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The Clinical Value of Exhaled Nitric Oxide in Asthma

Il Significato Clinico dell'Ossido Nitrico nell'Asma

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In memory of my father

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SUMMARY

Bronchial asthma is an inflammatory disease and measurement of biomarkers in exhaled breath has recently become an attractive approach to non-invasively monitor airway inflammation. In bronchial asthma, increased fractional exhaled nitric oxide (FeNO) concentration in exhaled breath has been shown to reflect the extent of eosinophilic inflammation. Moreover, the increase of FeNO levels are suppressed by inhaled corticosteroids (ICS).

Therefore, monitoring of FeNO is a useful marker of inflammation in asthma and it has proven to be very effective in the differential diagnosis of allergic asthma, predicting the response to ICS therapy.

Several FeNO analyzers are commercially available. Because of great advances in technology a wide range of hand-held FeNO analyzers and smaller less costly devices are now becoming available, making FeNO measurement a routine test in the primary care of asthmatic patients. In the first study we tested a new portable device to investigate correlation and agreement with the standard stationary device.

Nowadays, overweight and obesity are common conditions worldwide. In particular, the incidence of overweight and obesity in Italian people is estimated almost of 32% and 10%, respectively. Obesity has been considered as a proinflammatory state and therefore several studies have been investigated the relationship between elevated body mass index (BMI) and asthma. In the second study we aimed to investigate in a large cohort of Italian asthmatic patients whether or not overweight patients were different from normal weight subjects both in terms of clinical and functional features and in terms of airway inflammation, as assessed by FeNO measurement.

Although the clinical application of FeNO is still needed to be fully clarified, the use of this marker in clinical practice is providing a useful adjunct to conventional tools for the assessment and

management of the respiratory disease. There is a general agreement that the development of new techniques detecting the distinct patterns of biomarkers in exhaled breath heralds the beginning of the era of “Breathomics”. In this regard, future developments may include FeNO “breathograms” to aid early detection, diagnosis and follow-up of airways diseases.

SOMMARIO

L'asma bronchiale è una patologia infiammatoria delle vie aeree. Negli ultimi anni, la misurazione di biomarkers nell'aria esalata è diventata una metodica non invasiva di uso comune per la valutazione dell'infiammazione delle vie aeree.

Nell'asma bronchiale, è stato dimostrato che valori elevati della frazione dell'ossido nitrico esalato (FeNO) riflettono il grado di infiammazione eosinofila delle vie aeree. Inoltre, il trattamento con corticosteroidi inalatori (ICS) riduce i valori di FeNO.

Di conseguenza, la misurazione del FeNO è un utile biomarker dell'infiammazione nell'asma ed è risultato essere un parametro molto efficace nella diagnosi differenziale dell'asma allergica in quanto predice la risposta al trattamento con ICS.

In commercio sono disponibili diversi analizzatori del FeNO. Grazie al progresso della tecnologia sta diventando disponibile una grande varietà di analizzatori portatili, più piccoli e meno costosi, il che rende la misurazione del FeNO un test di routine nella pratica clinica dell'asma. Nel primo studio abbiamo testato un nuovo apparecchio portatile per valutarne la correlazione e la concordanza con l'apparecchio stanziale standard.

Al giorno d'oggi, sovrappeso e obesità sono condizioni molto diffuse in tutto il mondo. Nello specifico, nella popolazione italiana la percentuale di persone in sovrappeso e obese è stimata rispettivamente intorno al 32% e 11%. L'obesità è considerata come uno stato pro-infiammatorio, pertanto diversi studi hanno approfondito la relazione tra elevato indice di massa corporea (Body Mass Index, BMI) e asma. Nel secondo studio ci siamo posti l'obiettivo di verificare se, in un'ampia coorte di pazienti asmatici italiani, i pazienti in sovrappeso differivano dai soggetti normopeso in termini di aspetti clinico-funzionali e di infiammazione delle vie aeree stimata con la misurazione del FeNO.

Nonostante le applicazioni cliniche del FeNO non siano ancora completamente validate, l'utilizzo di questo marker nella pratica clinica fornisce un valore aggiuntivo agli strumenti convenzionali nella valutazione ed il monitoraggio delle malattie respiratorie. C'è un consenso generale riguardo al fatto che lo sviluppo di nuove tecniche in grado di determinare i differenti patterns dei biomarkers nell'esalato segna l'inizio dell'era della "Breathomics". A questo riguardo, futuri sviluppi potrebbero includere i "breathograms" del FeNO come metodica di supporto nella diagnosi precoce e nel follow-up delle malattie delle vie aeree.

ASTHMA and AIRWAY INFLAMMATION

Asthma is a leading medical problem worldwide, which involves almost 300 millions people of each age and all countries throughout the world.

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment (1).

Although asthma is increasingly recognised as a syndrome comprising a number of inflammatory phenotypes, the most common pathological feature is airway inflammation, sometimes associated with airway structural changes (remodelling) and hyperresponsiveness.

The airway inflammation involves upper and lower respiratory tracts. It is yielded by an increase of inflammatory cells, predominantly T lymphocytes, eosinophils, mast cells and also neutrophils, which release specific mediators of the asthmatic response.

In detail, the mucosal mast cell degranulation produces cysteinyl leukotrienes, prostaglandins D₂, a lot of pro-inflammatory cytokines (tumor necrosis factor alpha – TNF- α -, interleukins IL-3, IL-4) and histamine, acting on the hyperresponsive smooth muscle that induce airway obstruction and inflammation (2). Activated eosinophils release basic proteins, eotaxin, TNF- α , IL-3, IL-5, GM-CSF (granulocyte macrophage-colony stimulating factor) which amplify the inflammatory response and the airway remodelling (3). T CD4⁺ lymphocytes are present in an increased number, mainly Th₂, releasing cytokines such as IL-4, IL-5, IL-9, IL-13 that regulate the eosinophilic inflammation and IgE production by B lymphocytes (4). The macrophages induce inflammation by macrophage-derived chemokines (MDC) and stimulate the remodelling releasing growth factors, i.e. PDGF (platelet derived growth factor), b-FGF (basic fibroblasts growth factor) and TGF- β (transformation growth factor-beta) (5).

The pathogenesis of asthma also involve airway structural cells, such as epithelial cells, smooth muscle cells, endothelial cells, fibroblasts and myofibroblasts, that in turn produce inflammatory mediators and connective tissue components increasing airway narrowing, oedema, mucus hypersecretion and structural changes.

This pattern of inflammation is the responsible for the cascade of events linking the initial stimulus to the final abnormality in airway function. Furthermore, the local and systemic effects prompted by the mediators involved in this response yield a further activation and recruitment of other inflammatory cells into the airway. This vicious mechanism leads to parallel inflammatory pathways through the release of specific enzymes by activated cells. One of these is nitric oxide synthase Type 2 (NOS2, inducible or iNOS), that produces nitric oxide (NO), a marker of airway inflammation in asthma (6).

The clinical manifestations of asthma – symptoms, sleep disturbances, limitation of daily activity, impairment of lung function, use of rescue medications – can be controlled with appropriated treatment. When asthma is controlled, there should be no more than occasional recurrence of symptoms and severe exacerbations should be rare. (1)

Using techniques to monitoring airway inflammation has become a key area for research and may be helpful in the management, early detection of asthma flares, and to monitor long-term therapy.

Among the clinical features concurring to the diagnosis of asthma, symptoms and pulmonary function testing are easily performed. On the other hand, assessment of airway inflammation is more difficult and the gold-standard (bronchial bronchoscopy with lavage and biopsy) is too invasive and may be hazardous for the patient. Thus, a considerable number of potential non-invasive biomarkers identified in exhaled breath, sputum, blood and urinary samples are nowadays preferred. Induced or spontaneously produced sputum analysis allows quantification of inflammatory eosinophilic or neutrophilic cells and analysis of exhaled gas or breath condensate

allows the detection of several volatile organic compound (VOCs) and non-volatile markers of inflammation and oxidative stress. Furthermore, measurement of fractional exhaled nitric oxide (FeNO) has been suggested as a non-invasive marker of airway inflammation in asthma (7).

