

DIAGNOSIS

The Effect of Spirometry on Bronchial and Alveolar Nitric Oxide in Subjects with Asthma

LUIS PRIETO, M.D., PH.D.,^{1,*} LETICIA RUIZ-JIMENEZ, M.D.,² AND JULIO MARIN, M.D., PH.D.¹

¹Universidad de Valencia, Valencia, Spain.

²Servicio de Alergología, Hospital Clínico S Carlos, Madrid, Spain.

Objective. The effect of spirometric maneuvers on exhaled nitric oxide (NO) at the constant flow rate of 50 ml/s (FE_{NO}) has been studied with equivocal results. Furthermore, the effects of spirometry on bronchial NO flux (J'aw_{NO}) and alveolar NO (CA_{NO}), two measurements increasingly being used in clinical and research protocols, are unknown. The aim of this study was to evaluate the effect of spirometry on FE_{NO}, J'aw_{NO}, and CA_{NO} in adults with asthma. **Methods.** Forty-four adults with asthma were studied. To assess the impact of exhaled NO measurement itself on exhaled NO values, FE_{NO}, J'aw_{NO}, and CA_{NO} were obtained twice, at baseline and after a resting period of 10 min. Then spirometry (with or without bronchodilator) was performed followed by exhaled NO measurements at 10 min. **Results.** In the group with pre-bronchodilator study only ($n = 26$), mean (95% CI) values before spirometry were 37.3 ppb (22.2–52.4) for FE_{NO}, 2375 pl/s (1613–3137) for J'aw_{NO}, and 1.65 ppb (0.95–2.35) for CA_{NO}, compared with 35.5 ppb (21.1–49.0, $p = .10$), 2402 pl/s (1663–3141, $p = .85$), and 1.60 ppb (0.64–2.56, $p = .87$) after spirometry, respectively. Spirometry-induced changes in exhaled NO values were also not significant in the group with both pre- and post-bronchodilators ($n = 18$). Furthermore, changes in FE_{NO}, J'aw_{NO}, and CA_{NO} values were similar in the two groups. **Conclusions.** Our findings demonstrate that spirometry (with or without bronchodilator) does not induce significant changes in bronchial NO flux or alveolar NO values. Therefore, exhaled NO values may be obtained after spirometric maneuvers.

Keywords alveolar nitric oxide, asthma, bronchial nitric oxide, exhaled nitric oxide, spirometry

INTRODUCTION

Exhaled nitric oxide (NO) levels are increased in atopic subjects with asthma (1) and are correlated with the number of eosinophils in the lower respiratory tract (2). Therefore, exhaled NO has been proposed as a non-invasive marker of airway inflammation in asthma (3). The plateau NO concentration measured during exhalation at the constant flow rate of 50 ml/s, referred as fractional exhaled NO (FE_{NO}), has been extensively studied as a potential index of airway inflammation. However, phenotyping asthma inflammation is a relevant topic, and the interest extends beyond the identification of predominant inflammatory constituents to the localization of inflammation (4) (central vs. peripheral airways). Thus, special interest has been paid on the possibility of modeling NO production to calculate how much of the NO that is measured in exhaled air originates from small airways/alveolar (CA_{NO}) and from bronchial (J'aw_{NO}) sources (5).

Exhaled NO clinical and research protocols often incorporate measures of lung function, such as spirometry before and after inhaled bronchodilator administration. Previous reports have provided conflicting data on the effect of spirometric maneuvers upon FE_{NO} values. Initial studies performed in adults (6,7) and children (8) reported that repeated spirometry was associated with a significant decrease in FE_{NO} values. Additionally, inhaled albuterol (200 µg) produced a

significant mean increase of the order of 10 parts per billion (ppb) in FE_{NO} for 1 h when compared with placebo inhaler (7). By contrast, other investigations performed in adults (9,10) and children (10–12) with asthma reported that FE_{NO} values were not significantly affected by prior spirometry maneuver. In spite of these contradictory findings, guidelines from the joint European Respiratory Society (ERS)/American Thoracic Society (ATS) task force recommend to perform exhaled NO analysis before spirometry (13). Moreover, the effect of spirometric maneuvers on J'aw_{NO} and CA_{NO} values is not yet known.

The aim of the present study was to evaluate the effect of spirometry on FE_{NO}, J'aw_{NO}, and CA_{NO} in adults with asthma.

