

PROF. OLIVIER VANDENPLAS (Orcid ID : 0000-0002-4608-3310)

Article type : Original Article: Airway Diseases

ASSESSMENT OF EOSINOPHILIC AIRWAY INFLAMMATION AS A CONTRIBUTION TO THE DIAGNOSIS OF OCCUPATIONAL ASTHMA

Carolina Beretta^{1,2}, Catherine Riffart¹, Geneviève Evrard¹,

Jacques Jamart³, Joël Thimpont⁴, and Olivier Vandenplas^{1,4}

Affiliations:

¹ *Université catholique de Louvain, Centre Hospitalier Universitaire UCL Namur, Department of Chest Medicine, Yvoir, Belgium;*

² *Specialization School in Occupational Medicine, University of Pavia and Istituti Clinici Scientifici Maugeri, Unità Operativa di Medicina del Lavoro, Sezione di Allergologia, Pavia, Italy.*

³ *Université catholique de Louvain, Centre Hospitalier Universitaire UCL Namur, Scientific Support Unit, Yvoir, Belgium;*

⁴ *Fedris (Agence fédérale pour les risques professionnels), Service Médical, Brussels, Belgium*

Short title: Eosinophilic inflammation in occupational asthma

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13265

This article is protected by copyright. All rights reserved.

Correspondence author: Dr Olivier Vandenplas, Department of Chest Medicine, *Centre Hospitalier Universitaire UCL Namur*, B-5530 Yvoir, Belgium; Tel: +32-81 42 33 63;

E-mail: olivier.vandenplas@uclouvain.be

Funding: This work was supported by a grant from the *Fondation Louvain (Legs Pierre De Merre)*. Carolina Beretta was supported by the *Ministero dell'Istruzione, dell'Universita' e della Ricerca, Università degli Studi di Pavia*.

Conflict of interest: There is no conflict to declare in relation to this article. The sponsors had no role in the design of the study, the collection and analysis of the data, or in the preparation of the manuscript.

Authors' contribution: CB, JT, and OV were involved in the conception and design of the study, interpretation of the data, and drafting and reviewing of the manuscript. CB, CR, GE and JJ were involved in data collection, analysis of data and reviewing of the manuscript. OV supervised specific inhalation challenges and is the guarantor of the final content of the manuscript.

List of abbreviations:

FeNO: Fractional exhaled nitric oxide

FEV₁ : Forced expiratory volume in one second

ICS: Inhaled corticosteroids

OA: Occupational asthma

PC₂₀: Provocative concentration of histamine causing a 20% fall in FEV₁

Ppb: Parts per billion

SIC: Specific inhalation challenge

Abstract

Background: Ascertaining the presence of asthma through the assessment of nonspecific bronchial hyperresponsiveness (NSBH) is a key step in the diagnosis of occupational asthma (OA). We aimed at investigating whether indices of airway inflammation including fractional exhaled nitric oxide (FeNO) and sputum eosinophils would be useful adjuncts to the measurement of NSBH in diagnosing OA defined as a positive specific inhalation challenge (SIC).

Methods: The study included 240 consecutive subjects with a suspicion of OA who completed a SIC, of whom 133 showed a positive response. The sensitivity, specificity, and predictive values of NSBH, and FeNO, as well as sputum eosinophil counts assessed at baseline of the SIC were determined.

Results: A concentration of histamine inducing a 20% decline in FEV₁ (PC₂₀) ≤16 mg/mL showed a sensitivity of 87% and a specificity of 36%. A FeNO level ≥25 ppb and a sputum eosinophil count ≥ 2% provided lower sensitivity rates (47% and 39%, respectively) than the PC₂₀ value. Eight of the 17 subjects without baseline NSBH despite a positive SIC showed a sputum eosinophil count ≥2%, a FeNO level ≥25 ppb or both outcomes. Combining either a PC₂₀ value ≤16mg/mL or a FeNO ≥25 ppb increased the sensitivity to 91%. Using either a PC₂₀ ≤16mg/mL or a sputum eosinophil count ≥1% increased the sensitivity to 94%.

Conclusion: Adding the assessment of FeNO level and sputum eosinophils to NSBH improves the identification of subjects who may have OA and require further objective testing before excluding the possibility of OA.

Keywords: Asthma; bronchial provocation tests; eosinophils; nitric oxide; occupational diseases; sputum

INTRODUCTION

The diagnosis of sensitizer-induced occupational asthma (OA) remains challenging and most often requires a stepwise approach (1-6). It is widely recommended that the initial step in diagnosing OA is to document the presence of asthma through the demonstration of reversible airflow obstruction or increased nonspecific bronchial hyperresponsiveness (NSBH) in subjects without demonstrable airflow limitation (1-6). The presence of NSBH has a low specificity (~50%), but a reasonable sensitivity (73-84%) in establishing a diagnosis of OA (~55%) (1, 6, 7). Therefore, the latest American College of Chest Physicians guidelines stated that *“the absence of NSBH has a fairly high negative predictive value for asthma and, by inference, for OA (when assessed within 24h after exposure), and generally can be used to rule out active disease”* (3). This recommendation has been recently confirmed in a large retrospective study that further emphasized the importance of performing the assessment of NSBH while the subjects are still at work (8) since NSBH may improve rapidly and even return to normal after cessation of exposure to the causal agent (9-11). However, in current practice, assessment of NSBH while at work is unavailable in a substantial proportion (ranging from 53% to 85%) of subjects evaluated for possible OA (8, 12) because they are often removed from the offending environment before referral for diagnostic evaluation. In this setting, the sensitivity and the NPV of NSBH assessment decreased respectively to 67% and 82% (8).