EXHALED NITRIC OXIDE

Nitric oxide (NO) is a gaseous molecule that is present in virtually all human organ systems and freely diffuses across membranes. NO is synthesized by various cells types through the enzyme NO synthase (NOS), which is encoded by three different genes in the human genome and differentially expressed in the airways. NOS oxidizes L-arginine to L-citrulline with the generation of NO in a process both oxygen and NADPH dependents. There are three different isoenzymes of NOS: constitutive NOS (cNOS) isoforms, like neuronal NOS (nNOS or NOS1) - in neurons -, endothelial NOS (eNOS or NOS3) - in endothelial cells -, and inducible form (iNOS or NOS2) - in macrophages, T lymphocytes, neutrophils, eosinophils and epithelial cells -. Recently has been identified a fourth isoform, NOS4, a constitutive form potentially involved in end-stage renal disease and diabetic nephropathy (6). These isoforms differ in their capacity to produce NO and in the final effect of the NO produced. Both NOS1 and NOS3 are calcium and calmodulin-dependent. Agonists such as acetylcholine, bradykinin and histamine result in an increase of calcium ions inducing cNOS to release picomolar concentrations of NO, which acts as a local regulatory factor, such as neurotransmission, vasodilator tone and cardiac contractility. The constitutive forms are corticosteroid-resistant, therefore basal levels of NO are not affected by these drugs (8). In contrast, NOS2 is calcium/calmodulin-independent. During inflammatory or infective disorders it is transcriptionally upregulated by inflammatory cytokines, such as TNF α , IF γ , IL1 β , endotoxin, lipopolysaccharides, viruses, bacteria and allergens, regardless of Ca $^{2+}$ influx. Once induced, within several hours NOS2 produces largest concentrations (nanomolar) of NO in the airway epithelium having a pro-inflammatory effect. It remains stable in the gaseous phase constituting the

main source of level of NO in the exhaled breath. NOS2 is glucocorticoid-sensitive. Therefore, its expression is up-regulated in subjects with allergic asthma whereas is decreased in steroid-treated asthmatic patients (6). NO rapidly reacts with oxygen, superoxide anions, nucleotides, water, thiols, metalloproteins, amines and lipids to form nitrites, nitrates, peroxynitrite, S-nitrosothiols, S-nitrosamines, amplifying its physiological functions. In the lung, in addition to play a role in the inflammatory response, the chemical products of NO are involved in bronchodilatation, neurotransmission in bronchial smooth muscle, plasma exudation, oedema and vascular smooth muscle relaxation, through the release of cyclic guanosine monophosphate (cGMP). Moreover, NO participates in host defences due to its bactericidal and cytotoxic properties (9,10) (Fig.1).

CLINICAL APPLICATION OF EXHALED NITRIC OXIDE IN ASTHMA

Nitric oxide is a gas originally regarded damaging to human health as a toxic molecule of air pollution. After the first detection in coronary arteries of NO-free radical as endothelium-derived relaxing factor (EDRF) in 1987 (11,12), the advent of chemiluminescence analyzers in the early 1990s allowed the detection of NO in exhaled breath (13). Afterwards, a large body of scientific works has confirmed that NO is a signalling molecule implicated both in biological processes and in pathological conditions, so that became the “Molecule of the Year” in 1992 (14). Patients with asthma were found to have higher levels of the fractional orally exhaled NO as compared with healthy subjects (7,15,16) that decreased in response to treatment with corticosteroids (17,18). This quickly prompted the evaluation of exhaled NO as a potential non-invasive method to diagnose asthma and monitor the response to anti-inflammatory therapy. Indeed, almost two key points converge to provide the rationale for the use and interpretation of FeNO measurements. Firstly, in asthma increased FeNO (>45ppb) reflects eosinophilic-mediated inflammatory pathways underlying both at the central and/or peripheral site of the airway. Conversely, a low FeNO (< 25ppb) may be regarded as a valued predictive marker for the absence of eosinophilic bronchial

inflammation (19). Thus, FeNO is a good surrogate marker to distinguish eosinophilic (i.e. allergic asthma) from non-eosinophilic pathologies, providing a complementary tool in aid to the conventional pulmonary testing in the assessment of patients with undiagnosed respiratory symptoms. Secondly, due to the close relationship between airway eosinophilia and steroid responsiveness, FeNO may be used in clinical practice to predict the response to asthma controller therapy and identify the efficacy of inhaled corticosteroids (ICS) as well as to monitor compliance with ICS therapy or withdrawal in asthmatic patients (20). As such, it has been proved its potential use as a marker to predict asthma exacerbations (21,22).

It should be emphasized that FeNO is related with other outcomes in allergic asthma, closely correlating with the percentage of eosinophils in samples of induced sputum, blood, BAL and bronchial reactivity (23,24), but without a significant correlation with lung function parameters. In a consensus statement on the clinical applications of this technique for the diagnosis and management of asthma, FeNO was recommended to be included among the standard parameters used into clinical care (25). A recent comprehensive review (19) analyzes several design issues of five randomized controlled algorithm asthma trials that reported only equivocal benefits of adding FeNO measurements to traditional diagnostic methods, such as spirometry. In conclusion, Barnes et al. (19) defined FeNO as *“an important adjunct for diagnosis and management in selected cases of asthma...providing a non-invasive window into predominantly large-airway presumed eosinophilic inflammation”*. Lastly, in a review evaluating the technical issues and confounding factors related to FeNO measurements, Kostikas et al. (26) concluded that FeNO represents the only exhaled biomarker that has reached clinical practice even in primary care setting.

In comparison with other techniques, the measurement of exhaled NO provides immediate results, is easier to implement, reproducible and feasible also in young subjects and patients with severe airflow obstruction, for whom other tests are difficult or impossible to perform. Due to its non-invasive nature, it can be easily performed in outpatient visits during the follow-up of asthmatic

patients. It also represents a cheaper technique as compared to standard clinical tools. A major disadvantage of use of NO exhaled as diagnostic test for asthma is represented by the confounding factors that might influence the value of FeNO measurement, like infection of high airways, lung cancer or current medications.

Measurements of nitric oxide

In 1997, a taskforce of the European Respiratory Society (ERS) (27) and in 1999 the American Thoracic Society (ATS) (28) published statements, which defined the guidelines for adequate FeNO measurement, which have been updated in a joint document in 2005 (29,30). In clinical practice, there are two ways to measure the level of exhaled NO by either on-line or off-line methods, even though this last is considered obsolete and no longer recommendable. The on-line method uses techniques capable to evaluate the level of FeNO in exhaled air from a single breath exhalation, calculating FeNO value at the end-expiratory plateau. Exhalation should be performed at stable expiratory flow rate of 50ml/s. An immediate result is obtained and expressed in parts per billion (ppb), which is equivalent to nanoliters per liter.

OBJECTIVES

First Study

The most widely used approach to FeNO measurement is chemiluminescence. In 2003 a FeNO stationary chemiluminescence analyzer (Niox; Aerocrine AB, Solna, Sweden) was approved by U.S. Food and Drug Administration for the use in asthma management (31). Nevertheless, generally chemiluminescence devices tend to be rather expensive, large and poorly portable. Therefore, smaller cheaper portable equipments based on an electrochemical cell technique are now available, allowing the assay possible in daily practice by general physicians and, in the near future, at home by patients.

A new portable device (NObreath, Bedfont, Rochester, UK) has recently been developed but no published study has evaluated its reliability.

The aim of our study was to compare FeNO values obtained by the new portable device to those of the standard stationary FeNO analyzer (Niox) in a large cohort of asthmatics, to investigate correlation and agreement with the standard device.

Second Study

Available literature on airway disease highlights a linked association between obesity and asthma, and several studies aimed to investigate the relationship between elevated BMI and asthma control, degree of severity and airway inflammation.

In obese asthmatic patients, the characteristics of airway inflammation are complex. The adipose tissue may have a pro-inflammatory effects, by secreting cytokines, chemokines and adipokines, which likely generate a different pathways from the classic Th2 cytokine driven (32). However, discordant findings were reported on the relation between BMI and FeNO, since obesity was associated with lower, or higher or normal FeNO values (33).

Furthermore, in the obese patients some comorbidities, including gastroesophageal reflux disease (GERD) and sleep disorders breathing, which may mimic asthma symptoms, may occur, by

inducing diagnostic issues. Notably, up to now, no study has been specifically addressed to investigate the relation between asthma and overweight, a condition in which the effects on lung mechanics as well as comorbidity are likely to be less remarkable than in morbidly obese patients.

The aim of our study was to ascertain whether or not in a large cohort of asthmatic patients overweight patients were different from normal weight patients both in terms of clinical and functional features and in terms of airway inflammation, as assessed by FeNO measurement.

