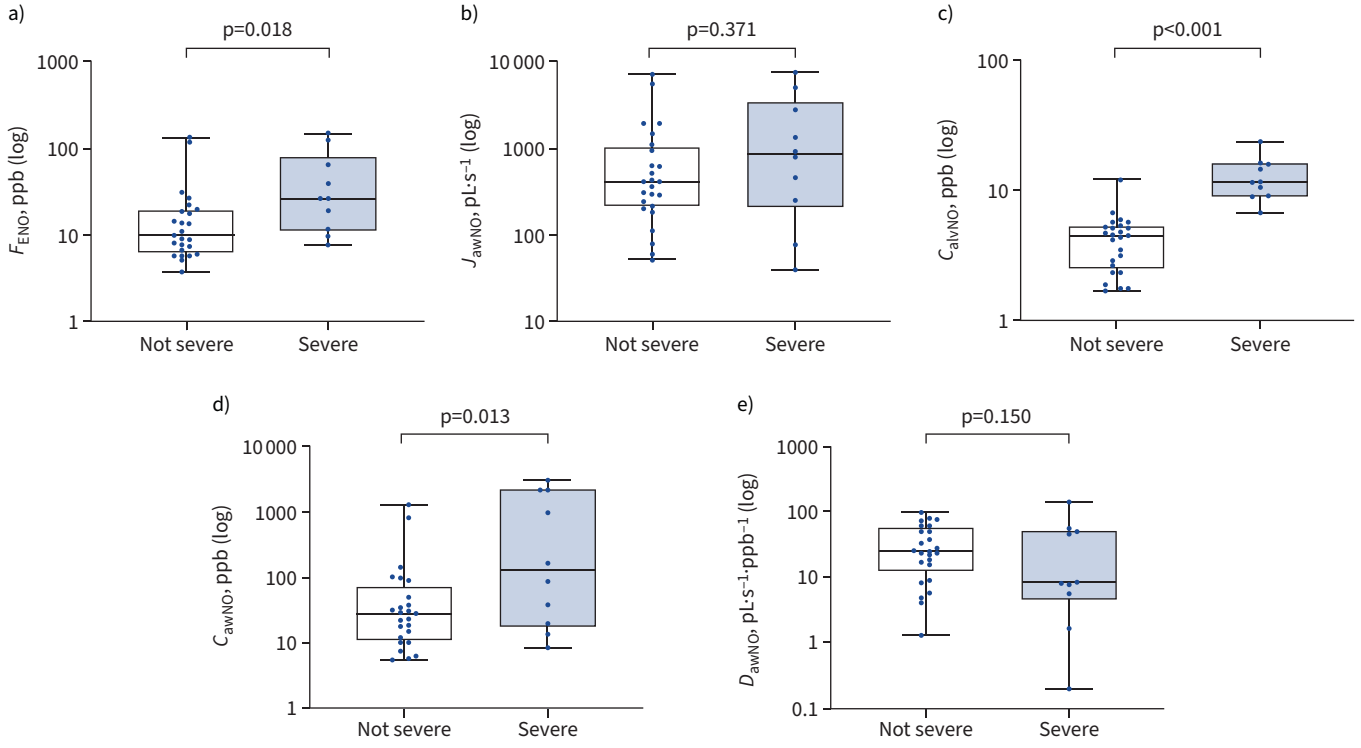

FIGURE 3 Bronchial inflammation parameters (log-transformed) according to severity of asthma exacerbation (n=35): a) exhaled nitric oxide fraction (F_{ENO}), b) bronchial flux of nitric oxide (J_{awNO}), c) alveolar concentration of nitric oxide (C_{alvNO}), d) airway wall concentration of nitric oxide (C_{awNO}) and e) transfer factor of nitric oxide (D_{awNO}). p-values were calculated with linear mixed modelling.

99% and positive LR 19.8, but sensitivity 20% and negative LR 0.8. Conversely, C_{alvNO} <4 ppb, for example, had lower ability to exclude an exacerbation within the next 4 months (sensitivity 71.4% and negative LR 0.48).

