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Running head: Occupational asthma without airway hyperresponsiveness

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ABSTRACT

BACKGROUND: The absence of nonspecific bronchial hyperresponsiveness (NSBH) has been documented in a substantial proportion of workers with occupational asthma (OA).

OBJECTIVE: We investigated the clinical and inflammatory characteristics associated with the absence of baseline NSBH in a cohort of subjects with OA ascertained by a positive specific inhalation challenge (SIC).

METHODS: A retrospective study was conducted among 1068 subjects who completed an SIC with various occupational agents at three tertiary centers.

RESULTS: Among 377 subjects with a positive SIC, 63 (16.7%) did not exhibit baseline NSBH. Upon post-challenge assessment of these 63 subjects, 45 developed NSBH, while 18 still lacked demonstrable NSBH. Multivariate analysis revealed that quiescent asthma —defined by untreated asthma associated with the absence of airway obstruction— was the predominant clinical feature associated with undetectable NSBH at baseline (odds ratio: 3.59, 95% confidence interval: 1.86–6.93). Subjects with and without baseline NSBH exhibited similar rates of post-challenge increases in fractional exhaled nitric oxide ≥13 ppb (47.8% and 43.6%, respectively) and sputum eosinophil count ≥2% (72.2% and 74.2%, respectively). Baseline NSBH assessment identified positive SICs with a substantially lower sensitivity (68%) and negative predictive value (71%) among subjects with quiescent asthma (n=211) than among those (n=857) with active disease (87% and 88%, respectively).

CONCLUSIONS: The absence of NSBH does not allow OA to be ruled out without further investigation in subjects with quiescent asthma. On the other hand, a negative NSBH test has a high negative predictive value for OA among subjects with active disease.

Abstract word count: 247 words

Keywords: Airway hyperresponsiveness; Fractional exhaled nitric oxide; Occupational asthma; Specific inhalation challenge; Sputum eosinophils.

HIGHLIGHT BOX

What is already known about this topic?

Occupational asthma has been documented in subjects without nonspecific bronchial hyperresponsiveness, but the clinical characteristics associated with this occurrence and their impact on diagnostic strategies have not yet been comprehensively delineated.

What does this article add to our knowledge?

Quiescent asthma—defined as untreated asthma and absence of airflow obstruction—is a stronger predictor for the absence of nonspecific bronchial hyperresponsiveness than removal from work exposure in subjects with occupational asthma.

How does this study impact current management guidelines?

The clinical feature of “quiescent asthma” allows to identify a subset of subjects with a history of work-related asthma symptoms in whom the absence of nonspecific bronchial hyperresponsiveness does not allow exclusion of occupational asthma without further investigation, while a negative assessment makes the diagnosis reasonably unlikely among those with active disease.

Abbreviations used

CI – Confidence interval

E-PHOCAS – European network for the PHenotyping of OCcupational ASthma;

FeNO – Fractional exhaled nitric oxide;

FEV_1 – Forced expiratory volume in one second;

FVC – Forced vital capacity

ICS – Inhaled corticosteroid

NPV – Negative predictive value

NSBH – Nonspecific bronchial hyperresponsiveness

OA – Occupational asthma;

OR – Odds ratio

PPV – Positive predictive value

SABA – Inhaled short-acting β_2-agonist

SIC – Specific inhalation challenge

INTRODUCTION

The objective documentation of asthma through the demonstration of reversible airflow obstruction or nonspecific bronchial hyperresponsiveness (NSBH) is a pivotal step in the diagnostic evaluation of occupational asthma (OA) [1-4]. Assessment of NSBH has a high sensitivity and negative predictive value (NPV) for diagnosing OA, indicating that this condition is highly unlikely in the absence of demonstrable NSBH [5]. However, Pralong et al. [5] reported that this approach is only valid in subjects who have been recently exposed to the causal agent, because NSBH may improve rapidly after cessation of exposure and may even return to levels observed in nonasthmatic individuals. In such cases, NSBH may reappear upon re-exposure to the causal sensitizing agent [5-7]. Nevertheless, NSBH values within the nonasthmatic range have been occasionally documented in some subjects both before and after asthmatic reactions elicited by specific inhalation challenges (SICs) with occupational agents [5, 7-12].