Figure Legend:

Figure 1. Schematic diagram of synthesis and functions of nitric oxide.

Key: NOS = nitric oxide synthase; NADPH = the reduced form of NADP; NADP = Nicotinamide Adenine Dinucleotide Phosphate; NO = nitric oxide; cGMP = cyclic guanyl monophosphate; NANC = noradrenergicnoncholinergic; DNA = deoxyribonucleic acid; Th2 = lymphocytes T-helper 2; IL = interleukin

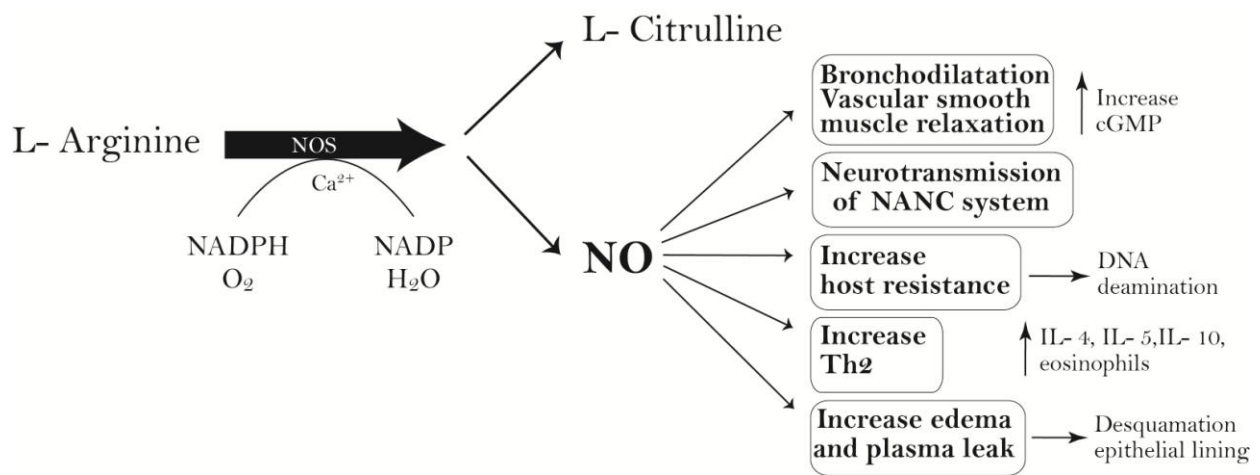


Figure 1.

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“MEASUREMENT OF FRACTIONAL EXHALED NITRIC OXIDE BY A NEW PORTABLE DEVICE. COMPARISON WITH THE STANDARD TECHNIQUE”

ABSTRACT

Background: Fractional exhaled nitric oxide (FeNO) measurement is a reliable non-invasive marker of airway inflammation. The use of portable FeNO analyzers could enable the introduction of airway inflammation assessment in primary care.

Objective: We compared FeNO values obtained by a new portable device (NObreath, Bedfont, UK) to those of the standard stationary analyzer (NIOX, Aerocrine, Sweden) in a large cohort of asthmatics.

Methods: 154 (age range: 14-83 years, FEV₁ range: 48 to 134% pred, asthma control test [ACT] range: 7-25) out of 168 recruited patients, completed the study. Each patient performed at least two valid FeNO measurements at a constant flow rate of 50ml/s on each of the two analyzers.

Results: A significant relationship between the FeNO values obtained by the two devices ($r = 0.95$, $p < 0.001$) was found. Altman-Bland plot confirmed this agreement. Within-patient repeatability was excellent in both devices. Intraclass Correlation Coefficients for NIOX and NObreath values were 0.925 and 0.967, respectively. By means of receiver operating characteristic curve analysis the FeNO cutoff points which better identified patients with $ACT \geq 20$ were 15 ppb (0.84 sensitivity and 0.42 specificity) by NIOX and 25 ppb (0.53 sensitivity and 0.69 specificity) by NObreath. Easiness to use of both devices, assessed by visual analogue scale was not different.

Conclusion: FeNO measurements obtained by the new portable FeNO analyzer are reliable because they are directly comparable with those obtained by the stationary standard device. The use of portable instruments may facilitate the FeNO measurement in primary care.

INTRODUCTION

The measurement of fractional exhaled nitric oxide (FeNO) is a reliable non-invasive marker of airway inflammation (1). FeNO has been shown to be increased in some airway diseases, such as asthma (2), allergic rhinitis (3), chronic rhinosinusitis (4) and chronic cough (5). Notably, FeNO measurement has been proved to play a role in asthma diagnosis (6) and management (7) and has become a clinical routine in asthmatic patients.

The chemiluminescence analyzer is currently considered as the standard technique for measuring FeNO and a FeNO stationary chemiluminescence analyzer (NIOX, Aerocrine AB, Solna, Sweden) has been approved by the U.S.A. Food and Drug Administration for use in asthma management (8). However, limitations of the stationary device, such as cost, size and frequent calibration requirement, may preclude its wide-scale introduction into clinical practice. Furthermore, a portable FeNO analyzer could be extremely useful in primary care as an additional tool to improve care of asthmatic patients.

A new portable device using electrochemical sensors (NObreath, Bedfont, Rochester, UK) has recently been developed but no published study has evaluated its reliability. The aim of the study was, therefore, to compare in a large group of asthmatic patients FeNO values obtained by the new portable device to those of the standard stationary chemiluminescence FeNO analyzer and to calculate a conversion equation. Additionally, we evaluated the within subject reproducibility of the FeNO values obtained by both devices as well as their capability to discriminate asthmatic patients with controlled disease from those with uncontrolled one. Lastly, we assessed the easiness to use of the two devices.

METHODS

Subjects

Asthmatic patients (14 years of age and older) with specialist-diagnosed asthma were eligible to take part in the study and were prospectively recruited over a 6-month period from our Asthma Outpatient Clinic. In each patient, duration of disease, atopy and smoking habit were recorded. No patient has undergone any FeNO measurement before the study.

We ensured that the patient had followed the pre-test instructions, i.e. had consumed nitrate-rich foods and beverages, including alcoholic ones, at least 1 h before the test, had not smoked tobacco products and had not practised exercise or tiring exertion for 1 h prior to testing and, finally, had not an acute respiratory infection in progress, as these factors can affect the test results. Moreover, all patients underwent FeNO measurement before lung function test. Only patients able to perform at least two acceptable FeNO measurements both on NIOX and on NObreath (up to six attempts per device) were included in the analysis.

The study was approved by local ethics committee and all patients gave their informed consent.

FeNO measurement

FeNO was measured according to the ATS/ERS guidelines (9) using a hand-held device NObreath (FeNO_{NObreath}) and a stationary NIOX (FeNO_{NIOX}). The order of the measurements was random. All tests were performed at the same time of day to allow a possible circadian rhythm effect. For both types of measurements, patients were seated in the upright position without a nose clip. In a subgroup of patients, the within subject reproducibility of FeNO_{NObreath} and FeNO_{NIOX} was assessed by repeating measurements on two separate days by 1 week. To participate to repeatability study, patients had to be in clinically stable conditions and had not to modify their therapeutic regimen and to show a change in FEV₁ greater than 10% on the two study occasions. The repeated measurements were performed during the same time of the day (± 2 h).

In detail, $\text{FeNO}_{\text{NObreath}}$ was obtained inviting the subjects to inhale as deeply as possible and after 3s, guided by an auditory cue, to exhale through the mouthpiece, keeping the ball in the flow indicator in the middle of the black band in the centre of the tube, at a constant flow rate of 50 ml/s. The required exhalation time is approximately 16s. To ensure a breath sample was taken at the correct flow rate, the monitor has been held upright at all times during the test. $\text{FeNO}_{\text{NIOX}}$ was performed asking the subjects to inhale nitric oxide-free air through a filter connected to the device deeply to total lung capacity and then to exhale for 10s at a constant pressure guided by a visual cue to stabilize flow rate. All tests were performed at an exhalation pressure of 10-20 cm H₂O, to maintain a fixed flow-rate of 50 ml/s. Measurements were repeated after a brief rest period until two acceptable values (± 2.5 p.p.b. for measurements < 50 p.p.b. and $\pm 5\%$ for measurements ≥ 50 p.p.b.) were performed (maximum six attempts). The mean of two adequate values for each subject was recorded for analysis. For NIOX the system calibration was performed every 14 days, whereas NObreath was set to zero every month.

