

Original article

Multicentre trial evaluating alveolar NO fraction as a marker of asthma control and severity

Background: Exhaled NO can be partitioned in its bronchial and alveolar sources, and the latter may increase in the presence of recent asthmatic symptoms and in refractory asthma. The aim of this multicentre prospective study was to assess whether alveolar NO fraction and FE_{NO} could be associated with the level of asthma control and severity both at the time of measurement and in the subsequent 3 months.

Methods: Asthma patients older than 10 years, nonsmokers, without recent exacerbation and under regular treatment, underwent exhaled NO measurement at multiple constant flows allowing its partition in alveolar (with correction for back-diffusion) and bronchial origins based on a two-compartment model of NO exchange; exhaled NO fraction at 50 ml/s ($FE_{NO,0.05}$) was also recorded. On inclusion, severity was assessed using the four Global initiative for asthma (GINA) classes and control using Asthma Control Questionnaire (ACQ). Participants were followed-up for 12 weeks, control being assessed by short-ACQ on 1st, 4th, 8th and 12th week.

Results: Two-hundred patients [107 children and 93 adults, median age (25th; 75th percentile) 16 years (12; 38)], 165 receiving inhaled corticosteroid, were included in five centres. The two-compartment model was valid in 175/200 patients (87.5%). Alveolar NO and $FE_{NO,0.05}$ did not correlate to control on inclusion or follow-up (either with ACQ /short-ACQ values or their changes), nor was influenced by severity classes. Alveolar NO negatively correlated to $MEF_{25-75\%}$ ($\rho = -0.22$, $P < 0.01$).

Conclusion: Alveolar and exhaled NO fractions are not indexes of control or severity in asthmatic children and adults under treatment.

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Several studies have evaluated the relationships between exhaled NO and asthma control. An increase in exhaled NO fraction (FE_{NO}) has been demonstrated in acute asthma and in presence of recent asthmatic symptoms (1–4), which clearly emphasizes the ability of exhaled NO to indicate major loss of asthma control. It has also been demonstrated that the sequential measurement of FE_{NO} could predict milder degrees of loss of control (5). The usefulness of a single measurement to describe subtle

degrees of asthma control at the time of measurement or its ability to predict further loss of control would constitute two more clinically relevant end-points, but remains controversial (6, 7).

Partitioning exhaled NO in its bronchial and alveolar sources deserves scrutiny. We and others previously demonstrated that FE_{NO} measured at 50 ml/s mainly assesses the bronchial origin of exhaled NO, whereas alveolar NO concentration, measured in the

compressible compartment, implicates deep lung inflammation, a process that is of importance in asthma (1, 4, 8). An increased alveolar NO concentration has been reported in steroid-resistant asthma (9) and in presence of nocturnal symptoms (10), which supports the hypothesis that its assessment could be linked to severity and control.

The aim of this multicentre prospective study was to assess whether alveolar NO fraction could be associated with the level of asthma control and severity both at the time of measurement and in the subsequent 3 months of follow-up in a hospital cohort of asthmatic children and adults. We evaluated whether an elevated alveolar NO concentration is associated with milder degrees of loss of asthma control and/or severe asthma, while a normal NO concentration indicates better control and/or a nonsevere asthma. Only patients under treatment and without overt symptoms of loss of control were included as we wanted to demonstrate the clinical relevance of alveolar NO in daily practice. A secondary objective was to assess whether FE_{NO} is also associated with milder degrees of loss of asthma control and/or severe asthma.

Methods

Participants

Patients older than 10 years were eligible if they were addressed for pulmonary function testing for the follow-up of an established asthma (diagnosis of asthma recorded in their general practitioner's notes and at least one prescription for any anti-asthma medication in the last 12 months) in one of the five participating centres. The study was restricted to never-smokers. Recent (within 2 weeks) exacerbation or anti-asthma treatment modification and other chronic respiratory disease were the non-inclusion criteria.

Approval for the study was given by our local ethics committee; all participants (and/or parents) gave written, informed consent.

Study design

This design is summarized in Fig. 1. On inclusion, patients underwent pulmonary function tests (see below) and an assessment of both asthma severity and control according to recent American Thoracic Society/European Respiratory Society (ATS/ERS) Statement (11). Asthma control was assessed at inclusion using Asthma Control Questionnaire [ACQ, including forced expiratory volume in 1 s (FEV_1)] (12) and on subsequent weeks using both phone interviews (ACQs-short without FEV_1) (13) and the Asthma Control Diary (ACD) (12). A peak flow meter (MiniWright®, Clement Clark International, Harlow, UK) was provided to each included patient for the whole follow-up. Severity is defined as the difficulty in controlling asthma with treatment and was assessed using the four-class of GINA guidelines (14). Two independent GINA classifications were combined (14), the first one based on symptoms and FEV_1 and the second one based on current medication, as previously done (15). Atopy (self-reported) was defined based on the knowledge of positive skin prick test, or a history of seasonal rhinitis /conjunctivitis. Participants were followed up

prospectively for 12 weeks or until the occurrence of (i) a frank modification of treatment [twofold increase or decrease of inhaled corticosteroid (ICS) for more than 3 days] or (ii) severe exacerbation (episode of increasing asthma symptoms requiring a course of oral steroids for more than 3 days) (11).