In this study, we aimed to further characterize the phenotypic characteristics of subjects who lack baseline NSBH but still develop asthmatic reactions after challenge exposure to occupational agents and to evaluate the impact of these characteristics on the accuracy of NSBH assessment for diagnosing OA.

METHODS

Study design and population

This retrospective study was conducted within the European network for the PHenotyping of OCcupational ASthma (E-PHOCAS) cohort which recruited subjects who completed a specific inhalation challenge (SIC) with the suspected workplace agents in European tertiary centers [13-15]. The present analysis was restricted to subjects from three centers (Lodz, Poland; Madrid, Spain; and Yvoir, Belgium) that performed NSBH assessment using the same bronchoprovocation method: the tidal breath method [16, 17].

This study included subjects with a positive SIC, who fulfilled the following criteria: a positive SIC result, defined by a ≥15% fall in forced expiratory volume in one second (FEV1) after challenge exposure to the causal agent (n=421); available measurement of baseline NSBH (n=386); and available post-SIC NSBH values from subjects who did not exhibit NSBH at the pre-SIC assessment (n=377). Subjects with a negative SIC (n=691) recruited from the same cohort were also included in the analysis to evaluate the ability of NSBH assessment to differentiate between subjects with a positive versus negative SIC. A negative SIC was defined by an FEV_1 change of <15% in the absence of a two-fold or greater increase in the post-challenge NSBH level compared with the baseline value [18].

Data collection

Data collected at the time of diagnostic evaluation were anonymously retrieved from each study site using a standardized spreadsheet, as previously described [13-15].

Ethics

This retrospective anonymized study was approved by the local Institutional Review Board of each participating center.

Specific inhalation challenges

The SIC methodology conformed with international recommendations, including the performance of a "control challenge" on a separate day prior to challenge exposure to the suspected occupational agents, and functional monitoring for at least 6 hours after the control and active challenges [18]. Inhaled corticosteroids were withheld for 3 to 7 days prior to the SIC procedure.

Challenge exposures to occupational agents were generated using a "work-simulation" approach aimed at reproducing the conditions of workplace exposure as closely as possible [19]. The collected data included the maximum fall in FEV_1 during the early and late components of the bronchial response expressed as the absolute percentage change from the pre-challenge value.

Assessment of nonspecific bronchial hyperresponsiveness

The level of NSBH was assessed through the inhalation of doubling concentrations of histamine (at two centers) or methacholine (at one center) from 0.03 mg/mL to 16 mg/mL at tidal breathing for 2-minute periods, as described by Cockcroft et al. [16, 17]. The results were expressed as the concentration of histamine/methacholine that induced a 20% decrease in FEV_1 (PC_{20}). The absence of NSBH was defined by a PC_{20} value >16 mg/m since this threshold value is associated with the highest NPV for asthma [17, 20]. NSBH was measured at the end of the control day (the baseline value) as well as 24 hours after the last challenge exposure to the offending agent (the post-challenge value).

Additionally, participating investigators were asked to review the medical files of the subjects without baseline NSBH, who were removed from exposure for over one week at the time of the SIC, in order to determine whether NSBH had been assessed prior to the SIC while the subjects were still exposed at their workplace.

Fractional exhaled nitric oxide

The FeNO levels were measured at two centers (Madrid, Spain and Yvoir, Belgium) at a flow rate of 50 mL/s in compliance with international recommendations [21]. Measurements were

performed using different analyzers based on either chemiluminescence or electrochemical detection [15].

For the present analysis, we used FeNO values obtained in the morning before challenge exposure to occupational agents (the baseline value), and at 24 hours after the last challenge (the post-challenge value). Changes in FeNO were computed as the absolute difference between the post-SIC and baseline values, expressed in parts per billion (ppb). A post-challenge FeNO increase of ≥13 ppb was considered significant [15].

