

Original article

Exhaled nitric oxide and air trapping correlation in asthmatic children

Exhaled nitric oxide (eNO) levels have been shown to correlate with atopy and with airway hyperresponsiveness but not with standard spirometry. The aim of our study was to evaluate the correlation between eNo levels and functional residual capacity (FRC), residual volume (RV), RV to total lung capacity (TLC) ratio, and pulmonary resistances in asthmatic children ages 6–13 years. Forty-nine patients (35 males) were enrolled in the study. Nineteen of them were not receiving inhaled corticosteroids. The eNO levels were measured by chemiluminescence's analyzer and lung function study were performed by body box plethysmography. As expected, there was no correlation between eNO levels and forced vital capacity (FVC); forced expiratory volume in the first second (FEV₁); mid respiratory flow between 25 and 75% of the vital capacity (MEF_{25–75}), FEV₁/FVC, and pulmonary resistances. Instead a correlation was found between eNO level and RV both considering all the study population together ($r = 0.51$, $P = 0.001$) and separately the asthmatic children not receiving ICS ($r = 0.6$, $P = 0.003$). In the patients receiving ICS the correlation was still present ($r = 0.43$, $P = 0.01$). The correlation between eNo levels and RV may reflect the effect of airway inflammation on NO production and diffusion as well as peripheral airway trapping and consequent RV.

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Exhaled nitric oxide (eNO) is a noninvasive test that measures airway inflammation in asthma (1). The eNO levels have been shown to correlate with atopy and increased airway hyperresponsiveness (2) with peripheral blood eosinophils, total IgE and serum eosinophil cationic protein (3) but not with FEV₁ and other forced expiratory flows (3–7).

The lack of a correlation between eNO levels and standard lung function tests was explained by the concept that eNO is a distinct component of the asthmatic state and particularly of the eosinophilic inflammation (1–7). Increasing concentrations of eNO have been demonstrated to function as predictive of asthma exacerbations (8). Since this has been suggested to be the case also for increased residual volume (RV) in patients with normal or near normal flow-volume values (9) we aimed to evaluate the correlation between eNo and measures of air trapping such as functional residual capacity (FRC), RV, and RV to total lung capacity (TLC) ratio (RV/TLC) in asthmatic children. This to our knowledge was not previously studied.

Patients and methods

Forty-nine subjects (35 males, 14 females) ranging in age from 6 to 13 years (mean $\pm$ SD = 10.4 ± 2.4 years), with a history of asthma according to the GINA guidelines (10) were enrolled in the study.

Thirty patients (25 sensitive to perennial allergens and five with no atopy) with mild-moderate asthma were regularly receiving inhaled corticosteroids (23 received also regular treatment with β_2 long acting, salmeterol or formoterol). The other 19 patients (14 sensitive to seasonal allergens and five with no atopy) with intermittent asthma were receiving no long-term treatment because were studied during remission out of the pollen season. Five subjects sensitive to perennial and/or seasonal allergens but with no symptoms and no clinical history of asthma and/or rhinitis served as controls.

Nitric oxide measurement

The eNO was measured with a fast response chemiluminescence's analyzer (NOA Tm280, Sievers Instruments Inc., Boulder, CO) by the online single-breath technique, according to the ATS and ERS recommendations for children (11, 12). The analyzer has an NO sampling rate of 200 ml/min, a detection range of <0.1 –500.000 parts per billion (p.p.b.), and a response time of 0.02 s for 90% full scale. Briefly, the subjects were asked to perform a single slow exhalation starting from TLC through a mouthpiece and a one-way nonrebreathing valve, against a resistance of 16 cm H₂O and under a visual biofeedback, to maintain a 50 ml/s steady flow. Subjects were at rest, refraining from eating and exercise from at least 1 h, sitting down, and breathed filtered NO-free air (air filter AFL 01410, Sievers Instruments Inc., Boulder, CO) without a nose clip before the single exhalation maneuver. Ambient air was recorded before and after each test, and was found always to be lower than 20 p.p.b. The analyzer was calibrated daily with a zero signal (air filter scrubber, potassium permanganate and charcoal, ACT 01400, Sievers Instruments Inc., Boulder, CO) and certified NO/N₂ calibration gases (Messer, Collegno, Italy) containing 108 and

10.3 p.p.m. The eNO levels were measured at the plateau (> 2 s during an exhalation > 6 s) of the end-exhaled reading and expressed as p.p.b. At least three measurements that varied by less than 10% or two measurements that varied by less than 5% were recorded, and the average of the respective plateau was taken as a value, according to guidelines (11, 12).

Lung function evaluation

Measurements were carried out using a constant volume (830 l) whole body pletismography (Master Lab, E. Jaeger GmbH, Würzburg, Germany). The equipment was calibrated daily with a 3 l syringe with a displacement that is in the same order of magnitude as the vital capacity of the subject. The airway opening pressure transducer was physically calibrated daily. Breathing was continually monitored by the operator, in order to establish a stable baseline representing FRC. The airway was usually occluded by closure of a shutter valve when the subject was at FRC. The subject made a series of gentle panting maneuvers at a frequency of about 1 Hz. A series of 3–5 panting maneuvers were carried out, after which, the shutter was opened and the subject was instructed to expire maximally followed by a slow inspiratory vital capacity (VC) maneuver up to TLC (13). All children performed the panting maneuvers adequately with 'full co-operation' and with reproducible values (variation between different performances no greater than 5%).