Pulmonary function tests

At inclusion, exhaled NO fraction was measured before spirometry at multiple constant flows (between 50 and 250 ml/s, four to six expiratory flows of which the 50 ml/s flow: $FE_{NO,0.05}$) using the same chemiluminescence analyser (ENDONO 8000; SERES, Aix en Provence, France) in all centres. The alveolar NO fraction ($C_{alv,NO}$, ppb), the maximum NO flux from conducting airways ($J_{aw,NO}$, nl/min) based on a two-compartment model of NO exchange dynamics (4, 16) and the Pearson correlation coefficient between NO output and expiratory flow were calculated automatically. The relationship between NO output and expiratory flow was deemed linear when $r \geq 0.80$, as previously described (17); when linearity was not observed, only $FE_{NO,0.05}$ was recorded. Validity criteria of each FE_{NO} and analyser calibration were as recommended by international guidelines (18). Thus, we obtained the following exhaled NO variables: $C_{alv,NO}$, $J_{aw,NO}$, $FE_{NO,0.05}$ and $C_{alv-cor,NO}$, the latter being the value of alveolar NO concentration corrected for back-diffusion of NO from conducting airways (19). Spirometry was performed according to international guidelines (20), under anti-asthma treatment.

Statistical analysis

We hypothesized that half of the patients would be controlled. We calculated that a sample size of 100 would have more than 90% power to detect a difference between the expected $C_{alv-cor,NO}$ mean of 5 ppb among stable patients and 7 ppb among the nonwell-controlled patients, assuming a standard deviation of 3 ppb and using a two-sided two-group *t*-test with a significance level of 0.05. We chose *a priori* a target sample size of 150 patients, to take into account an expected 50% of patients who may be lost to follow-up at 3 months.

Because exhaled NO variables and asthma control scores were not distributed normally, we used nonparametric methods. Quantitative variables were compared between groups using Kruskal–Wallis tests. First, we compared exhaled NO variables measured at inclusion between the different levels of asthma control and severity at that time. Second, we compared the exhaled NO variables measured at inclusion between (i) well-controlled and nonwell-controlled patients at each follow-up visit and (ii) the groups of patients whose control improved, worsened or did not change at each follow-up visit. Two cut-off values for control were assessed using an ACQ or ACQs score < 0.75 (to be confident that a patient has well-controlled asthma) or 1.5 (to be confident that the patient has inadequately controlled asthma) as recommended (21). Improvement of asthma control was defined by an absolute decrease in the ACQs score of 0.5 or more, while worsening was defined by an increase of 0.5 or more, as a 0.5 change in ACQ is considered to be the minimum change that is clinically relevant (5).

Agreement between the daily diary (ACD) and the weekly telephonic asthma control (ACQs) was assessed using Bland and Altman methodology, including the difference plot and calculation of 95% limits of agreement (22). Two-tailed *P*-values were deemed significant when inferior to 0.05. Data analysis was performed using SAS v9.1 software (SAS France, Grégy sur Yerres, France) by independent investigators (L. T. and M. J.).