Children with C_{alvNO} >7 ppb had a high probability of experiencing an exacerbation within the next 4 months (table 4); they also had more exacerbations (50.0% *versus* 21.9%; p<0.001), more severe

TABLE 3 Probability of exacerbation

	Exploratory models		Multivariable model	
	OR (95% CI)	p-value	OR (95% CI)	p-value
cACT	0.70 (0.34–7.60)	0.350		
FEV_1	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
F_{ENO}	1.18 (0.80–1.88)	0.406	1.13 (0.57–2.25)	0.720
J_{awNO}	0.99 (0.80–1.24)	0.950		
C_{alvNO}	1.46 (1.13–1.88)	0.004	1.65 (1.07–2.53)	0.023
C_{awNO}	0.73 (0.39–1.37)	0.326		
D_{awNO}	1.03 (0.79–1.33)	0.847		

cACT: Childhood Asthma Control Test; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; ΔFEV_1: % change in FEV_1 after administration of 400 µg salbutamol inhaler; F_{ENO}: exhaled nitric oxide fraction; J_{awNO}: bronchial flux of nitric oxide; C_{alvNO}: alveolar concentration of nitric oxide; C_{awNO}: airway wall concentration of nitric oxide; D_{awNO}: transfer factor of nitric oxide. Repeated measures (mixed effects) logistic regression with adjustment for sex and controller therapy (inhaled corticosteroids and leukotriene receptor antagonists). In exploratory models the effect of each factor was assessed separately. The multivariable model presents the combined effect of C_{alvNO}, F_{ENO} and ΔFEV_1.

