Simultaneously increased fraction of exhaled nitric oxide levels and blood eosinophil counts relate to increased asthma morbidity

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Background: We have previously described that fraction of exhaled nitric oxide (FENO) levels and blood eosinophil counts offer additive information in relation to asthma and asthma exacerbations when analyzing data from a large population study.

Objective: We sought to investigate increased FENO levels and blood eosinophil counts in relation to lung function, bronchial hyperresponsiveness (BHR), and asthma control in a cohort of young asthmatic patients.

Methods: Measurements of FENO levels and blood eosinophil counts were available in 406 subjects (208 women) aged 10 to 35 years. Asthma control was assessed through the Asthma Control Test. Moderate-to-severe BHR was defined as a cumulative dose of methacholine of less than 0.3 mg causing an FEV_1 decrease of 20%.

Results: Subjects with simultaneously increased FENO levels (≥20-25 ppb) and blood eosinophil counts (≥0.3 × 10^9/L) had a higher prevalence of uncontrolled asthma (Asthma Control Test score, <20) than subjects with singly increased blood eosinophil counts (40.5% vs 21.1%, $P = .01$). This difference remained significant ($P = .006$), and a significant difference was also found between subjects with both increased FENO levels and blood eosinophil counts and subjects with normal FENO levels and blood eosinophil counts ($P = .02$) after adjusting for confounders. Having increased FENO levels and blood eosinophil counts related to a higher prevalence of moderate-to-severe BHR than having normal FENO levels and blood eosinophil counts or singly increased FENO levels or blood eosinophil counts (85.7% vs 35.8% or 63.3% or 60%, $P < .05$ all comparisons).

Conclusion: We have shown that simultaneously increased local (FENO) and systemic (blood eosinophil) markers of type 2 inflammation related to a higher likelihood of BHR and uncontrolled asthma in a large cohort of young asthmatic patients. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Exhaled nitric oxide, blood eosinophil count, asthma control, lung function, bronchial responsiveness*

Asthma is a disease that affects more than 200 million persons globally. Inflammation is a major component of asthma, and medication to control asthma is primarily anti-inflammatory. Despite this, at present, assessment of inflammatory markers plays only a limited role in diagnosing and monitoring asthma in international guidelines (http://www.ginasthma.org/documents/4). However, the need for finding relevant biomarkers in asthma is generally acknowledged, and there is intense ongoing research to find useful biomarkers in clinical practice.[1]

Fraction of exhaled nitric oxide (FENO) is probably the biomarker that is most widely used in clinical practice at present. FENO is a local marker of airway inflammation, which is primarily triggered by IL-4 and IL-13 acting on respiratory epithelium.[2] Clinical guidelines on the use of FENO have been published by the American Thoracic Society (ATS).[3] The use of FENO has also been recommended recently in the United Kingdom by the National Institute of Health Care Excellence (http://www.nice.org.uk/guidance/dg12) when diagnosing and monitoring asthma. Despite this, the use of FENO in the diagnosis and monitoring of asthma remains controversial (http://www.ginasthma.org/documents/4).

Measurement of blood eosinophil counts is sometimes used in clinical practice when monitoring asthmatic patients. Eosinophilia is primarily triggered by IL-5.[4] Measurement of blood eosinophil counts has gained in importance since it has been found that asthmatic patients with higher blood eosinophil counts are more likely to improve when using anti–IL-5 blocking antibodies, such as mepoluzimab.[5,6] Furthermore, a high blood eosinophil count has been shown to be a risk factor for future asthma exacerbations in adults with persistent asthma.[7]

FENO has traditionally been considered a surrogate marker for eosinophilic inflammation.[3] However, we have recently discussed the dissociation between FENO levels and eosinophil counts and have suggested an explanatory model for this dissociation.[2] In line with this, for the first time, we have, through analysis of data from a large population study,[8] described that FENO levels and blood eosinophil counts associate independently with asthma, wheeze, and asthma attacks. The aim of the present study was to investigate whether FENO levels and blood eosinophil counts also have an additive value in relation to lung function, bronchial responsiveness, and asthma control in a cohort of young patients with asthma.