After $\text{FeNO}_{\text{NObreath}}$ and $\text{FeNO}_{\text{NIOX}}$ patients rated the easiness to use of both devices on an interval scale, which was a 100-mm horizontal visual analogue scale (VAS). The VAS consisted of a horizontal ruler without any mark on the patient's side with the words "easy to use" and "not easy to use" on the left and right end, respectively. Easiness to use ratings were expressed in mm from 0 to 100 and corresponded to the distance of the marker from the left end of the visual analogue scale.

Lung function testing

Lung function was measured by a flow-sensing spirometer connected to a computer for data analysis (CPFS/D Spirometer, MedGraphics, St Paul, MN, USA) which met American Thoracic Society (ATS) standards. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), and FEV_1/FVC ratio were recorded. FVC and FEV_1 are expressed as percent of predicted value (10), FEV_1/FVC as percent.

Asthma Control Assessment

Asthma control was assessed using the Italian version of the Asthma Control Test (11). Patients subjectively evaluated the degree of impairment caused by their disease during the preceding 4 weeks by responding to five questions using a five-point-scale. The ACT is reliable, valid, and responsive to changes in asthma control over time (11). The sum of the scores of the five questions gave the total ACT score (range 5–25). A cutoff score of 20 or more identifies patients with well controlled asthma.

Statistical Analysis

The distribution of variables was assessed by means of Kolmogorov-Smirnov Goodness-of-Fit test. To normalize the distribution, FeNO data were log-transformed for analysis and reported as geometric mean \pm GSEM. Other numerical variables were expressed as mean \pm SD, unless otherwise specified. Paired t-test and unpaired t-test were used for comparisons, when appropriate. The relationship between measures was estimated by Pearson's correlation coefficient (r) and linear regression analysis. The agreement between measures was assessed by the method of differences against the means according to Bland and Altman (12) and the Spearman correlation coefficient (r_s) was used to identify any potential tendency for the separation of agreement at higher or lower values. The repeatability of measures was expressed as intraclass correlation coefficient (r_I) (13). The receiver operating characteristic (ROC) curve method (14) was used to plot the true positive rate (sensitivity) in function of the false positive rate (100-specificity) for discriminating patients with asthma under control from those with poorly controlled asthma for each device. A p value \leq 0.05 was considered as significant.

RESULTS

One hundred sixty-eight asthmatic patients were enrolled. Fourteen patients were excluded after failing to perform acceptable FeNO measurements (six for NIOX, five for NObreath, one for both NIOX and NObreath, two unapproved values). A total of one hundred fifty-four consecutive patients (age range: 14-83 years, 54 males, 18 current smokers, 112 atopics) completed the study (Table 1). One hundred and seven patients (69%) were receiving therapy. In all patients, spirometry ranged from a severe ventilatory defect to normal value (FEV₁ range: 48% to 134%) (Table 1).

FeNO_{NIOX} and FeNO_{NObreath} values were significantly different (24.6 ppb ± 1.073 vs 22.6 ppb ± 1.075, p= 0.0002) (Table 2) and were significantly related ($r = 0.95$, $p < 0.001$) with the following regression equation $\text{Log FeNO}_{\text{NIOX}} = 0.287 \text{ (SE=0.078)} + 0.935 \text{ (SE = 0.024)} \cdot \text{Log FeNO}_{\text{NObreath}}$ ($r^2 = 0.91$, $p < 0.001$) (Figure 1). The Bland and Altman plot showed a high degree of agreement between the devices (Figure 1) and Spearman correlation coefficient confirmed the lack of bias at either end of the range of values ($r_s = -0.088$, $p = 0.275$).

In the subgroup of 20 patients (age range: 19-70 years; 6 males), who performed the FeNO_{NObreath} and FeNO_{NIOX} reproducibility, spirometry did not significantly change in the two occasions (FVC = 111% pred ± 21 vs 106% pred ± 16; FEV₁ = 100 % pred ± 19 vs 96 % pred ± 17; FEV₁/FVC = 76 % pred ± 10 vs 76 % pred ± 10). In this subgroup of patients, FeNO_{NIOX} and FeNO_{NObreath} values obtained in the two occasions were respectively 21.8 ppb ± 1.18 and 21.0 ppb ± 1.20 ($r_I = 0.925$) and 19.1 ppb ± 1.20 and 19.9 ppb ± 1.19 ($r_I = 0.967$) (Figure 2).

Ninety-six patients out of 154 had well controlled asthma (ACT ≥ 20) and lower FeNO_{NIOX} and FeNO_{NObreath} values, as compared to patients with poorly controlled asthma (20.9 ppb ± 1.08 vs 31.8 ppb ± 1.13, $p = 0.004$ and 19.9 ppb ± 1.08 vs 27.6 ppb ± 1.13, $p = 0.041$). According to the

ROC curve method, the plot of the true-positive rate in function of the false-positive rate for different cutoff points of FeNO_{NIOX} and FeNO_{NObreath} values with respect to a ACT \geq 20, as threshold value, showed respectively 0.644 (95% confidence interval [CI], 0.562 to 0.719; p = 0.002) and 0.607 (95% CI, 0.525 to 0.684; p = 0.0251) area under curve (AUC) values. Pairwise comparisons of the ROC curves revealed a difference in the AUC of 0.0369 (95% CI, 0.004 to 0.0697; p = 0.028) between the FeNO_{NIOX} and FeNO_{NObreath} values. The FeNO_{NIOX} and FeNO_{NObreath} cutoff points, which maximized sensitivity and specificity were 15 ppb (0.84 sensitivity and 0.42 specificity) and 25 ppb (0.53 sensitivity and 0.69 specificity), respectively (Figure 3). Well controlled and poorly controlled patients did not differ in FEV₁ values (95% \pm 15 vs 93% \pm 20, p = 0.477).

FeNO_{NIOX} required a significant greater number of attempts to obtain two acceptable values, as compared to FeNO_{NObreath} (p = 0.01). However, easiness to use of both devices, assessed by means of VAS, was not different (Table 2).

DISCUSSION

The results of the present study show that FeNO measurements obtained by NObreath, a new portable FeNO analyzer, are reliable because they are directly comparable with those obtained by NIOX, the stationary device currently considered as the standard for measuring FeNO. Furthermore, because of its manageability and easiness to use the portable device could be suitable for home-monitoring of asthma as well as for epidemiological studies.

It is important to appreciate that FeNO measurements in both healthy subjects and asthmatic patients are right-skewed (15,16) and require appropriate transformation prior to statistical analysis. This may imply some difficulties for interpreting measurements obtained in clinical setting. In this

regard, we found a significant difference between FeNO values obtained by the two devices and the regression line in figure 1 does not dissect the origin of the axis, confirming a consistently higher value from the NIOX device compared with the NObreath device. We also provided the regression equation to convert FeNO values measured by NObreath into those obtained by NIOX. However, it is of note that the difference between the values measured by the two devices amounts to approximately 2 ppb, which would not be clinically significant.

In the present study, Bland-Altman plot demonstrated agreement between both devices and, importantly, the difference between values obtained by the NObreath and NIOX did not change with increasing FeNO values. This finding is of clinical relevance since measurements with NObreath can be reliably performed in any asthmatic patient regardless of the degree of airway inflammation. Moreover, the reliability of NObreath device is further supported by its excellent degree of within-subject repeatability over time (Intraclass Correlation Coefficient > 0.9). Furthermore, the repeatability of NObreath was similar to that of the stationary standard analyzer. Our data are in line with previous reports comparing agreement (15,16) and repeatability (16) between the stationary standard analyzer and the MINO, a portable device of FeNO measurement.

In this study, we also assessed the capability to discriminate poorly controlled asthmatic patients from well controlled ones, by using the two FeNO measurement devices. As expected, we found that well controlled patients had significantly lower FeNO values, as compared to poorly controlled patients. Interestingly, this difference was found with both devices, even if the magnitude of the difference was greater with the standard device. Additionally, in our cohort of patients a FeNO value greater than 15 ppb obtained by NIOX or greater than 25 ppb obtained by NObreath had a high likelihood to be associated to poorly controlled asthma. According to the ROC curve analysis, NIOX sensitivity was higher than that of NObreath, which conversely showed higher specificity. The ROC AUC value generated using the NIOX device was higher than that of

NObreath. Taken together, our findings suggest that the portable device has a slightly lower discriminating power than the standard device, at least in terms of controlled versus uncontrolled asthma. Interestingly, our results are similar to those of a previous paper (15), which reported a slightly lower discriminating power of MINO, as compared to NIOX, to separate asthmatic patients from healthy subjects.