Approval for the study was obtained from the Local Hospital Ethical committee and written parental consent was obtained.

Statistical analysis

Results are expressed as mean $\pm$ SD. A $P < 0.05$ was regarded as statistically significant.

Correlation between eNO, FRC, RV, RV/TLC, total and specific resistances, MEF_{25-75} , FEV_1 , and FEV_1/FVC were evaluated using Spearman's rank correlation. Correlation between eNO, FRC, RV, and RV/TLC justified for the presence of atopy were tested in both ICS-treated and ICS-naïve patients separately.

All statistical calculations were performed using SPSS Version 9.0 software for windows (SPSS Inc., Chicago, IL) package for personal computer.

Results

General characteristics of the study population, lung function tests and eNO values are shown in Table 1. As expected control subjects showed better lung function and both lower FRC, RV, RV/TLC ratio, and eNO compared with asthmatics. Considering all the 49 asthmatic subjects together, a significant correlation between eNO and RV was found ($r = 0.51$, $P = 0.001$) (Fig. 1), between FRC and eNO ($r = 0.35$, $P < 0.05$) and between RV/TLC and eNO ($r = 0.42$, $P = 0.003$) while no correlation was found between eNO and resistances, MEF_{25-75} , or FEV_1 and FEV_1/FVC . Analyzing the patients with intermittent asthma and steroids naïve the correlation between eNO and RV ($r = 0.6$, $P = 0.003$) was present as well as between FRC and eNO ($r = 0.34$, $P = 0.05$), RV/TLC and eNO ($r = 0.5$, $P = 0.008$). In the 30 patients receiving steroids the correlation between eNO and RV was still

Table 1. General characteristics and lung function of the study population

	Patients received ICS	Patients ICS naïve	Controls
<i>n</i>	30	19	5
Age (years)	10.5 $\pm$ 2.9	10.1 $\pm$ 2.3	11.2 $\pm$ 2.4
FVC (%)	102.7 $\pm$ 11.9	95.6 $\pm$ 9.4	99.2 $\pm$ 7.1
FEV_1 predicted (%)	107.1 $\pm$ 14.9	97.6 $\pm$ 14.2	110.2 $\pm$ 10.9
MEF_{25-75}	99.8 $\pm$ 28.2	87.4 $\pm$ 32.1	126.2 $\pm$ 42.1
FEV_1/FVC (%)	103.9 $\pm$ 7.4	102.1 $\pm$ 13.9	110.7 $\pm$ 5.0
RV (%)	112.3 $\pm$ 37.7	109.6 $\pm$ 38.7	82.2 $\pm$ 18.0
FRC (%)	106.8 $\pm$ 23.7	102.7 $\pm$ 18.9	93.0 $\pm$ 40.8
eNO (p.p.b.)	23.7 $\pm$ 15.8	25.8 $\pm$ 14.3	10.0 $\pm$ 3.5

Data are presented Mean $\pm$ SD.

FVC, forced vital capacity; FEV_1 , forced expiratory volume in the first second; MEF_{25-75} , mid respiratory flow between 25 and 75% of the vital capacity; eNO, exhaled nitric oxide; FRC, functional residual capacity; RV, residual volume.

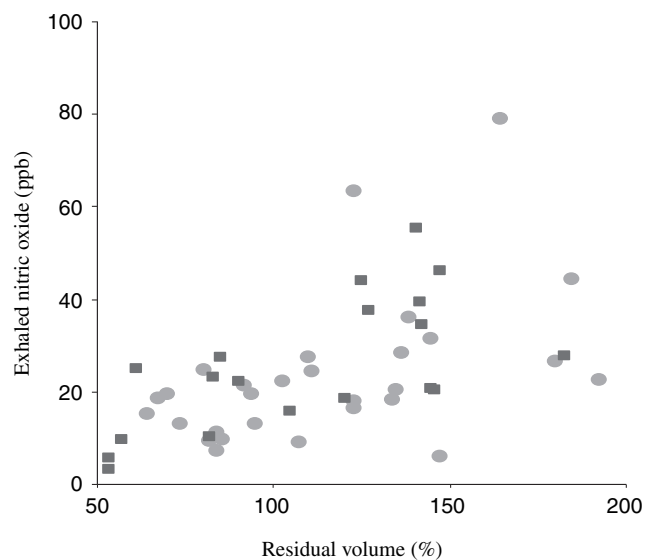


Figure 1. Correlation between RV and eNO (square indicated patients ICS naïve, circle indicated patients treated with ICS). Some patients have very similar values; therefore the number of dots appears reduced compared with the number of studied subjects.

significant ($r = 0.43$, $P = 0.01$) while it just failed to be significant for both FRC and RV/TLC ($P = 0.06$). No other correlation between eNO and the other lung functions variables were found in the two subgroups.