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The MIDAS study was performed within an industry-academy collaboration framework initiated by the Swedish Governmental Agency for Innovation Systems (VINNOVA, SAMBIO program), where Aerocrine AB (producer of exhaled NO devices) and Thermo Fisher Scientific, Immunodiagnostics (producer of allergy tests), were partners and cofinanced the program. A.M. had support from Uppsala University Hospital, the Swedish Heart and Lung Foundation, Bror Hjerpstedt's Foundation, and the Asthma and Allergy Foundation.

Disclosure of potential conflict of interest: M. Borres is an employee of Thermo Fischer Scientific, Immunodiagnostics, and participated in the study as adjunct professor at Uppsala University. K. Alving is a former employee and has served as a board member of Aerocrine (producer of exhaled NO devices) and has received equipment, stock options, and travel support from Aerocrine, as well as reagents from Thermo Fisher Scientific. The rest of the authors declare that they have no relevant conflicts of interest.

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Abbreviations used
ACT: Asthma Control Test
ATS: American Thoracic Society
BHR: Bronchial hyperresponsiveness
FENO: Fraction of exhaled nitric oxide
FVC: Forced vital capacity
ICS: Inhaled corticosteroid
LLN: Lower limit of normal
LTRA: Leukotriene receptor antagonist

METHODS

Subjects

The study was based on subjects who participated in a project run within the framework of an industry-academy collaboration on minimally invasive diagnostic procedures in allergy, asthma, or food hypersensitivity (the MIDAS study). Subjects were recruited from both primary and specialist care in Uppsala, Sweden. A total of 410 subjects aged 10 to 35 years with physician-diagnosed asthma and daily treatment with inhaled corticosteroids (ICSs), oral leukotriene receptor antagonists (LTRAs), or both during at least 3 of the preceding 12 months were included in the present study.[9,10] Subjects were recruited from both primary and secondary care in Uppsala, Sweden, between March 2010 and March 2012. Exclusion criteria were other chronic respiratory diseases, active tuberculosis, and recorded blood-borne disease.

The size of the study was selected to have a large group that could be studied in relation to minimally invasive and noninvasive diagnostic tools for asthma and allergic sensitization. The evidence about independent roles of blood eosinophils and FENO was not available before the study was completed.[8]

Clinical asthma characteristics

Subjects responded to questions regarding asthma symptoms in the preceding 12 months.[11] The degree of asthma control was assessed by using the Asthma Control Test (ACT).[12] Having an ACT score of less than 20 was defined as having uncontrolled asthma. Asthma attacks were self-reported. Each subject's use of ICSs and LTRAs was recorded through an interview. Information on the prescribed daily dose of ICS was collected from the subjects' medical records.

Exhaled nitric oxide

FENO values were measured in accordance with the ATS/European Respiratory Society recommendations by using a chemiluminescence analyzer at a flow rate of 50 mL/s (NIOX Flex; Aerocrine AB, Solna, Sweden).[13] FENO levels were regarded as normal if they were less than 20 ppb for a patient younger than 18 years or less than 25 ppb for a patient 18 years or older, whereas values of 20 ppb or greater and 25 ppb or greater, respectively, were considered increased.[3] An analysis was also performed using a higher cutoff of 50 ppb for adults (aged ≥18 years) and 35 ppb for patients less than 18 years of age.[3]

Lung function

Flow-volume curves were obtained in accordance with the ATS recommendations,[14] with a Masterscope spirometer (Jaeger Master, Wurzberg, Germany). The lower limit of normal (LLN) for the ratio between FEV_1 and forced vital capacity (FVC) was calculated in accordance with the method of Hankinson et al.[15] Subjects were subdivided as having normal or impaired lung function values (FEV_1 <80% or ≥80%, FVC <80% or ≥80% of predicted value, or FEV_1/FVC ratio less than the LLN or at the LLN or greater). For subjects less than 18 years of age, Solymar reference values for lung function were used,[16] whereas Hedenström reference values were used for subjects older than 18 years.[17,18]