We found that the number of attempts to achieve the required acceptable measurements was slightly, but significantly lower for NObreath than that for NIOX. This result is consistent with a previous report (17), which compared NIOX and MINO and reported a significantly less number of attempts needed to achieve the acceptable measurements by using MINO. This finding could at least partly be explained by the fact that some measurements in the NIOX may be discarded after a linear regression analysis of the NO plateau has been performed, even though the number of regression failures was not recorded in the present study. The linearized plateau must not deviate more than 10% from the horizontal axis according to current guidelines (9). Notably, the difference in the number of attempts between the devices was not perceived by the patients in terms of different easiness to use of NIOX in comparison with NObreath. Easiness to use ratings assessed by the visual analogue scale were not different between NIOX and NObreath.

In conclusion, this study shows that there is clinically acceptable agreement between the new portable NObreath and the stationary standard device NIOX. The repeatability of measurements obtained by NObreath was similar to those obtained by NIOX. In addition, FeNO measurements with both devices was a reliable method of differentiating well controlled from poorly controlled asthmatic patients. Lastly, more than 90% of the subjects of our large cohort of asthmatics with a wide spectrum of age were able to successfully use both devices. The use of portable instruments will enable the introduction of FeNO measurement in the primary health care.

Table 1. Characteristics of 154 patients with asthma

Age (years)	43 ± 16
Sex (M/F)	0.54
BMI (kg/m ²)	25 ± 5
Atopy (Yes/No)	2.67
Disease duration (years)	11 ± 11
Smoking habit (Yes/No)	0.13
ACT (0-25)	21 (17-23)
FVC (% of pred)	105 ± 17
FEV ₁ (% of pred)	94 ± 17
FEV ₁ /FVC (% of pred)	75 ± 10

Values are expressed as mean ± SD, ratio or median (25%-75% percentile)

ACT = Asthma Control Test; BMI = Body Mass Index; FEV₁= Forced Expiratory Volume at 1st second; FEV₁/FVC = Forced Expiratory Volume at 1st second/Forced Vital Capacity ratio; FVC = Forced Vital Capacity

Table 2. FeNO values obtained by NIOX and NObreath devices, number of attempts to obtain at least two acceptable FeNO values by the devices and easiness to use of the devices.

	NIOX	NObreath	<i>p</i>
FeNO (ppb)	24.6 ± 1.073	22.6 ± 1.075	0.002
Attempts (No.)	3 (2-4)	2 (2-3)	0.01
Easiness (VAS, mm)	10 (0-22)	10 (0-22)	0.93

Values are expressed as geometric mean ± GSEM or median (25%-75% percentile)

VAS = Visual Analogue Scale.

Figures Legends

Figure 1. Linear regression with 95% confidence interval (*upper panel*) and Altman-Bland plot (*lower panel*) of NObreath vs NIOX values in 154 asthmatic patients

Figure 2. Within-subject repeatability of NIOX values (*upper panel*) and NObreath (*lower panel*) in 20 asthmatic patients. The intraclass correlation coefficients were 0.925 and 0.967, respectively. The continuous line is the line of identity.

Figure 3. Receiver operating characteristic (ROC) curve analysis for different cutoff points of FeNO measurements obtained by NIOX (blue line) and by NObreath (green line) with respect to an Asthma Control Test score ≥ 20 , as threshold value. Area under curve (AUC) values of NIOX and NObreath measurements were 0.644 ($p = 0.002$) and 0.607 ($p = 0.0251$), respectively. Pairwise comparisons of the ROC curves revealed a difference in the AUC of 0.0369 ($p = 0.028$).

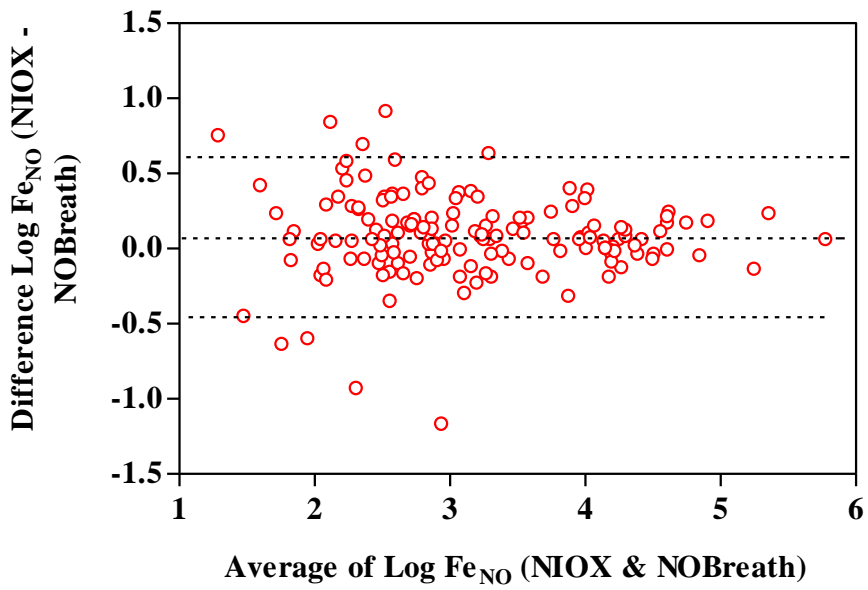
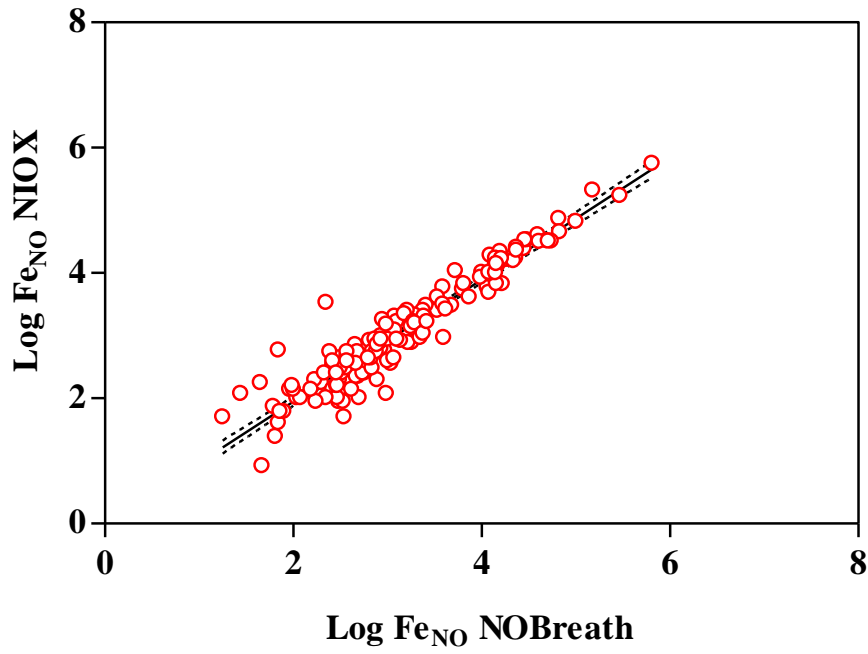