In the 23 out of 30 children with perennial asthma and receiving a combined treatment with ICS plus β_2 long acting the correlation between eNO and RV was maintained ($r = 0.44$, $P = 0.03$). In the remaining seven asthmatic subjects receiving only ICS the trend towards a correlation between the two variables was present, but not statistically significant ($r = 0.18$) due to the limited number of subjects. There was no effect of atopy on the correlation between eNO and different measures of air trapping in either ICS-treated in ICS-naïve asthmatic

children. When the correlation between VR and eNO was controlled by age, this variable did not influence the results. Specifically there were no relationships between age and eNO.

Discussion

In this group of asthmatic children we found a correlation between eNo and different measures of air trapping such as FRC, RV, and RV/TLC. This is in agreement with the expected relation between increasing airways inflammation and increasing air trapping in the lung. This makes sense physiologically. Unequal ventilation increases with lung volume (14) and this is associated with regional perfusion defects (15). This is expected to increase alveolar NO concentration due to a reduced binding of NO by hemoglobin and consequently to increase in eNO diffusion (16). The major site of NO diffusion in the normal subjects is the large airways, but because of ventilation perfusion mismatching also the periphery of the lung may be recruited in asthmatic patients (17). Furthermore the increase in lung volume tends to increase airway caliber expanding the luminal surface area over which diffusion is occurring (18).

Since increases in FRC, RV and RV/TLC are thought to be due to early airway closure from either airway wall or luminal pathology as from loss of radial traction (19) and since all these events are present in airway inflammation in asthma (20) it is not surprising to find a correlation between increasing measures of gas trapping and eNO.

This association has also a clinical meaning as an increased eNO may be present even in subjects whose asthma has remitted (21) and increased FRC and RV can be the only baseline pulmonary abnormality in asthmatics during long symptom-free intervals (22, 23). This was the case either for eNO and FRC, RV, and RV/TLC in our study population with intermittent seasonal asthma evaluated when asymptomatic out of the pollen season. In those patients we found the best correlation due to the lack of any interference done by anti-inflammatory treatment.

Furthermore, in asthmatic children, the presence of hyperinflation, even if with normal or near normal FEV₁ and with disease apparently under control, may be associated with more severe asthma exacerbations (9). At this regards increasing levels of eNO has been demonstrated to have a positive predictive values that ranged from 80 to 90% for loss of asthma control associated with increase in sputum eosinophils and in airways hyperresponsiveness (8).

Moreover distal lung dysfunction and inflammation at night was demonstrated in patients with nocturnal asthma (24) and pulmonary gas trapping was recently shown to increase in asthmatic children with supine position and this was considered a possible factor contributing to nocturnal worsening of asthma (25). The lacking effect of

atopy on the correlation between different measures of gas trapping and eNO is very likely due to ICS treatment in symptomatic patients and to the timing of evaluation (out of the pollen season) in the intermittent asthmatics. Furthermore, the absence of any correlation between FEV₁ and eNO can be explained by the recently demonstrated, limited value of this parameter of lung function in determining asthma severity especially in children receiving asthma controller therapy (26). The same can be, reasonably, speculated for pulmonary resistances.

One possible limitation of our study is the fact that we evaluated eNO at the single lung flow (50 ml/s steady flow) suggested by the combined ERS/ATS statement (12). This doesn't allow distinguishing between alveolar and bronchial contribution in eNO in agreement with two-compartment model (27). However, it was considered that eNO is released within conductive airways, whereas alveolar NO levels are negligible (28). In particular, by measuring NO in exhaled air at multiple exhalation flow rates, no difference was observed in the alveolar concentration of eNO between asthmatic and healthy children (28) confirming previous data of normal alveolar NO concentration in adult asthmatics (29, 30). Increased alveolar eNO was observed in patients with allergic alveolitis suggesting that eNO measurement at different flow rates can be important in airways diseases different from asthma (30). Even if, a very recent study documented an alveolar NO production in asthmatics (31), it was, however, suggested that this might be due to back diffusion of NO from the airways to the alveoli (32).

Our findings suggest that an increased recruitment of smaller airways and an expanded luminal surface area, associated with increasing FRC, RV, and RV/TLC are very likely responsible of the increased eNO. Once that this has been observed, future studies employing the evaluation of eNO at different flow will better characterize the different contribution of central and peripheral airways in eNO values. Although, no correlation are generally found between spirometry and eNO, our observation of a correlation between different measures of gas trapping and eNo seems to have a physiological as well as a clinical meaning and fits well with the recent observation that thoracic gas volume and TLC are superior to forced expiratory maneuvers in evaluating distal lung inflammation (33). Obviously the ease of measurement of eNO and its significant positive correlation with other markers of inflammation (3) as well as with FRC and RV further expand the utility and meaning of eNO measurement in asthmatic children.

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