Induced sputum cell counts

At 2 centers (Lodz, Poland and Yvoir, Belgium), induced sputum samples were obtained at the end of the control day (the baseline value) and at 24 hours after the SICs (the post-challenge value) through the inhalation of increasing concentrations of hypertonic saline [15]. Changes in sputum eosinophils were computed as the difference between the post-challenge and the baseline value, expressed as the absolute percentage of total nonsquamous cell counts. A post-challenge increase in sputum eosinophils of 2% or greater compared with the pre-challenge value was considered as an “eosinophilic response” [15, 22].

Data analysis

Continuous variables are presented as medians (25^{th}–75^{th} percentiles) and categorical variables as numbers and proportions of available data. Comparisons between subjects were made using the chi-squared or Fisher’s exact test for categorical variables, and the nonparametric Kruskal-Wallis rank-sum test for numerical variables. The Wilcoxon signed-rank test with continuity correction was used to compare markers of airway inflammation before and after the SIC, within the same subjects. Multivariate logistic regression analysis was conducted using a binomial generalized linear model to identify the clinical characteristics that were significantly associated with the absence of baseline NSBH. The baseline clinical and functional variables incorporated into the regression model were selected based on a *P* value ≤0.1 in univariate comparisons, including no use of a reliever inhaled short-acting β_2-agonist

(SABA); no treatment with an inhaled corticosteroid (ICS); a FEV_1 to forced vital capacity (FVC) ≥0.70; and removal from workplace for more than one week. These variables were subsequently summarized into a single composite feature—"quiescent asthma"—defined as untreated asthma without airflow obstruction. Additional variables investigated in previous studies on NSBH were also included in the regression model, including the type of causal agent (low-molecular-weight vs. high-molecular-weight agents) and the duration of asthma symptoms at work (i.e., ≥24 months vs. <24 months). Airway inflammatory biomarkers were not included in the multivariate regression analysis, because these data were unavailable in a large fraction of the subjects.

Sensitivity, specificity, and the positive predictive value (PPV) and NPV of baseline NSBH assessment were evaluated among the 377 subjects with a positive SIC and 691 subjects with a negative SIC, and in subsets of subjects having quiescent or active disease. Statistical analyses were performed using the R software version 4.5.0 (*www.r-project.org*, Vienna, Austria). A *P* value <0.05 was considered significant.

RESULTS

Characteristics of subjects without baseline nonspecific bronchial hyperresponsiveness

Among the 377 subjects with a positive SIC included in this analysis, 63 (16.7%, 95% confidence interval [CI]: 13.5–20.1%) failed to demonstrate baseline NSBH. Among these 63 subjects without baseline NSBH, 45 developed NSBH in the asthmatic range at 24 hours post-SIC, whereas 18 (4.9%, 95% CI: 3.0–7.4%) subjects failed to demonstrate NSBH both before and after the positive SIC.

The workplace agents that caused a positive SIC are summarized in Table E1 (available in this article's Online Repository). Table I presents the clinical and functional characteristics of the subjects with a positive SIC. Compared to the subjects who exhibited baseline NSBH, those without baseline NSBH less frequently used asthma medication, including inhaled SABA and ICS, and were less likely to show a baseline FEV_1/FVC ratio <0.70. The proportion of quiescent asthma—defined by no need of any asthma medication and a FEV_1/FVC ratio ≥0.70—was significantly higher among subjects without demonstrable baseline NSBH (38.1%) than in those with baseline NSBH (16.6%, *P*<0.001).

The median (25^{th}-75^{th} percentiles) delay between cessation of workplace exposure and the SIC procedure did not differ between the 63 subjects without baseline NSBH (3.5 months [0.1–11.8]) and those who demonstrated baseline NSBH (2 months [0.1–11], *P*=0.290). Compared to those with baseline NSBH, subjects without baseline NSBH were slightly more likely to be removed from workplace exposure for more than one week at the time of the SIC procedure; however, this difference did not reach statistical significance (71.0% vs. 58.8%, *P*=0.087) (Table I). Subjects without baseline NSBH exhibited a higher rate of isolated late asthmatic reactions during the SIC.

Univariate associations with negative baseline NSBH are detailed in Table II. The multivariate logistic regression analysis (Table II) revealed that quiescent asthma at the time of evaluation

was the predominant factor associated with undetectable NSBH at baseline of the SIC procedure (odds ratio [OR]: 3.59, 95% CI: 1.86–6.93, *P*<0.001).

