

ORIGINAL ARTICLE

Asthma and Lower Airway Disease

Exhaled Nitric Oxide and Sputum Eosinophils Are Complementary Tools for Diagnosing Occupational Asthma

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Keywords: fractional exhaled nitric oxide | occupational asthma | specific inhalation challenge | sputum eosinophils

ABSTRACT

Background: Exposure-related changes in exhaled nitric oxide (FeNO) and sputum eosinophils have not been thoroughly compared in the investigation of occupational asthma.

Objective: This study aimed at comparing the accuracies of the changes in FeNO concentrations and sputum eosinophil counts in identifying asthmatic reactions induced by occupational agents during specific inhalation challenges (SICs).

Methods: This retrospective multicenter study included 321 subjects who completed an assessment of FeNO and sputum eosinophils before and 24 h after SICs with various occupational agents, of whom 156 showed a positive result.

Results: Post-challenge changes in FeNO and sputum eosinophils showed similar accuracies, with areas under the receiver operating characteristics curve of 0.78 (95% confidence interval [95% CI], 0.72–0.83) and 0.81 (95% CI, 0.76–0.86), respectively. Increases

Abbreviations: AUC, area under the curve; CI, confidence interval; E-PHOCAS, European network for the PHenotyping of Occupational Asthma; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; HMW, high-molecular-weight; ICS, inhaled corticosteroid; LMW, low-molecular-weight; NPV, negative predictive value; NSBH, nonspecific bronchial hyperresponsiveness; OA, occupational asthma; OR, odds ratio; PPV, positive predictive value; ROC, receiver operating characteristics; SIC, specific inhalation challenge.

Virginie Doyen and Nicolas Migueres have contributed equally to this study.

The E-PHOCAS investigators are listed in Appendix S1 of this article's supporting information.

in FeNO level ≥ 13 ppb and sputum eosinophils $\geq 1.25\%$ were identified as the optimal threshold values for differentiating positive from negative SICs. Using these thresholds, the changes in FeNO and sputum eosinophils each achieved a $\geq 95\%$ specificity but a low sensitivity (55% and 62%, respectively). FeNO and sputum eosinophils showed discordant increases in 38% of subjects with a positive SIC. Combining either a rise in FeNO ≥ 13 ppb or an increase in sputum eosinophils $\geq 1.25\%$ increased the sensitivity to 77%.

Conclusions: Increases in FeNO concentration and/or sputum eosinophils after exposure to occupational agents strongly support a diagnosis of occupational asthma. The assessment of both markers of airway inflammation should be regarded as a reliable complementary tool to spirometry for identifying bronchial responses to occupational agents.

1 | Introduction

Making an accurate diagnosis of sensitizer-induced occupational asthma (OA) is a key step for minimizing the adverse health and socio-economic impacts of the disease. However, no simple test would allow for diagnosing the condition with a sufficiently high level of confidence [1, 2]. A substantial proportion of asthmatic reactions induced by occupational agents is associated with an increase in sputum eosinophil counts [3–5]. As a result, investigation of airway inflammation through the induced sputum technique has been highlighted as an effective aid to lung function tests in the investigation of OA upon exposure to occupational agents at work [6] and during specific inhalation challenges (SIC) in the laboratory [4, 7]. However, the analysis of sputum cells is time-consuming, requires technical expertise, is not readily available in most clinical settings, and may be unsuccessful in a substantial fraction of those tested [8]. In contrast, the measurement of fractional exhaled nitric oxide (FeNO) concentration as a surrogate marker for eosinophilic airway inflammation is fast, feasible in almost all patients, and provides immediate results [8–10]. Several factors other than eosinophilic inflammation can affect FeNO levels, however, including sex, obesity, atopy, smoking, and treatment with inhaled corticosteroids (ICS) [9–11].

A number of studies have documented a delayed increase in FeNO after asthmatic reactions induced by various high-molecular-weight (HMW) and low-molecular-weight (LMW) agents [12–25]. Nevertheless, these studies provided inconsistent findings regarding the usefulness of measuring FeNO in the clinical investigation of OA [21, 23–25]. In addition, exposure-related changes in FeNO have not been thoroughly compared to the changes in sputum eosinophil counts as markers of OA [17].

The aim of this study was to assess and compare the accuracy of post-challenge changes in FeNO and sputum eosinophil counts in differentiating positive from negative SICs in a large multi-center cohort with the ultimate goal of determining whether measurement of these changes offers a useful adjunct to lung function tests for diagnosing OA when the SIC provides indeterminate functional changes or is not feasible or not available.