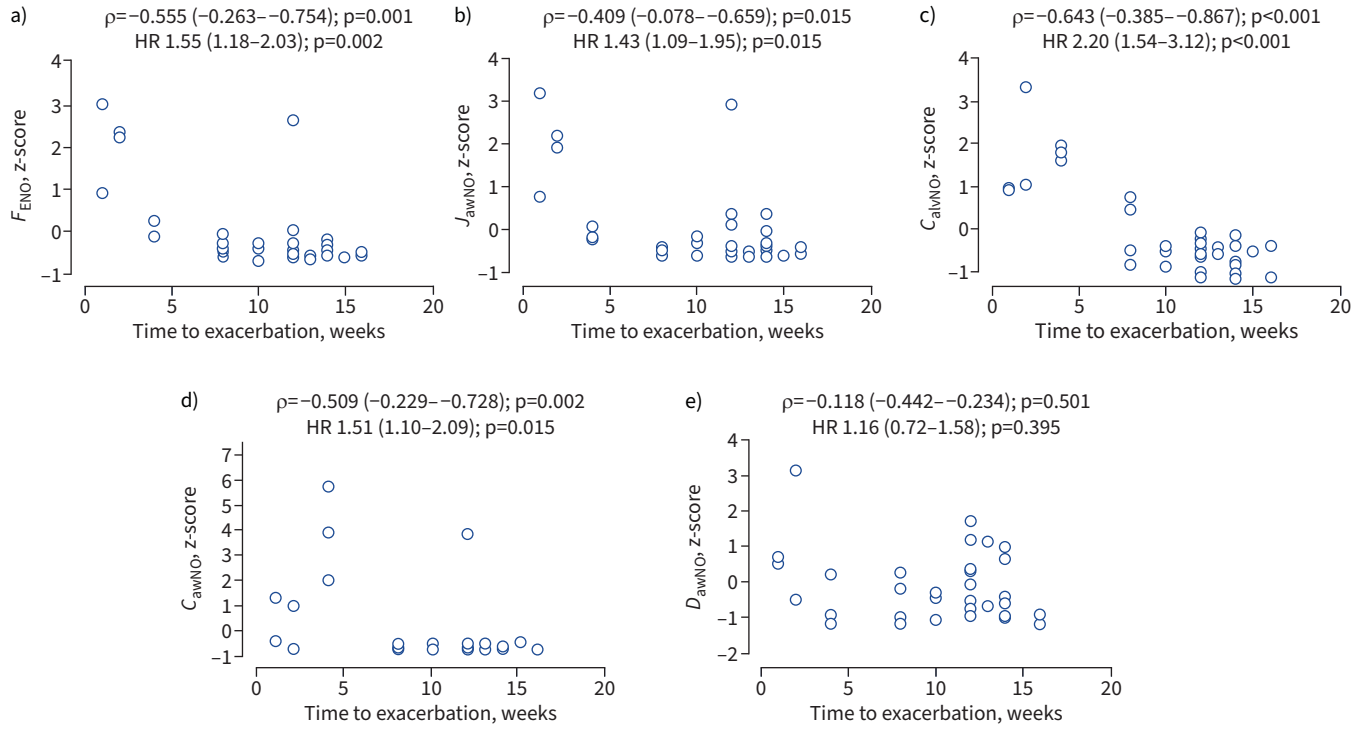

FIGURE 4 Bronchial inflammation indices (z-scores) and time to exacerbation: a) exhaled nitric oxide fraction (F_{ENO}), b) bronchial flux of nitric oxide (J_{awNO}), c) alveolar concentration of nitric oxide (C_{alvNO}), d) airway wall concentration of nitric oxide (C_{awNO}) and e) transfer factor of nitric oxide (D_{awNO}). Spearman's ρ and Cox's hazard ratio (HR) values (with their 95% confidence intervals) are presented.

exacerbations (90.0% *versus* 0.9%; p<0.001) and shorter time to exacerbation (4.2±2.9 *versus* 12.5±2.1 weeks; p<0.001). The corresponding differences in cACT and FEV_1 were not significant, but participants with C_{alvNO} >7 ppb presented lower forced mid-expiratory flows (table 4).

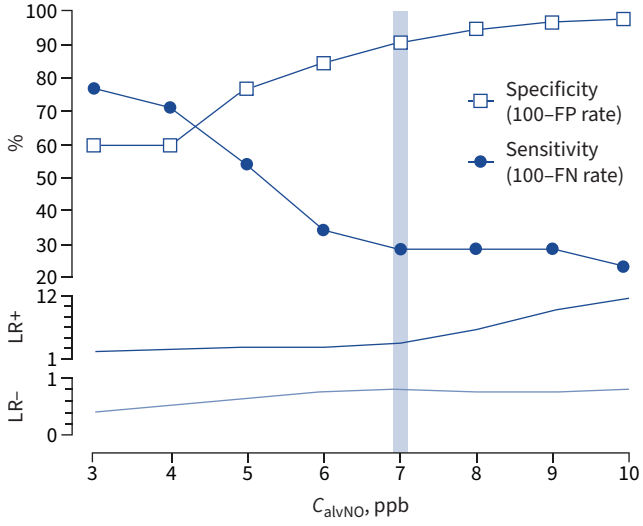

FIGURE 5 Predictive characteristics of different alveolar concentration of nitric oxide (C_{alvNO}) levels. The lower C_{alvNO} value with LR+ >3 (high-risk cut-off) is marked. FP: false-positive; FN: false-negative; LR+: positive likelihood ratio; LR−: negative likelihood ratio.

TABLE 4 Characteristics of children with increased alveolar concentration of nitric oxide (C_{alvNO})

	C_{alvNO} <7 ppb (n=114)	C_{alvNO} ⩾7 ppb (n=20)	p-value
cACT	25.1±3.1 (25 (14–27))	25.0±4.1 (27 (15–27))	0.434
FEV_1, % pred	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
$FEF_{25–75\%}$, % pred	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
F_{ENO}, ppb	21.9±30 (11.4 (3.8–209.0))	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)	<0.001
Time to exacerbation, weeks	12.5±2.1 (13 (8–16))	4.2±2.9 (4 (1–8))	<0.001

Data are presented as mean±SD (median (range) or range) or n (%), unless otherwise stated. C_{alvNO}: alveolar concentration of nitric oxide; cACT: Childhood Asthma Control Test; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of FVC; ΔFEV_1: % change in FEV_1 after administration of 400 µg salbutamol inhaler; F_{ENO}: exhaled nitric oxide fraction. Comparisons were performed using the Mann–Whitney U-test or the Chi-squared test, as appropriate.