The characteristics of the subjects with a positive SIC and quiescent asthma (n=76) at the time of evaluation are presented in Table E2 (available in this article's Online Repository). These subjects were slightly younger, exposed more often to low-molecular-weight agents, and less frequently atopic than those with "active asthma" (n=301). Interestingly, subjects with quiescent asthma reported a shorter median (25^{th}-75^{th} percentiles) duration of work-related asthma symptoms prior to the SIC (14 months [8–35]) vs. 36 months [18–79], *P*<0.001) and were more frequently removed from work exposure (75.0% vs. 57.2%, *P*=0.004) compared to subjects with active disease.

The characteristics of the 18 subjects without NSBH both pre- and post-SIC are detailed in Table E3 available in this article's Online Repository and compared to the 45 subjects without baseline NSBH who developed NSBH after a positive SIC in Table E4. These subjects without NSBH both before and after a positive SIC differed from those who developed NSBH after the SIC only by a higher proportion of males (77.8% vs. 46.7%, *P*=0.029) and less frequent use of a SABA on demand (22.2% vs. 48.9%, *P*=0.025).

A total of 62 subjects without baseline NSBH were removed from workplace exposure at the time of the SIC procedure. For 44 of these subjects, information regarding NSBH assessment prior to the SIC was available from their medical files. An assessment of NSBH while exposed at work had been completed for only 9 (20.4%) of these 44 subjects tested prior to the SIC, and was documented as being in the asthmatic range for 5 of them, including 4 subjects who lacked NSBH at both the pre- and post-SIC assessments.

Airway inflammatory markers in subjects without baseline nonspecific bronchial hyperresponsiveness

FeNO measurements were available from 244 subjects (64.7%) with a positive SIC (Table III). The median baseline FeNO value was similar in subjects without baseline NSBH and those

who exhibited baseline NSBH; however, the proportion of subjects with baseline FeNO ≥50 ppb was slightly lower among subjects without baseline NSBH than in those showing baseline NSBH (5.1% vs. 18.0%, *P*=0.054). The positive SIC response was associated with a significant post-challenge increase in FeNO ≥13 ppb in similar proportions of subjects with and without baseline NSBH (47.8% and 43.6%, respectively).

Sputum eosinophil counts were available from 200 (53.1%) subjects who showed a positive SIC (Table III). Sputum data were available in similar proportions of subjects with (169/314, 53.8%) and without (31/63, 49.2%) NSBH at baseline, but in only 5 of 18 subjects who failed to demonstrate NSBH both before and after the SIC (Table IV). The median eosinophil count was slightly lower among subjects without baseline NSBH compared with those who demonstrated baseline NSBH, although the proportion of subjects with an eosinophil count ≥2% was similar between these two groups (41.9% and 46.3%) (Table III). Both subjects with and without baseline NSBH exhibited significant rates of post-challenge changes in sputum eosinophils, with increases ≥2% occurring in 72.2% and 74.2%, respectively. Among subjects lacking baseline NSBH, the changes in sputum eosinophils could not be reliably compared between the subsets of subjects with and without post-challenge NSBH due to the small numbers of subjects with sputum data (Table IV).

Sensitivity, specificity, and predictive values of baseline nonspecific bronchial hyperresponsiveness

Within the entire population of 377 subjects with a positive SIC and 691 subjects with a negative SIC (Table E5 available in this article's Online Repository), assessment of baseline NSBH identified a positive SIC result with a sensitivity of 83% (95% CI: 79–87%) and a specificity of 51% (95% CI: 47–54%), with a PPV of 48% (95% CI: 44–52%) and a NPV of 85% (95% CI: 81–88%) (Table V). When considering only the 211 subjects with quiescent asthma, assessment of baseline NSBH showed substantial decreases in sensitivity to 68% (95% CI: 57–79%) and NPV to 71% (95% CI: 60–80%) (Table V). In contrast, among subjects with active disease, the presence of baseline NSBH still provided high sensitivity (87%, 95% CI: 83–91%)

and NPV (88%, 95% CI: 84–91%). The ability of baseline NSBH to differentiate positive from negative SIC results did not significantly differ between subjects removed from workplace exposure for more than one week at the time of the SIC (n=623) and those who were still exposed at work (n=436) (Table V).