2 | Methods

2.1 | Study Design and Population

This retrospective observational study was conducted among consecutive subjects who completed a SIC with the workplace agent suspected of causing their work-related asthma symptoms between 2006 and 2018 in 14 tertiary centers participating to

the European network for the PHenotyping of OCCupational Asthma (E-PHOCAS) [22, 26]. Eligible subjects for this analysis were those with available FeNO measurements and sputum eosinophil counts both before and 24 h after the SIC procedure, as detailed in Appendix S2 and Figure S1.

2.2 | Ethics

Approval for this retrospective study was obtained from local Institutional Review Boards, the “Comité Consultatif sur le Traitement de l’Information en Matière de Recherche dans le Domaine de la Santé” and the “Commission Nationale de l’Informatique et des Libertés”.

2.3 | Data Collection

The demographic, occupational, and clinical characteristics of the subjects at the time of the diagnostic evaluation were entered in the database [22, 26]. Information was gathered about associated work-related disorders including rhinitis, urticaria, and contact dermatitis which were defined based on symptoms reported by the patients. We also collected baseline blood eosinophil counts and induced sputum cell counts and FeNO levels measured at baseline and 24 h after challenge exposures, when available. The anonymized information was entered into a standardized Excel spreadsheet at each participating center and pooled together in a central database held at the Strasbourg University.

2.4 | Procedures

2.4.1 | Specific Inhalation Challenges

The decision-making policy for performing SICs in each participating center is summarized in Appendix S3. The SIC methodology conformed with international recommendations in terms of the performance of a control (placebo) test on a separate day before challenging the subjects with occupational agents and functional monitoring for at least 6 h after the control and active challenges [27]. In all centers, asthma medications were withdrawn according to their duration of action and ICS were withheld three to 7 days before the SIC. Challenge exposure to occupational agents was generated using “realistic” approaches that are further described in Appendix S3 [28]. A SIC result was considered positive when a fall in $FEV_1 \geq 15\%$ from pre-challenge value was recorded at any time point during the functional monitoring [27].

2.4.2 | Measurements of Nonspecific Bronchial Hyperresponsiveness

The level of NSBH was measured at baseline before the SIC procedure (i.e., at the end of the “control day”) and 24 h after the last challenge exposure. The degree of NSBH was expressed as the concentration or dose of the pharmacological agent inducing a 15% or 20% drop in FEV₁ according to the bronchoprovocation method used in each center. NSBH was graded a posteriori as “absent”, “mild”, or “moderate-to-severe” according to available recommendations or a consensus Delphi approach among investigators [22, 29]. Post-challenge changes in the level of NSBH were expressed as the ratio of NSBH at baseline divided by the NSBH value measured at 24 h after the last exposure to the offending agent.

2.4.3 | Fractional Exhaled Nitric Oxide

The FeNO levels were measured using different devices based on chemiluminescence and/or electrochemical detection and reported as parts per billion (ppb). Measurements were made at a flow rate of 50 mL/s in compliance with the recommendations of the European Respiratory Society and the American Thoracic Society [30]. The FeNO values used in this analysis were those obtained in the morning before challenge exposure to occupational agents (i.e., baseline value) and 24 h after the last challenge. The changes in FeNO were expressed as the post-challenge value minus the baseline value in ppb.

2.4.4 | Induced Sputum Analysis

Sputum cell analysis was performed before and 24 h after the SICs in eight of the 14 participating centers [5, 31]. Sputum was induced through slightly different methods in participating centers, including the inhalation of nebulized isotonic saline in one center, a single concentration of hypertonic solution (i.e., 3% or 4%) in two centers, and in the other five centers, increasing concentrations of hypertonic solutions (i.e., 3%, 4%, and 5% in four centers or 10% and 20% in one center) for a maximum cumulative duration of 15–30 min [5]. Processing of sputum samples was carried out either by selecting viscid portions from the expectorate (four centers) [32] or using the whole expectorate (four centers) [33]. Homogenization of the sample was achieved by adding dithiothreitol (0.1%). All centers applied quality criteria based on the cell viability ($\geq 40\%$) and the level of contamination by squamous cells ($< 20\%$ in five centers; $< 30\%$ in two centers; and $< 50\%$ in two centers). Differential cell counts were determined by counting a minimum of 400 non-squamous cells. Changes in sputum eosinophils were computed as the difference between the post-challenge and the baseline values expressed as percent of non-squamous cells.