There is considerable interest in the non-invasive assessment of eosinophilic airway inflammation through sputum cell analysis and measurement of fractional exhaled nitric oxide (FeNO) as complementary tools to lung function tests in the diagnosis of asthma (13, 14) and OA (15). Nevertheless, sputum induction and processing are time consuming, require technical expertise, and are unsuccessful in a substantial fraction of the subjects. By contrast, the measurement of FeNO as a surrogate marker for eosinophilic airway inflammation is simple, fast, and feasible in almost all patients (14). In addition, current evidence highlights an important degree of discordance between NSBH, sputum

eosinophilia, and FeNO level, indicating that these indices reflect different dimensions of asthma (14, 16-19). Although the changes in sputum eosinophils and FeNO induced by exposure to occupational sensitizing agents have been thoroughly investigated (15, 20-22), there is little information on whether the assessment of these indices of airway inflammation could help the clinicians to predict a diagnosis of OA as compared to the result of a SIC (7, 23).

The aim of this study was to evaluate whether the assessment of FeNO and sputum eosinophils could provide additional useful information to the measurement of NSBH in the diagnostic evaluation of OA, especially in those subjects who fail to demonstrate NSBH at initial evaluation.

METHODS

Study Design and Subjects

This prospective observational study included consecutive subjects who had been investigated for possible OA in a tertiary centre (*Centre Hospitalier Universitaire UCL Namur*) through the performance of a SIC from June 2006 to July 2016. In our centre, SICs with occupational agent(s) are routinely performed to diagnose OA irrespective of the presence or absence of baseline NSBH, provided that the baseline FEV₁ is equal to or above 60% of predicted value. The subjects were referred by either their attending physician or the Belgian Workers' Compensation Board (*Fedris*) (69% of the subjects). All Belgian French-speaking workers who submit a claim for work-related asthma are referred to our centre in order to perform an SIC procedure. Subjects were included in this study if they had performed a baseline assessment of FeNO; 26 subjects were excluded from analysis because FeNO measurement was lacking due to technical failure.

Ethics

The study protocol was approved by the *Comité d'Ethique Médicale* of the *Centre Hospitalier Universitaire UCL Namur* (approval number B03920072360). The participants signed a statement of informed consent.

Procedures

Specific inhalation challenges

SICs were completed according to a previously described protocol (20, 22, 24) as well as international recommendations (25). Briefly, occupational agents were generated in specifically dedicated challenge rooms using a 'realistic' approach aimed at mimicking the condition of exposure at work based on the assessment of an occupational hygienist of the Belgian Workers' Compensation Board. Asthma medications were withdrawn according to their duration of action, while ICS were halted 72 hours prior to the tests.

All subjects completed a first day of testing during which they were exposed to a 'control' substance for 30 minutes to ensure that fluctuations in forced expiratory volume in one second (FEV₁) were $\leq 12\%$ of the baseline value. Spirometry (26) was measured at baseline and serially for at least 6 hours after exposure. Assessment of baseline NSBH to histamine and 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 to the suspected occupational agents was gradually increased until a $\geq 20\%$ fall in FEV₁ occurred or a cumulative exposure of two hours on the same day was completed. Those subjects who did not demonstrate a $\geq 20\%$ fall in FEV₁ during the first active challenge day systematically completed a second challenge for a maximum of two hours on the following day. An SIC was considered positive when a reproducible fall in FEV₁ of 20% or more as compared to pre-challenge value was recorded. Additional challenges

were proposed when there was a significant (>3-fold) decrease in the post-challenge histamine PC₂₀ value as compared to the baseline value (27).

Assessment of nonspecific bronchial hyperresponsiveness

The concentration of histamine inducing a 20% fall in FEV₁ (PC₂₀) was assessed at the end of the control day (i.e. the baseline value) through the tidal breathing method (28). The presence of NSBH was defined as a PC₂₀ value ≤16 mg/mL (29). After the histamine challenge test, the subjects were administered an inhaled bronchodilator (salbutamol 400 µg) and sputum was induced.

Sputum induction and processing

Sputum induction was carried out at the end of the control day by inhaling increasing concentrations (3%, 4%, and 5%) of nebulized hypertonic saline as previously described (20, 24, 30). Cut-off values of 1%, 2%, and 3% were used to define significant sputum eosinophilia (7, 31).

FeNO measurements

The baseline level of FeNO was measured before challenge exposure using a chemoluminescence analyser or an electrochemical sensor-based device (NIOX and NIOX MINO, Aerocrine AB, Solna, Sweden) at a flow rate of 50 mL/s in accordance with international recommendations (32, 33). The FeNO values were categorized as low (<25 ppb) and high (≥50 ppb) as recommended by the American Thoracic Society clinical practice guidelines (13).

Analysis of data

Quantitative data are presented as median with 25th and 75th percentiles. Comparison between subgroups of subjects was made using Wilcoxon rank sum test, chi-square, or Fisher's exact test as appropriate. Multivariate logistic regression analysis with backward

Accepted Article

selection based on Wald test was conducted among subjects with a positive SIC to assess whether smoking habits, atopy, treatment with ICS, time elapsed between the last work exposure to the offending agent and the SIC procedure (≤ 2 days vs. > 2 days), level of exposure to the suspected agent at the time of SIC (“persistent exposure” vs. “reduced exposure” vs. “no exposure”), and type of agent (high-molecular-weight vs. low-molecular-weight agents) were associated with a baseline histamine PC₂₀ value ≤ 16 mg/mL, a FeNO level ≥ 25 ppb, and a sputum eosinophil count $\geq 2\%$ using the IBM SPSS Statistics V.19.0 software (SPSS Inc, Chicago, IL, USA). The sensitivity, specificity, PPV, and NPV with 95% confidence intervals (CI) of baseline NSBH, FeNO level, and sputum eosinophil counts as compared to the SIC result were calculated using the MedCalc 9.4.2.0 software (MedCalc, Mariakerke, Belgium). A P-value < 0.05 was considered significant.