SUBJECTS AND METHODS

Study Subjects

The study was approved by the ethics committee of Dr Peset University Hospital, and informed consent was obtained from all participants. Forty-four adults with at least 1-year history of physician-diagnosed asthma were recruited from the Allergy Clinic from our institution. All subjects had mild to moderately persistent asthma well controlled with albuterol as needed or inhaled corticosteroids (ICSs) as monotherapy or in combination with a long-acting β_2 -agonists. Bronchodilators were withheld for 6 h (short acting) and 24 h (long acting) before the tests. Asthma was diagnosed by the presence of symptoms of wheeze, breathlessness, or cough plus methacholine

*Corresponding author: Luis Prieto, M.D., PH.D., Departamento de Medicina, Facultad de Medicina y Odontología, Universidad de Valencia, Av. Blasco Ibáñez 15, 46010 Valencia, Spain; Fax: +34961622501; E-mail: jesus.l.prieto@uv.es

airway hyperresponsiveness ($PC_{20} \leq 8\text{mg/ml}$) or an improvement in FEV_1 from baseline $\geq 15\%$ after 400 μg inhaled albuterol.

Study Design

The study was performed in 1 day. To assess the impact of exhaled NO measurement itself on exhaled NO values, FE_{NO} , $J'aw_{NO}$, and CA_{NO} were obtained twice, at baseline and after a resting period of 10 min. Then spirometry (with or without bronchodilator) was performed followed by FE_{NO} , $J'aw_{NO}$, and CA_{NO} measurements at 10 min. The number of forced vital capacity (FVC) maneuvers was recorded.

Pulmonary Function

Lung function (flow–volume curves) was measured using a calibrated pneumotachograph (Jaeger MasterScope; Erich Jaeger GmbH, Würzburg, Germany) according to the standardized guidelines (14). Baseline forced expiratory maneuvers were repeated until three consecutive maneuvers with $\leq 5\%$ difference between the highest and the lowest FEV_1 were achieved. In a group of 18 subjects, a dose of 400 μg of albuterol was then inhaled and spirometry was recorded 15–20 min later, using the same procedures. Reference values were those of the European Community for Coal and Steel (15).

Nitric Oxide Measurements

Online exhaled NO measurements were performed using a chemiluminescence technique (NiOx; Aerocrine, Stockholm, Sweden). In this study, exhaled NO at 50 ml/s was referred to as FE_{NO} and was obtained according to a standardized method (12). Exhaled NO was also measured at expiratory flow rates of 100, 200, and 300 ml/s. To enable the subjects to achieve the target flow rate, dynamic flow resistors were used (Aerocrine). The exhalation times varied from 6 to 10 s, depending on the exhalation flow rate, and exhaled NO was always measured during the last 3 s of the exhaled NO maneuver. The average of two values performed at each of three flow rates was recorded. The technique of Tsoukias and George (16) was used to calculate $J'aw_{NO}$ in pl/s (intercept with the y-axis) and steady-state CA_{NO} concentration in ppb (slope) by applying linear least squares to a plot of the output of NO (exhaled NO values times the expired flow) versus flow.

CA_{NO} was adjusted for the trumpet shape of the airway tree (increasing cross-sectional area with distance to the lungs) and axial diffusion (17,18) from central to peripheral airways ($CA_{NO\text{cor}}$) using the method described by Condorelli et al. (18), i.e., $CA_{NO\text{cor}}(\text{ppb}) = CA_{NO}(\text{ppb}) - J'aw_{NO}(\text{pl/s})/860$, where CA_{NO} and $J'aw_{NO}$ are small airway/alveolar and large airway NO concentration, respectively, computed by the method of Tsoukias and George (16). $J'aw_{NO}$ was corrected by multiplying the original value by 1.7 (18).

Statistical Analysis

The primary outcome of the study was the CA_{NO} value. Secondary outcomes were $J'aw_{NO}$ and FE_{NO} values. The sample size requirement of this study was calculated

considering a 1-ppb change in CA_{NO} after spirometry as clinically relevant on the basis of previous studies (19). Based on these assumptions and using a repeated-measures design, it was estimated that 18 patients would be required to provide 80% power at the two-sided 0.05 level.