DISCUSSION

The results of this multicenter study demonstrated that the absence of baseline NSBH in subjects with ascertained OA was predominantly associated with the absence of any asthma medication and airway obstruction at the time of evaluation—characteristics that were summarized under the term “quiescent asthma”. Although this finding might be expected from the relationship between the severity of asthma and the level of NSBH, this study is, to our knowledge, the first to highlight “quiescent asthma” as a simple clinical marker identifying a subset of patients with a history of work-related asthma symptoms in whom the absence of NSBH may not exclude a diagnosis of OA.

Our findings confirmed that the absence of ICS use and a FEV_1/FVC ratio ≥0.70 were associated with NSBH values in the nonasthmatic range in subjects with OA, in line with the results of prior studies [5, 7]. However, these studies did not evaluate asthma symptoms or the need for a rescue bronchodilator. The data-driven construct of “quiescent asthma” was introduced to aggregate the clinical and functional characteristics that were significantly associated with the absence of NSBH into a single composite feature that would be meaningful for clinicians. Remarkably, our data indicated that quiescent asthma at the time of assessment was more closely associated with a negative NSBH test than removal from the workplace, which contrasted with the study by Pralong et al. [5]. These investigators reported that the sensitivity and NPV of NSBH was substantially lower in subjects who were no longer exposed to their workplace at the time of assessment (67% and 82%, respectively) compared with those who were still at work (95% and 95%, respectively). They concluded that a negative methacholine challenge makes the diagnosis of OA very unlikely only in subjects still exposed at work. Within our cohort, the assessment of baseline NSBH showed a sensitivity of 88% (95% CI: 81–93%) and NPV of 89% (95% CI: 83–93%) in differentiating positive from negative SICs among subjects still at work at the time of assessment, which did not significantly differ from the sensitivity (81%, 95% CI: 75–86%) and NPV (82%, 95% CI: 77–87%) among subjects removed from workplace exposure (Table V). Our data indicate that the clinical feature of

"quiescent asthma" is a stronger predictor of undetectable baseline NSBH than removal from the workplace. The time elapsed between the last work exposure and the diagnostic evaluation should likely be regarded as an indirect reflection of decreasing NSBH and asthma severity after avoidance of exposure [23-25]. This is further supported by the finding that subjects with quiescent asthma were more frequently removed from work than those with clinically active disease (Table E2 available in this article's Online Repository). The discrepancy between studies pertaining to the effect of "removal from exposure" is likely to result from an inaccurate evaluation of exposure to the causal agent while the subjects were still at work. As outlined by Pralong et al. [5], determining whether a subject is removed or still exposed to the causal agent based on the available working status may not reflect the actual level of exposure at work, because the subject might have been relocated to a non-exposed or less exposed job at her/his workplace. The level of exposure to the causal agent might also have been reduced by improved workplace hygiene measures, engineering changes, substitution of the causal agent by a less sensitizing compound, or use of personal protective equipment [26], an information that is most often unavailable in retrospective studies. A systematic review found that reduction of exposure may be associated with symptom improvement or recovery in 60% and 18%, respectively, of the subjects with OA and with a decrease or recovery of NSBH in 39% and 15%, respectively [27]. This may also explain—at least partly—why 13% of the subjects with OA still working at the time of the diagnostic evaluation were categorized as having quiescent asthma (Table I).

Actually, the results of our study reinforce the statement that "*the absence of NSBH has a fairly high NPV for current symptomatic asthma, and generally can be used to rule out active disease*" issued in the latest American College of Chest Physicians consensus statement on the diagnosis of work-related asthma [2]. NSBH assessment provided a substantially lower sensitivity (68%, 95% CI 57–79%) and NPV (71%, 95% CI: 60–80%) for differentiating positive from negative SIC results among subjects with quiescent asthma. Accordingly, the absence of NSBH in subjects with quiescent asthma at the time of testing should prompt further