RESULTS

Population characteristics

The study included 240 subjects, of whom 133 (55%) showed a positive SIC response. The demographic and clinical characteristics of the subjects with positive and negative SIC results are presented in Table 1. Subjects with a positive SIC were more often atopic (56% vs. 40%, $p=0.013$) and showed a slightly lower baseline FEV₁/forced vital capacity ratio (median [25th-75th percentiles]: 73% [66-79] vs. 75% [69-81], $p=0.019$). HMW agents were more often involved in positive (60%) than in negative SICs (26%, $p < 0.001$).

Baseline nonspecific bronchial hyperresponsiveness

A histamine PC₂₀ value ≤ 16 mg/mL showed a sensitivity of 87% and a specificity of 36% for a positive SIC, with a PPV of 63% and an NPV of 69% (Table 2). The multivariate logistic regression analysis conducted among the subjects with a positive SIC showed that a baseline PC₂₀ value ≤ 16 mg/mL was associated with ICS use (odds ratio [OR]: 8.0; 95% confidence interval [CI]: 1.8-34.5; $p=0.002$), a FEV₁/FVC ratio $< 70\%$ (OR: 10.2; 1.1-90.9;

p=0.009), exposure to a HMW agent (OR: 7.2; 1.4-37.7; p=0.008), a delay since last work exposure ≤ 2 days (OR: 20.4; 1.9-200), and persistent exposure to the causal agent (OR: 5.8; 1.6-20.5).

Baseline level of fractional exhaled nitric oxide

When compared to the PC₂₀ value (Table 2), FeNO levels ≥ 25 ppb and ≥ 50 ppb yielded lower sensitivity (47% and 20%, respectively), but higher specificity (71% and 94%, respectively) for a positive SIC result. The multivariate logistic regression analysis among subjects with a positive SIC showed that the likelihood of a FeNO level ≥ 25 ppb increased when the subjects were atopic (OR: 2.9; 1.4-6.0; p=0.004) and never smokers (OR: 2.5; 1.2-5.0; p=0.01).

Baseline sputum eosinophils

Seventy-nine of 133 (59%) subjects with a positive SIC and 59 of 107 (55%) subjects with a negative SIC provided a suitable sputum sample at baseline. Subjects who produced a suitable sputum sample did not differ from those who could not do so with regard to baseline histamine PC₂₀ value (median [25th-75th percentile]: 4.0 mg/mL [1.3-14.0] vs. 4.0 mg/mL [1.4-15.1]) and FeNO level (19 ppb [10-37] vs. 17 ppb [10-33]).

As shown in Table 2, baseline eosinophil counts $\geq 1\%$, 2% , and 3% provided lower sensitivity rates (72%, 39%, and 29%, respectively), but higher specificity rates (46%, 68, and 78%, respectively) as compared to the PC₂₀ value. Sputum eosinophil counts showed a moderate, though significant correlation with FeNO levels ($r_s=0.41$; p <0.001). In the logistic regression analysis, none of the *a priori* selected clinical characteristics were significantly associated with a sputum eosinophil count $\geq 2\%$ at baseline assessment.

Combining the assessment of NSBH and indices of airway inflammation

Coupling a histamine PC₂₀ ≤16mg/mL with a high FeNO level increased the specificity of the PC₂₀ value for a positive SIC result from 36% to 78% for a cut-off value of 25 ppb and 94% for a cut-off value of 50 ppb, but decreased the sensitivity from 87% to 44% and 20%, respectively (Table 2). Combining a PC₂₀ ≤16 mg/mL with a sputum eosinophil count ≥2% or ≥3% also increased the specificity to 76% and 81%, respectively, but reduced the sensitivity to 33% and 27%.

By contrast, using either a PC₂₀ ≤16mg/mL or a FeNO ≥25 ppb increased the sensitivity for a positive SIC from 87% by using the PC₂₀ value alone to 91%, while the specificity decreased to 29% (Table 2). Using either a PC₂₀ ≤16mg/mL or a sputum eosinophil count ≥1% and ≥2% also increased the sensitivity to 94% and 91%, respectively, while the specificity decreased to 17% and 25%. The sensitivity and specificity of a PC₂₀ value ≤16 mg/mL and the markers of eosinophilic airway inflammation, either alone or in combination, for predicting the result of the SIC were not significantly affected by the patient's work exposure status (i.e. complete removal, reduced exposure, persistent exposure) at the time of the SIC procedure (Online Table 1).

Characteristics of subjects without baseline NSBH

Among the 133 subjects with a positive SIC, 17 (13%) failed to demonstrate a baseline histamine PC₂₀ value in the asthmatic range. The results of the assessments of NSBH and indices of airway inflammation in these subjects are detailed in Table 4. Five of them showed a baseline FeNO level ≥25 ppb. Twelve of the 17 subjects were able to produce a suitable sputum sample and seven of them showed a baseline eosinophil count ≥1%. Altogether, ten (59%) of the 17 subjects showed either a FeNO level ≥25 ppb, a sputum eosinophil count ≥2%, or both of these outcomes (n=3).