Figure 1

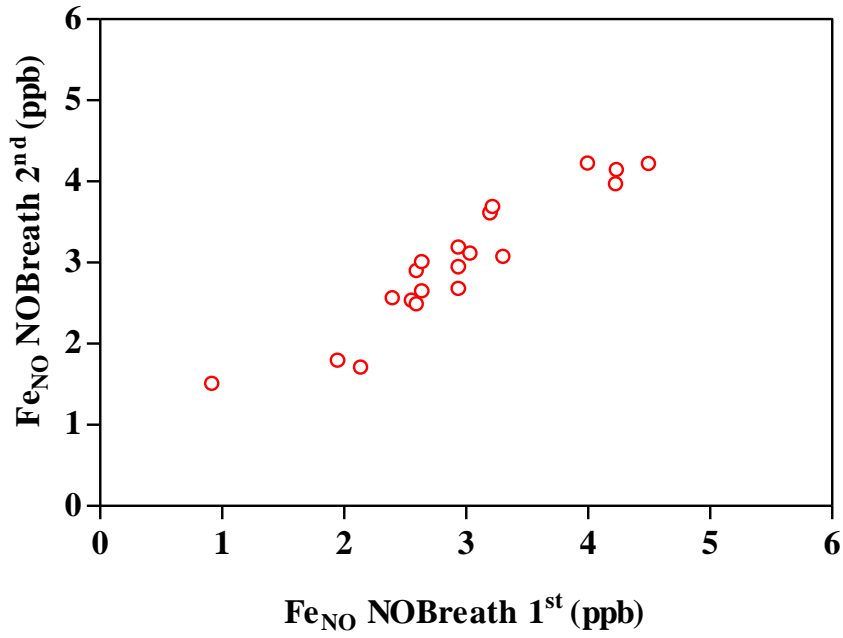
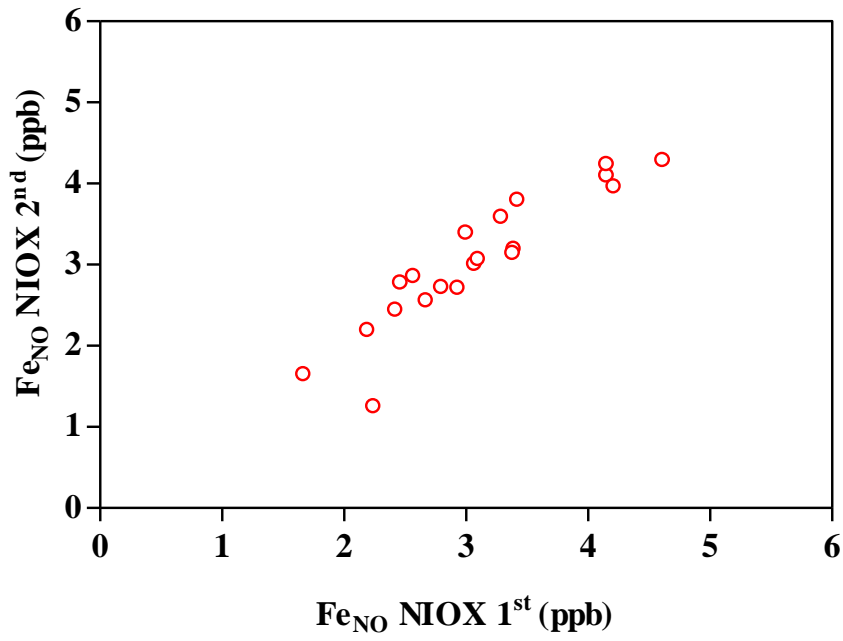


Figure 2

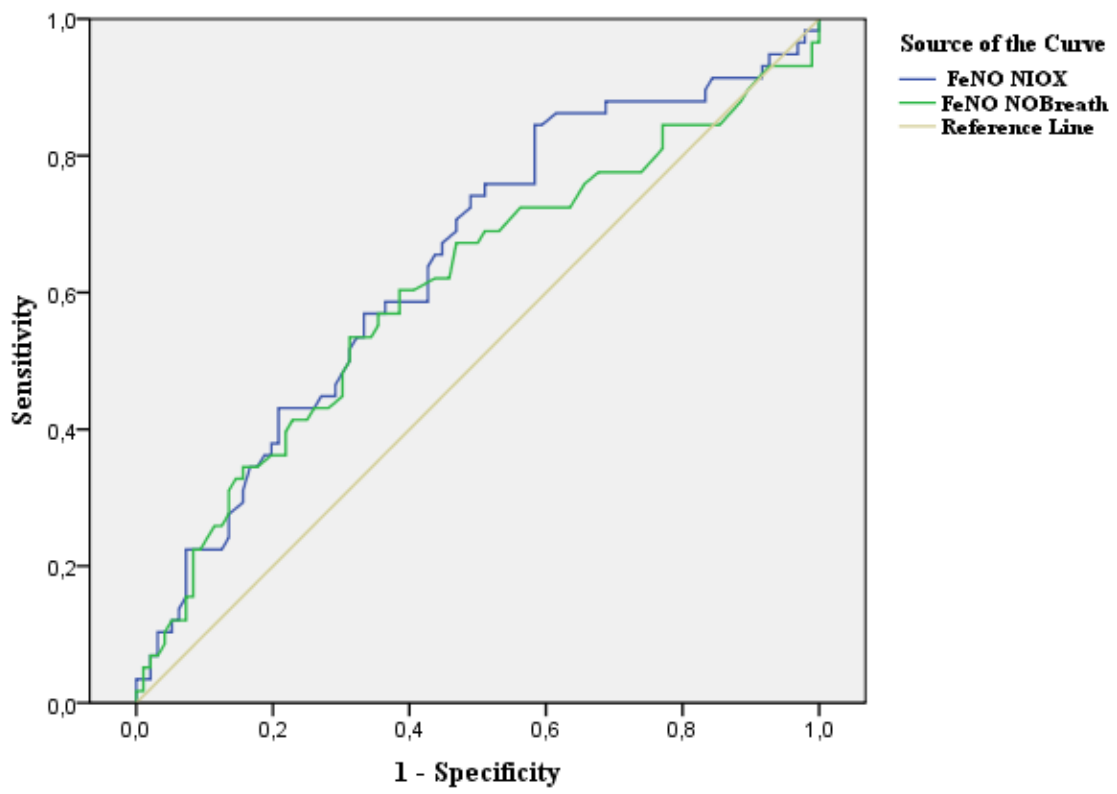


Figure 3

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“OVERWEIGHT IS ASSOCIATED WITH AIRFLOW OBSTRUCTION AND POOR DISEASE CONTROL BUT NOT TO EXHALED NITRIC OXIDE CHANGE IN AN ASTHMATIC POPULATION”

ABSTRACT

Background: The association between elevated body mass index (BMI) and asthma remains controversial.

Objective: To investigate the relationship between overweight (BMI > 25 ≤ 30 kg/m²), lung function, disease control and airway inflammation in an asthmatic population.

Methods: We consecutively studied 348 patients (43±16 yr; 211 F). In all patients, BMI, spirometry, Asthma Control Test (ACT) and Fractional Exhaled Nitric Oxide (FeNO, ppb) were measured.

Results: 145 patients were overweight and, as compared to those with normal BMI, had lower values of FVC, FEV₁ and FEV₁/FVC and of FEF₂₅₋₇₅, even when normalized for FVC ($p < 0.05$ for each comparison). The ratio between the number of patients with well controlled asthma (ACT ≥ 20) and that of patients with poor controlled asthma (ACT <20) was significantly lower in overweight patients (1.07 vs 1.84; $\chi^2 = 6.030$, $p < 0.01$). In overweight patients, the odds ratio of uncontrolled asthma expressed by logistic regression analysis was 1.632 (95% CI = 1.043-2.553), independently from gender, atopy, smoke and inhaled steroid therapy. No difference was observed in FeNO values between overweight and normal weight patients (39 ± 34 vs 40 ± 34 ppb).

Conclusions: Our results shows that in an asthmatic population, the overweight is associated with airflow obstruction and poor disease control, but not to any FeNO change. The finding of the

present study support the view that other factors than airway inflammation alone may explain the relationship between asthma and elevated BMI

INTRODUCTION

In Western countries, asthma and obesity are chronic and prevalent conditions (1). Epidemiologic investigations have shown an association between obesity or elevated body mass index (BMI) and prevalent and incident asthma (1). However, the role of obesity in asthma control and severity remains unclear. As compared with normal weight patients, severe asthma was found to be more prevalent in obese patients (2), and obesity has been recognised as a risk factor for poor disease control (3). On the other hand, elevated BMI was associated with worse asthma control and quality of life, but not to asthma severity (4). Furthermore, among adults presenting to the emergency department with acute asthma, asthma exacerbations among obese and nonobese adults were similar (5). Lastly, using four validated asthma control questionnaires, Clerisme-Beaty et al (6) failed to find an association between obesity and asthma control in an urban population with asthma.

It is also of note that the value of the airway inflammation in asthmatic patients with elevated BMI remains to be fully clarified (7). The measurement of fractional exhaled nitric oxide (FeNO), a reliable non-invasive marker of airway inflammation (8-11) which has become a clinical routine (12,13) in asthmatic patients, has provided conflicting results in patients with asthma, when categorized by BMI. As compared with normal weight patients, obese asthmatic patients showed lower (14,15), as well as higher (16,17) or similar (18,19) FeNO values.