investigation before excluding OA. Whenever possible, these subjects should be returned at work to reevaluate NSBH when they experience work-related asthma symptoms, preferably coupled with serial measurements of peak expiratory flows and NSBH and/or airway inflammatory markers (i.e., FeNO and/or sputum eosinophils) at work and away from work [2, 4, 15, 22]. Alternatively, a SIC with the suspected workplace agent in a specialized center should be considered whenever feasible, since this test remains the “reference standard” for diagnosing OA [1, 2, 4, 18]. On the other hand, our data confirmed a high sensitivity (87%, 95% CI: 83–91%) and NPV (88%, 95% CI: 84–91%) of NSBH assessment in subjects with active disease. In these subjects, the absence of NSBH allows for ruling out the diagnosis of OA with a high level of confidence, although clinicians may also take into account the clinical history and results of immunological tests if available to decide whether there is a need to perform serial measurements of peak expiratory flows, NSBH and/or airway inflammatory markers at work or a SIC with the suspected agent.

We did not refer to the concept of “asthma remission” which include criteria ascertained over a defined prolonged period of observation whereas, inherent to the retrospective design of our study, quiescent asthma was defined at a single time-point assessment. Current definitions of asthma remission usually refer to either “clinical remission” (i.e., no asthma symptoms or medication use for a specific period) or “complete” (or “pathophysiological”) remission which also includes normal lung function, NSBH, and/or inflammatory markers) [28]. In our retrospective cohort, the need for an inhaled bronchodilator for symptom relieve was used as a proxy for the frequency and severity of asthma symptoms [14, 29]. This might have led to an underestimation of symptom burden due to poor perception or nonrecognition of respiratory symptoms that could be relieved by an inhaled bronchodilator, similar to what has been described in subjects with asymptomatic NSBH in the general population [30]. Our findings should be further refined using validated instruments for assessing the level of asthma control [31, 32]. Although our definition of quiescent asthma included the absence of airflow obstruction on spirometry, this feature should not be considered as reflecting “complete

remission" since 68% of OA subjects with quiescent asthma exhibited a baseline histamine/methacholine PC_{20} <16 mg/mL and approximately half of them showed increased levels of airway inflammatory markers (Table E2 available in this article's Online Repository).

The sensitivity and specificity of baseline NSBH found in our cohort (83% [95% CI: 79–87%] and 51% [95% CI: 47–54%], respectively) were in line with the pooled estimates derived from a meta-analysis of diagnostic tests, which found that a single assessment of NSBH had a sensitivity of 84% (95% CI: 67–93%) and a specificity of 48% (95% CI: 26–72%) for the diagnosis of OA caused by various high- and low-molecular-weight agents [1]. Our findings further confirm that a substantial proportion of subjects with OA ascertained by a positive SIC (16.7%, 95% confidence interval: 13.5–20.1%) fail to demonstrate baseline NSBH. This rate is consistent with that reported in the retrospective monocentric study published by Pralong et al. (55 of 278; 19.8%) [5], but slightly higher than in the study by Beretta et al. (17 of 133; 12.7%) [7]. Within our multicenter cohort, the absence of NSBH was documented both before and after a positive SIC in 18 of 377 (4.8%, 95% CI: 3.0–7.4%) subjects with a positive SIC, which is in line with the rates reported by Pralong et al. (23/278, 8.3%) [5] and Beretta et al. (8/133, 6.0%) [7]. We acknowledge that our retrospective study does not allow for determining whether a PC_{20} values above 16 mg/mL in subjects with OA reflects a "false negative" result related to clinically quiescent asthma from a "genuine" negative NSBH test because this would have required the measurement of NSBH during prolonged exposure to the causal agent at work. Nevertheless, a substantial proportion (45/63, 71%) of our subjects without baseline NSBH developed a histamine/methacholine PC_{20} value in the asthmatic range after a positive SIC, a higher proportion than those reported by Pralong et al. (22/55, 40% [5] and Beretta et al. (9/17, 53%) [7]. In addition, these investigators documented that a substantial fraction (11/23) of subjects without NSBH before and after SIC showed an NSBH value within the asthmatic range when assessed during workplace exposure [5]. NSBH assessment at work was available only in a small number (5/18, 28%) of our subjects without NSBH both before and after the SIC, but the level of NSBH was documented in the asthmatic range in four of these five subjects while