2.5 | Data Analysis

Continuous variables were summarized as medians and 25th–75th percentiles and categorical variables by their frequencies and proportions. Comparisons between subjects were made using the chi-squared or Fisher's exact test for categorical variables and non-parametric tests for numerical variables. Receiver operating characteristics (ROC) curves were built to evaluate

the accuracy of the post-challenge increase in FeNO and sputum eosinophil counts to differentiate positive from negative SICs. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the ROC curve (AUC), and Youden index (i.e., sensitivity + specificity – 1) with their 95% confidence intervals (95% CIs) were determined. The “optimal” cut-off value was identified as the change in FeNO or sputum eosinophils that yielded the highest Youden index value.

Multivariate logistic regressions were conducted to identify the factors that were associated with a high (i.e., ≥ 25 ppb) baseline FeNO and increases in FeNO and sputum eosinophils above the threshold values. These multivariate regressions were carried out using a binomial generalized linear model and a stepwise procedure based on the Akaike information criterion to select the most parsimonious model. The independent variables incorporated into these multivariate regressions were selected on the basis of a p value < 0.10 in univariate analysis.

Statistical analyses were performed using the R software version 4.3.2 (www.r-project.org, Vienna, Austria) and $p < 0.05$ was considered significant.

3 | Results

3.1 | Characteristics of the Subjects

The baseline characteristics of the subjects are summarized in Table 1 and further detailed in Appendix S4 and Table S1. Among the 321 subjects included in this analysis, 156 (49%) showed a $\geq 15\%$ decline in FEV1 during the SIC.

When compared to those with a negative SIC, the subjects who developed an asthmatic response during SIC more frequently experienced concomitant work-related rhinitis and more often showed baseline airway obstruction and NSBH. Subjects with a positive SIC had a higher median baseline FeNO level as well as higher blood and sputum eosinophil counts than those with a negative SIC (Table 1).

A multivariate logistic regression analysis was conducted in order to identify the factors associated with a baseline FeNO ≥ 25 ppb (Table S2). The final multivariate model revealed that a baseline FeNO level ≥ 25 ppb was associated with male sex, atopy, the presence of baseline NSBH, and a final diagnosis of OA (i.e., a positive SIC). Current smoking was associated with a lower likelihood of a FeNO level ≥ 25 ppb.

There was a significant increase in the median FeNO concentration and sputum eosinophil count measured at 24 h after challenge exposure in subjects with a positive SIC, whereas subjects with a negative SIC had no significant changes (Table 1).

3.2 | Post-Challenge Changes in FeNO

The ROC analysis (Figure 1) showed that the accuracy of the changes in FeNO for differentiating positive from negative SICs expressed as the AUC was 0.78 (95% CI, 0.72–0.83). A post-challenge FeNO increase of ≥ 13 ppb was the optimal threshold

TABLE 1 | Characteristics of the subjects with positive and negative inhalation challenges.

