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The effect of bronchodilation and spirometry on fractional exhaled nitric oxide (FeNO50), bronchial NO flux (JawNO) and alveolar NO concentration (C_ANO) in children and young adults

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ABSTRACT

Objective: Fractional exhaled nitric oxide (FeNO), bronchial nitric oxide (JawNO) and alveolar nitric oxide (C_ANO) are biomarkers of eosinophilic inflammation, usually measured simultaneously with spirometry and bronchodilation. Our aim was to investigate the effect of bronchodilation and spirometry on FeNO, C_ANO and JawNO in children and young adults with well-controlled asthma and in healthy volunteers. **Methods:** FeNO was measured in 95 subjects (62 controls, 33 asthmatics). C_ANO and JawNO were assessed in 41 of the subjects (35 healthy, 6 asthmatics). Measurements were performed before spirometry (1), right after spirometry (2), 20 min after the first spirometry and bronchodilation (3), right after the post-bronchodilation spirometry (4) and 30 min after the last spirometry (5). **Results:** The presence of well-controlled asthma was not associated with different pattern of reaction after spirometry and bronchodilation. A statistically significant difference was observed only between FeNO4 and FeNO5, as well as between C_ANO1 and C_ANO3 (19.14 ± 1.68 vs 20.62 ± 1.85 ppb, $p = 0.001$ and 4.42 ± 0.40 vs 3.09 ± 0.32 ppb, $p = 0.001$, respectively). **Conclusions:** Spirometry and bronchodilation have an insignificant effect on FeNO and JawNO. Even if a slight change occurs in FeNO and JawNO, this does not modify clinician's decision and therapeutic strategy. C_ANO values (C_ANO1) are significantly decreased 20 min after spirometry and bronchodilation.

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Asthma; fractional exhaled nitric oxide; spirometry; bronchodilation

1. Introduction

Exhaled nitric oxide (eNO) represents a marker of eosinophilic inflammation of the respiratory system [1]. Asthma is a chronic inflammatory airway disorder, mainly characterized by airway eosinophilia, hyperresponsiveness and reversibility. Despite that, it is both diagnosed and managed according to patient's symptoms and lung function [2–4]. As there was a pressing need for an easily measured inflammatory marker, eNo became a part of the clinical practice [5–9].

Fractional exhaled nitric oxide (FeNO) values reduce after exposure to many factors including corticosteroids, exercise and cigarette smoking, whereas factors associated with increased values are uncontrolled asthma, poor air quality, hay fever, dietary exposures, height and male gender [10–18].

However, studies investigating the effect of spirometry and bronchodilation on FeNO yielded controversial results [19–21]. There are both reports of reduction to

FeNO due to spirometry and studies reporting no significant effect [19,20]. Similarly, there are studies reporting an increase to FeNO due to beta2-agonists and others showing an insignificant effect [20,21].

Bronchial flux (JawNO) and alveolar concentration (C_ANO) of NO can be assessed by measuring FeNO at multiple exhalation flow rates and applying mathematical models of pulmonary NO dynamics [22]. JawNO is closely correlated with FeNO50, while C_ANO reflects distal airway inflammation and is elevated in severe or poorly controlled asthma as well in other diseases including cystic fibrosis. C_ANO and JawNO are increasingly used in clinical practice, simultaneously with FeNO and spirometry. Nonetheless, data investigating the effect of spirometry and bronchodilation on C_ANO and JawNO are still limited.

Therefore, the aim of this study was to investigate the effect of spirometry and bronchodilation on FeNO, bronchial (JawNO) and alveolar (C_ANO) nitric oxide in healthy and asthmatic children and young adults.

2. Methods

Subjects

A total of 95 subjects (aged between 6 and 26) were recruited. The study group consisted of 62 healthy volunteers and 33 patients with asthma (Table 1). Children with asthma (aged 6–14, $n = 20$) were recruited from our pediatric clinic and young adults with asthma (aged 18–26, $n = 13$) were the students of our university. The study was approved by the Committee of Alexandroupolis Hospital, Democritus University of Thrace. The parents of the participating children were also informed, and they approved the whole procedure.