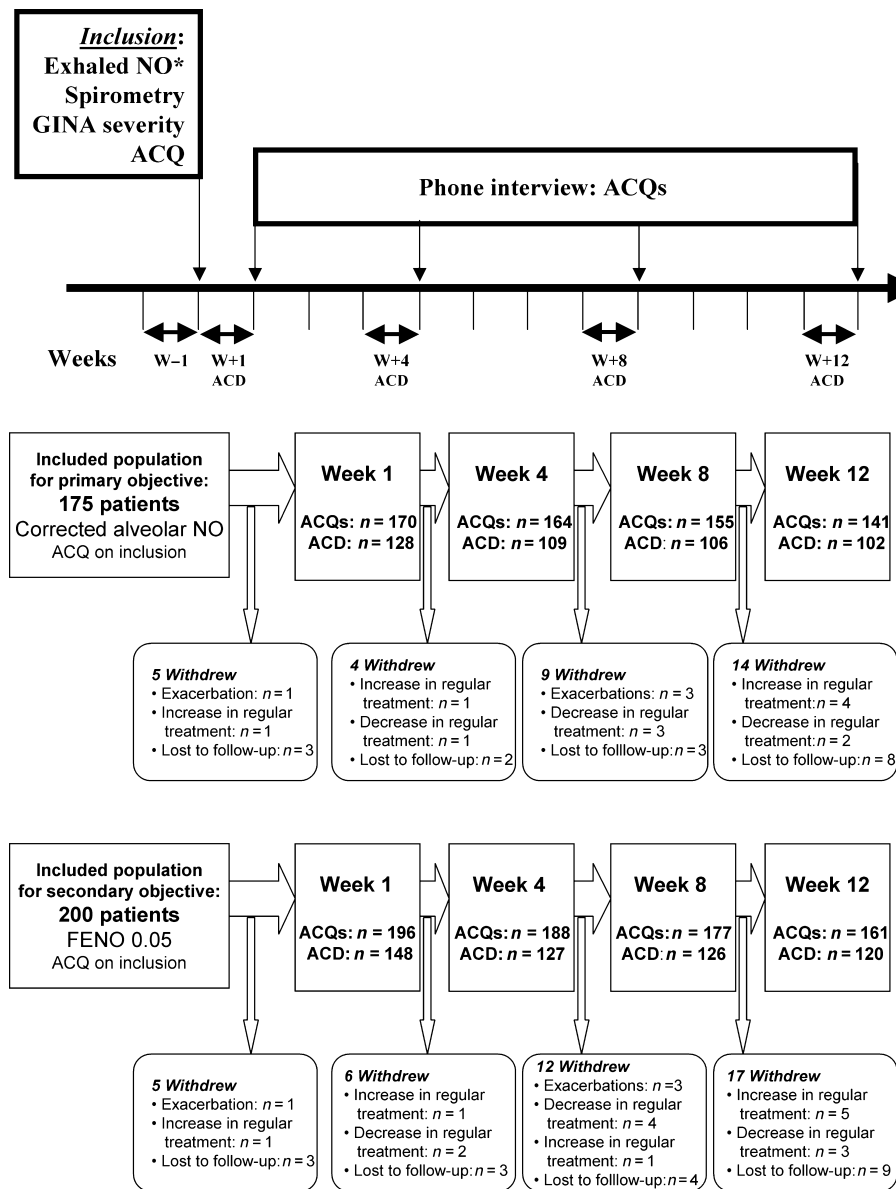


Figure 1. Study design and flow-chart of the participants. The upper panel describes the study design. *Exhaled NO was measured before spirometry at multiple constant flow rates allowing, when the linearity of the relationship between NO output and expiratory flow rate was confirmed (Pearson correlation coefficient $r \geq 0.80$), its partition in alveolar NO concentration ($C_{\text{alv,NO}}$), which was further corrected for back-diffusion ($C_{\text{alv-cor,NO}}$) and maximum flux of NO from conducting airways ($J'_{\text{aw,NO}}$). When r value was < 0.80 , the sole $FE_{\text{NO},0.05}$, obtained in all patients, was recorded. ACQ denotes Asthma Control Questionnaire, ACQs denotes its shortened version (without FEV_1 measurement) and ACD denotes Asthma Control Diary. GINA severity denotes the four classes of asthma severity (intermittent, persistent mild, moderate, severe) as previously used by Liard et al. (14) The middle panel shows the flow of the 175/200 participants who had alveolar NO measurement (primary objective). Reasons for study exit are detailed. Only four asthma exacerbations (0.09 per patient per year (95% CI 0.02–0.23) were observed. The lower panel shows the flow of the 200 participants who had $FE_{\text{NO},0.05}$ measurement (secondary objective).

Results

Description of the population

From June 2006 to December 2007, 200 patients were included. Figure 2 shows the flow-chart of patients throughout the study. The clinical and functional char-

acteristics of the patients at the time of inclusion are described in Table 1.

Validity of exhaled NO values

The Pearson correlation coefficient between NO output and expiratory flow was superior to 0.80 in 175 out of

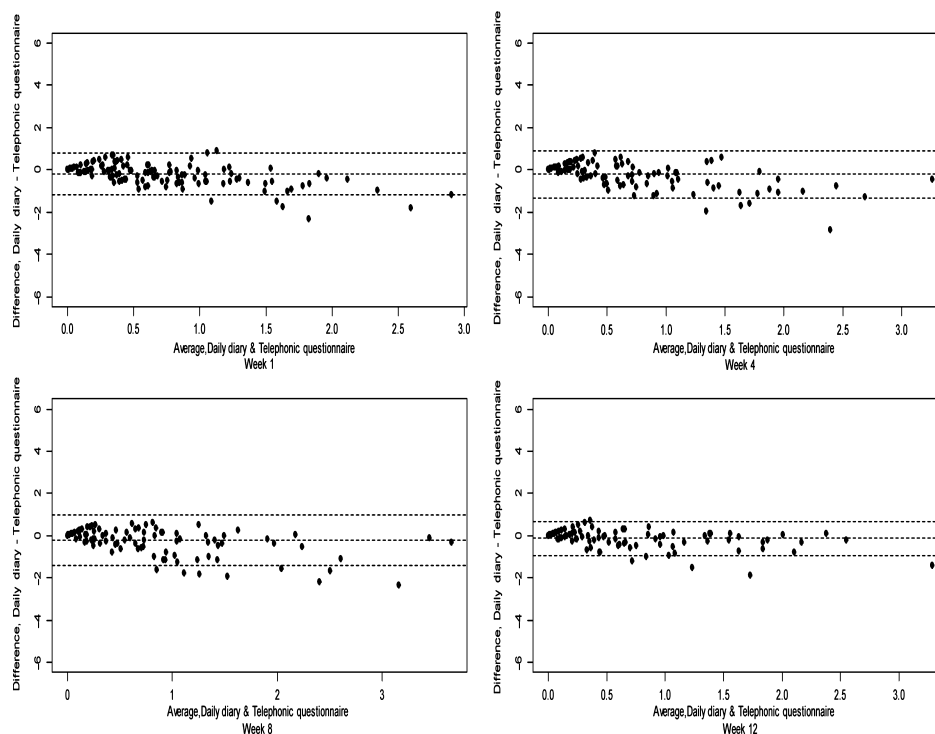


Figure 2. Bland–Altman analyses of ACD and ACQs over time. Asthma control was assessed using several criteria for two reasons. As we expected that some patients would be lost of follow-up, we planned to have both shortened version of ACQ (ACQs) and ACD to verify internal consistency of control assessment and to ensure that at least one mean of control assessment will be obtained. A good agreement between the two questionnaires is demonstrated. A constant difference over time is evidenced, ACQs scores being higher than ACD scores. The number of patients evaluated at each time-point is given in Fig. 1.