Negative CA_{NO} values before spirometry were recorded in eight subjects. These values have been replaced with 0. To evaluate normality of distributions, the Kolmogorov–Smirnov test was used. The distributions of all numerical variables were not significantly different from a standard normal distribution; hence, all numerical variables are reported as arithmetic means with 95% confidence intervals (CIs). Changes from baseline in FE_{NO} , $J'aw_{NO}$, and CA_{NO} values after repeated spirometry were assessed by repeated-measures analysis of variance (ANOVA). Post hoc testing was performed using Dunnett's method to compare each time point to baseline. Comparison of the characteristics of subjects with pre-bronchodilator spirometry only and those with pre- and post-bronchodilator spirometry was performed by unpaired Student's *t*-tests for continuous data and by Fisher exact tests for binary variables. *P*-values are two-sided, and values <0.05 were considered statistically significant. Data were analyzed with a statistical software package (Prism 5; GraphPad Software, San Diego, CA, USA).

RESULTS

The demographic, clinical and pulmonary function data for the 44 subjects studied are presented in Table 1. A mean of four (range 3–8) spirometric maneuvers were performed. Thirty-seven subjects (88%) had atopic asthma and 33 (75%) used ICS associated or not associated with long-acting β_2 -agonists.

Characteristics of subjects with pre-bronchodilator spirometry only and those with pre- and post-bronchodilator spirometry are presented in Table 2. As expected, the baseline pulmonary function was lower and the number of spirometric maneuvers was higher in the group with pre- and post-bronchodilator spirometry. Measurements of NO at multiple flow rates

TABLE 1.—Subject characteristics*.

Characteristics	Data
Age, year	41 (36–47)
Male/female gender, No	17/27
Smoker/non-smoker, <i>n</i>	7/37
Atopic/non-atopic, <i>n</i>	37/7
Treatment	
ICS	10
ICS + LABA	23
Albuterol as needed	11
FEV_1 , % predicted	87 (82–93)
FVC, % predicted	101 (96–106)
FEV_1 /FVC, %	73 (70–76)

Notes: *Data are presented as mean (95% CI) unless otherwise indicated.

Abbreviations: FEV_1 , forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroids; LABA, long-acting β -agonists.

TABLE 2.—Baseline characteristics and changes in exhaled nitric oxide values in subjects with pre-bronchodilator study only and in those with study both pre- and post-bronchodilators.

	Pre-bronchodilator (<i>n</i> = 26)	Pre- and post-bronchodilators (<i>n</i> = 18)	<i>p</i>
No. of maneuvers	2 (3–6)	5 (4–8)	<.0001
Age, years	39 (34–44)	44 (35–53)	.28
Male/female gender	7/19	10/8	.07
Duration of symptoms, years	16 (12–20)	19 (14–24)	.22
Smoker/non-smoker, <i>n</i>	5/21	2/16	.68
Atopic/non-atopic, <i>n</i>	20/6	17/1	.21
ICS treatment (yes/no), <i>n</i>	22/4	11/7	.09
FEV ₁ , % predicted	95 (89–101)	77 (69–84)	.0005
FVC, % predicted	106 (100–112)	93 (86–101)	.0069
FEV ₁ /FVC, %	76 (73–79)	68 (64–73)	.0034
Change in FE _{NO} , ppb	−2.3 (−4.7 to 0.1)	1.5 (−0.5–3.4)	.11
Change in J'aw _{NO} *, pl/s	27 (−270 to 323)	243 (−365–849)	.50
Change in CA _{NO} *, ppb	0.05 (−0.49 to 0.60)	0.53 (−0.24–1.24)	.30

Notes: *In 19 subjects with pre-bronchodilator study alone and in 12 with both pre- and post-bronchodilators in which J'aw_{NO} and CA_{NO} could be calculated. Abbreviations: FE_{NO}, fractional exhaled NO at a flow of 50 ml/s; J'aw_{NO}, bronchial NO flux; CA_{NO}, alveolar NO. For other abbreviations, see Table 1.

TABLE 3.—Exhaled nitric oxide values obtained before and after spirometry, in the group with pre-bronchodilator spirometry only and in the group with both pre- and post-bronchodilator spirometry*.

