Respiratory Medicine (2010) **104**, 801–807

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Comparison of eNO and histamine hyperresponsiveness in diagnosing asthma in new referrals

P. Munnik [a,c], I. van der Lee [b,c,*], J. Fijn [b], L.J. van Eijsden [b], J.-W.J. Lammers [a], P. Zanen [a]

[a] *University Medical Centre, Department of Pulmonary Diseases, 2130 AT Hoofddorp, Utrecht, The Netherlands*
[b] *Spaarne Hospital, Department of Pulmonary, Hoofddorp, The Netherlands*

Received 22 December 2008; accepted 4 December 2009
Available online 29 December 2009

KEYWORDS
Asthma;
Exhaled nitric oxide;
Histamine provocation test;
FEV1;
Receiver operator curve

Summary
The mainstay of the diagnosis of asthma is the presence of reversible airway obstruction. Exhaled NO levels are increased in asthma, in close relationship with the amount of airway inflammation, and may be used for monitoring the disease and adjusting therapy. In this study we investigated the role of eNO as a diagnostic for asthma, compared with the FEV1-reversibility and the PC20 (20% decrease of the FEV1 in the bronchial histamine provocation test), in two independent centers, on an unselected population. ENO measurements were performed with chemoluminesence technique in one center and with an electrochemical device in the other. Only after correction for so-called nuisance factors (allergy, use of inhaled steroids, recent infection, smoking, sex and the use of nitrate food) the eNO appeared as a diagnostic with equal power as the FEV1-reversibility and the PC20.

Therefore, screening for asthma in our study population, with the eNO measurement, is a simple, fast and safe strategy.

Introduction

Asthma is a chronic inflammatory disorder, characterised by recurrent respiratory symptoms against a background of increased bronchial responsiveness to external stimuli, giving rise to variable airflow limitation which is reversible either spontaneously or with treatment.[1] Asthma is very common in Europe, its prevalence in young adults is estimated about 20%.[2] Diagnosis of asthma is based on symptoms and evidence of reversible airway obstruction, shown in pulmonary function tests. This may not always be straightforward and sometimes confirmation is needed by bronchial provocation tests (histamine or metacholine provocation tests). These tests are time-consuming and constitute a certain risk for patients as they can lead to severe bronchoconstriction.

* Corresponding author. Spaarne Hospital, Department of Pulmonology, PO Box 770, 2130 AT Hoofddorp, The Netherlands. Tel.: +31 238907220; fax: +31 238907221.
E-mail address: vdlee@tiscali.nl (I. van der Lee).
[c] Authors contributed equally.

0954-6111/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2009.12.002

Exhaled NO (eNO) is increased in patients with asthma and can be used to monitor the therapeutic settings in asthma.[3,4] ENO also harbors the potential to serve as a diagnostic separating asthma from non-asthma.[5] The measurement of eNO is easy and safe to perform in both adults and children; most school-aged children are able to perform the test.[6] Airway eosinofilia, eNO and bronchial hyperresponsiveness are well correlated, as all are influenced by the same inflammatory process.[6,7] ENO levels are a strong predictor for steroid responsiveness in subjects with undiagnosed respiratory symptoms.[8] In a general population, eNO levels are strongly correlated with atopy.[9] Replacement of provocation tests by an eNO measurement would be advantageous, because the eNO test is quicker to perform and induces no bronchoconstriction.

The diagnostic role of eNO, however, is not well defined. Many researchers only correlated parameters, but did not test the equivalence of eNO to other diagnostic tests.[10,11] Especially comparisons of the histamine provocation test and eNO are scarce and suffer from spectrum or selection bias.[12,13] Therefore, data generated by these studies cannot simply be extrapolated to a population of possible asthmatics. In most studies with asthmatic subjects and eNO measurements, steroid naïve subjects were included. In daily practice general practitioners often start inhaled corticosteroid (ICS) therapy and subsequently refer to the pulmonologist. Therefore, the diagnostic quality of eNO in an unselected sample remains to be assessed.

This study examines the diagnostic role of eNO in comparison with the histamine provocation test and FEV1-reversibility, in two unselected samples of new referrals to a pulmonary outclinic from two different hospitals.

