Respiratory Medicine (2007) **101**, 2416–2421

ELSEVIER

respiratoryMEDICINE

Diagnostic utility of inflammatory biomarkers in asthma: Exhaled nitric oxide and induced sputum eosinophil count

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Received 31 January 2007; accepted 29 May 2007
Available online 21 August 2007

KEYWORDS
Asthma;
Inflammation;
Diagnosis;
Nitric oxide;
Induced sputum

Summary
Background: Even though an inflammatory process is known to be the underlying cause of asthma, diagnosis is based on clinical history, reversible airway obstruction and bronchial hyperresponsiveness according to international guidelines. The fraction of exhaled nitric oxide (FE_{NO}) and induced sputum eosinophil count (Eos%) have been used as non-invasive inflammatory biomarkers.
Objectives: The aim of this study was to compare the sensitivity and specificity of FE_{NO}, Eos% and spirometry and to assess whether their combined use in clinical practice would improve diagnostic yield.
Methods: In 50 patients with asthma symptoms we performed spirometry, a methacholine challenge test, FE_{NO} measurement and assessment of Eos% in induced sputum. The standard diagnosis of asthma followed the guidelines of the Global Initiative for Asthma.
Results: Twenty-two of the 50 patients were diagnosed with asthma. The sensitivity and diagnostic accuracy were higher for FE_{NO} measurement (77%; area under the receiver operating curve [AUC], 0.8) than for spirometry (22%; AUC, 0.63). The sensitivity and specificity of Eos% in induced sputum were 40% and 82%, respectively, and the diagnostic accuracy of Eos% was lower (AUC, 0.58). When both inflammatory biomarkers were used together specificity increased to 76%.
Conclusions: The diagnostic accuracy of FE_{NO} measurement was superior to that of the standard diagnostic spirometry in patients with symptoms suggestive of asthma. The use of

Abbreviations: FE_{NO}, fraction of exhaled nitric oxide; Eos%, eosinophil count expressed as a percentage; PPV, positive predictive value; NPV, negative predictive value; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; ppb, parts per billion; PD_{20}, the dose of methacholine producing a 20% fall in FEV_1; ROC, receiver operating characteristic curve; AUC, area under the receiver operating characteristic curve

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0954-6111/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.rmed.2007.05.019

FE_{NO} measurement and induced sputum Eos% together to diagnose asthma in clinical practice is more accurate than spirometry or FE_{NO} assessment alone and easier to perform.

Introduction

Asthma is a chronic airway disease whose diagnosis is based on clinical history, reversible airway obstruction and bronchial hyperresponsiveness.[1] However, the real cause of these functional disorders is a chronic inflammatory process in which mastocytes and eosinophils play a major role.[2] Because conventional approaches to diagnosing asthma do not involve an assessment of airway inflammation, the fraction of exhaled nitric oxide (FE_{NO}) and the induced sputum eosinophil count expressed as a percentage (Eos%) have been proposed as inflammatory biomarkers that are useful in this setting.[3,4] Several studies have demonstrated that each of these biomarkers is more accurate than standard approaches to the diagnosis of asthma,[5,6] but the accuracy of both used together has not been assessed. We therefore hypothesised that diagnostic yield in terms of specificity and sensitivity might improve if both tests were used together.

Our aim was to analyse the sensitivity, specificity and positive and negative predictive values of FE_{NO} measurement and Eos% in comparison with conventional diagnostic tests (spirometry, bronchodilator response and methacholine challenge) in the diagnosis of asthma. We also sought to evaluate whether using both inflammatory biomarkers together would provide greater diagnostic accuracy in patients with a clinical history suggestive of asthma.

Material and methods

Patients

Fifty-seven consecutive patients were recruited for prospective study. All were referred to our hospital-based respiratory medicine outpatient clinic for diagnosis with a clinical history suggestive of asthma (dry cough, wheezing, and shortness of breath) from October 2004 to November 2005. We excluded patients with conditions that could affect FE_{NO} or Eos% measurement for reasons other than asthma: subjects with symptoms of respiratory tract infection in the previous 6 weeks or with systemic manifestations of atopy (rash, digestive symptoms, etc.) and patients who had received treatment with inhaled or oral corticosteroids in the last 4 weeks. All patients enrolled agreed to participate voluntarily and gave written informed consent. The institutional review board of our hospital approved the study.

Study design

The tests in this prospective study were conducted on 2 consecutive days. The first day the patient filled in a clinical symptoms questionnaire[7] and underwent FE_{NO} measurement, spirometry with bronchodilator response and collection of induced sputum. The next day a methacholine challenge test was performed. All the procedures were carried out at the same hour of each day and in an order that guaranteed that the results of one test did not interfere with the next. Trained members of our lung function laboratory staff executed the tests.

Study procedures

The tests of reference for the diagnosis of asthma were the conventional lung function tests (spirometry and bronchodilator response) and methacholine challenge test following guidelines of the Global Initiative for Asthma (GINA).[1] Spirometry was performed following international guidelines[8] with a Datospir 120 (Sibelmed, Barcelona, Spain). A forced expiratory volume in 1 s (FEV_1) $\geqslant$ 80% of predicted and/or a ratio of FEV_1 to forced vital capacity (FVC) $\geqslant$ 75% were considered to lie within normal limits.

Spirometry results lying outside the reference limits were classified as mild obstruction (60% $\leqslant$ FEV_1 $\geqslant$ 74%), moderate (59% $\leqslant$ FEV_1 $\geqslant$ 40%), or severe (FEV_1 $\leqslant$ 39%). A positive bronchodilator response was defined as an increase in FEV_1 $\geqslant$ 15% and/or $\geqslant$ 200 mL from baseline after inhalation of 400 μg of salbutamol.[8]

The methacholine challenge was performed according to international guidelines as a dose–response test of increasing doses of methacholine chlorohydrate (0.1–32 mg/mL) every 5 min.[9] The test was stopped when the highest concentration (32 mg/mL) was tolerated, or if a fall of 20% in FEV_1 from baseline was induced after methacholine was inhaled. The results were expressed as the dose of methacholine provoking a 20% fall in FEV_1 (PD_{20}). A methacholine challenge test was considered positive if the PD_{20} was $\leqslant$ 16 mg/mL.[9] A subject who presented a clinical history suggestive of asthma and a positive methacholine challenge test was diagnosed with asthma following the GINA guidelines.[1]

Inflammatory biomarkers

FE_{NO} measurement was performed with a conventional chemoluminescence analyser (SIR N-6008, Madrid, Spain) according to international guidelines.[10,11] The standardised single breath technique was used; each patient inhaled to total lung capacity once and then exhaled at a constant flow rate of 50 mL/s for approximately 10 s. A resistance with a pressure above 5–20 cm H_2O was provided to ensure velum closure and to exclude contamination from nasal NO. To interpret FE_{NO} recordings, we took only the valid NO plateau of the exhalation curve (held for $>$ 3 s and with a variation of $<$ 10%). The mean value of FE_{NO} from three technically valid measurements was recorded. The cutoff for a positive result was defined as a FE_{NO} $\geqslant$ 20 parts per billion (ppb).[12]

Sputum induction was carried out following described procedures.[13] Briefly, each patient gave a sputum sample