Discrepancies in reports on asthma-obesity relationship may be partly due to the fact that obesity may be associated with lung volume changes and comorbidity, such as gastroesophageal

reflux disease, which in obese patients may mimic asthma and may determine inaccuracy both in diagnosis and in severity grading of asthma. Notably, up to now, no study has been specifically addressed to investigate the relation between asthma and overweight, a condition in which the effects on lung mechanics as well as comorbidity are likely to be less remarkable than in morbidly obese patients.

The aim of the present study was to ascertain whether or not in a large cohort of asthmatic patients, recruited in an Italian tertiary care asthma clinic, overweight patients were different from normal weight patients both in terms of clinical and functional features and in terms of airway inflammation, as assessed by FeNO measurement.

METHODS

Subjects

Patients (14 years of age and older) with asthma diagnosis according to the international guidelines (20) were eligible to take part in the study and were prospectively recruited over 18-month period, from April 2010 to September 2011, from our Asthma Outpatient Clinic. In each patient, BMI, duration of disease, smoking habit and asthma therapy were recorded.

BMI was defined as the weight in kilograms divided by the square of height in meters. In each subject it was calculated from patients' self-reported height and weight. The international standard definition of obesity was used (21). Patients were classified as underweight (BMI <18.5 kg/m²) normal (18.5 ≤ BMI ≤ 25 kg/m²), overweight (25 > BMI ≤ 30 kg/m²) and obese (BMI >30 kg/m²).

Atopy was assessed by skin prick tests with a battery of 10 common inhalant allergens. All patients underwent FeNO measurement, completed the Asthma Control Test (ACT) and underwent spirometry, as part of their visit.

The study was approved by local ethics committee and all patients gave their informed consent.

FeNO measurement

We ensured that the patients were not affected by any acute respiratory infection and had followed the pre-test instructions, i.e. no nitrate-rich foods and beverages, including alcoholic ones, no tobacco smoking and no exercise within 1 h preceding the test, as these factors can affect the test results. Moreover, all patients underwent FeNO measurement before lung function test. Only patients able to perform at least two acceptable FeNO measurements were included in the analysis. FeNO was measured according to the ATS/ERS guidelines (22) using a FeNO stationary chemiluminescence analyzer (NIOX, Aerocrine AB, Solna, Sweden).

All FeNO measurements were performed at the same time of day (± 2 h) to allow a possible circadian rhythm effect. In detail, patients were seated in the upright position without a nose clip and were asked to inhale nitric oxide-free air through a filter connected to the device deeply to total lung capacity and then to exhale for 10s at a constant pressure guided by a visual cue to stabilize flow rate. All tests were performed at an exhalation pressure of 10-20 cm H₂O, to maintain a fixed flow-rate of 50 ml/s. Measurements were repeated after a brief rest period until two acceptable values (± 2.5 ppb for measurements < 50 ppb and ± 5 % for measurements ≥ 50 ppb) were performed (maximum six attempts). The mean of two adequate values for each subject was recorded for analysis. The system calibration was performed every 14 days.

Asthma Control Assessment

Asthma control was assessed using the Italian version of the Asthma Control Test (23). Patients subjectively evaluated the degree of impairment caused by their disease during the preceding four weeks by responding to five questions using a five-point-scale. The ACT is reliable, valid, and responsive to changes in asthma control over time (23, 24). The sum of the scores of the five questions gave the total ACT score (range 0–25). A cutoff score of 19 or less identifies patients with poor controlled asthma.

Lung function testing

Lung function was measured by a flow-sensing spirometer connected to a computer for data analysis (CPFS/D Spirometer, MedGraphics, St Paul, MN, USA) which met American Thoracic Society (ATS) standards. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV₁), FEV₁/FVC ratio, forced expiratory flow after 25% of the FVC has been exhaled (FEF₂₅), forced expiratory flow rate over the middle 50% of the FVC (FEF₂₅₋₇₅) were recorded. FVC, FEV₁ and FEF₂₅₋₇₅ are expressed as absolute value and as percent of predicted value (25), FEV₁/FVC as percent.

Statistical Analysis

The distribution of variables was assessed by means of Kolmogorov-Smirnov Goodness-of-Fit test. Variables are expressed as mean \pm SD, unless otherwise specified. Because of their non Gaussian distribution, FeNO values were log-transformed before analysis. Unpaired t-test, Mann Whitney test and Pearson chi square test were used for comparisons, when appropriate. To examine relationships between measures Pearson's correlation coefficient (r) and Spearman rank order correlation coefficient (r_s) were used, when appropriate. Logistic regression analysis was performed to test the association between the presence of overweight (dependent variable) and gender, smoking habit, atopy, poor controlled asthma and inhaled steroid treatment, as independent

variables. Odds ratios are presented with 95% confidence intervals. A p value ≤ 0.05 was considered as significant.

RESULTS

Of the 422 patients who agreed to participate in the study, 14 had a BMI ≤ 18.5 kg/m² (3.3%), 203 had BMI > 18.5 and ≤ 25 kg/m² (44.6%) and 205 had BMI > 25 kg/m² (48.6%). Out of 205 patients with elevated BMI, one hundred forty-five patients were overweight (34.3%) and 60 were obese (14.2%). Underweight and obese patients were excluded from the study, leaving 348 patients suitable from the final analysis (Table 1).

The overweight patients were significantly older than patients with normal BMI ($p < 0.001$). The majority of the patients were atopic (75%) with no difference between the two subgroups of patients (Table 1). Patients with normal BMI did not also differ from patients with increased BMI in gender distribution, disease duration and smoking habit (Table 1). In the two subgroups of patients, the ratio between the number of patients treated with inhaled steroids and that of untreated patients tended to be significantly higher in overweight patients, as compared to normal weight patients (104/41 vs 130/73; chi square= 2.268, $p=0.082$) (Table 1). In all patients, spirometry ranged from a severe obstructive ventilatory defect to normal value (FEV₁/FVC range: 45 to 98 % and FEV₁ range: 43% to 137%) (Table 1).

The spirometry values were significantly lower in overweight patients, as compared to the corresponding ones in normal weight patients (Table 1). Notably, FEF₂₅₋₇₅ values, even when normalized for FVC, were significantly lower in overweight patients (Table 1). Additionally, in all patients an inverse significant correlation was found between BMI and FEV₁ ($r = -0.154$; $p = 0.004$), FEV₁/FVC ($r = -0.233$; $p < 0.001$), and FEF₂₅₋₇₅ ($r = -0.152$; $p < 0.005$).

The ACT score was significantly different between overweight and normal weight patients (Table 1). In the two subgroups of patients, the ratio between the number of patients with well controlled asthma ($ACT > 20$) and that of patients with poor controlled asthma ($ACT \leq 19$) was significantly lower in overweight patients, as compared to patients with normal BMI (1.07 vs 1.84; chi square= 6.030, $p=0.01$) (Figure 1). In the whole study population, the ratio between the number of well controlled and poor controlled patients was 1.46. Moreover, in all patients ACT score values were inversely related to BMI values ($r_s = -0.170$; $p < 0.001$). Logistic regression analysis showed that overweight was significantly associated to poor controlled asthma, but not to gender, smoking habit, atopy and inhaled steroids (Table 2).

No difference was observed in FeNO values between overweight and normal weight patients (Table 1).

DISCUSSION

The main finding of the present study is that overweight patients with asthma had worse spirometry and disease control, as compared with normal weight patients, regardless of the gender, smoking habit and atopy. The percent of patients who were receiving inhaled steroids tended to be higher among overweight patients than normal weight patients. Importantly, no significant difference in airway inflammation, assessed by FeNO measurement, was found between the two groups of patients. Lastly, as it has been reported in the Italian general population (26), we found that increased BMI becomes more common with increasing age, additionally our results also indicate a high rate of obese among adult asthmatics seen in an Italian tertiary care asthma clinic.

Physiologically, an elevated BMI may affect lung volumes, with no direct effect on airway caliber (27). Spirometric variables, such as FVC and FEV_1 , tend to decrease with increasing BMI

with no change or increase in FEV₁/FVC (26). Expiratory flows decrease with increasing weight in proportion to the lung volume and, in obese patients, specific airway resistance, calculated by adjusting for the lung volume at which the measurement were made, is in the normal range (27). Whether or not asthma and elevated BMI may interact on lung function has been so far little investigated. In a large group of subjects with normal values for airway function, but a wide range of BMI, including subjects with working diagnosis of asthma, Jones et al. (28) showed a significant linear relationship between BMI and lung volumes with no change in FEV₁/FVC. The study did not provide any separate analysis for asthmatic and non asthmatic subjects. In a large cohort of young adults aged 28-30 yrs, King et al (29) found that after adjusting data for smoking and asthma, elevated BMI was associated with reduced lung volume, which was in turn linked with airway narrowing, as assessed by the airway conductance. Interestingly, this study showed that the reduction in airway calibre was only partly related to the reduction in lung volume, suggesting other nonvolume-related mechanisms.