Patients with asthma

Patients were included in the asthmatic group according to the following criteria: (1) doctor diagnosis of asthma based on history of at least two of the following: wheezing, cough, shortness of breath, chest tightness; (2) increase in FEV1 20 min after bronchodilation $> 12\%$; and (3) exclusion of other diseases that were part of the differential diagnosis. All patients had controlled asthma at the time of assessment and at least 4 months before.

Healthy subjects had normal pulmonary function tests before and after bronchodilation, no history or clinical evidence of acute or chronic respiratory disease and negative prick test to ten common aeroallergens.

Pulmonary function testing

A dry rolling-seal spirometer was used for the pulmonary function tests (MIR Spirodoc, Italy) based on the ERS/ATS criteria [23]. Every subject performed three efforts and the best values of forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were recorded.

Nitric oxide testing

eNO was measured using an analyzer (ECO-MEDICS, CLD88sp, Switzerland) according to ERS/ATS guidelines [24]. All subjects were rested and had not eaten foods that influence the results before the test. Subjects inhaled air free of nitric oxide and then exhaled for at least 7 sec through a mouthpiece connected to the analyzer. Subjects were guided through the procedure by especially trained staff and computer visual graphics. Any effort that did not meet the ERS/ATS criteria was excluded. Initially, baseline FeNO was measured for every subject after three repeated exhalations agreeing to within 10%. Four different flows were used to measure FeNO (30, 50, 100, 300 ml/sec). Bronchial NO flux (JawNO) and alveolar NO concentration (C_A NO) were calculated using a

mathematical model by measuring eNO in three different flows (30, 100, 300 ml/sec) [22].

Study design (Figure 1)

Baseline FeNO (FeNO1) was measured in all 95 subjects. Baseline bronchial NO flux (JawNO1) and alveolar NO concentration (C_A NO1) were measured from 41 subjects (6 asthmatics, 35 healthy volunteers). Then, every subject performed a spirometry. FeNO was measured again straight after spirometry, in order to investigate the effect of spirometry on FeNO (FeNO2, JawNO2 and C_A NO2). The next step was the guided administration of bronchodilation (four puffs of salbutamol) to the subjects, in order to minimize or avoid the misuse [25]. After a waiting period of 20 min, we measured FeNO again (FeNO3, JawNO3 and C_A NO3), so as to investigate the effect of salbutamol on FeNO. Afterwards, FeNO was measured again (FeNO4, JawNO4 and C_A NO4). Finally, after 30 min of rest, we measured FeNO5, to test whether a resting period of 30 min modifies FeNO levels (FeNO5, JawNO5 and C_A NO5).

Statistics

For analysis of variance of multiple depended variables in relation with multiple independent variables (either fixed factors or covariates), a multivariate general linear model was used. For comparisons concerning continuous variables between paired samples, Wilcoxon Signed Ranks Test was performed; however, in cases where

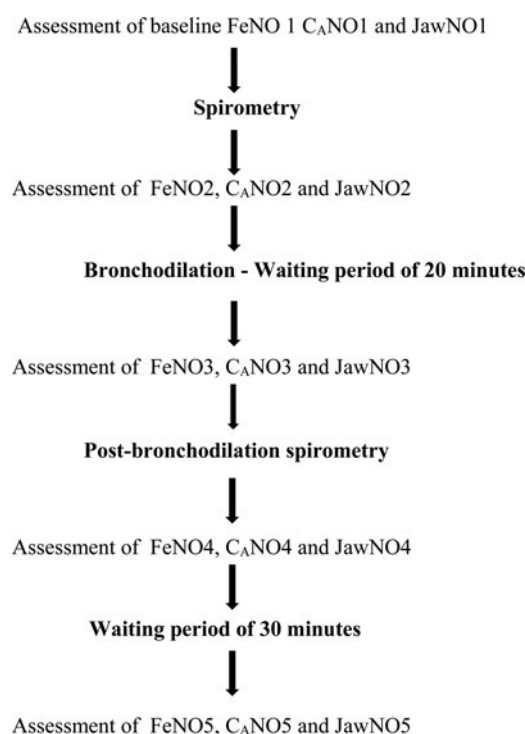


Figure 1. Schematic representation of the study design.