Characteristics	Missing values	Negative SIC (<i>n</i> = 165)	Positive SIC (<i>n</i> = 156)	<i>p</i> values
Type of workplace agent, HMW agent, <i>n</i> (%)	0	29 (17.6)	67 (42.9)	< 0.001
Age, year ^a	0	44 (35–53)	42 (34–51)	0.408
Sex, male, <i>n</i> (%)	0	100 (60.6)	102 (65.4)	0.376
Body mass index, kg/m ^{2a}	0	26.8 (24.3–30.8)	26.6 (23.7–31.4)	0.723
Smoking status, <i>n</i> (%)				
Current smoker	0	37 (22.4)	46 (28.5)	0.890
Never and ex-smoker		128 (77.6)	110 (70.5)	
Atopy ^b , <i>n</i> (%)	0	82 (49.7)	85 (54.5)	0.391
History of childhood asthma, <i>n</i> (%)	2/4	10 (6.1)	10 (6.6)	0.871
Duration of work exposure before onset of work-related asthma, mo ^a	5/5	52 (12–183)	72 (32–201)	0.065
Duration of work-related asthma symptoms at work, mo ^a	3/10	24 (9–54)	24 (12–48)	0.796
Interval since last work exposure, mo ^a	1/1	2 (0.1–13)	5 (0.1–12)	0.780
Use of an inhaled short-acting beta ₂ -agonist, <i>n</i> (%)	0/6	81 (49.1)	82 (54.7)	0.323
Treatment with an inhaled corticosteroid, <i>n</i> (%)	0	84 (50.9)	75 (48.1)	0.612
Work-related rhinitis, <i>n</i> (%)	3/1	79 (48.8)	102 (65.8)	0.002
Work-related urticaria, <i>n</i> (%)	1/5	17 (10.4)	12 (7.9)	0.458
Work-related contact dermatitis, <i>n</i> (%)	1/5	18 (11.0)	20 (13.2)	0.537
Baseline spirometry				
FVC, % pred ^a	0	101 (93–113)	102 (93–111)	0.888
FEV ₁ , % pred ^a		93 (84–104)	91 (80–100)	0.011
FEV ₁ /FVC, % ^a		77 (72–81)	73 (66–79)	< 0.001
Airflow obstruction, <i>n</i> (%) ^c		13 (7.9)	32 (20.5)	0.001
Baseline NSBH, <i>n</i> (%)	0/5	69 (41.8)	113 (74.8)	< 0.001
Blood eosinophils				
Cells/μL ^a	20/24	180 (100–244)	240 (135–360)	< 0.001
> 300/μL, <i>n</i> (%)		24 (16.6)	51 (38.6)	< 0.001
Baseline FeNO				
Ppb ^a	0	14 (9–23)	22 (12–39)	< 0.001
≥ 25 ppb, <i>n</i> (%)		34 (20.6%)	72 (46.2%)	< 0.001
Post-challenge change in FeNO, ppb ^{a,d}	0	1 (–1–4)	14 (2–39)	< 0.001
Baseline sputum eosinophils, % ^a	0	0.5 (0–2.0)	1.4 (0.3–4.1)	< 0.001
Post-challenge sputum eosinophils, % ^a	0	0.5 (0–1.5)	5.0 (1.5–15.1)	< 0.001
Post-challenge change in sputum eosinophils				
% ^{a,e}	0	0 (–0.6–0)	2.4 (0–9.9)	< 0.001
≥ 2%, <i>n</i> (%)		4 (2.4)	89 (57.0)	< 0.001

Note: Data are presented as numbers (% of available data) or medians (interquartile ranges). *p* values in boldface are statistically significant.

Abbreviations: FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; HMW, high molecular weight, NSBH, nonspecific bronchial hyperresponsiveness; SIC, specific inhalation challenge.

^aMedian and 25th–75th percentiles.

^bAtopy defined by the presence of a positive skin-prick test to at least one common allergen.

^cAirflow obstruction defined by an FEV₁ < 80% predicted value and an FEV₁/FVC ratio < 0.70.

^dDifference between the post-challenge value measured 24 h after the last challenge exposure and the pre-challenge value expressed in ppb.

^eChanges in sputum eosinophil counts computed as the difference between the post-challenge assessment performed 24 h after the last challenge exposure and the baseline value expressed as percent of nonsquamous cells.

value for identifying an asthmatic reaction during the SIC, yielding a high specificity (95%; 95% CI, 90%–98%) and PPV (90%; 95% CI, 82%–96%), but a low sensitivity (55%; 95% CI, 46%–62%) and NPV (68%; 95% CI, 62%–74%) (Table 2).

Univariate associations with a post-challenge FeNO increase ≥ 13 ppb are detailed in Table S3. The multivariable logistic regression analysis found that, aside from a positive SIC result (odds ratio [OR], 19.3; 95% CI, (9.01–46.40); $p < 0.001$),