Table 1. Clinical and functional characteristics of the patients

Characteristic	Children (<i>n</i> = 107)	Adults (<i>n</i> = 93)	Whole population (<i>n</i> = 200)
Gender ratio, F/M	43/64	52/41	95/105
Age, years	12 [11; 14]	42 [29; 55]	16 [12; 38]
Duration of asthma, years	9.0 [6.0; 11.0]	18.0 [9.0; 27.0]	10.0 [7.0; 17.0]
Self-reported atopy, <i>n</i> (%) [*]	89 (85)	73 (79)	162 (82)
Asthma medications, <i>n</i> †			
short acting β 2-agonist ‡	55	43	98 (49)
ICS	4	5	9 (4.5)
ICS + long acting β 2-agonist	89	67	156 (78)
leukotriene modifier	31	14	45 (22.5)
BEDD < 500 μ g	50	20	70 (35)
BEDD $\geq$ 500 μ g	43	52	95 (47.5)
Pulmonary function tests			
PEF, % predicted	95 [85; 106]	95 [81; 110]	95 [84; 106]
FEV ₁ , % predicted	100 [92; 109]	93 [82; 101]	97 [87; 106]
MEF _{25–75} %, % predicted	85 [70; 103]	68 [49; 87]	80 [61; 92]
MEF ₅₀ %, % predicted	84 [71; 101]	70 [50; 86]	78 [60; 93]
Exhaled NO variables			
C _{alv} NO, ppb (175 patients)	5.2 [3.4; 8.3]	7.3 [4.1; 12.1]	5.8 [3.7; 9.4]
C _{alv} -corNO, ppb (175)	3.1 [1.6; 5.5]	4.8 [2.1; 10.3]	3.6 [1.7; 6.5]
J _{aw} NO, nl/min (175)	49.8 [25.6; 111.4]	60.5 [31.8; 93.5]	58.4 [28.2; 103.1]
FE _{NO,0.05} , ppb (200)	28.0 [12.8; 49.9]	31.8 [19.6; 46.8]	30.0 [15.9; 48.1]

Data are summarized using median (25% percentile; 75% percentile) BEDD, beclomethasone equipotent daily dose; ICS, inhaled corticosteroid.

^{*}Three missing data (one adult, two children). †Three missing data (two adults, one child). ‡Either on demand or systematic administration.

200 patients (87.5%). Patients ($n = 25$) in whom the linearity of the relationship between NO output and expiratory flow was not observed were predominantly adults (adults: 17/25 vs 76/175, $P = 0.02$) with a $FE_{NO,0.05}$ of 40.0 ppb [21.4; 80.0] vs 29.1 ppb [13.9; 46.4] for the other 175 patients ($P = 0.01$), which does not seem related to atopy as a trend toward the opposite result was evidenced (with atopy: 17/25 vs 145/175, $P = 0.09$).

Consequently, flow-independent exhaled NO parameters were calculated for 175 patients (Table 1). A significant relationship was observed between J'_{awNO} and $C_{alv,NO}$ ($\rho = 0.35$, 95% CI 0.21–0.47). When alveolar NO was corrected for back-diffusion the statistical significance of this relationship was lost (J'_{awNO} and $C_{alv-cor,NO}$: $\rho = -0.02$, 95% CI -0.17 to 0.12).

Atopic asthmatic patients ($n = 145$) vs nonatopic patients ($n = 27$) had lower (trend) $C_{alv-cor,NO}$ (3.5 ppb [1.7; 6.2] vs 5.8 ppb [2.8; 11.3], $P = 0.06$), higher J'_{awNO} (60.1 nl/min [29.8; 103.0] vs 36.6 nl/min [15.4; 64.5], $P = 0.05$) and similar $FE_{NO,0.05}$ (30.9 ppb [16.2; 48.2] vs 24.2 ppb [12.8; 45.4], $P = 0.29$).

Patients receiving ICS ($n = 140$) vs those without steroid treatment ($n = 32$) had similar $C_{alv-cor,NO}$ (3.5 ppb [2.0; 6.3] vs 4.6 [0.2; 7.2], $P = 0.86$) but lower values of both J'_{awNO} (48.6 nl/min [24.0; 89.6] vs 95.7 [49.9; 154.8], $P = 0.002$) and $FE_{NO,0.05}$ (28.0 ppb [13.5; 42.0] vs 47.5 [24.3; 62.2], $P = 0.002$).

Exhaled NO measurements and asthma control

There was a good agreement between the daily diary (ACD) and the weekly telephonic asthma control (ACQs) questionnaires when considering weekly assessments separately as well as the multiple assessments per patient (mean bias: -0.19; 95% limits of agreement: -1.43 to 1.05). Figure 2 shows the Bland-Altman analyses for the two scores obtained at the different time-points. It has to be noted that ACQs was systematically higher than ACD.

On inclusion, well-controlled patients represented 106/200 (53%) when choosing $ACQ < 0.75$ and 147/200 (73%) when choosing an $ACQ < 1.5$. Figure 3 shows that all exhaled NO parameters were non significantly different at inclusion and during the whole follow-up for