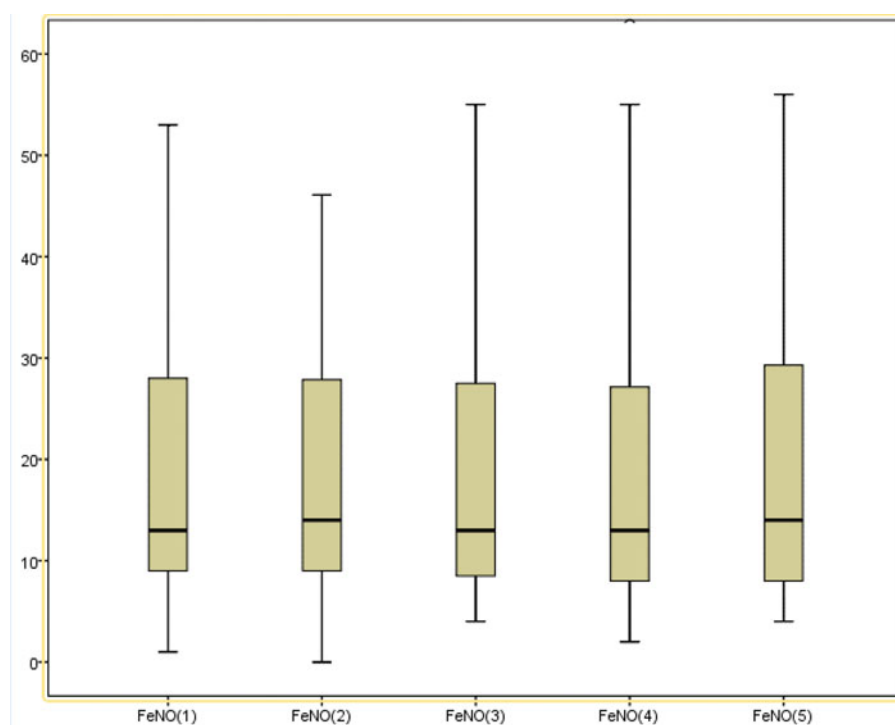


Figure 2. FeNO values. A statistically significant difference between FeNO (4) and FeNO (5) was observed ($p = 0.001$). No difference was observed between FeNO (1) and FeNO (2) ($p = 0.606$), FeNO (2) and FeNO (3) ($p = 0.324$), FeNO (3) and FeNO (4) ($p = 0.248$); as four multiple comparisons were performed, the level of statistical significance was set to $0.05/4 = 0.0125$ due to Bonferroni correction.

Table 1. Baseline characteristics.

Subjects	N	Sex (M/F)	FEV1 pre	FEV1 post	FVC
Normal	62	31/31	102.59 (1.48)	104.86 (1.48)	100.66 (1.74)
Asthmatic	33	16/17	104.28 (2.87)	109.40 (2.46)	99.30 (2.67)

homogeneity of variances had not been rejected using Levene's test between groups, the more robust parametric Student's *t*-test for paired samples was additionally performed. The level of statistical significance was set to $\alpha = 0.05$. In case of μ multiple comparisons, Bonferroni correction was applied; thus, the level of statistical significance was corrected to α/μ . All statistical tests were two-tailed. Mean values are accompanied with their standard errors. All numerical values are given with at least two significant digits.

3. Results

Mean FeNO

A multivariate general linear model was used to analyze variance between four dependent variables (FeNO2,

FeNO3, FeNO4 and FeNO5) and independent ones. With regard to independent variables, fixed factors included gender and presence of well-controlled asthma and covariates FeNO1, baseline FEV1, post-spirometry FEV1, FVC and age. Levene's test rejected null hypothesis that the error variance of the dependent variables is equal across groups. No statistically significant effect on FeNO2, FeNO3, FeNO4 or FeNO5 was attributed to FeNO1, baseline FEV1, post-spirometry FEV1, FVC, gender, age or the presence of well-controlled asthma.