At post-challenge assessment completed 24 hours after the end of exposure to the occupational agent (Table 4), eight of the 17 subject demonstrated a histamine PC₂₀ value ≤16 mg/mL and one subject showed airway obstruction with a >15% improvement after an inhaled bronchodilator. A post-challenge measurement of FeNO was available in 16 subjects and 12 of them showed a FeNO level ≥25 ppb. Twelve of the 17 subjects had sputum induction performed before and after the SIC; all of them showed a post-challenge eosinophil count ≥1%. The eight subjects with a post-challenge PC₂₀ value ≥16 mg/mL displayed a post-challenge sputum eosinophil count ≥1% and three of them showed a post-challenge FeNO level ≥25 ppb (Table 4).

DISCUSSION

This study showed that a substantial proportion (59%) of subjects with ascertained OA who failed to demonstrate baseline NSBH displayed either a FeNO level ≥25 ppb or a sputum eosinophil count ≥1%. Therefore, indices of eosinophilic airway inflammation increase the sensitivity of the assessment of NSBH alone in identifying subjects who may suffer from OA. To our knowledge, this is the first study that investigated the usefulness of both the FeNO level and sputum eosinophil count in addition to the level of NSBH in identifying OA as compared to the SIC result. Although the FeNO level and sputum eosinophil count alone provided overall lower sensitivity rates than the measurement of NSBH (Table 2), combining either a histamine PC₂₀ ≤16mg/mL or a FeNO ≥25 ppb increased the sensitivity for identifying subjects with OA from 87% (95% CI: 80-92%) for the PC₂₀ value alone to 91% (95% CI: 85-95%). Similarly, using either a PC₂₀ ≤16mg/mL or a sputum eosinophil count ≥1% increased the sensitivity to 94% (95% CI: 86-98%). These sensitivity rates are similar to those reported by Pralong et al. for the methacholine challenge (i.e. 95% to 98%) in subjects exposed at work at the time of NSBH assessment (8). Accordingly, combining either a histamine PC₂₀ ≤16mg/mL or a FeNO ≥25 ppb or a sputum eosinophil count ≥1% should

have a high NPV in settings where the pre-test probability of OA is low, especially in secondary respiratory health centers or in workplace health surveillance programs.

The sensitivity and specificity of baseline assessment of the histamine PC₂₀ value in our cohort (87% and 36%, respectively) were in line with the pooled estimates derived from a meta-analysis of studies reporting the sensitivity (84% [95% CI: 67-93%]) and specificity (48% [95% CI: 26 to 72%]) of a single assessment of NSBH in the diagnosis of OA caused by various HMW and LMW agents (1). Our figures were also consistent to the sensitivity (80%) and specificity (47%) recently reported by Pralong et al. (8) in a tertiary center in Quebec, in which 58% of the subjects were no longer exposed at work. Of note, the NPV of NSBH reported in their whole cohort was substantially higher (86%) than in our population (69% [95% CI: 55-81%]) because of the lower prevalence of positive SICs in their cohort (28%) as compared to ours (55%). If the sensitivity of the histamine challenge found in our study was to be applied to the population investigated by Pralong et al. (8), the NPV would be similar (i.e. 88%) to the figure reported by these investigators.

There is currently scarce information on whether assessment of airway inflammation could help the clinicians to predict a diagnosis of OA as compared to the result of a SIC. The sensitivity and specificity of baseline sputum eosinophil counts in this study (Table 2) were consistent with those reported by Malo et al. (7). In this large cohort, a baseline eosinophils count $\geq 1\%$ and $\geq 3\%$ yielded sensitivity rates of 50% and 29%, respectively, and specificity rates of 67% and 86%. Coupling the information on sputum eosinophils to the result of the methacholine challenge increased the specificity from 51% for a PC₂₀ value ≤ 16 mg/mL alone to 81% and 91% using cut-off eosinophil thresholds $\geq 1\%$ and $\geq 3\%$, respectively. In our cohort, the specificity of sputum eosinophils was lower, reaching only 81% (95% CI: 69-90%) when a histamine PC₂₀ value ≤ 16 mg/mL was combined with an eosinophil count $\geq 3\%$.

A recent meta-analysis concluded that the overall specificity (82% [95%CI: 76-86%]) of FeNO for making the diagnosis of asthma was higher than the sensitivity (65% [95% CI: 58-72%]), indicating a higher diagnostic potential for ruling in than for ruling out the diagnosis of asthma (34). So far, only one study investigated the usefulness of a single FeNO measurement in predicting the response to the SIC (23). These investigators documented a specificity of 80% and a sensitivity of 60% for baseline FeNO level ≥ 25 ppb, but they did not compare the diagnostic performance of FeNO and NSBH. In our cohort, a high FeNO level ≥ 50 ppb (13) yielded a high specificity of 94% (95% CI: 88-98%) for diagnosing OA, although the PPV was only 82% (95% CI: 64-93%) and would even be lower in populations with a lower prevalence of OA.