Pre-bronchodilator	Before spirometry	After spirometry	<i>p</i>
FE _{NO} , ppb	37.3 (22.2–52.4)	35.0 (21.1–49.0)	.10
J'aw _{NO} , pl/s	2375 (1613–3137)	2402 (1663–3141)	.85
CA _{NO} , ppb	1.65 (0.95–2.35)	1.60 (0.64–2.56)	.87
Pre- and post-bronchodilators			
FE _{NO} , ppb	29.7 (17.7–41.6)	31.2 (17.8–44.3)	.13
J'aw _{NO} , pl/s	2770 (1006–4533)	3012 (681–5343)	.40
CA _{NO} , ppb	1.26 (0.36–2.14)	1.78 (0.45–3.12)	.12

Notes: *Values are presented as mean (95% CI). For abbreviations, see Tables 1 and 2.

did not fit the linear model in seven subjects of the group with pre-bronchodilator spirometry alone and in 6 subjects from the group with pre- and post-bronchodilator spirometry. Therefore, J'aw_{NO} and CA_{NO} values could only be calculated in 19 and 12 subjects, respectively. Spirometry-induced changes in exhaled NO (FE_{NO}, J'aw_{NO} and CA_{NO}) values in each group were not significant (Table 3 and Figures 1–3) and these changes were similar in the two groups of subjects studied (Table 2).

DISCUSSION

The results of the present study confirm that, in clinical practice, FE_{NO} values identified in subjects with asthma are not significantly affected by prior FVC maneuvers, such as those that occur during the serial performance of spirometry. The new finding is that spirometry (with or without bronchodilator) does not induce significant changes in bronchial NO flux or alveolar NO values.

In the present study, we quantified the effect of a single spirometric session (3–8 FVC maneuvers) on single flow exhaled NO measurement. Our data demonstrate the lack

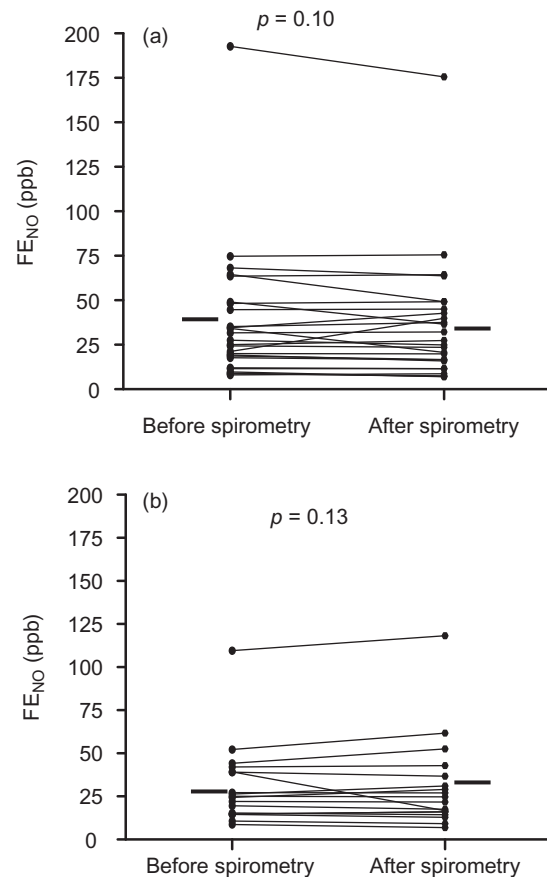


FIGURE 1.—Individual values for fractional exhaled nitric oxide concentration at a constant flow rate of 50 ml/s (FE_{NO}) measured before and 10 min after spirometry in (a) subjects with pre-bronchodilator only (*n* = 26) and (b) those with both pre- and post-bronchodilators (*n* = 18). Horizontal lines are means.

of influence of spirometric maneuvers on FE_{NO} values at 10 min. These findings are consistent with those reported by Tee and Hui (9), which showed a lack of influence of spirometric maneuvers on FE_{NO} levels measured at 5 min. In addition, our results suggest that if repeated