As we proved that the presence of well-controlled asthma was not associated with different pattern of reaction after these maneuvers, we present FeNO values of the whole study group in Figure 2. Demographics and separate FeNO values of normal and asthmatics are presented in Tables 1 and 2, respectively.

All five FeNO values (FeNO1, FeNO2, FeNO3, FeNO4 and FeNO5) were compared with each other. Statistically significant result was observed only between FeNO4 and FeNO5 (19.14 ± 1.68 ppb and 20.62 ± 1.85 ppb, respectively, Wilcoxon Signed Ranks Test $p < 0.001$; level of statistical significance after Bonferroni correction 0.0125). No statistically significant effect of spirometry,

Table 2. Effect on FeNO.

Subjects	FeNO (1)	FeNO (2)	FeNO (3)	FeNO (4)	FeNO (5)
Normal	20.11 (1.89) ppb	20.39 (1.92) ppb	20.00 (1.80) ppb	19.64 (1.72) ppb	21.41 (1.94) ppb
Asthmatic	19.19 (3.78) ppb	19.10 (3.67) ppb	18.78 (3.66) ppb	18.25 (3.56) ppb	19.22 (3.81) ppb

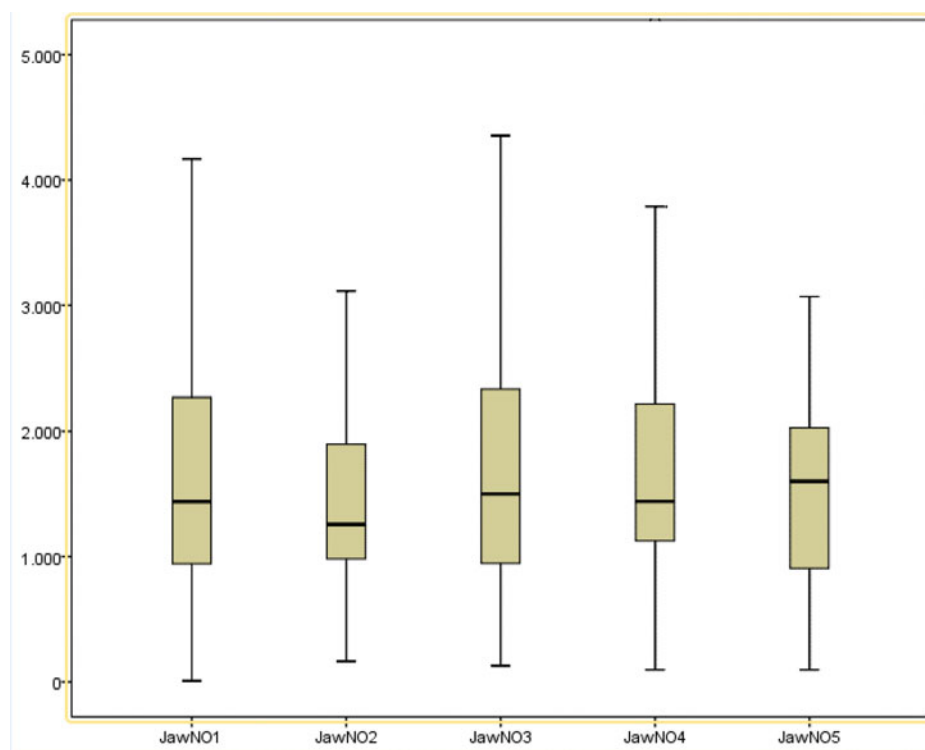


Figure 3. JawNO values. No difference was observed between JawNO (1) and JawNO (2) ($p = 0.422$), JawNO (2) and JawNO (3) ($p = 0.228$), JawNO (3) and JawNO (4) ($p = 0.656$), JawNO (4) and JawNO (5) ($p = 0.882$); as four multiple comparisons were performed, the level of statistical significance was set to $0.05/4 = 0.0125$ due to Bonferroni correction.

bronchodilation or post-bronchodilation spirometry was observed ($p = 0.606$, $p = 0.324$, $p = 0.248$).