Among the 17 subjects without baseline NSBH despite a diagnosis of OA ascertained by a positive SIC (Table 4), 59% showed a sputum eosinophil count $\geq 1\%$ and/or a FeNO level ≥ 25 ppb. However, the predictive values of FeNO levels and sputum eosinophil counts were low when considering all subjects without baseline NSBH. Among these subjects, only 42% of those with a FeNO level ≥ 25 ppb and 41% of those with an eosinophil count $\geq 1\%$ showed a positive SIC response (Table 3). Conversely, 72% of those with a FeNO level < 25 ppb and 67% of those with eosinophils $< 1\%$ showed a negative SIC. Of note, eight (6%) of the 17 subjects without baseline NSBH still showed a PC₂₀ value in the nonasthmatic range after the positive SIC response. Of these, all displayed a post-challenge sputum eosinophil count $\geq 1\%$ and three of them also showed a FeNO level ≥ 25 ppb (Table 4). Pralong et al. reported a similar proportion (8%) of subjects with OA who failed to show NSBH both before and after the SIC. These investigators also found that most of their subjects who had sputum induction showed post-challenge sputum eosinophilia. These findings further highlight the discordances between NSBH and markers of eosinophilic airway inflammation that have already been well documented in the diagnosis of non-occupational asthma (14, 16-19) and OA (24, 35), indicating that these indices measure different underlying pathophysiologic mechanisms.

Accepted Article

Several considerations deserve attention for interpreting our findings. The main limitation of this study results from the higher proportion (55%) of positive SIC as compared to other tertiary centers. Notably, this proportion was 28% in the Quebec cohort (8) and ranged from 21% to 61% (median, 28%) in seven other European centres where SICs were performed routinely over the last 10 years (F. de Blay, unpublished data, European Academy of Allergy and Clinical Immunology Task Force on Occupational Asthma). It is likely that our population included more selected subjects who were “prescreened” by chest or occupational physicians before seeking specialised medical advice or filing a claim for compensation. Accordingly, the predictive values of NSBH and indices of airway inflammation derived from our cohort cannot be extrapolated to other healthcare settings since NPVs and PPVs depend on the prevalence of the condition in the tested population (i.e. the pre-test probability of the disease) (1, 3). In addition, the limited number of subjects without baseline NSBH precluded reliable comparison of the respective diagnostic performance of FeNO and sputum eosinophils in this subset of patients. Notwithstanding, the major strength of this study is that NSBH and markers of eosinophilic airway inflammation were prospectively assessed over a 10-year period in a tertiary referral unit where SIC were performed routinely using the same protocol (25). Another strength is that we assessed the diagnostic performance of NSBH and markers of eosinophilic airway inflammation using pre-established thresholds providing either high sensitivity or high specificity which are more useful for clinicians than the ‘optimal’ cut-off values derived from receiver-operating curves since they allow for ruling-out (high sensitivity required) or ruling-in (high specificity required) a diagnosis of OA in a triage approach.

In conclusion, depending on the pre-test probability of OA in the target population, the assessment of FeNO and/or sputum eosinophils may provide a signal that is helpful in identifying patients who are likely to develop an asthmatic response on exposure to the suspected occupational agent and should complete further investigation before excluding a diagnosis of OA (1-6). FeNO and sputum eosinophils should be assessed in subjects

Accepted Article

evaluated for work-related asthma who fail to demonstrate NSBH and cannot be returned to their usual workplace. Further prospective studies are required to validate these findings and develop diagnostic probabilistic models that combine clinical features, NSBH and markers of eosinophilic inflammation in order to improve the diagnostic approach of OA.

Table 1. Demographic, clinical, and functional characteristics of the subjects

Characteristic	Negative SIC (n=107)	Positive SIC (n=133)	P-value [†]
Gender (male), n (%)	57 (53)	83 (62)	0.154
Age, yr*	41 (33-51)	40 (33-49)	0.480
Never/ever smoker, n (%)	52/55 (49/51)	77/56 (58/42)	0.151
Atopy [†] , n (%)	43 (40)	75 (56)	0.013
Preexisting asthma, n (%)	26 (24)	38 (29)	0.457
Work-related rhinitis, n (%)	73 (68)	101 (76)	0.183
Referral by workers' compensation board, n (%)	61 (57)	96 (72)	0.014
Duration of work exposure before asthma onset, mo*	84.0 (37.8-221.0) (n=106)	77.5 (36.0-203.8) (n=130)	0.559
Duration of symptoms at work, mo*	19.0 (8.0-53.0) (n=103)	26.0 (12.5-57.0) (n=129)	0.166
Delay between last exposure and SIC:			0.984
Median value, mo*	3.0 (0.1-15.0)	6.0 (0.1-13.0)	
≤2 days, n (%):	34 (32)	47 (35)	0.672
Level of exposure to the suspected agent, n (%):			0.323
Complete avoidance	54 (50)	58 (44)	
Reduction	26 (24)	44 (33)	
Persistent	27 (25)	31 (23)	
Medication, n (%):			
Short-acting beta ₂ -agonist	62 (58)	73 (55)	0.635
ICS, n (%)	70 (65)	79 (59)	0.339
ICS, daily dose, mg*	500 (0-1000)	500 (0-1000)	0.803
Long-acting beta ₂ -agonist	57 (53)	75 (56)	0.629
FEV ₁ , % pred*	89 (82-99)	88 (79-100)	0.403
FEV ₁ <80% pred, n (%)	21 (20)	34 (26)	0.277
FEV ₁ /FVC, %*	75 (69-81)	73 (66-79)	0.019
FEV ₁ /FVC <70%, n (%)	33 (31)	48 (36)	0.393
Airway obstruction, n (%)*	16 (15)	25 (19)	0.432
Histamine PC ₂₀ :			
Median value, mg/ml*	7.6 (3.1-32.0)	2.6 (1.0-7.6)	<0.001
>16 mg/ml, n (%)	38 (36)	17 (13)	<0.001
HMW agents, n (%):			<0.001
Flour, cereals	28 (26)	80 (60)	
Latex	19 (18)	52 (39)	
Enzymes	6 (6)	16 (12)	
Plant-derived products	2 (2)	7 (5)	
Plant-derived products	1 (1)	5 (4)	
LMW agents, n (%):			
Quaternary ammonium compounds	79 (74)	53 (40)	
Disinfectants, cleaning agents (NOS)	8 (7)	14 (10)	
Persulfate salts	14 (13)	8 (6)	
Isocyanates	13 (12)	5 (4)	
Resins, glues, paints (NOS)	6 (6)	10 (8)	
Woods	14 (13)	2 (2)	
Acrylate compounds	12 (11)	4 (3)	
Metals	4 (4)	2 (2)	
Welding	1 (1)	4 (3)	
Miscellaneous LMW agents	2 (2)	2 (2)	
Miscellaneous LMW agents	5 (5)	2 (2)	
Pattern of asthmatic reaction, n (%):			
Immediate	-	79 (59)	
Dual	-	20 (15)	
Atypical	-	34 (26)	