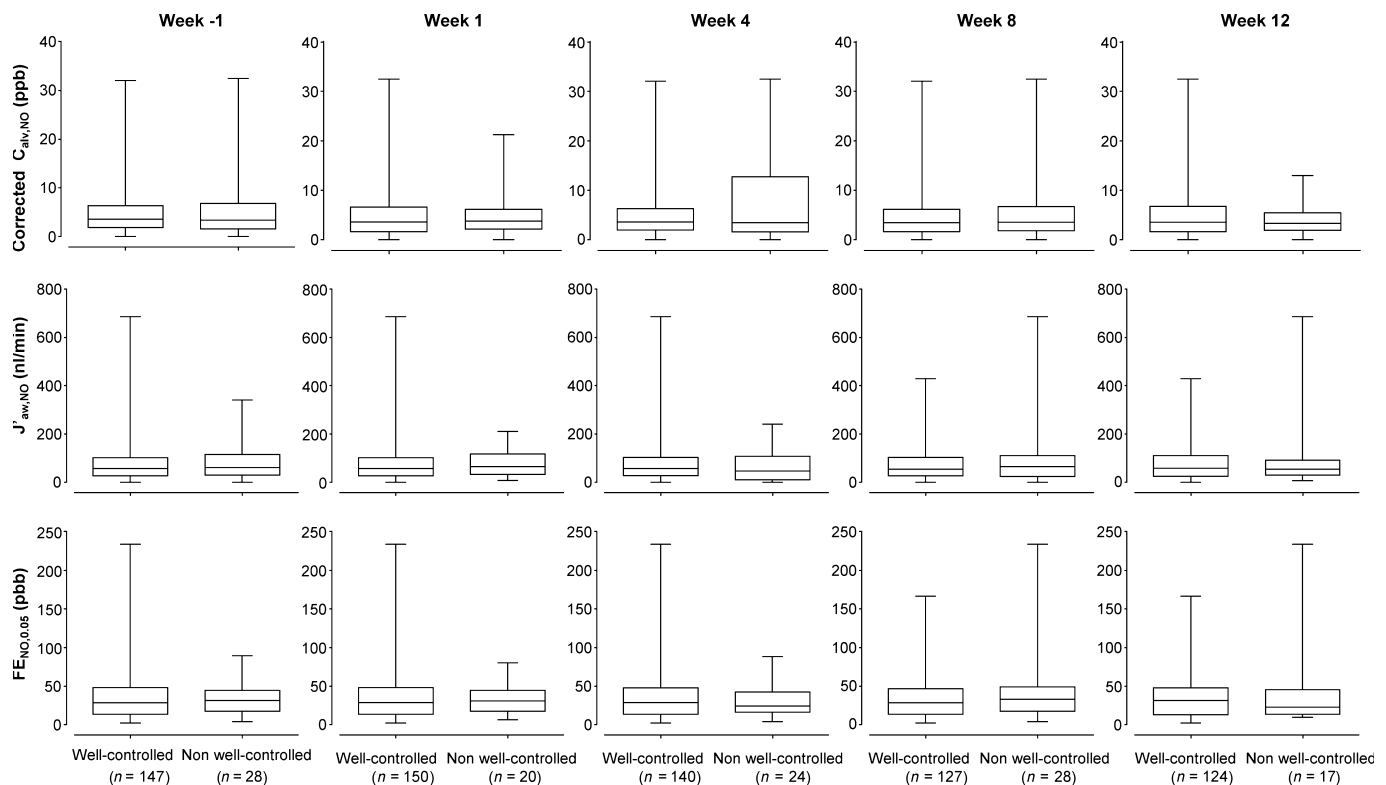


Figure 3. Exhaled NO parameters and asthma control over time. Asthma control questionnaire scores were ACQ on inclusion and ACQs on subsequent weeks (see *Methods*). Patients were classified according to their level of asthma control (well-controlled, ACQ or ACQs < 1.5 ; nonwell-controlled, ACQ or ACQs ≥ 1.5). The number of patients in each group of control is given. Exhaled NO parameters (upper panel, corrected alveolar NO fraction [corrected $C_{alv,NO}$]; middle panel, maximum bronchial NO flux [$J'_{aw,NO}$]; lower panel, exhaled NO fraction measured at 50 ml/s expiratory flow [$FE_{NO,0.05}$]) obtained at inclusion are shown in the two levels of control over time (inclusion, 4th, 8th, 12th week). No significant difference is demonstrated. Box and whisker plots show median, 25th and 75th percentiles, and minimum and maximum values.