The predictive characteristics of a change in C_{alvNO} between visits were assessed in children with an exacerbation after visit 2 but without an exacerbation between visits 1 and 2, since treatment with systemic corticosteroids could have affected nitric oxide measurements. The median (range) C_{alvNO} change was −0.1 (−3.6–5.8) ppb or −5.4% (−56.3–150%) in those who did not exacerbate *versus* 1.2 (0.2–4) ppb or 60% (3.6–480%) in those who experienced an asthma exacerbation (p<0.001) (figure 6). C_{alvNO} increase was a better risk predictor of future exacerbation (AUC 0.939 (95% CI 0.832–0.987)) than a single C_{alvNO} measurement (AUC 0.650 (95% CI 0.507–0.776); p<0.001). An increase of C_{alvNO} of 0.5 ppb from visit 1 to visit 2 had sensitivity 92%, specificity 92%, positive LR 11.8 and negative LR 0.08 for the identification of an exacerbation within the next 4 months. Similarly, the AUC for the relative C_{alvNO} change between the two visits was also informative (0.931 (95% CI 0.817–0.984)); a 10% increase of C_{alvNO} from the previous visit had sensitivity 92.3%, specificity 88.2%, positive LR 7.9 and negative LR 0.09 in predicting a future exacerbation. Given the small numbers, we did not subdivide further to explore whether the predictive power was greater within 1 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 4 months. Specifically, a marker of distal inflammation, C_{alvNO}, was the best predictor of risk of all the parameters measured. We found decreased forced mid-flows in children with high C_{alvNO} levels (*i.e.* >7 ppb), which seems to support the value of C_{alvNO} as a marker of small airway disfunction in asthma [7]. More important, C_{alvNO} >7 ppb was highly specific but not very sensitive for a subsequent exacerbation and C_{alvNO} <4 ppb excluded risk of an attack also with high specificity but low sensitivity.

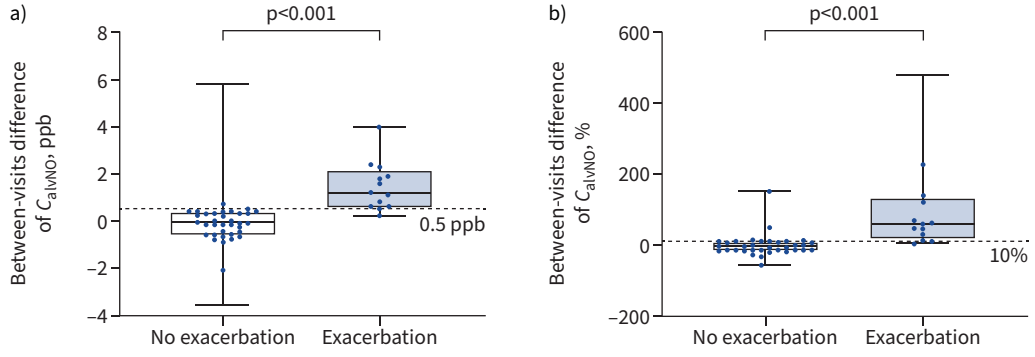

FIGURE 6 Comparison of a) absolute and b) relative alveolar concentration of nitric oxide (C_{alvNO}) differences between visits 1 and 2 in relation to exacerbation occurrence after visit 2. The dotted line marks the cut-off value with the best sensitivity/specificity combination in predicting an exacerbation (from receiver operating characteristic curve analysis).

Even more sensitive, in a small subgroup, was an increase in C_{alvNO} of >0.5 ppb between visits 4 months apart. As expected, neither symptoms (cACT) nor FEV_1 or airway reversibility tests were useful in the assessment of risk.