Bronchial NO flux (JawNO)

A multivariate general linear model was used to analyze variance between four dependent variables (JawNO2, JawNO3, JawNO4 and JawNO5) and independent ones. With regard to independent variables, fixed factors included gender and presence of asthma and covariates JawNO1, FeNO1, baseline FEV1, post-spirometry FEV1, FVC and age. Levene's test rejected null hypothesis that the error variance of the dependent variables is equal across groups. No statistically significant difference effect on JawNO2, JawNO3, JawNO4 and JawNO5 was attributed to JawNO1, baseline FEV1, post-spirometry FEV1, FVC gender, age or the presence of asthma. However, FeNO1 is strongly covariates (observed power 1.000) at a statistically significant extent ($p < 0.001$) with all JawNO measurements: JawNO1 (partial eta-squared 0.853), JawNO2 (partial eta-squared 0.762), JawNO3

(partial eta-squared 0.604), JawNO4 (partial eta-squared 0.608) and JawNO5 (partial eta-squared 0.546).

As we proved that the presence of well-controlled asthma was not associated with different pattern of reaction after these maneuvers, we present JawNO values of the whole study group in Figure 3. Separate JawNO values of asthmatics and healthy volunteers are presented in Table 3. JawNO was calculated in 35 of 62 healthy subjects and in 6 of 33 patients with asthma. All five JawNO values (JawNO1, JawNO2, JawNO3, JawNO4 and JawNO5) were compared with each other. No statistically significant effect of spirometry, bronchodilation, post-bronchodilation spirometry or rest on JawNO was observed ($p = 0.422$, $p = 0.228$, $p = 0.656$, $p = 0.882$).

Alveolar NO concentration (C_A NO)

A multivariate general linear model was used to analyze variance between four dependent variables (C_A NO2, C_A NO3, C_A NO4 and C_A NO5) and independent ones. With regard to independent variables, fixed factors

Table 3. Effect on JawNO.

Subjects	JawNO (1)	JawNO (2)	JawNO (3)	JawNO (4)	JawNO (5)
Normal	2008.6 $\pm$ 492.2 pl/sec	1632.3 $\pm$ 167.2 pl/sec	1719.7 $\pm$ 168.5 pl/sec	1711.9 $\pm$ 187.2 pl/sec	1664.9 $\pm$ 162.9 pl/sec
Asthmatic	1983.8 $\pm$ 819.8 pl/sec	1946.6 $\pm$ 810.6 pl/sec	2094.4 $\pm$ 768 pl/sec	1887.6 $\pm$ 691.9 pl/sec	2274.6 $\pm$ 733.2 pl/sec