Bronchial responsiveness

Methacholine provocation was performed with the Aerosol Provocation System (Viasys Healthcare GmbH, Hoechberg, Germany) using a simplified protocol described in detail elsewhere.[9] Bronchial responsiveness was defined as normal when the methacholine cumulative dose causing a decrease in FEV_1 (PD_{20}) was greater than 1.0 mg, borderline to mild at 0.3 to 1.0 mg, and moderate to severe at less than 0.3 mg in accordance with the methods of Schulze et al.[19]

Blood eosinophil counts

Blood eosinophils were counted at the Department of Clinical Chemistry at Uppsala University by using a routine method (Cell-Dyn 4000; Abbott, Abbott Park, Ill). Subjects were divided into 2 groups based on blood eosinophil counts: normal ($<0.3 \times 10^9$/L) or increased ($\geq 0.3 \times 10^9$/L).[7,20] An analysis was also performed by using a higher cutoff of 0.5×10^9/L, which has been suggested to define eosinophilia.[21]

Atopy

IgE levels against a mix of aeroallergens (grass, tree, and weed pollen and animal, mite, and mold allergens; Phadiatop, Immunodiagnostics; Thermo Fisher Scientific, Uppsala, Sweden) and a mix of food allergens (fx5, Immunodiagnostics, Thermo Fisher Scientific)[22] were measured in all but 3 subjects. Subjects were defined as atopic if they had IgE antibodies against either Phadiatop of 0.35 kU_A/L or greater or fx5 of 0.35 kU_A/L or greater.

Statistics

Statistical analyses were performed with STATA/IC 14.1 software (StataCorp LP, College Station, Tex). Group-wise differences between subjects with singly increased blood eosinophil counts or FENO levels or simultaneously increased FENO levels and blood eosinophil counts and subjects with FENO levels and blood eosinophil counts at normal levels were primarily studied. *t* Tests were used for continuous variables with normal distribution, and Mann-Whitney *U* tests were used for continuous nonnormally distributed variables. χ^2 Tests were used to assess differences in categorical variables, such as prevalence of abnormal lung function or moderate-to-severe bronchial hyperresponsiveness (BHR) or uncontrolled asthma and having frequent asthma attacks. The Pearson regression coefficient was used to assess the correlation between FENO levels and blood eosinophil counts (both log-transformed). Multiple logistic regression models with the outcome variable abnormal lung function, moderate-to-severe BHR, or uncontrolled asthma were used to estimate the likelihood of these events in relation to FENO and blood eosinophil categories. Furthermore, a logistic regression model with increased FENO levels and increased blood eosinophil counts as independent predictors was also tested to assess the independent effects of having increased FENO levels and blood eosinophil counts on the studied asthma outcomes. These models were adjusted for age, sex, weight group, atopy, smoking habits, current dose of ICS, and current use of LTRA. A *P* value of less than .05 was considered statistically significant. A *P* value of less than .0166 (ie, .05/3) was used as an indicator of statistical significance in the models in which we compared having singly increased or simultaneously increased biomarkers versus having normal levels of both biomarkers to adjust for multiple comparisons.

Ethics

The Uppsala Regional Ethical Review Board approved the study (approval no. 2009/349), and all subjects and their legal guardians provided written informed consent.

RESULTS

Patients' characteristics

Patients' characteristics are presented in Table I. A lower proportion of women was found in the group with singly increased blood eosinophil counts compared with the group with normal FENO levels and normal blood eosinophil counts ($P = .01$). Subjects with simultaneously increased FENO levels and blood eosinophil counts were younger than subjects in any of the other 3