Occupational asthma phenotypes identified by increased fractional exhaled nitric oxide after exposure to causal agents

Catherine Lemiere, MD, MSc,[a] Sebastien NGuyen, MD,[a] Francesco Sava, MD, MSc,[a] Vinciane D'Alpaos, MSc,[b] François Huaux, MD,[d] and Olivier Vandenplas, MD, PhD[b,c] *Montreal, Quebec, Canada, and Yvoir and Brussels, Belgium*

Background: The added value of fractional exhaled nitric oxide (FENO) remains controversial in the investigation of occupational asthma (OA).

Objective: We sought to assess whether or not the increase of FENO levels following positive specific inhalation challenge (SIC) was restricted to phenotypes of subjects sharing common clinical characteristics by using a statistical cluster analysis.

Methods: Subjects were investigated for possible OA in a tertiary center using SICs from 2006 to 2012. FENO levels and sputum eosinophil counts were assessed at baseline and 24 hours after SIC. We performed a 2-step cluster analysis of the subgroup of subjects with OA. A multivariate logistic regression was performed in order to identify the variables associated with an increase in FENO in subjects with OA.

Results: One hundred and seventy-eight subjects underwent SIC; 98 had a positive test. The cluster analysis performed in the OA subgroup identified 3 clusters. Despite a positive SIC, there was no increase in the FENO levels after exposure to occupational agents in Cluster 3, in which subjects were only exposed to low-molecular-weight (LMW) agents. The molecular weight of the agent (high molecular weight vs LMW) was the only factor associated with an increase in FENO (OR: 4.2 [1.1-16.8]) in subjects with a positive SIC.

Conclusion: An increase in FENO after exposure to agents causing OA seems to occur more consistently in subjects with OA caused by high molecular weight than in those with OA due to LMW. (J Allergy Clin Immunol 2014;134:1063-7.)

Key words: *Asthma, bronchial provocation tests, eosinophils, exhaled nitric oxide, occupational diseases, sputum*

Abbreviations used
AHR: Airway hyperresponsiveness
FENO: Fractional exhaled nitric oxide
HMW: High molecular weight
ICS: Inhaled corticosteroid
LMW: Low molecular weight
OA: Occupational asthma
SIC: Specific inhalation challenge
ROC: Receiver operating characteristics

Establishing or excluding a diagnosis of immunologically mediated (or sensitizer-induced) occupational asthma (OA) requires a high level of accuracy, because the condition is associated with significant health and socioeconomic impacts.[1] Over the past 2 decades, there has been growing interest in the noninvasive assessment of eosinophilic airway inflammation through sputum cell analysis and the measurement of fractional exhaled nitric oxide (FENO) as complementary tools to conventional lung function tests in the diagnosis and management of asthma.[2] Sputum cell counts have been shown to be useful as an additional tool in the investigation of OA.[3] However, sputum induction and processing are time-consuming and require technical expertise and thus are available in only a limited number of centers. The measurement of FENO as a surrogate marker for eosinophilic airway inflammation is simple and feasible in almost all patients and provides immediate results, but it is more sensitive to confounding factors, such as smoking, atopy, and treatment with inhaled corticosteroids (ICS), as compared with sputum eosinophil counts.[4] Its added value in the investigation of OA remains controversial due to conflicting data published in the literature.[5] One of the reasons for the discrepancies between the studies may pertain to the different phenotypes of patients included in those studies. As previously demonstrated, the atopic status of the subjects, as well as their treatment with ICS, are likely to influence the results obtained. Some studies looked at OA induced by a variety of agents,[6] whereas others focused on OA due to a single agent such as isocyanates.[7]

The aim of this study was to assess whether or not the increase of FENO levels following positive specific inhalation challenge (SIC) was restricted to phenotypes of subjects sharing common clinical characteristics by using a statistical cluster analysis.