Legend: FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; HMW: high molecular weight agents; LMW: low-molecular weight agents. NOS: not otherwise specified; PC₂₀: concentration of histamine inducing a 20% fall in FEV₁; SIC: specific inhalation challenge.

* Median value with 25th-75th percentiles in parentheses;

† Atopy is defined by a positive skin-prick test reaction to one or more common inhalant allergens ‡

¥ Airway obstruction defined by a FEV₁ <80% predicted value and a FEV₁/FVC ratio <70%.

Table 2. Sensitivity, specificity, and predictive values of histamine challenge, FeNO, and sputum eosinophils

Baseline assessment	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Histamine PC ₂₀ : ≤16 mg/ml	87 (80-92)	36 (26-45)	63 (55-70)	69 (55-81)
FeNO level: ≥25 ppb	47 (39-56)	71 (62-79)	67 (57-76)	52 (44-60)
≥50 ppb	20 (14-28)	94 (88-98)	82 (64-93)	49 (42-56)
Sputum eosinophil count (n=138): ≥1%	72 (61-82)	46 (33-59)	64 (53-74)	55 (40-69)
≥2%	39 (28-51)	68 (54-79)	62 (47-75)	46 (35-56)
≥3%	29 (19-40)	78 (65-88)	64 (46-79)	45 (35-55)
Histamine PC ₂₀ ≤16mg/ml AND: FeNO ≥25 ppb	44 (35-52)	78 (68-85)	71 (62-78)	53 (48-57)
FeNO ≥50 ppb	20 (13-27)	94 (88-98)	81 (65-91)	49 (46-51)
Sputum eosinophils ≥1%	63 (52-74)	63 (49-75)	69 (61-77)	56 (47-64)
Sputum eosinophils ≥2%	33 (23-44)	76 (63-86)	65 (52-76)	46 (41-51)
Sputum eosinophils ≥3%	27 (17-38)	81 (69-90)	66 (50-78)	45 (41-50)
Histamine PC ₂₀ ≤16 mg/ml OR: FeNO ≥25 ppb	91 (85-95)	29 (21-39)	61 (58-65)	72 (58-83)
FeNO ≥50 ppb	88 (81-93)	36 (26-45)	63 (59-66)	70 (58-80)
Sputum eosinophils ≥1%	94 (86-98)	17 (8-29)	60 (57-63)	67 (42-85)
Sputum eosinophils ≥2%	91 (83-96)	25 (15-38)	62 (58-66)	68 (48-83)
Sputum eosinophils ≥3%	87 (78-94)	30 (19-44)	63 (58-67)	64 (47-78)

Legend: 95% confidence intervals are presented between parentheses; FeNO: fractional concentration in nitric oxide; PC₂₀: provocative concentration of histamine causing a 20% fall in FEV₁.

Table 3. Indices of airway inflammation at baseline of SIC

Baseline assessment	Negative SIC (n=107)			Positive SIC (n=133)		
	PC ₂₀ >16 mg/ml	PC ₂₀ ≤16 mg/ml	P-value [†]	PC ₂₀ >16 mg/ml	PC ₂₀ ≤16 mg/ml	P-value [†]
FeNO:	(n=38)	(n=69)		(n=17)	(n=116)	
Median value, ppb*	14.4 (7.8-22.2)	14 (8.7-33.0)	0.316	18 (17.0-35.3)	25.2 (10.9-46.0)	0.751
≥25 ppb, n (%)	7 (18)	24 (35)	0.074	5 (29)	58 (50)	0.112
≥50 ppb, n (%)	0	6 (9)	0.061	1 (6)	26 (22)	0.114
Sputum eosinophils:	(n=20)	(n=39)		(n=12)	(n=67)	
Median value, %*	0.8 (0.2-1.8)	1 (0.5-3.5)	0.273	1.3 (0-2.4)	1.3 (0.8-3.8)	0.232
≥1%, n (%)	10 (50)	22 (56)	0.640	7 (58)	50 (43)	0.246
≥2%, n (%)	5 (25)	14 (36)	0.396	6 (50)	26 (39)	0.852
≥3, n (%)	2 (10)	11 (28)	0.110	2 (17)	21 (31)	0.303

Legend: FeNO: fractional concentration in nitric oxide; PC₂₀: provocative concentration of histamine causing a 20% fall in FEV₁; SIC: specific inhalation challenge.

