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

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**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

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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 $\chi^2$ 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 $\chi^2$ 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.

**TABLE I.** Characteristics of the subjects

| | Positive SIC | Negative SIC | *P* value |
|---|---|---|---|
| n | 98 | 80 | |
| Age | 40.05 ± 10.3 | 41.44 ± 11.2 | .4 |
| Symptoms duration (months) | 38.07 ± 46.9 | 55.5 ± 71.5 | .06 |
| Asthma preceding current employment (n, %) | 21 (21.4) | 14 (17.5) | .5 |
| Sex, male (n, %) | 60 (61.2) | 46 (57.5) | .6 |
| Atopy (n, %) | 62 (63.3) | 34 (44.2) | .01 |
| Aeroallergens (n) | Mites (32), cat (18), dog (22), tree pollen (16), weeds (34), grass (10), molds (11) | Mites (23), (cat 9), dog (9), tree pollen (14), weeds (16), grass (8), molds (7) | |
| Smoking habits, Never/current/ex (n, %) | 57 (58.2)/24 (245)/17 (17.3) | 36 (45)/22 (27.5)/22 (27.5) | .16 |
| Type of agent, HMW (n, %) | 61 (62.2) | 20 (25) | <.001 |
| Treatment with ICS (n, %) | 55 (56.1) | 52 (65) | .3 |
| $FEV_1$ (% predicted) | 87.4 ± 13.1 | 90.1 ± 12.7 | .2 |
| FVC (% predicted) | 101.1 ± 12.8 | 99.7 ± 12.4 | .4 |
| Baseline $PC_{20}$ (mg/mL) | 2.5 (0.8-9.05) | 10.9 (3.9-32.0) | <.001 |
| $PC_{20}$ (mg/mL post-SIC) | 1.12 (0.5-4.9) | 8.8 (4.6-18.5) | <.001 |
| Baseline FENO (ppb, n = 162) | 28 (14.1-40,8) | 13.7 (9.3-27.7) | .002 |
| Post-SIC FENO (n = 138) | 43.0 (24.5-77.3) | 17 (10.7-30.2) | <.001 |
| Baseline eosinophils (%, n = 113) | 1.5 (0.9-3.9) | 1.0 (0.3-2.4) | .06 |
| Post-SIC eosinophils (%, n = 104) | 7.5 (3.4-12.8) | 1.0 (0.5-2.2) | <.001 |

Results of continuous variables are represented as means ± SDs, except for $PC_{20}$, FENO, and sputum cell counts, which are presented as median (25th to 75th percentiles).
*IQR*, Interquartile range.

One hundred and thirty-five subjects performed NO analysis before and after SIC. Suitable sputum samples before and after SIC were available in 104 subjects.

There was a statistically and clinically significant difference in FENO levels ($P < .001$), sputum eosinophil counts ($P < .001$), and $PC_{20}$ ($P < .001$) before and after SIC in subjects with positive challenge. There was no change in sputum eosinophil counts after SIC ($P = .36$) in subjects with negative SIC. Although there was a statistically significant difference between FENO levels ($P = .02$) before and after challenge, these changes were minimal and were not clinically meaningful (Table I).

Overall, there was a weak though significant correlation between the change in sputum eosinophil counts and FENO levels before and after exposure to the offending agent ($\rho = 0.4$; $P < .001$).

**ROC for assessing the association of changes in FENO after exposure to the offending agent and positivity of SIC.** We performed an ROC analysis to determine the diagnostic efficiency of changes in FENO before and after SIC. A post-challenge increase in FENO levels of 17.5 ppb or more was associated with a positive SIC with a specificity of 90% and a sensitivity of 45.3% (area under the curve: 0.7 ± 0.45; $P < .001$) (Fig 1).