This is the first study prospectively evaluating C_{alvNO} as a marker of risk in children. The strengths of the study include its prospective design and the fact that children were assessed with varying levels of asthma severity, although all participants had well-controlled asthma at recruitment. The use of the improved Eco Medics device for measuring exhaled nitric oxide enabled us to measure C_{alvNO} and J_{awNO} in all our subjects, unlike our previous experience [16]. However, inevitably there are some weaknesses. There were some potential markers of risk that we did not include; in particular, well-known risk factors for an asthma exacerbation include a previous severe exacerbation, over-use of short-acting β_2-agonists and under-use of ICSs [18]. 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 fine-particle ICSs reduced the risk of exacerbations in those with high C_{alvNO}. We had no data on lung diffusion capacity, and we could not assess the effect of other factors such as cardiac output, haemoglobin concentration and airway lining fluid pH on C_{alvNO} levels [6]. Finally, our findings ideally need to be 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 airways inflammation, as measured by C_{alvNO}, underscores the importance of distal as well as proximal airways disease in the pathophysiology of asthma. As a clinical test, 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_{alvNO} in the present data form a subgroup who are at high risk and need a focused reassessment of risk factors. Those children with an increase in C_{alvNO} between visits also merit this approach, although the usefulness of a change in C_{alvNO} is limited by the need to make measurements at two time-points. Conversely, those with a very low C_{alvNO} and no change over time would not be expected to have exacerbations, and if exacerbations are reported, the paediatrician might consider whether symptoms are being over-reported [19].

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 ICSs improve outcomes when added to the treatment regime of those suffering acute exacerbations and have an elevated or increasing C_{alvNO}.

Conflict of interest: None declared.

References

1 Telenga ED, van den Berge M, ten Hacken NH, *et al.* Small airways in asthma: their independent contribution to the severity of hyperresponsiveness. *Eur Respir J* 2013; 41: 752–754.
2 Sutherland ER, Martin RJ, Bowler RP, *et al.* Physiologic correlates of distal lung inflammation in asthma. *J Allergy Clin Immunol* 2004; 113: 1046–1050.
3 Kraft M, Martin RJ, Wilson S, *et al.* Lymphocyte and eosinophil influx into alveolar tissue in nocturnal asthma. *Am J Respir Crit Care Med* 1999; 159: 228–234.
4 Kraft M, Djukanovic R, Torvik J, *et al.* Evaluation of airway inflammation by endobronchial and transbronchial biopsy in nocturnal and nonnocturnal asthma. *Chest* 1995; 107: 3 Suppl., 162S.
5 Kraft M, Djukanovic R, Wilson S, *et al.* Alveolar tissue inflammation in asthma. *Am J Respir Crit Care Med* 1996; 154: 1505–1510.
6 Marozkina NV, Gaston B. Nitrogen chemistry and lung physiology. *Annu Rev Physiol* 2015; 77: 431–452.
7 Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. *J Appl Physiol* 1998; 85: 653–666.
8 Cohen J, Douma WR, ten Hacken NH, *et al.* Ciclesonide improves measures of small airway involvement in asthma. *Eur Respir J* 2008; 31: 1213–1220.
9 Hodgson D, Anderson J, Reynolds C, *et al.* A randomised controlled trial of small particle inhaled steroids in refractory eosinophilic asthma (SPIRA). *Thorax* 2015; 70: 559–565.
10 Petsky HL, Cates CJ, Kew KM, *et al.* Tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils): a systematic review and meta-analysis. *Thorax* 2018; 73: 1110–1119.
11 Malinovschi A, Fonseca JA, Jacinto T, *et al.* Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subjects. *J Allergy Clin Immunol* 2013; 132: 821–827.

12 Reddel HK, Taylor DR, Bateman ED, *et al.* An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009; 180: 59–99.

13 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005; 26: 319–338.

14 Högman M, Meriläinen P. Extended NO analysis in asthma. *J Breath Res* 2007; 1: 024001.

15 Dweik RA, Boggs PB, Erzurum SC, *et al.* An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (F_{ENO}) for clinical applications. *Am J Respir Crit Care Med* 2011; 184: 602–615.

16 Paraskakis E, Brindicci C, Fleming L, *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: 260–267.

17 Faul F, Erdfelder E, Lang AG, *et al.* G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; 39: 175–191.

18 Buelo A, McLean S, Julious S, *et al.* At-risk children with asthma (ARC): a systematic review. *Thorax* 2018; 73: 813–824.

19 Nagakumar P, Gambir N, Sanghani N, *et al.* Role of a prolonged inpatient admission when evaluating children with problematic severe asthma. *Eur Respir J* 2018; 51: 1701061.