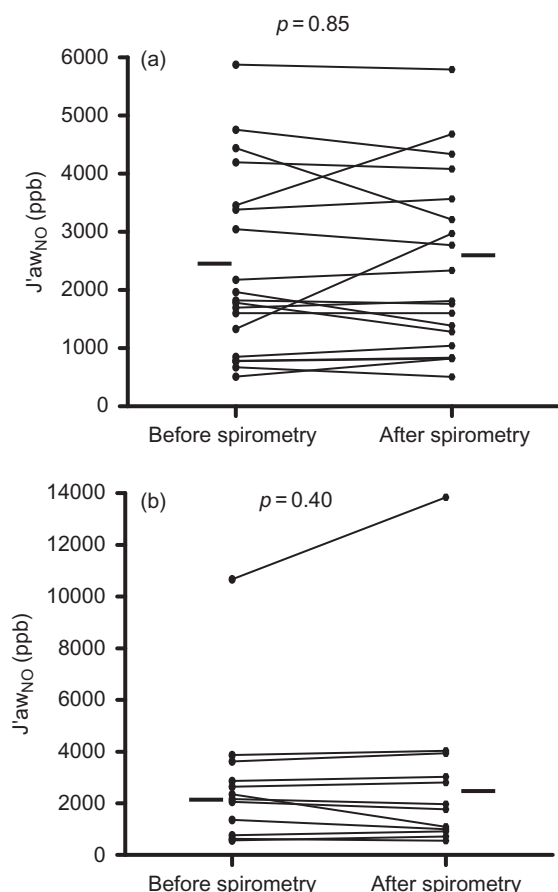


FIGURE 2.—Individual values for bronchial nitric oxide flux (J'_{awNO}) measured before and 10 min after spirometry in (a) subjects with pre-bronchodilator only ($n = 19$) and (b) those with both pre- and post-bronchodilators ($n = 12$) in which J'_{awNO} could be calculated. Horizontal lines are means.

spirometry has an immediate effect on FE_{NO} , as reported by Silkoff et al. (7), an interval of 10 min seems to be sufficient to revert the values to pre-spirometric levels. By contrast, Deykin et al. (20) reported that repeated spirometry was associated with a significant decrease in FE_{NO} values; this effect was significant at 15 min, maximal at 30 min and lasted several hours. The authors speculated that repeated spirometric maneuvers reduced NO production through an effect on bronchodilating, non-adrenergic, non-cholinergic nerves within the lower airways. The discrepancy between our results and those reported by Deykin and coworkers (20) may be explained, at least in part, by differences in subject characteristics and method for NO measurement. Deykin et al. (20) studied subjects with mild atopic asthma not treated with ICS for at least 6 weeks, whereas a significant proportion of our patients (75%) were treated with ICS. The use of ICS by our patients might have suppressed, at least theoretically, the NO generation from the source of NO, which may be sensitive to forced respiratory maneuvers. We do not believe, however, that our findings could have been influenced by ICS treatment because similar findings were obtained in the group of 11 patients not treated with ICS (data not

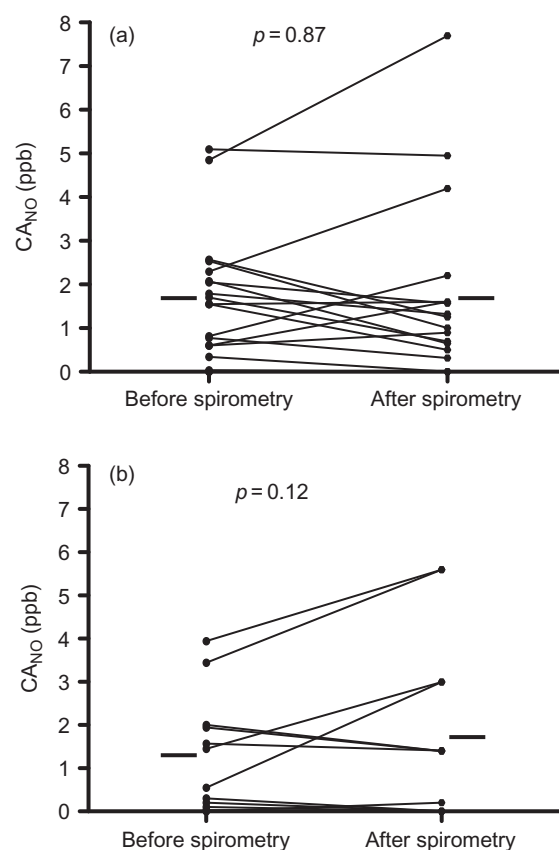


FIGURE 3.—Individual values for alveolar nitric oxide (CA_{NO}) measured before and 10 min after spirometry in (a) subjects with pre-bronchodilator only ($n = 19$) and (b) those with both pre- and post-bronchodilators ($n = 12$) in which CA_{NO} could be calculated. Horizontal lines are means.

shown). Furthermore, in the study by Deykin et al. (20), exhaled air from tidal breathing was collected into bags for analysis (offline method), whereas we used an online method for NO assessment. It has been reported (21,22) that collection techniques have a significant impact on measured NO values and, therefore, we cannot reject that significant FE_{NO} changes might be observed using the offline method. This should be studied in future investigations.