Methods

Centres

Two centres were involved in this study: the Spaarne Hospital, Hoofddorp, the Netherlands, which is a hospital serving the general community and the University Medical Center, Utrecht, the Netherlands (UMCU), which is a referral-type hospital, but also serves the general community.

Subjects

Random samples from all newly referred outpatients were drawn before a diagnosis was made. Immediately after a first visit to the outpatient clinic, subjects were invited to undergo full lung function testing, including measurement of the eNO levels and histamine provocation thresholds. Current medication was not a selection criterion, nor age or sex. Only those subjects referred for a suspected diagnosis in which measurement of eNO clearly is of no value were excluded (e.g. lung cancer). Subjects referred for a second opinion or in whom a diagnosis was already established were also excluded. Subjects gave written informed consent and the local medical ethical committees approved the study.

Pulmonary function measurements

Total lung capacity (TLC) and residual volume (RV) were determined by whole body plethysmography and spirometry/flow-volume curves via pneumotachography (Jaeger, Wurzburg and ZAN, Oberthulba, both in Germany) according to ERS-guidelines.[14] The V_A (alveolar volume), via single breath methane dilution, was measured as part of the determination of the TL_{CO} (Transfer factor of the lung for carbon monoxide).

At arrival, subjects first rested for $\pm$15 min after which the baseline lung function was determined. Three consecutive spirometry/flow-volume curves measurements were done and the flow-volume loop with the highest value of the FVC and FEV_1 was selected. Measurement of the bronchodilator response was done on a protocol basis.[14] On a second visit the eNO, allergy test and bronchoprovocation test was done consecutively. Patients refrained from using short-acting and long-acting bronchodilators 8 or 12 h prior to testing, respectively. All subjects received 400 μg salbutamol via MDI plus a spacer device, 15 min later spirometry was repeated, to measure/determine reversibility.

Measurement of exhaled NO

ENO measurements were performed in accordance with the ERS/ATS guidelines.[15] In the UMCU eNO levels were measured with a ECO MEDICS CLD 88 in conjunction with DENOX 88 (Eco Physics, Dürnten, Switserland). For measurements of eNO the subjects exhaled from total lung capacity to residual volume. The exhalation was controlled with a biofeedback monitor, and the subjects were asked to control the flow at 50 ml/s. Total exhalation time was 12 s. NO as well as CO_2 were measured. The point at which the CO_2 level reached 90% of its maximum was taken to determine the average NO over the next 5 s. Average of three measurements within 5% of each other were taken.

In the Spaarne Hospital eNO was measured with the Niox Mino device (Aerocrine, New Providence, United States of America), in which the NO is measured with an electrochemical cell, using an exhalation flow of 50 ml/s, with a total exhalation time of 10 s.

Measurement of histamine hyperresponsiveness

In the UMCU histamine diphosphate was administered using a DeVilbiss no. 646 handheld nebuliser (DeVilbiss Health Care Inc. Somerset, PA) in doubling doses from 0.25 to 16 mg/ml. Histamine and phosphate-buffered saline acted as positive and negative controls, respectively. The test was stopped at the moment the FEV_1 fell by more than 20%. Salbutamol aerosol was administered to aid recovery. The concentration of histamine that provoked a 20% fall in FEV1 (PC_{20}) was estimated by interpolation. Airway hyperresponsiveness was defined as a PC_{20} of $<$8 mg/ml.

In the Spaarne hospital the same protocol was used, with a Spira® Dosimeter (Spira Respiratory Care Center, Hämeenlinna, Finland), measurements were made on V_{max} Spectra 20 (Jaeger, Bilthoven, The Netherlands).

Skin prick testing

Sensitization to common allergens was measured by skin prick test reactions on the volar side of the forearm. In the UMCU fourteen allergens were tested (ALK-Abello AS Nieuwegein Netherlands). Mixed grasses 10.000 BU/ml, Mugwort (*Artemisia vulgaris)* 10.000 BU/ml, Mixed Tree pollen (birch, alder and hazel) 10.000 BU/ml, Dog (*Canis*