**Cluster analysis in the occupational asthma subgroup.** Twenty-five subjects were not kept into the cluster analysis due to missing variables. Three clusters were identified in the subjects with OA (Table II). Cluster 1 and 2 were mainly composed of subjects exposed to HMW agents, whereas Cluster 3 was exclusively composed of subjects exposed to LMW agents. Cluster 1 was composed of subjects with higher $PC_{20}$, a trend towards a higher $FEV_1$, and no subjects taking ICS, compared with Clusters 2 and 3. Clusters 1 and 2 comprised more atopic subjects and more subjects reporting rhinitis than Cluster 3. No significant increase in FENO levels after exposure was seen in Cluster 3, whereas an increase in sputum eosinophil counts was observed in the 3 clusters. Overall, there was a higher increase in FENO levels after exposure to HMW (28.8 ± 27.1 ppb) than after exposure to LMW (9.5 ± 18.4 ppb; $P = .001$) in subjects with OA.

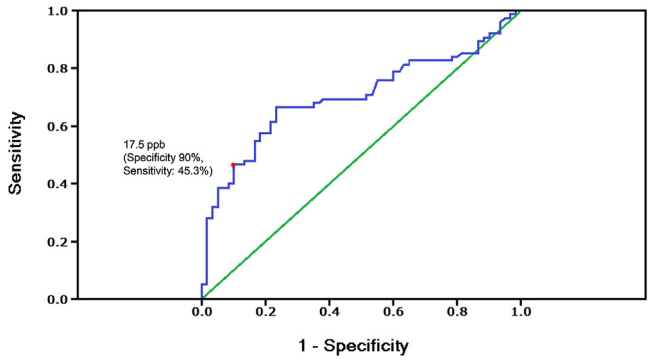


**FIG 1.** ROC curve assessing the association of changes in FENO before and after exposure to the offending agent and positivity of SIC. The *green line* represents the reference line and the *blue line* is the actual ROC curve showing the sensitivity and specificity of each change in FENO levels associated with a positive SIC. The *red dot* represents the value of the change in FENO levels associated with a high specificity for a positive SIC.

The multivariate logistic regression performed in order to identify the factors associated with an increase in FENO in subjects with OA retained only the molecular weight of the agent (HMW) as a factor associated with an increase in FENO (Table III).

## DISCUSSION

To the best of our knowledge, this study is the first that attempted to identify the factors associated with a change in FENO in subjects with work-related asthma after exposure to the causal agent. Previous studies have observed that the increase in FENO was not uniform among subjects who experienced a positive asthmatic reaction during SIC.[20] Piipari et al showed that subjects with elevated baseline levels and late asthmatic reactions had an increase in FENO levels after SIC. However, this study included only 14 subjects with OA, and results were not adjusted for confounding factors.[21]