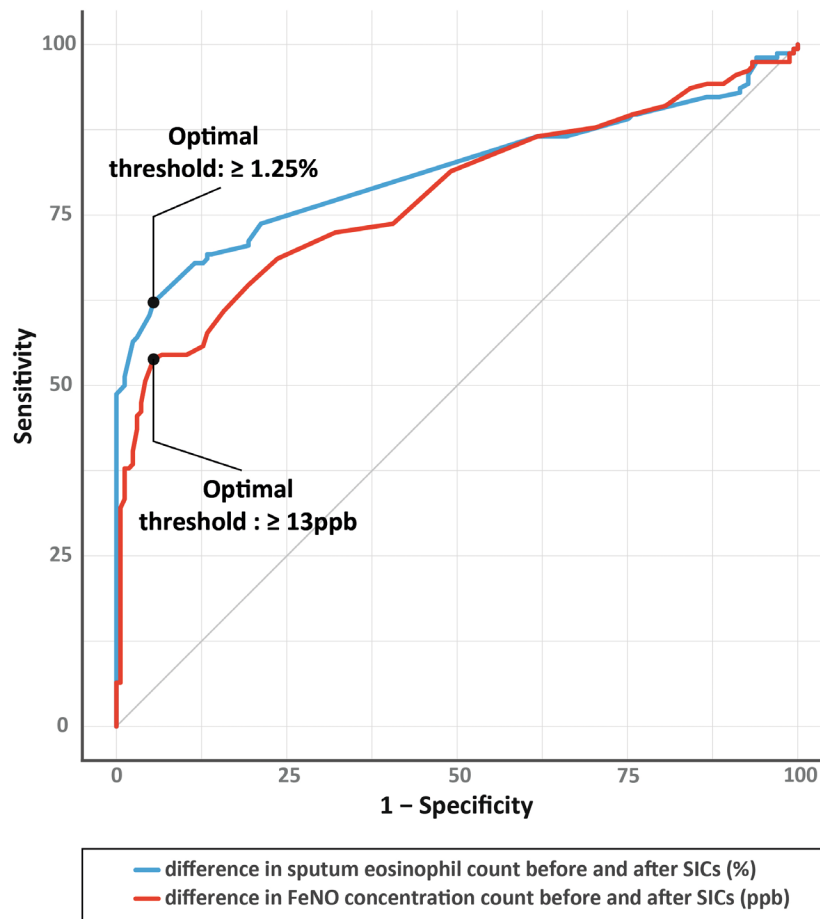


FIGURE 1 | Receiver operating characteristic (ROC) plots for the differences in the absolute percentage change in sputum eosinophils (blue line) and the concentration in FeNO expressed in ppb (red line) between baseline and 24h after challenge exposure for identifying asthmatic reactions. The optimal cut-off value was identified by the highest Youden index value (i.e., sensitivity + specificity – 1). FeNO, fractional exhaled nitric oxide; SIC, specific inhalation challenge.

TABLE 2 | Sensitivity, specificity, and predictive values of post-challenge changes in FeNO and sputum eosinophils.

	FeNO increase ≥ 13 ppb	Sputum eosinophil increase $\geq 1.25\%$	FeNO i increase ≥ 13 ppb or sputum eosinophil increase $\geq 1.25\%$
Sensitivity, % (95% CI)	55 (46–62)	62 (54–70)	77 (70–83)
Specificity, % (95% CI)	95 (90–98)	95 (90–97)	89 (83–93)
Positive predictive value, % (95% CI)	90 (82–96)	92 (84–96)	87 (80–92)
Negative predictive value, % (95% CI)	68 (62–74)	73 (66–78)	80 (74–86)
AUC, (95% CI)	0.78 (0.72–0.83)	0.81 (0.76–0.86)	NA
Youden Index, (95% CI)	0.48 (0.43–0.55)	0.57 (0.51–0.62)	0.66 (0.61–0.71)

Abbreviations: 95% CI, 95% confidence interval; AUC, area under the receiver operating characteristic curve; FeNO, fractional exhaled nitric oxide; NA, not applicable.

work-related rhinitis was the most significant determinant of an increase in post-challenge FeNO ≥ 13 ppb (OR, 2.62; 95% CI, 1.27–5.55; $p < 0.001$), with obesity showing a weak negative association (OR, 0.51; 95% CI, 0.25–0.98; $p = 0.047$) (Table 3).

TABLE 3 | Multivariate analysis of factors associated with a post-challenge increase in FeNO ≥ 13 ppb.

Independent variables	Odds ratio (95% CI)	<i>p</i> value
Initial model		
SIC result, positive vs. negative	19.3 (9.01–46.40)	<0.001
HMW vs. LMW agents	1.50 (0.76–2.95)	0.239
Sex, male vs. female	1.44 (0.76–2.75)	0.265
Body mass index ≥ 30 kg/m ² vs. < 30 kg/m ²	0.50 (0.25–0.98)	0.047
Work-related rhinitis, yes vs. no	1.74 (0.89–3.43)	0.104
Baseline NSBH, present vs. absent	0.66 (0.33–1.31)	0.246
Baseline FeNO ≥ 25 ppb vs. < 25 ppb	1.22 (0.65–2.29)	0.529
Final model		
SIC result, positive vs. negative	19.0 (9.35–43.9)	<0.001
Work-related rhinitis, yes vs. no	2.11 (1.14–3.98)	0.019
Body mass index ≥ 30 kg/m ²	0.51 (0.25–0.98)	0.047

Note: The multivariate logistic regression analysis included 312 subjects. The independent variables incorporated into the initial regression model were selected on the basis of $p < 0.10$ in univariate analysis (Table 3). *p* values in boldface indicated statistical significance.