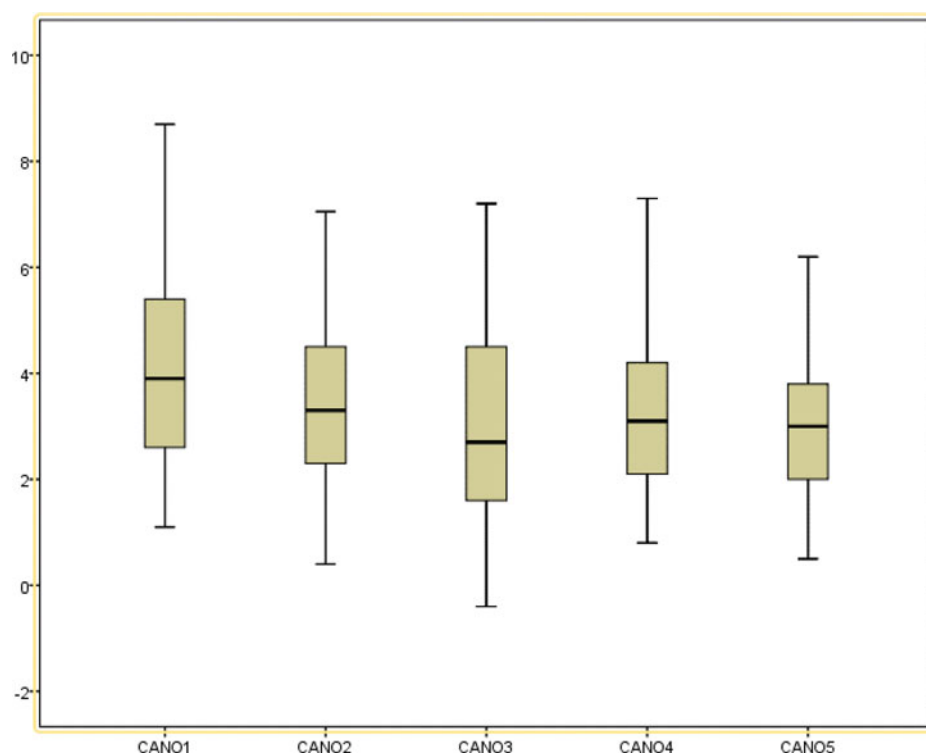


Figure 4. $C_A NO$ values. No difference was observed between $C_A NO$ (1) and $C_A NO$ (2) ($p = 0.032$), $C_A NO$ (2) and $C_A NO$ (3) ($p = 0.063$), $C_A NO$ (3) and $C_A NO$ (4) ($p = 0.645$), $C_A NO$ (4) and $C_A NO$ (5) ($p = 0.885$); as four multiple comparisons were performed, the level of statistical significance was set to $0.05/4 = 0.0125$ due to Bonferroni correction.

included gender and presence of asthma and covariates $C_A NO1$, $FeNO1$, baseline FEV1, post-spirometry FEV1, FVC and age. Levene's test rejected null hypothesis that the error variance of the dependent variables is equal across groups. No statistically significant effect on $C_A NO2$, $C_A NO3$, $C_A NO4$ and $C_A NO5$ was attributed to independent variables.

As we proved that the presence of well controlled asthma was not associated with different pattern of reaction after these maneuvers, we present $C_A NO$ values of the whole study group in Figure 4. Separate $C_A NO$ values of asthmatics and healthy volunteers are presented in Table 4. All five $C_A NO$ values ($C_A NO1$, $C_A NO2$, $C_A NO3$, $C_A NO4$ and $C_A NO5$) were compared between each other. No statistically significant effect of spirometry, bronchodilation, post-bronchodilation spirometry or rest on $C_A NO$ was observed ($p = 0.032$, $p = 0.063$, $p = 0.645$, $p = 0.885$), respectively. Alveolar NO concentration after spirometry and bronchodilation ($C_A NO3$) was significantly lower as compared to the baseline

alveolar NO concentration ($C_A NO1$) (3.09 ± 0.32 vs 4.42 ± 0.40 ppb, respectively, $p = 0.001$).

4. Discussion

To the best of our knowledge, this is the first study of the effect of spirometry, bronchodilation and rest during the same day on bronchial NO flux (JawNO) and alveolar NO concentration ($C_A NO$) in children and young adults. The main finding of our study was that spirometry and bronchodilation have a clinically insignificant effect on the values of $FeNO$ of young healthy and well-controlled asthmatic subjects. Similar results were recorded for the bronchial NO flux of the healthy subjects and a small group of asthmatics, whereas a significant reduction was recorded in alveolar NO concentration of the same group 20 min after spirometry and bronchodilation. Additionally, it was shown that the NO dynamics regarding the effect of the spirometry and bronchodilation for the three measured parameters ($FeNO$, JawNO

Table 4. Effect on $C_A NO$.