**TABLE II.** Cluster analysis in subjects with occupational asthma

| | Cluster 1 | Cluster 2 | Cluster 3 | *P* value |
|---|---|---|---|---|
| n | 21 | 29 | 23 | |
| Age | 35.9 ± 9.8 | 40.2 ± 10.4 | 45.8 ± 8.7 | .05 |
| Sex, male (n, %) | 14 (66.7) | 19 (65.5) | 10 (43.5) | .2 |
| Atopy (n, %) | 16 (76.2) | 22 (75.9) | 8 (34.8) | .003 |
| Asthma preceding current employment (n, %) | 6 (28.6) | 8 (27.6) | 3 (13.0) | .4 |
| Nonsmokers (n, %) | 16 (76.2) | 15 (51.7) | 14 (60.9) | .1 |
| ICS treatment (n, %) | 0 (0) | 27 (93.1) | 15 (65.2) | <.001 |
| Asthma symptom duration (mo) | 52.6 ± 56.5 | 51.4 ± 46.3 | 59.2 ± 101.3 | .9 |
| Rhinitis (n, %) | 18 (85.7) | 28 (96.6) | 11 (47.8) | <.001 |
| Agent (HMW, %) | 16 (76.2) | 29 (100) | 0 (0) | <.001 |
| Occupational agents | Flour (H): 9<br>Enzymes (H): 2<br>Latex (H): 4<br>Linen (H): 1<br>Welding (L): 1<br>Cobalt (L): 1<br>Isocyanates (L): 1<br>Persulfate (L): 1<br>Platinum (L): 1 | Flour: 18<br>Latex: 7<br>Tomato: 1<br>Cereals: 1<br>Enzymes: 2 | Isocyanates: 3<br>Aldehyde: 1<br>Wood: 3<br>Welding: 1<br>Cleaning agents: 10<br>Persulfate: 1<br>Glue: 1<br>Cobalt: 1<br>Acrylate: 1<br>Ethyl acetate: 1 | |
| Type of reaction I, D, L (n, %) | 16 (76.2), 3 (27.3), 2 (9.5) | 15 (51.7), 6 (20.7), 8 (27.6) | 10 (43.5), 2 (8.7), 11 (47.8) | .06 |
| $FEV_1$ (% predicted) | 90.8 ± 13.4 | 83.8 ± 11.6 | 89.9 ± 15.6 | .1 |
| Baseline $PC_{20}$ (mg/mL) | 12.2 (1.3-30) | 1.4 (0.6-8.0) | 1.8 (0.8-3.7) | <.001 |
| $PC_{20}$, last day (mg/mL) | 5.8 (1.0-12.2) | 1.1 (0.6-4.6) | 0.7 (0.1-2.3) | .015 |
| Maximum $FEV_1$ (fall, %) | 34.3 ± 12.7 | 34.6 ± 14.8 | 29.0 ± 7.1 | .2 |
| FENO baseline (ppb) | 24.6 (16.2-38.0) | 28.0 (14.4-58.3) | 22.2 (10-33.5) | .2 |
| FENO last day (ppb) | 66.0 (31.9-106,4) | 48.3 (30.0-81.5) | 29.0 (12.5-43.0) | .001 |
| FENO increase ≥17.5 ppb (n, %) | 13 (61.9) | 17 (58.6) | 4 (14.4) | .003 |
| Baseline eosinophil counts (%) | 1.0 (0.4-1.8) | 2.0 (1.0-3.5) | 1.7 (1.0-5.5) | .1 |
| Eosinophil counts post-exposure (%) | 6.2 (1.8-9.0) | 7.2 (4.5-12.0) | 7.4 (2.0-11.9) | .6 |

Results of continuous variables are represented as means ± SDs, except for $PC_{20}$, FENO, and sputum cell counts, which are presented as median (25th-75th percentiles).
*H*, High-molecular-weight agent; *L*, low-molecular-weight agent.

**TABLE III.** Factors associated with a change in FENO greater than 17.5 ppb after exposure to the offending agent in subjects with OA

| Determinant variables for a change in FENO >17.5 ppb | OR (95% CI) | *P* value |
|---|---|---|
| Duration of exposure (min) | 0.99 (0.9-1.0) | .2 |
| Maximum fall in $FEV_1$ | 1.02 (0.9-1.1) | .5 |
| Baseline $FEV_1$ (ref: FEV < 80%) | 2.3 (0.7-8.1) | .2 |
| ICS (ref: no treatment with ICS) | 1.02 (0.3-3.1) | .9 |
| Type of agent (ref: HMW) | 4.2 (1.1-16.8) | .04 |
| Smoking (ref: never a smoker) | 1.5 (0.5-4.3) | .5 |
| Atopy (ref: nonatopic) | 2.0 (0.5-7.5) | .3 |
| Baseline FENO (ref: ≤25 ppb) | 0.5 (0.2-1.5) | .2 |