Abbreviations: 95% CI, 95% confidence interval; FeNO, fractional exhaled nitric oxide; HMW, high molecular weight, LMW, low molecular weight; NSBH, nonspecific bronchial hyperresponsiveness; SIC, specific inhalation challenge.

3.3 | Post-Challenge Changes in Sputum Eosinophils

The AUC for the difference between the post-challenge and the baseline percentage of sputum eosinophils was 0.81 (95% CI, 0.76–0.86) (Figure 1). The ROC curve identified a post-challenge increase $\geq 1.25\%$ from baseline value in sputum eosinophils as the optimal threshold. Using this cut-off, the specificity and PPV of the changes in sputum eosinophils as compared with the SIC results were 95% (95% CI, 90%–97%) and 92% (95% CI, 84%–96%), respectively, with a sensitivity of 62% (95% CI, 54%–70%) and a NPV of 73% (95% CI, 66%–78%) (Table 2). The multivariate logistic regression analysis revealed that a $\geq 1.25\%$ increase in sputum eosinophils was significantly and independently associated with a positive SIC (OR, 28.68; 95% CI, 13.75–67.89; $p = 0.026$) and challenge exposure to HMW agents (OR, 2.08; 95% CI, 1.11–3.96; $p = 0.026$) (Table S4). We conducted logistic regression analyses among SICs with HMW and LMW agents separately (Table S4). Among HMW agents, a $\geq 1.25\%$ increase in sputum eosinophils was associated with a positive SIC result (OR, 60.8; 95% CI, 13.9–446.7; $p < 0.001$) and work-related rhinitis (OR, 8.23; 95% CI, 1.85–46.02; $p = 0.008$), while among LMW agents, the main determinants for an increase in sputum eosinophils were a positive SIC response (OR, 23.81; 95% CI, 10.0–66.50; $p < 0.001$) and a baseline sputum eosinophil count $\geq 3\%$ (OR, 2.75; 95% CI, 1.22–6.37; $p = 0.016$).

3.4 | Relationship Between Changes in FeNO and Sputum Eosinophils

There was a statistically significant—though weak—correlation between the difference in FeNO and sputum eosinophil counts assessed before and after exposure to the offending agent (Spearman correlation coefficient, 0.334; $p < 0.001$). The changes in FeNO and sputum eosinophils showed concordant increases in only 97 out of the 156 (62%) subjects with a positive SIC (Table 4). In the positive SIC group, 36 (23%) subjects demonstrated a $\geq 1.25\%$ increase in sputum eosinophil count without changes in FeNO whereas 23 (15%) had a ≥ 13 ppb rise in FeNO without changes in sputum eosinophilia. Comparison of these two subgroups revealed only a trend toward a higher frequency of baseline NSBH in the 36 “eosinophilic-only responders” (88%) as compared with the 23 “FeNO-only responders” (65%, $p = 0.077$) (data not detailed).

TABLE 4 | Concordance between changes in FeNO and sputum eosinophils.

	Positive SIC			Negative SIC		
	Change in Eos $\geq 1.25\%$	Change in Eos $< 1.25\%$	Total	Change in Eos $\geq 1.25\%$	Change in Eos $< 1.25\%$	Total
Change in FeNO ≥ 13 ppb	61	23	84	0	9	9
Change in FeNO < 13 ppb	36	36	72	9	147	156
Total	97	59	156	9	156	165

Abbreviations: Eos, sputum eosinophils; FeNO, fractional exhaled nitric oxide; SIC, specific inhalation challenge.

In line with the discordance between changes in FeNO and sputum eosinophils, using either a rise in FeNO ≥ 13 ppb or an increase $\geq 1.25\%$ in sputum eosinophils for identifying a positive SIC response increased the sensitivity to 77% (95% CI, 70%–83%) (Table 2).

4 | Discussion

4.1 | Main Findings

To the best of our knowledge, this study is the first that compares the changes in FeNO with those of sputum eosinophils in a large, multicenter cohort of subjects challenged with a wide variety of occupational agents. The findings indicate that post-exposure changes in both FeNO and sputum eosinophils above the threshold values achieve a high specificity and PPV for identifying asthmatic reactions induced by occupational sensitizers, but a low sensitivity and NPV. Nevertheless, combining the assessment of both inflammatory markers increases the sensitivity compared to either of them alone.