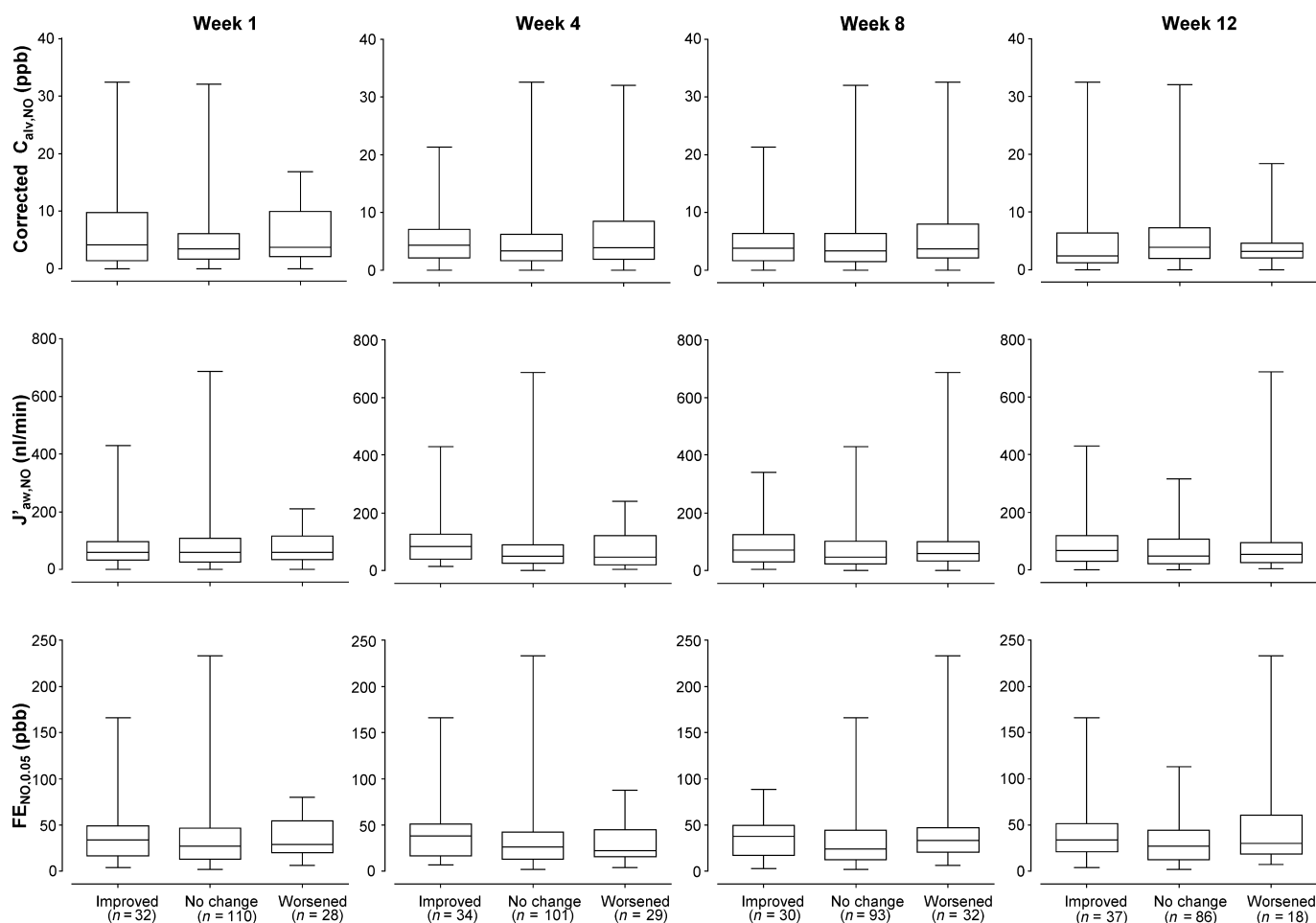


Figure 4. Changes in exhaled NO parameters over time. We hypothesized that exhaled NO parameters on inclusion may be linked to subsequent stability of asthma control. The changes in the level of control over time are described according to values of exhaled NO parameters (upper panel, corrected alveolar NO fraction [corrected $C_{\text{alv,NO}}$]; middle panel, maximum bronchial NO flux [$J'_{\text{aw,NO}}$]; lower panel, exhaled NO fraction measured at 50 ml/s expiratory flow [$\text{FE}_{\text{NO},0.05}$] obtained at inclusion. An improvement (improved group) of asthma control is defined by a decrease ≥ 0.5 of asthma control questionnaire (ACQ) on inclusion and ACQs on subsequent weeks while a worsening (worsened group) is defined by an increase ≥ 0.5 of asthma control questionnaire. No significant differences were evidenced. The number of patients in each group of control is given. Box and whisker plots show median, 25th and 75th percentiles, and minimum and maximum values.

well- (ACQ/ACQs < 1.5) and non well-controlled (ACQ/ACQs ≥ 1.5) asthmatic patients. There was no significant relationship between modifications of ACQ/ACQs scores over time (improvement or deterioration) and initial exhaled NO parameters (Fig. 4).

When choosing a cut-off value of 0.75 for ACQ/ACQs to define well-controlled *vs* nonwell-controlled patients, similar results were observed, for instance on inclusion: $C_{\text{alv-cor,NO}}$ (well-controlled: 3.6 ppb [1.9; 6.4], $n = 106$ *vs* 3.8 [1.7; 6.8], $n = 69$, $P = \text{NS}$).

Exhaled NO measurements and asthma severity

Exhaled NO values at inclusion were similar for the four classes of asthma severity (GINA) (Fig. 5).

Nevertheless, a significant relationship was observed between $C_{\text{alv-cor,NO}}$ and $\text{MEF}_{25-75\%}$ ($\rho = -0.22$, 95% CI -0.36 to -0.06 , $P < 0.01$).

Discussion

We and others suggested that alveolar NO fraction could be a noninvasive marker of both asthma control and severity. Nevertheless, these results had mostly been obtained in a limited number of selected patients, and strenuous criteria of asthma control (loss of control) and severity (refractory asthma) were often used. Consequently, we designed the first multicentre longitudinal study that specifically addressed the clinical potential of