In this study, we found that flow-volume curve parameters, such as FVC, FEV₁ and FEF₂₅₋₇₅, were significantly lower in overweight asthmatic patients than those of normal weight patients. Interestingly, when compared to normal weight patients, the reductions in FVC and in FEV₁ were not affected to the same extent in overweight patients, since in these patients the FEV₁/FVC ratio was significantly lower. In addition, even when normalized for FVC, the FEF₂₅₋₇₅ values were significantly lower in overweight patients. Taken together these findings indicate that overweight patients, as compared to normal weight patients, had higher degree of both proximal and peripheral airflow obstruction, which was greater than expected on the basis of the reduction in lung volume.

The increased airflow obstruction, which we found in overweight patients, cannot refer to an increase in airway inflammation, as assessed by FeNO measurement, since these patients did not differ from normal weight patients in this parameter. Asthmatic patients with elevated BMI, as

compared to normal weight patients do not appear to have increased airway cellular inflammation [30]. Notably, it seems also likely that the elevated BMI does not contribute to asthma through conventional Th type 2-mediated inflammatory pathways (30). In obese patients, it has been also hypothesised that airway structures could be remodelled by exposure to proinflammatory adipokines or damaged by the continual opening and closing of small airways throughout the breathing cycle (30) and one cannot exclude that even in overweight asthmatic patients these mechanisms may play a role.

In the present study, overweight patients had worse disease control and tended to take more inhaled steroids, as compared to normal weight patients. Patients with elevated BMI may experience wheezing and breathlessness due to their excess weight (31) and, consequently, may falsely attribute weight-related respiratory symptoms to asthma, causing increased medication use (32). However, this explanation could be only partly taken into account for our results, since overweight patients had a greater degree of airflow obstruction, to which the asthmatic symptoms could be attributed. In this context, it is conceivable that in overweight patients inhaled steroid therapy even increased, as compared to normal weight patients, was not enough to the disease control. It is of note that an increase in BMI was associated with an increase in the delay/avoidance of health care (33). In other words, in overweight patients behavioral factors could condition self-management both in terms of asthma control and in terms of diet and physical activity.

Previous studies provided discordant results on the relationship between asthma and elevated BMI, showing a poor (3,4) or similar (6) asthma control in obese patients, in comparison to normal weight patients. Notably, studies showing poor control (3,4) recruited patients in outpatient clinics, whereas the study showing no difference (6) recruited their patients in community-based outpatients primary care practices. Moreover, there are some difference between all these study and ours. These studies (3,4,6) were mainly addressed to obese patients and did not provide any

information on the airway inflammation of the patients. Notably, only in one study (4) the patients were assessed by spirometry, which showed a reduction of FVC values in obese patients, when compared to normal weight patients.

As compared to the Italian general population (26), in our cohort of patients we found a higher rate of obese patients. Our data are consistent with the results of a previous Canadian report (4) and suggest that in industrialized countries, obesity may be more common among adult tertiary care asthmatics than in the general population. This finding could be due to the fact that asthmatic patients followed at a tertiary care hospital may have likely more co-morbidity than patient followed by primary care physicians.

We acknowledge that this study has some limitations. Firstly, we defined normal weight and overweight only in terms of BMI and we cannot exclude that other measures of excess body weight, such as waist-hip ratio or waist circumference, might better clarify the impact of increasing body fat on asthma. However, what is the best risk factor among adiposity measures is still debatable (34). Additionally, we calculated the BMI using self-reported height and weight. It has been reported that, despite the high correlation between measured and self-reported data, the prevalence of overweight calculated from measured values was higher than that calculated from self-reported values among older adults (35). When calculated with self-reported height, BMI was one unit lower than when calculated from measured height for persons older than 70 years (35). As the most of our patients was young adults (median age 41 yrs, 30-54 yrs 25%-75% percentile), we are confident in our estimates of BMI.

In conclusion, although we cannot infer any cause-effect relationship between adiposity and clinical and functional features of asthma from our data, the present study shows that preobese asthmatic patients are at increased risk of airflow obstruction and of poor disease control. This study

also supports the view that other factors than airway inflammation alone may explain the relationship between elevated BMI and asthma and further underlines the relevance which behavioural factors may have in the management of asthma.

Table 1. Patient's Characteristics by Body Mass Index Category

	Overall (n= 408)	BMI > 18. 5 ≤ 25 kg/m ² (n=203)	BMI > 25 ≤ 30 kg/m ² (n=145)	<i>p value</i>
Age (years)	43 ± 16	37 ± 15	48 ± 16	<0.001
Gender (M/F)	0.65	0.65	0.65	0.537
Atopy (Yes/No)	3.00	3.41	2.54	0.143
Disease duration (years)	11 ± 12	11 ± 12	11 ± 11	0.347
Smoking habit (Yes/No)	0.18	0.19	0.17	0.432
Steroids (Yes/No)	2.05	1.78	2.53	0.082
FVC (% pred)	105 ± 16	107 ± 15	103 ± 18	0.045
FVC (L)	3.93 ± 1.14	4.16 ± 1.09	3.61 ± 1.12	<0.001
FEV ₁ (% pred)	94 ± 17	97 ± 15	91 ± 19	0.001
FEV ₁ (L)	2.98 ± 0.94	3.21 ± 0.89	2.66 ± 0.90	<0.001
FEV ₁ /FVC (%)	75 ± 9	77 ± 9	73 ± 9	<0.001
FEF ₂₅₋₇₅ (% pred)	76 ± 29	80 ± 27	71 ± 30	0.005
FEF ₂₅₋₇₅ (% L/sec)	2.57 ± 1.27	2.87 ± 1.25	2.15 ± 1.17	<0.001
FEF ₂₅₋₇₅ /FVC	0.64 ± 0.26	0.68 ± 0.26	0.59 ± 0.25	0.001
ACT (0-25)	20 (17-23)	21 (18-24)	20 (16-23)	0.012
FeNO (ppb)	39 ± 34	40 ± 34	39 ± 34	0.927

Values are expressed as mean ± SD, ratio or median (25%-75% percentile)

Table 2. Odds Ratios (95% Confidence Intervals) by regression logistic analysis of female gender, smoking habit, atopy, inhaled steroid therapy and poor controlled disease for overweight patients with asthma

	OR (95% CI)	<i>p value</i>
Female Gender	0.903 (0.577-1.412)	0.653
Smoking Habit	0.934 (0.508-1.718)	0.827
Atopy	0.735 (0.446-1.211)	0.227
Inhaled Steroids	1.292 (0.803-2.081)	0.291
Poor Controlled Asthma	1.632 (1.043-2.553)	0.032

Figure Legend

Figure 1. Percent of asthmatic patients, categorized by BMI, with female gender, smoking habit, atopy, inhaled steroid therapy and poor controlled asthma.

Key: NW = Normal Weight; OW = Overweight. * $p = 0.012$ by chi-square

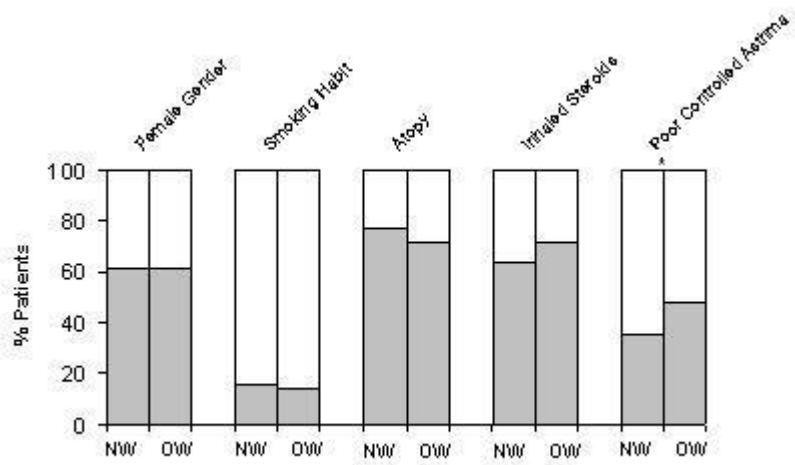


Figure 1.

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