* Median value with 25th-75th percentiles in parentheses;

Table 4. Characteristics of subjects without NSBH at baseline assessment

Subject no.	Causal agent	Prechallenge assessment			Postchallenge assessment*		
		Histamine PC ₂₀ (mg/ml)	FeNO (ppb)	Sputum eosinophils (%)	Histamine PC ₂₀ (mg/ml)	FeNO (ppb)	Sputum eosinophils (%)
1	Flour	24.0	32	2.0	28.0	78	15.5
2	Flour	>32	18	2.5	26.0	26	6.5
3	Flour	>32	23	4.2	32.0	22	38.0
4	Flour	>32	18	1.5	>32	24	3.0
5	Platinum salt	>32	17	0	30.5	45	3.5
6	Flour	>32	15	0	>32	NA	2.5
7	Herbal tea	20.6	7	0	30.4	9	7.5
8	Isocyanate	>32	17	0	>32	24	2.5
9	Latex	28.2	48	2.5	9.8	104	8.5
10	Persulfate salts	23.4	41	2.0	12.2	66	19.8
11	Latex	19.3	39	0	7.7	138	7.5
12	Flour	>32	269	NA	NA [†]	342	NA
13	Flour	>32	17	7.8	16.0	92	48.5
14	Flour	>32	17	NA	3.6	94	NA
15	Latex	24.5	18	NA	7.0	126	NA
16	Pancreatin	>32	12	NA	14.0	95	NA
17	Flour	29.7	20	NA	7.1	30	NA

Legend: FeNO: fractional concentration in nitric oxide; NA: not available; PC₂₀: provocative concentration of histamine causing a 20% fall in FEV₁.

* Assessment performed 24 hours after the end of the challenge exposure that induced a ≥20% fall in FEV₁.

[†] Airway obstruction with >15% reversibility after an inhaled bronchodilator.

REFERENCE

1. Beach J, Russell K, Blitz S, Hooton N, Spooner C, Lemiere C, et al. A systematic review of the diagnosis of occupational asthma. *Chest*. 2007;131(2):569-78.
2. Nicholson P, Cullinan P, Burge P, Boyle C. Occupational asthma: Prevention, identification & management: Systematic review & recommendations: British Occupational Health Research Foundation. London; 2010.
3. Tarlo SM, Balmes J, Balkissoon R, Beach J, Beckett W, Bernstein D, et al. Diagnosis and management of work-related asthma: American College Of Chest Physicians Consensus Statement. *Chest*. 2008;134(3 Suppl):1S-41S.
4. Baur X, Sigsgaard T, Aasen TB, Burge PS, Heederik D, Henneberger P, et al. Guidelines for the management of work-related asthma. *Eur Respir J*. 2012;39(3):529-45.
5. Tarlo SM, Lemiere C. Occupational asthma. *N Engl J Med*. 2014;370(7):640-9.
6. Vandenplas O, Suojalehto H, Cullinan P. Diagnosing occupational asthma. *Clin Exp Allergy*. 2017;47(1):6-18.
7. Malo JL, Cardinal S, Ghezzo H, L'Archeveque J, Castellanos L, Maghni K. Association of bronchial reactivity to occupational agents with methacholine reactivity, sputum cells and immunoglobulin E-mediated reactivity. *Clin Exp Allergy*. 2011;41(4):497-504.
8. Pralong JA, Lemiere C, Rochat T, L'Archeveque J, Labrecque M, Cartier A. Predictive value of nonspecific bronchial responsiveness in occupational asthma. *J Allergy Clin Immunol*. 2016;137(2):412-6.
9. Cockcroft DW, Mink JT. Isocyanate-induced asthma in an automobile spray painter. *Can Med Assoc J*. 1979;121(5):602-4.

- Accepted Article
10. Lemièrè C, Cartier A, Malo JL, Lehrer SB. Persistent specific bronchial reactivity to occupational agents in workers with normal nonspecific bronchial reactivity. *Am J Respir Crit Care Med*. 2000;162(3 Pt 1):976-80.
 11. Malo JL, Ghezzo H. Recovery of methacholine responsiveness after end of exposure in occupational asthma. *Am J Respir Crit Care Med*. 2004;169(12):1304-7.
 12. Padoan M, Pozzato V, Simoni M, Zedda L, Milan G, Bononi I, et al. Long-term follow-up of toluene diisocyanate-induced asthma. *Eur Respir J*. 2003;21(4):637-40.
 13. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184(5):602-15.
 14. Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med*. 2014;108(6):830-41.
 15. Quirce S, Lemiere C, de Blay F, Del Pozo V, Gerth Van Wijk R, Maestrelli P, et al. Noninvasive methods for assessment of airway inflammation in occupational settings. *Allergy*. 2010;65:445-59.
 16. Lemiere C, Ernst P, Olivenstein R, Yamauchi Y, Govindaraju K, Ludwig MS, et al. Airway inflammation assessed by invasive and noninvasive means in severe asthma: eosinophilic and noneosinophilic phenotypes. *J Allergy Clin Immunol*. 2006;118(5):1033-9.
 17. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. *J Allergy Clin Immunol*. 2013;132(1):72-80.