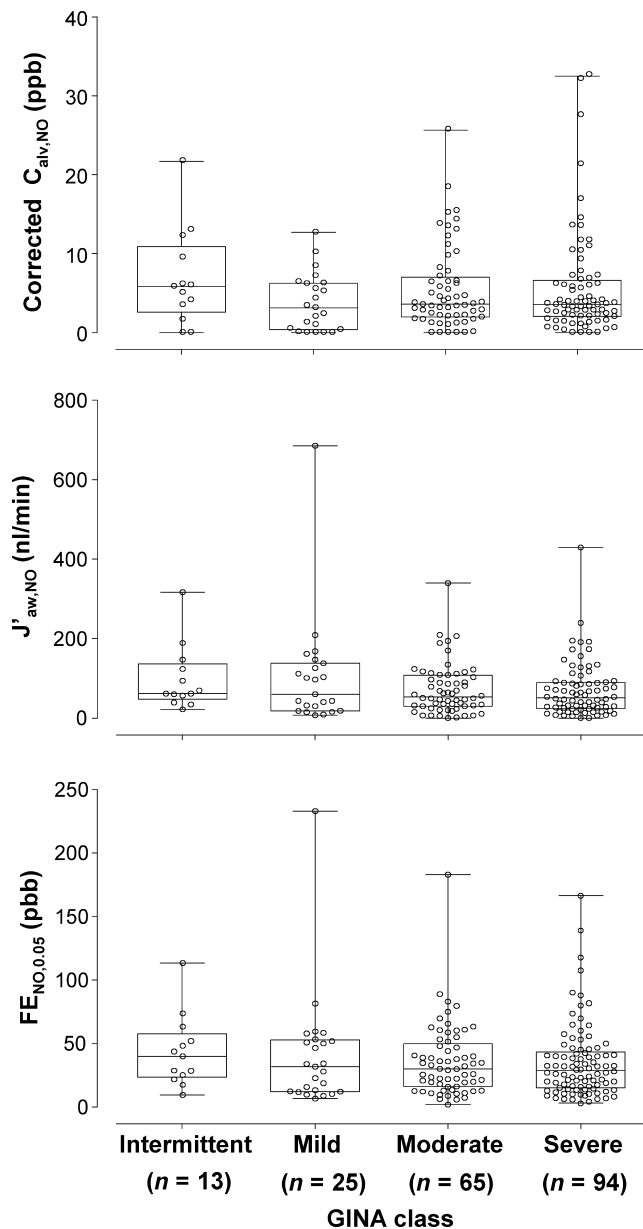


Figure 5. Exhaled NO parameters according to severity. Asthma severity was based on the four GINA classes (see Methods). No significant difference is demonstrated for exhaled NO parameters (upper panel, corrected alveolar NO fraction [corrected $C_{\text{alv,NO}}$]; middle panel, maximum bronchial NO flux [$J'_{\text{aw,NO}}$]; lower panel, exhaled NO fraction measured at 50 ml/s expiratory flow [$FE_{\text{NO},0.05}$]) obtained at inclusion. Box and whisker plots show median, 25th and 75th percentiles, and minimum and maximum values.

partitioning exhaled NO in a large hospital cohort of asthmatic children and adults under treatment, mainly with persistent asthma. Our study demonstrates that alveolar NO concentration and $FE_{\text{NO},0.05}$ are neither associated with asthma control evaluated by Asthma Control Diary and Questionnaire or severity evaluated

by GINA classes in this specific sample of asthma patients [mainly (~80%) receiving ICS plus long acting β_2 -agonist].

Validity of the exhaled NO measures

The five centres used the same analyser, which has specifically been designed for the multiple constant flow rate measurement, and the data from 12.5% of our subjects did not fit to the two-compartment model of NO exchange in the lung, accordingly to the results of two previous studies (17, 23). We further demonstrate that alveolar NO correction for back-diffusion is critical in the asthma setting as previously shown (19), as in its absence $J'_{\text{aw,NO}}$ clearly influenced $C_{\text{alv,NO}}$ values (giving a positive correlation). As expected, atopic patients and patients without ICS had higher values of $J'_{\text{aw,NO}}$. These results confirm the validity of exhaled NO measurements in our multicentre trial.

Exhaled NO parameters and asthma control

Asthma control was assessed using several parameters for two reasons. As we expected that some patients would be lost to follow-up (see Fig. 2), we planned to have both shortened version of ACQ and ACD to verify internal consistency of control assessment and to ensure that at least one mean of control assessment will be obtained. The results of these two scores were logically correlated but interestingly, ACQ systematically over-estimated the ACD score, as previously demonstrated by Juniper et al. (12).

Frank symptoms at the time of assessment have clearly been associated with elevated exhaled NO (1, 2). This latter result is in accordance with the further increase in FE_{NO} that is observed in acute asthma (3). Whether exhaled NO reflects more subtle degrees of control remains debated (6, 7, 24). Senna et al. (25) found a correlation between $FE_{\text{NO},0.05}$ and Asthma Control Test in 27 newly diagnosed asthma while their patients did not receive any treatment. Michils et al. (5) showed that the ability of FE_{NO} to reflect asthma control was reduced in patients using high doses of inhaled corticosteroids that may explain these discrepant results. Finally, two recent studies concluded that FE_{NO} is weakly or nonsignificantly linked to various asthma control scores (6, 7).

Few studies used the partitioning of exhaled NO in asthma. In 44 nonsmoking asthma patients in a stable condition receiving ICS plus long-acting β_2 -agonist Gelb et al. (26) have shown that an abnormal $C_{\text{alv,NO}}$ increased the relative risk of severe exacerbation by 3.0 (95% CI 0.9–9.9) and abnormal $J'_{\text{aw,NO}}$ by 2.4 (1.0–5.6), suggesting a link between alveolar NO and control. Nevertheless, their $C_{\text{alv,NO}}$ values were overestimated as they were not corrected for back-diffusion. Moreover, their end-point was severe exacerbation, which combines components of both asthma control

and severity (11). Our result confirms the findings of Berry et al. (27) who did not find a correlation between alveolar NO and Juniper asthma control score in more severe patients. Our sample size was calculated for a 90% power to detect a 2 ppb difference in alveolar NO (corrected for back-diffusion); consequently, we can conclude that alveolar NO does not constitute a sensitive marker of asthma control in asthmatic patients under treatment (approximately 80% receiving inhaled corticosteroid plus long-acting β_2 -agonist). We deliberately chose to include patients without recent exacerbation because our question was whether alveolar NO could reflect subtle levels of control that can be difficult to assess clinically. The present study was not designed to detect whether exhaled NO parameters were sensitive markers of subsequent exacerbations (the observed severe exacerbation rate was approximately 0.10/patient/year, similar to that observed in a previous study that enrolled patients receiving ICS) (28).