Previous studies have produced discordant results regarding the accuracy of FeNO changes in differentiating positive from negative SICs with occupational agents [8]. The AUCs estimated from ROC curves ranged from 0.59 to 0.90 [17–21, 23], and optimal thresholds for the changes in FeNO have been reported from 8 ppb [18] to 13 ppb [21], 17.5 ppb [20], and 25 ppb [19]. Overall, these thresholds have provided a high specificity (90%–92%) for identifying a positive FEV₁ response to occupational agents, with the exception of the study by Sastre and coworkers [19] who reported a specificity of 80%.

HMW agents have been associated with a greater increase in FeNO than LMW agents [20–22], although a marked increase in FeNO has also been documented after exposure to some LMW agents, including isocyanates [14, 16], acrylate compounds [34], and platinum salts [35]. By contrast, we found no role of the molecular weight category of the agents in the outcome and instead identified coexisting work-related rhinitis as a major determinant of the post-challenge changes in FeNO (Table 3). This finding suggests a strong interaction of the upper airways in the production of FeNO in the lower airways. Of note, concomitant work-related rhinitis has not been examined previously as a potential co-factor in FeNO changes after challenge exposure to occupational agents [12, 14–25]. Work-related rhinitis has been strongly associated with OA due to HMW agents [22], however, which might explain the reported associations between these agents and changes in FeNO in studies that did not account for the potential role of the upper airways [20–22].

Demographic and clinical characteristics have been consistently associated with higher (i.e., male sex, atopy) or lower (i.e., smoking, obesity, treatment with ICS) baseline FeNO values [9–11]. Our findings provide evidence that increases in FeNO after an asthmatic reaction induced by occupational agents are not significantly impacted by atopy and smoking (Table S3 and Table 3). In contrast, the multivariate analysis revealed that baseline FeNO levels in our cohort were significantly affected by the same

factors that influence baseline FeNO in nonoccupational asthma (i.e., male sex, current smoking, and atopy) (Table S2). On the other hand, it has been shown that treatment with ICS at the time of the SIC may blunt the post-exposure increase in FeNO [13, 17]. However, this inhibitory effect is not observed when ICS are withdrawn 72 h [17] prior to the challenge, which may account for the absence of an ICS treatment effect in our cohort. Obesity was the only demographic characteristic associated with a suppressive effect on post-exposure increases in FeNO, which is in line with earlier findings on baseline FeNO [11].

The accuracy of the post-challenge changes in FeNO has been compared with the accuracy of sputum eosinophils changes in a limited series only [17]. In that study, changes in FeNO yielded a poor discriminating value with an AUC of 0.6 (95% CI, 0.5–0.8) compared with the changes in sputum eosinophils (AUC 0.8 [95% CI, 0.7–0.9]). However, increases in FeNO might have been blunted by ICS as these medications were not stopped before the SIC in all subjects. In contrast, post-challenge increases in FeNO and sputum eosinophils were similarly highly accurate in our cohort, in which ICS were stopped before the SIC. However, the changes in these two markers were discordant in 38% of the subjects with a positive SIC (Table 4). These data further highlight that the underlying biological pathways leading to airway eosinophilia can be substantially different from those determining FeNO production. Such discordances have been consistently documented for baseline sputum eosinophil counts and FeNO levels in nonoccupational asthma [11, 36–39], but to our knowledge, no available data reported on the dissociation between the FeNO and sputum eosinophil responses after challenge exposure to common aeroallergen. In our cohort, the phenotypic characteristics of the “FeNO-only responders” did not significantly differ from those of the “eosinophilic-only responders”, with the exception of a nonsignificant trend toward more frequent baseline NSBH in the latter group. Determining the biological pathways leading to a FeNO versus an eosinophilic response after allergen exposure require further investigation.

4.2 | Clinical Implications

Although SIC is still acknowledged as the “reference standard” for demonstrating a causal relationship between asthma and exposure to an occupational agent [2, 27, 40], these tests may result in false-negative results, especially when specific bronchial reactivity to the causal agent has declined after cessation of workplace exposure [41]. Accordingly, there is a need to identify markers of bronchial reactivity to sensitizing occupational agents that are more sensitive than the changes in FEV₁ [27]. It has been previously documented that an increase in sputum eosinophils may precede functional changes during SIC [7] and a post-challenge increase in sputum eosinophil counts has a high accuracy for differentiating positive from negative SICs with an AUC of 0.86 [95% CI, 0.8–0.9] [4]. Our findings strongly suggest that the assessment of exposure-related changes in both FeNO levels and sputum eosinophil counts may provide useful supportive evidence of a significant bronchial response during SICs that produce equivocal changes in FEV₁ without the need for performing additional challenge exposure in the laboratory and/or at the workplace.