METHODS

Study design and population

This was a prospective observational study that included consecutive subjects who had been investigated for possible OA in a tertiary center

From [a]the Hôpital du Sacré-Cœur de Montréal, Université de Montréal; [b]the Department of Chest Medicine, Centre Hospitalier Universitaire de Mont-Godinne, Université Catholique de Louvain, Yvoir; [c]Fonds des Maladies Professionnelles, Brussels; and [d]the Industrial Toxicology and Occupational Medicine Unit, Université Catholique de Louvain, Brussels.

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Corresponding author: Catherine Lemiere MD, MSc, Department of Chest Medicine, Sacré-Coeur Hospital, 5400 Gouin West, Montreal, Quebec, Canada, H4J 1C5. E-mail: catherine.lemiere@umontreal.ca.

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(CHU Mont-Godinne) through the performance of a SIC from 2006 to 2012. There was no exclusion criteria, as the intent of this real-life situation study was to include the whole population of subjects investigated during a 6-year time frame in order to have a representative sample of a day-to-day practice in a center specializing in the field of OA. Measurements of airway hyperresponsiveness (AHR) to histamine and assessment of FENO and sputum eosinophil counts were performed at baseline and 24 hours after inhalation challenge exposures to a control substance and the suspected occupational agent. The study protocol was approved by the Comité d'Éthique Médicale of the Centre Hospitalier Universitaire de Mont-Godinne (approval number B03920072360). The subjects signed a statement of informed consent.

Procedures

Specific inhalation challenges. SICs were carried out according to a previously described protocol.[8-11] Briefly, occupational agents were generated in 5 m^3 cubicles using a realistic approach.[11,12] The realistic approach aims to mimic the work environment as much as possible. For example, a baker is asked to toss flour from one tray to the other to produce airborne particles. This approach for performing SICs has been shown to be safe and rarely induce severe asthmatic reactions requiring administration of systemic steroids.[9] The concentrations of the agents generated during the SIC procedures were not quantified, with the exception of SIC with isocyanates, in which the concentrations were kept below the short-term limit value of 20 ppb. Asthma medications were withdrawn according to their duration of action,[13] while inhaled corticosteroids were halted 72 hours prior to the tests.

On the first day, the subjects were exposed to a "control" agent for 30 minutes to ensure that FEV_1 fluctuations were ≤12% of the baseline value. The "control" non-sensitizing substance was selected according to the nature of the occupational agent suspected of causing OA; for instance, lactose powder for SIC with agents in powder form (flour, drugs, persulphates), pine dust for SIC with wood dusts, vinyl gloves for SIC with latex gloves, and diluents for polyurethane products and other resins.[11] Spirometry[14,15] was measured at baseline and serially for at least 6 hours after exposure. Assessment of baseline AHR to histamine and evaluation of inflammatory cells in induced sputum were performed at the end of the control day.

On the following day, the subjects were challenged with the suspected occupational agent(s). Spirometry was measured according to the same schedule as on the control day. The duration of exposure was gradually increased (ie, 1, 4, 10, 15, 30, and 60 minutes) until a ≥20% fall in FEV_1 occurred or a cumulative exposure of 2 hours on the same day was completed. Those subjects who did not demonstrate a ≥20% fall in FEV_1 during the first active challenge day systematically completed a second challenge for a maximum of 2 to 3 hours on the following day. Additional challenges were proposed when there was a significant (>3-fold) decrease in the post-challenge PC_{20} value[16] or when an increase in sputum eosinophils >3% was found,[8] as compared with the control day values. An SIC was considered positive when a reproducible fall in FEV_1 of 20% or more as compared to pre-challenge value was recorded.

Assessment of nonspecific airway hyperresponsiveness. The level of AHR was assessed through the inhalation of doubling concentrations of histamine at tidal breathing for 2-minute periods, as described by Cockcroft et al.[17] The results were expressed as the concentration of histamine inducing a 20% decrease in FEV_1 (PC_{20}). A histamine PC_{20} value ≤16 mg/mL was considered as reflecting significant AHR. The histamine PC_{20} was assessed at the end of the control day (ie, the baseline value) and reassessed 6 to 8 hours after each active challenge as well as 24 hours after the last challenge exposure, provided that FEV_1 was ≥90% of the pre-challenge value. After the histamine bronchoprovocation, the subjects were administered an inhaled bronchodilator (salbutamol 400 μg) and sputum was induced.