Exhaled NO parameters and asthma severity

Assessment of asthma severity is a complex issue (29). It has recently been stated that the most clinically useful concept of asthma severity is based on the intensity of treatment required to achieve good asthma control, i.e. severity is assessed during treatment (29). We chosen to assess asthma severity based on GINA guidelines combining both the level of control and treatment as done by Liard et al. (15). These authors, in a study involving 4362 patients (16–45 years, 42% with ICS) enrolled by 545 chest specialists in France assessing the severity of asthma, showed that the repartition was 13% intermittent, 27% mild persistent, 36% moderate persistent and 24% severe persistent (15). Our population of asthma patients was more severe, probably because of the hospital selection bias. We observed no significant relationship between exhaled NO and severity (GINA definition), and severe persistent asthmatic patients had similar alveolar NO concentration than intermittent asthmatic patients. In contrast, recent studies suggested that alveolar NO concentration is elevated in refractory asthma, unresponsive to ICS treatment and associated with peripheral airway dysfunction (9, 27, 30). Alveolar

NO could have been a marker of distal inflammation and/or remodelling as we found significant relationships between alveolar NO and both bronchoalveolar lavage eosinophilic cationic protein and $MEF_{25-75\%}$ (30). In a *post hoc* analysis, we similarly found that alveolar NO negatively correlated to $MEF_{25-75\%}$, suggesting that alveolar NO is a marker of peripheral airway dysfunction.

In conclusion, this first multicentre trial has addressed the clinical potential in term of asthma control and severity of partitioning exhaled NO into its alveolar and bronchial sources. We conclude that neither alveolar NO nor $FE_{NO,0.05}$ (or bronchial NO) constitute relevant markers of control or severity of asthma in children and adults under regular asthma treatment.

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Competing interests

All the authors declare no competing interest, excepting CD2 who received a free NO analyser (ENDONO 8000) from SERES company for the development of the multiple constant flow analysis software.

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References

1. 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**:1012–1018.
2. Mahut B, Peiffer C, Thibaudon M, Chevalier-Bidaud B, Defrance-Hutinet MF, Trinquart L et al. What does a single exhaled nitric oxide measurement tell us in asthmatic children? *J Asthma* in press.
3. Delclaux C, Sembach N, Claessens YE, Dolbeau G, Chevalier-Bidaud B, Renaud B et al. Offline exhaled nitric oxide in emergency department and subsequent acute asthma control. *J Asthma* 2008;**45**:867–873.

4. Delclaux C, Mahut B, Zerah-Lancner F, Delacourt C, Laoud S, Cherqui D et al. Increased nitric oxide output from alveolar origin during liver cirrhosis versus bronchial source during asthma. *Am J Respir Crit Care Med* 2002;**165**:332–337.
5. Michils A, Baldassarre S, Van Muylem A. Exhaled nitric oxide and asthma control: a longitudinal study in unselected patients. *Eur Respir J* 2008;**31**:539–546.
6. Shirai T, Furuhashi K, Suda T, Chida K. Relationship of the asthma control test with pulmonary function and exhaled nitric oxide. *Ann Allergy Asthma Immunol* 2008;**101**:608–613.
7. 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**:124–129.
8. Lehtimäki L, Kankaanranta H, Saarelainen S, Hahtola P, Jarvenpää R, Koivula T et al. Extended exhaled NO measurement differentiates between alveolar and bronchial inflammation. *Am J Respir Crit Care Med* 2001;**163**:1557–1561.
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**:737–741.
10. 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**:841–845.
11. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW 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.
12. Juniper EF, O'Byrne PM, Ferrie PJ, King DR, Roberts JN. Measuring asthma control. Clinic questionnaire or daily diary? *Am J Respir Crit Care Med* 2000;**162**:1330–1334.
13. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. *Respir Med* 2005;**99**:553–558.
14. From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA). 2002 Available from: <http://www.ginasthma.org>
15. Liard R, Leynaert B, Zureik M, Beguin FX, Neukirch F. Using Global Initiative for Asthma guidelines to assess asthma severity in populations. *Eur Respir J* 2000;**16**:615–620.
16. George SC, Hogman M, Permutt S, Silkoff PE. Modeling pulmonary nitric oxide exchange. *J Appl Physiol* 2004;**96**:831–839.
17. Mahut B, Louis B, Zerah-Lancner F, Delclaux C. Validity criteria and comparison of analytical methods of flow-independent exhaled NO parameters. *Respir Physiol Neurobiol* 2006;**153**:148–156.
18. 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**:912–930.
19. 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**:918–924.
20. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A et al. Standardisation of spirometry. *Eur Respir J* 2005;**26**:319–338.
21. 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**:616–621.
22. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res* 1999;**8**:135–160.
23. 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**:260–267.
24. Sippel JM, Holden WE, Tilles SA, O'Hollaren M, Cook J, Thukkani N et al. Exhaled nitric oxide levels correlate with measures of disease control in asthma. *J Allergy Clin Immunol* 2000;**106**:645–650.
25. Senna G, Passalacqua G, Schiappoli M, Lombardi C, Wilcock L. Correlation among FEV₁, nitric oxide and asthma control test in newly diagnosed asthma. *Allergy* 2007;**62**:207–208.
26. Gelb AF, Flynn Taylor C, Shinar CM, Gutierrez C, Zamel N. Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics. *Chest* 2006;**129**:1492–1499.
27. 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**:986–991.
28. 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**:2163–2173.
29. Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB et al. A new perspective on concepts of asthma severity and control. *Eur Respir J* 2008;**32**:545–554.
30. 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**:252–256.