It has been shown that the flow-independent parameters of exhaled NO exchange, i.e., airway NO flux and alveolar NO concentration, can be calculated by measuring exhaled NO at multiple exhalation flows, and then fitting a regression line to the plot of the elimination rate against the exhalation flow rate. The intercept and the slope of the regression line are estimates of the airway flux and alveolar NO concentration, respectively (5,16). Studies reported to date suggest that monitoring alveolar NO may provide benefits for evaluation of peripheral airway inflammation in nocturnal asthma (23) and in refractory asthma which is difficult to control with inhaled corticosteroids (24). To the best of our knowledge, this is the first study investigating the effect of spirometric tests on J'_{awNO} and CA_{NO} in subjects with asthma. Our results clearly showed that spirometry does not affect J'_{awNO} and CA_{NO} readings.

Furthermore, the lack of effect of spirometry on exhaled NO values was observed both in subjects with pre-bronchodilator spirometry alone and in those with pre- and post-bronchodilator maneuvers, despite the number of maneuvers in this last group was significantly greater. Hence, the number of FVC maneuvers or use of bronchodilator should not influence the interpretation of exhaled NO values. However, $J'aw_{NO}$ and CA_{NO} could be calculated in a small number of subjects ($n = 12$) with pre- and post-bronchodilator spirometry, and a type 2 error cannot be ruled out. Our results in this group with pre- and post-bronchodilator spirometry should, therefore, be confirmed in future investigations performed on a greater number of subjects.

The fact that $J'aw_{NO}$ and CA_{NO} measurements could only be made on 70% of the subjects studied is an important methodological problem and a potential weakness of this study. To overcome this problem, Mohat et al.(25) used the non-linear model described by Silkoff and colleagues (26) to attempt to calculate $J'aw_{NO}$ and CA_{NO} in the subjects in whom the data did not fit the linear model. We do not attempt this solution because exhaled NO levels using the very low flows recommended in this model (26) were greater than the maximum detection limit of the NIOX system (200 ppb) in more than 20% of our subjects with asthma or allergic rhinitis.

What are the clinical implications of our findings? The lack of difference between FE_{NO} , $J'aw_{NO}$, and CA_{NO} values measured before and after spirometry is of practical relevance. Asthmatic patients often have spirometric measurements as well as exhaled NO determinations to characterize disease control during a clinic visit. Guidelines from the joint European Respiratory Society/American Thoracic Society (4) task force recommend to perform exhaled NO analysis before spirometric tests to avoid the influence of forced expiratory maneuvers on exhaled NO levels in healthy and asthmatic subjects. Our results demonstrate that, at least in asthmatic patients, exhaled NO measurements can be performed 10 min after spirometric studies. Therefore, exhaled NO investigations can be performed next to spirometry and at a single clinic visit, which appears more convenient in a clinical setting. However, it has been demonstrated that, at least in children, repeated valsalva maneuvers performed to determine maximal expiratory pressures are consistently associated with a significant decrease in FE_{NO} levels (12). Therefore, although repeated spirometry leaves FE_{NO} , $J'aw_{NO}$, and CA_{NO} values unchanged, the influence of other pulmonary function test measurements on exhaled NO levels might be different and should be studied in future investigations.

In summary, the findings of the present study demonstrate that exhaled NO levels are not affected by prior FVC maneuvers. Hence, exhaled NO measurement may be performed 10min after baseline spirometry or spirometry pre- and post-bronchodilators, without significant changes in FE_{NO} , $J'aw_{NO}$, and CA_{NO} values.

CONCLUSIONS/KEY FINDINGS

Fractional exhaled NO, alveolar NO, and bronchial NO values are not affected by prior FVC maneuvers. Therefore, exhaled NO investigations can be performed next to spirometry and at a single clinic visit, which appears more convenient in a clinical setting.

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DECLARATION OF INTEREST

The authors declare that they have no conflict of interest, financial or otherwise, related to this study.

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