Sputum induction and processing. Sputum was induced by inhaling increasing concentrations (3%, 4%, and 5%) of nebulized hypertonic saline as previously described.[6,8,18] Total cell counts and cell viability were assessed using the trypan blue exclusion method in a Burker haemocytometer. The sample was considered adequate for analysis when there were fewer than 20% squamous cells and viability was more than 40%. Differential cell counts were determined by counting 400 nucleated non-squamous cells per slide on cytospin preparations stained with May-Grünwald-Giemsa. The results were expressed as the percentage of total non-squamous cells and as the absolute number of cells in millions per mL of sputum. Sputum samples were collected 6 to 8 hours after the end of control and active challenge exposures, as well as 24 hours after the end of the last challenge exposure.

FENO measurements. FENO was measured using an online chemiluminescence analyzer (NIOX, Aerocrine AB, Solna, Sweden) at a flow rate of 50 mL/s, in accordance with international recommendations.[19] The FENO measurement was performed before active challenges and repeated 24 hours after the challenges. These 2 values were used to compute the increase in FENO levels during SICs based upon previous data showing that the post-challenge increase in FENO becomes significant only at this time point.[6,7] FENO was always measured prior to the performance of any procedure such as spirometry, histamine challenge, sputum induction, or administration of bronchodilators.

Analysis of data

Continuous variables were reported as mean and standard deviation except for PC_{20}, FENO, and sputum cell counts, which were expressed as median and 25th to 75th percentiles. A Student *t* test for normally distributed continuous variables, a Mann-Whitney *U* test for non-normally distributed continuous variables, and a χ^2 test for categorical variables were used to compare the variables of interest between groups of subjects with positive and negative SIC. Receiver operating characteristics (ROC) curves were built in order to identify what changes in FENO (post-exposure value – baseline value) provided the optimal sensitivity and specificity associated with positive SIC. A 2-step cluster analysis was performed because categorical and continuous variables were used to form groups of subjects. This procedure includes a preclustering and a hierarchical clustering of the preclusters. Standardization of all continuous variables was made before clustering, and log-likelihood criterion was used as distance measures. To determine the number of clusters, Schwarz Bayesian criterion change was used, and a minimum of 10% of subjects in the smallest cluster composition had to be observed in order to retain the final solution. This analysis was performed using the following baseline variables: sex, age, atopy (defined by a positive skin prick test to at least 1 of 21 common aeroallergens), smoking habits, ICS treatment, and the change in FENO before and after exposure in subjects with a positive SIC. The variables included in the cluster analysis represented relevant clinical characteristics of subjects at baseline except for the changes in FENO after exposure, which was the variable of interest. Another model including airway responsiveness produced the same results but was not kept, because it decreased our sample size due to missing variables. Sputum eosinophils could not be entered in the cluster analysis, due to the many missing data for this variable. ANOVA for normally distributed continuous variables, a Kruskal-Wallis test for non-normally distributed continuous variables, and the χ^2 analysis for categorical measures were used to compare the variables of interest between clusters. Correlation analyses were performed using a Spearman rank test.

A multivariate logistic regression analysis was conducted to assess whether atopy (nonatopic vs atopic), smoking habits (never vs ever a smoker), treatment with ICS (yes vs no), duration of exposure to the offending agent during SIC, maximum fall in FEV_1 during SIC, type of agent (HMW vs LMW), baseline levels of FENO (levels below or equal to 25 ppb vs greater than 25 ppb), and baseline FEV_1 (lower than 80% vs 80% or greater) were associated with clinically significant changes in the levels of FENO after SIC as determined by the ROC analysis in subjects with OA. The statistical analysis was performed with the IBM SPSS statistical software (version 19.0.0), IBM Corporation (Somers, NY). Significance was accepted when $P \leq .05$.

RESULTS

One hundred and seventy-eight subjects underwent SIC, of whom 98 showed a positive response. The characteristics of the subjects with positive and negative SIC are presented in Table I.