Subjects	$C_A NO$ (1)	$C_A NO$ (2)	$C_A NO$ (3)	$C_A NO$ (4)	$C_A NO$ (5)
Normal	4.35 ± 0.45 ppb	3.76 ± 0.37 ppb	3.13 ± 0.35 ppb	3.19 ± 0.27 ppb	3.45 ± 0.31 ppb
Asthmatic	4.83 ± 0.88 ppb	3.38 ± 0.67 ppb	2.85 ± 0.89 ppb	3.35 ± 0.55 ppb	2.13 ± 1.32 ppb

and $C_{A\text{NO}}$) were similar for healthy and well-controlled asthmatic subjects.

With regard to spirometry, our results showed that the effect of spirometry on FeNO is insignificant. In line with our notion, Kissoon et al. found no significant effect of spirometry on children with asthma [21]. They also observed that spirometry increased FeNO slightly but insignificantly, in healthy children [21].

However, some previous studies reached different conclusion. Deykin et al. recruited ten healthy non-smoking subjects and showed that spirometry decreases FeNO values [26]. The same conclusion was conducted in another study of Deykin et al. for patients with asthma [27]. Silkoff et al. recruited eighteen subjects and also found a reduction of FeNO values due to spirometry both in asthmatics and healthy individuals [19]. The results yielded from the study of Gabriele et al. are in contrast to ours, as FVC maneuvers reduced FeNO in 24 asthmatic children [28]. Finally, data arising from a new large study of Eckel et al. demonstrate that spirometry does not alter FeNO values on healthy volunteers, but reduces its value in children with asthma [29].

The discrepancy of the results cannot be fully explained. Deykin et al. suggested that spirometry might reduce NO through non-adrenergic non-cholinergic effect [27]. Kissoon et al. speculated that increased NO release is a result of changes in lung volume during spirometry, which triggers neural mechanisms [21]. It seems also that lower flow rates are more sensitive to NO production [22]. Another possible explanation of the discrepancies among studies might be the small number of subjects in some studies, which could lead to erroneous interpretations. The technique might also affect the result. Finally, we could speculate that FeNO of a patient on corticosteroids, who has a well-controlled asthma, might not change due to spirometry, similarly with healthy individuals. In contrast, spirometry might alter FeNO production of a patient with uncontrolled asthma, since these patients exhibit excessive obstruction, air trapping and increased airway vascularity and it is well-known that NO is both flow dependent and its production is affected by the airway vascularity [4,5,10]. Collectively, the majority of studies show that even if a significant change exists, it is of small magnitude. Furthermore, based on the new ATS guidelines, significant is only a change greater than 20% for values over 50 ppb or a change higher than 10 ppb for values of FeNO lower than 50 ppb [24]. Therefore, such small changes do not affect neither clinicians's strategy.

With regard to the effect of bronchodilation on FeNO, data arising from our study show that it does not cause any significant effect on asthmatics and healthy volunteers. Our findings are in agreement with those

of Prieto et al. [20], Garnier et al. [30] and Baraldi et al. [10]. The findings of Silkoff et al. and Kissoon et al. are not in line with our notion [19,21]. Silkoff et al. presented a mean FeNO increase of 10% [19]. Kissoon et al. reported an increase of similar magnitude for patients with poorly controlled asthma, while there was also a smaller but significant increase for well-controlled asthmatics [21]. Yates et al. showed that a single high dose of salbutamol did not alter significantly FeNO in patients with mild asthma, who were not on corticosteroids, while a high-dose of salbutamol increased FeNO at 15 and 30 min, but this was also not statistically significant [31]. Finally, a recently published study reported that albuterol did not increase significantly FeNO in ICS-treated asthmatics and in patients with COPD, while it caused an increase in FeNO in steroid-naïve asthmatics [32]. Collectively, there is evidence showing that FeNO values are associated with changes in airway caliber [33]. In patients with poorly controlled asthma, FeNO possibly increases due to the fact that bronchodilation increases airway surface area. On the contrary, healthy volunteers and patients with well-controlled asthma are not likely to have such an increase in airway surface area. Furthermore, corticosteroids inhibit inducible nitric oxide synthase (iNOS), which is the enzyme responsible for the production of larger amounts of NO [34]. Therefore, treatment with corticosteroids might alter the effect of bronchodilation on FeNO in patients with asthma.