- Accepted Article
18. Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. *The lancet Respiratory medicine*. 2015;3(4):290-300.
 19. Nickels AS, Lim KG. Evaluation of exhaled nitric oxide's ability to predict methacholine challenge in adults with nonobstructive spirometry. *Ann Allergy Asthma Immunol*. 2016;117(4):365-9 e1.
 20. Lemiere C, D'Alpaos V, Chaboillez S, Cesar M, Wattiez M, Chiry S, et al. Investigation of occupational asthma: Sputum cell counts or exhaled nitric oxide? *Chest*. 2010;137(3):617-22.
 21. Prince P, Lemiere C, Dufour MH, Chaboillez S, Boulet LP. Airway inflammatory responses following exposure to occupational agents. *Chest*. 2012;141(6):1522-7.
 22. Lemiere C, NGuyen S, Sava F, D'Alpaos V, Huaux F, Vandenplas O. Occupational asthma phenotypes identified by increased fractional exhaled nitric oxide after exposure to causal agents. *J Allergy Clin Immunol*. 2014;134(5):1063-7.
 23. Sastre J, Costa C, del Garcia Potro M, Aguado E, Mahillo I, Fernandez-Nieto M. Changes in exhaled nitric oxide after inhalation challenge with occupational agents. *J Investig Allergol Clin Immunol*. 2013;23(6):421-7.
 24. Vandenplas O, D'Alpaos V, Heymans J, Jamart J, Thimpont J, Huaux F, et al. Sputum eosinophilia: an early marker of bronchial response to occupational agents. *Allergy*. 2009;64(5):754-61.
 25. Vandenplas O, Suojalehto H, Aasen TB, Baur X, Burge PS, de Blay F, et al. Specific inhalation challenge in the diagnosis of occupational asthma: consensus statement. *Eur Respir J*. 2014;43(6):1573-87.

26. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-38.
27. Vandenplas O, Delwiche JP, Jamart J, Van de Weyer R. Increase in non-specific bronchial hyperresponsiveness as an early marker of bronchial response to occupational agents during specific inhalation challenges. *Thorax*. 1996;51(5):472-8.
28. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy*. 1977;7(3):235-43.
29. Cockcroft DW. Direct challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest*. 2010;138(2 Suppl):18S-24S.
30. Djukanovic R, Sterk PJ, Fahy JV, Hargreave FE. Standardised methodology of sputum induction and processing. *Eur Respir J Suppl*. 2002;37:1s-2s.
31. Balbi B, Pignatti P, Corradi M, Baiardi P, Bianchi L, Brunetti G, et al. Bronchoalveolar lavage, sputum and exhaled clinically relevant inflammatory markers: values in healthy adults. *Eur Respir J*. 2007;30(4):769-81.
32. ATS/ERS Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005. *Am J Respir Crit Care Med*. 2005;171(8):912-30.
33. Alving K, Janson C, Nordvall L. Performance of a new hand-held device for exhaled nitric oxide measurement in adults and children. *Respiratory research*. 2006;7:67.
34. Karrasch S, Linde K, Rucker G, Sommer H, Karsch-Volk M, Kleijnen J, et al. Accuracy of FENO for diagnosing asthma: a systematic review. *Thorax*. 2016.
35. Lemiere C, Chaboilliez S, Trudeau C, Taha R, Maghni K, Martin JG, et al. Characterization of airway inflammation after repeated exposures to occupational agents. *J Allergy Clin Immunol*. 2000;106(6):1163-70.

Online Table 1. Sensitivity and specificity of histamine challenge, FeNO, and sputum eosinophils according to exposure status at the time of SIC

Baseline assessment	Sensitivity (%)	Specificity (%)
Histamine PC₂₀ ≤16 mg/ml		
Complete removal from exposure at the time of SIC (n=112)	91 (81-97)	37 (24-51)
Reduced exposure at the time of SIC (n=70)	82 (67-92)	42 (23-63)
Exposure at the time of SIC = usual (n=58)	87 (70-96)	26 (11-46)
FeNO level ≥25 ppb		
Delay between last exposure and SIC >2 days (158)	45 (35-56)	69 (57-80)
Complete removal from exposure at the time of SIC (n=112)	48 (35-62)	72 (58-84)
Reduced exposure at the time of SIC (n=70)	39 (24-54)	62 (41-80)
Persistent exposure at the time of SIC (n=58)	58 (39-75)	78 (58-91)
Sputum eosinophils ≥1%		
Complete removal from exposure at the time of SIC (n=66)	64 (46-79)	47 (28-66)
Reduced exposure at the time of SIC (n=37)	69 (48-86)	45 (17-77)
Persistent exposure at the time of SIC (n=35)	94 (71-100)	44 (22-69)
Sputum eosinophils ≥2%		
Complete removal from exposure at the time of SIC (n=66)	33 (19-51)	77 (58-90)
Reduced exposure at the time of SIC (n=37)	31 (14-52)	64 (31-89)
Persistent exposure at the time of SIC (n=35)	65 (38-86)	56 (31-78)
Histamine PC₂₀ ≤16mg/ml OR FeNO ≥25 ppb		
Complete removal from exposure at the time of SIC (n=112)	93 (83-98)	31 (20-46)
Reduced exposure at the time of SIC (n=70)	89 (75-96)	35 (17-56)
Persistent exposure at the time of SIC (n=58)	90 (74-98)	19 (6-38)
Histamine PC₂₀ ≤16mg/ml OR sputum eosinophils ≥1%		
Complete removal from exposure at the time of SIC (n=102)	96 (88-100)	11 (4-24)
Reduced exposure at the time of SIC (n=61)	95 (83-99)	20 (6-44)
Persistent exposure at the time of SIC (n=54)	97 (83-100)	4 (0-21)

Legend: 95% confidence intervals are presented between parentheses; FeNO: fractional concentration in nitric oxide; PC₂₀: provocative concentration of histamine causing a 20% fall in FEV₁; SIC: specific inhalation challenge with the suspected occupational agent(s).