Cluster analysis allowed identification of 3 distinct phenotypes. Cluster 1 included milder asthmatic subjects than Clusters 2 and 3. Cluster 3 contrasted greatly with Clusters 1 and 2, with lower number of atopic subjects, no exposure to HMW, and greater number of late reactions. Furthermore, Cluster 3 differed the most from Clusters 1 and 2 in terms of changes in FENO observed during SICs; FENO changes during SICs were restricted to Clusters 1 and 2, in which the subjects were mainly exposed to HMW, whereas no significant changes in FENO were observed in Cluster 3, which was composed of subjects only exposed to LMW. In subjects exposed to HMW agents, the increase in FENO was significantly associated with a positive SIC result. Our results are consistent with those of Pedrosa et al, who showed a high positive predictive value of a >12% increase in FENO for a positive SIC in a small group of subjects challenged with common allergens or HMW agents.[22]

The strength of our study was to include a large cohort of subjects, including a variety of sensitizers, which allowed to us to identify various phenotypes among this cohort. However, this study has several limitations that are inherent to its design as a prospective observational study conducted in a clinical practice. Our population included a highly heterogeneous population of subjects with work-related asthma, with a large number of subjects taking ICS as well as smokers. Although it has been clearly shown that FENO measurements were influenced by those factors, these variables did not seem to affect FENO results in a significant way in our multivariate analysis. Our data suggest that the changes in FENO after challenge exposure to various occupational agents are largely affected by the type of occupational agents, and thus probably by the different immunological mechanisms involved in OA caused by HMW and LMW agents. However, the study was not designed to characterize the pathophysiological mechanisms underlying the observed differences between the changes in FENO induced by LMW and HMW agents. The association between an increase in FENO and exposure to HMW agents may be due to the underlying IgE-mediated mechanism rather

than to the type of agent itself. FENO has been shown to be correlated with total serum IgE in asthmatic subjects.[23,24] The cohorts in which FENO had a high predictive value for asthma were composed of subjects exposed to HMW agents or common allergens.[22] However, several studies have shown an increase in FENO after exposure to isocyanates.[7,25] Interestingly, Barbinova et al showed an increase in FENO in subjects showing specific IgE to isocyanates.[25] Unfortunately, only 4 subjects had isocyanate-induced asthma in our cohort, and only 3 of them performed measurements of FENO before and after exposure, preventing us from making any conclusion regarding this specific agent. The methodology of SIC using a realistic approach does not allow taking formally into account the magnitude of the bronchial response to the occupational agent as a factor that could influence the changes in FENO. Nevertheless, in the multivariate logistic regression analysis, the threshold duration of exposure required to elicit a 20% decline in $FEV_1$ during SICs and the maximum fall in $FEV_1$ did not seem to significantly affect the FENO response. Other potential confounding factors, such as genetic polymorphism, could not be assessed in this study but may have clinical implications.[26]

Sputum eosinophil counts have been shown to increase in the majority of subjects with OA after exposure to the offending agent. Although there was a correlation between the change in sputum eosinophil counts and the FENO levels before and after exposure to the offending agent, the correlation was weak in our population. Interestingly, the 3 clusters with OA had an increase in sputum eosinophil count after exposure, whereas an increase in FENO was not found in Cluster 3, which included only OA due to LMW agents. Sputum eosinophil counts have been shown to have a better predictive value than FENO for diagnosing OA caused by various agents.[6] However, sputum cell counts could not be obtained in 37% of our subjects. This finding illustrates the limitation of sputum cell counts and the need to have access to a non-invasive measure of inflammation that can be done in the majority of subjects. This study indicates that, when restricted to HMW, FENO represents a reliable and suitable alternative to sputum eosinophil counts during the investigation of OA.

In conclusion, an increase in FENO was strongly associated with asthmatic reactions induced by HMW agents. Although the mechanisms underlying these different patterns of FENO response between HMW and LMW agents remain to be explored, the results of this study provide useful information for the clinical investigation of work-related asthma using FENO measurements as an indirect marker of airway inflammation.

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**Clinical implications: The assessment of FENO levels after challenge or workplace exposure is likely to be more helpful in the investigation of occupational asthma caused by high-molecular-weight agents than by low-molecular-weight agents.**

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