Finally, we found a statistically significant change on FeNO only after 30 min of rest. However, this change was also small and insignificant according to the ATS guidelines [24]. This slight increase in FeNO could be either result of the resting period or result of the bronchodilation. The fifth measurement, where FeNO is slightly increased, was performed 60 min after the bronchodilation. This is in agreement with the other studies, mentioned above, showing an increase in FeNO due to bronchodilation [19,21]. Possibly FeNO values do not alter statistically significantly 20 min after bronchodilation, but 60 min after the action of bronchodilation. However, this change is not considered important according to the ATS guidelines [24].

In the context of bronchial NO flux (JawNO), no statistically significant difference after spirometry or bronchodilation was found. In the group of patients with asthma, bronchial NO flux (JawNO) was reduced insignificantly after spirometry. Bronchodilation increased bronchial NO flux (JawNO), but there was also no statistically significant difference. In the group of healthy volunteers, spirometry reduced bronchial NO flux (JawNO) and bronchodilation increased it, but the difference was also not statistically significant. We should mention that

in our study the effect of bronchodilation was similar to healthy and well-controlled asthmatic subjects, which seems to be attributed to the fact that the asthmatics with good control of the disease have a relative stable airway caliber with less episode of bronchoconstriction that could affect the flow and accordingly both FeNO and JawNO.

With regard to $C_A\text{NO}$, when it was assessed right after spirometry, its values were insignificantly lower, while 20 min after spirometry and bronchodilation a significant decrease was recorded. This decrease of $C_A\text{NO}$ should probably be considered as a late effect of spirometry, which is not affected by bronchodilation. Our results seem to be similar with those of the study of Eckel et al., which demonstrated that $C_A\text{NO}$ values of asthmatic children were decreased due to spirometry when assessed (≤ 15 min) after spirometry, while it was not affected in healthy subjects [29]. Similar to our findings, Prieto et al. found that $C_A\text{NO}$ measured right after spirometry (≤ 10 min) was not decreased significantly [20]. It should be mentioned that in the study of Prieto et al., the asthmatic group was severe adult asthmatics, while in the study of Eckel et al. the asthmatics were steroid naive mild asthmatic children [20,29]. Therefore, a different bronchodilation effect on the NO production is anticipated and the comparison between these studies is difficult. Despite that, it seems that there is a late effect of spirometry in $C_A\text{NO}$ as we and Eckel et al. have shown, which is not significant if the assessment is performed right after spirometry [29]. It seems that the effect of spirometry on FeNO and JawNO is quite different from that on $C_A\text{NO}$. This could be attributed to the fact that there is no correlation between $C_A\text{NO}$ and FeNO values, since they represent a different anatomic site of nitric oxide production. Finally, a recently published study did not report a significant change after beta-2 agonist-inhalation on $C_A\text{NO}$ and JawNO values in patients with COPD [35]. Taken together, data regarding the effect of bronchodilation and spirometry on $C_A\text{NO}$ and JawNO are still limited. Future studies regarding this issue are sorely needed.

A limitation of our study is the small number of asthmatic subjects in whom $C_A\text{NO}$ and JawNO were assessed, despite the fact that the effect of spirometry, bronchodilation and rest was not different between healthy and well-controlled asthmatics. Nonetheless, this is the first study addressing the question of the simultaneous effect of the above factors in FeNO, $C_A\text{NO}$ and JawNO as measured in a real life medical visit.

In summary, data arising from our study show that spirometry, bronchodilation and rest have an insignificant effect on FeNO and JawNO of patients with well-controlled asthma and healthy volunteers. Even if a slight change occurs, that does not modify clinician's decision

and therapeutic strategy. On the contrary, spirometry seems to have a late lowering effect on $C_A\text{NO}$ and this should be considered in future studies.

Conflicts of interest

None.

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None.

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