The accuracy of exposure-related changes in FeNO and sputum eosinophils during SIC found in our cohort suggests that their measurements away from work and 24 h after a work shift could complement or even be an alternative to peak expiratory flow recordings when SICs are either unavailable or unfeasible. Girard and coworkers [6] found that an increase in sputum eosinophils $\geq 2\%$ when at work had a sensitivity of 52% (95% CI, 33%–71%) and a specificity of 80% (95% CI, 61%–91%) in predicting a positive SIC result. In contrast, the usefulness of serial assessments of FeNO at and away from work has been suggested only by a few case reports [42–44] and one case series [45], although the results of FeNO measurements were not validated against SICs in most of these cases [42, 43, 45]. However, determining the accuracy of FeNO measurements and sputum cell counts at and away from work would require further prospective validation against SICs.

4.3 | Limitations of the Study

We acknowledge the need for caution in interpreting the predictive values of the changes in FeNO and sputum eosinophils that may have limited generalizability given the high prevalence (i.e., the pre-test probability) of the condition in the tested population (49%). The diagnostic algorithm for performing a SIC varied among centers (Appendix S3). However, the vast majority (80%) of the subjects included in this analysis were recruited from four tertiary centers where all subjects referred for possible OA completed a SIC with the suspected agent(s) regardless of the results of other diagnostic tests, with no statistical difference in the proportion of positive SICs among the participating centers. Nevertheless, we cannot exclude a selection bias toward the referral to tertiary centers of subjects with a high pre-SIC clinical suspicion of OA. The potential for this referral bias is further supported by a higher rate of positive SIC among subjects challenged with HMW agents (70%) compared to those exposed to LMW agents (43%). This difference suggests a selection bias likely driven by the results of immunological tests for HMW agents that are available in primary and secondary care. However, a prospective study including SIC in a large sample of subjects with work-related asthma symptoms recruited in primary or secondary care would be almost unfeasible.

Given the retrospective study design, FeNO measurement and sputum processing were not completely standardized among participating centers. However, it seems unlikely that methodological aspects might have significantly affected the findings of this study. Studies comparing various chemiluminescence analyzers and electrochemical sensors have shown a high degree of correlation between measurements across all devices [46]. Available evidence also indicates that differences in sputum sample processing do not substantially affect the differential sputum cell counts [47–50].

The optimal threshold values for the post-challenge changes in FeNO (i.e., 13 ppb) and sputum eosinophils (i.e., 1.25%) identified in this cohort are very low and may question their relevance in clinical practice. The threshold value of 13 ppb for the changes in FeNO is in line with previous studies reporting thresholds ranging from 8 ppb [18] to 13 ppb [21], 17.5 ppb [20], and 25 ppb [19] after inhalation challenges with occupational agents. Our threshold value of 1.25% for the changes in sputum

eosinophils is lower than the cutoff of 2% usually considered to be the most discriminant value for predicting the development of a 20% fall in FEV₁ [51]. Girard et al. also identified a $\geq 1\%$ increase during periods at work compared with periods away from work as the optimal threshold for predicting a positive SIC in the laboratory [6]. Nevertheless, these authors recommended to use a cutoff of 2% as being more clinically significant. The low threshold values found in our cohort likely resulted from the very narrow changes in post-challenge FeNO and sputum eosinophils in negative SICs (Table 1), indicating a high reproducibility of these measures when performed at a 24-h interval. Further prospective studies are, however, required to determine the optimal thresholds for the changes in FeNO and sputum eosinophils when these indices are measured over weeks in subjects evaluated at and away from work [6].

5 | Conclusion

Despite the limitations inherent to the retrospective design of the study, the data indicate that exposure-related changes in FeNO and sputum eosinophils above threshold values strongly support a diagnosis of OA. Assessment of both FeNO and sputum eosinophils should be regarded as reliable complementary tools to lung function tests for identifying bronchial responses to occupational agents during SICs and workplace exposures.

Author Contributions

All authors contributed to the conception and design of the study as well as the acquisition, analysis and interpretation of the data. They provided input into the drafting of the manuscript, critical feedback, and final approval for submission of the manuscript for publication. V.D is the guarantor of the final content of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Research data are not shared.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.