they were exposed at work prior to the SIC. Altogether, these data suggest that only a minority of negative NSBH tests are “true negative” results confirmed after symptomatic exposure to the causal agent at work. It is also possible that some of these subjects without baseline and post-challenge NSBH may have developed a significant increase in the level of NSBH though remaining in the non-asthmatic range, as reported in a case report by Hargreave et al. [8]. However, we could not evaluate the changes in NSBH level above the histamine/methacholine PC_{20} threshold of 16 mg/mL, since this threshold was the highest concentration delivered to the subjects. Nevertheless, the lack of NSBH assessment at work should not affect the diagnostic implications of our findings since our retrospective cohort reflects the “real-world” clinical practice at specialized centers, in which about half of the subjects are removed from the offending work environment without NSBH assessment, and often cannot be returned to their workplace for further investigation [5, 7, 15].

Almost half of our subjects without baseline NSBH exhibited a baseline FeNO level ≥25 ppb and/or a sputum eosinophil count ≥2%, further indicating that subjects without NSBH may show Type 2 airway inflammation, as suggested in previous reports [5, 7, 10, 12]. Interestingly, the development of asthmatic reactions in subjects without baseline NSBH was associated with post-challenge increases in FeNO and/or sputum eosinophils similar to those observed in subjects with baseline NSBH. Overall, these data highlight discordances between measurements of NSBH, FeNO, and sputum eosinophilia, further indicating that these indices reflect different dimensions of asthma [33-36].

Our data showed that the clinical characteristics of the subjects without NSBH at both pre- and post-challenge assessments did not differ significantly from those of subjects without baseline NSBH ~~but~~ who developed post-SIC NSBH (Table E4 available in this article’s Online Repository). Notably, significant post-challenge increases in FeNO concentrations were documented even in subjects lacking NSBH at both pre- and post-SIC assessments (Table III). However, sputum data were available for only a small number of subjects without pre- and

post-SIC NSBH, precluding reliable conclusions about changes in airway eosinophilic inflammation in this subset of subjects.

Limitations of the study

The strengths of this study are its large sample size and the homogeneous criteria used for identifying NSBH and diagnosing OA, although several potential limitations should be further considered. This study was restricted to centers using the tidal breath bronchoprovocation method, which may limit the generalizability of the findings. However, the tidal breath method is the most thoroughly standardized and validated method for identifying clinically relevant NSBH [16, 20]. The other centers participating to the E-PHOCAS cohort used dosimeter methods based on various histamine/methacholine bronchoprovocation protocols [3, 17]. A preliminary analysis of the E-PHOCAS cohort indicated that the tidal breath method with a threshold concentration of 16 mg/mL provided a more conservative estimate of the prevalence of negative NSBH compared with the dosimeter methods. Accordingly, there is a need to further validate our findings using a standardized dosimeter method [37].

We acknowledge that caution should be applied in interpreting the predictive values of NSBH, because our estimates were obtained in a population recruited from tertiary centers with a high prevalence (i.e., pretest probability) of a positive SIC result (35.3%), although the proportion of positive SICs was similar to that reported in the study by Pralong et al. (27.5%) [5]. However, the NPV of NSBH assessment should be even higher in settings where the pretest probability of OA is lower, especially when the test is applied in secondary respiratory health centers or in workplace surveillance programs.

Another potential limitation of our data could result from the unavailability of FeNO and data sputum samples in 35% and 47%, respectively, of the subjects with a positive SIC. We acknowledge that sputum data should be interpreted cautiously in the subset of subjects without NSBH before and after the SIC due to the small number of available sputum samples, but it is unlikely that our findings on airway inflammatory markers would have been affected by selection biases. FeNO measurements and sputum analysis were performed systematically in

centers where these procedures were available. The investigators participating in this retrospective study completed questionnaires inquiring about the methodology and decision policy for performing these procedures. None of the Investigators declared applying any selection of the subjects who completed FeNO measurements or induced sputum procedure based on clinical criteria. Missing values resulted solely from the availability of the procedure in each of the three centers, technical failure of the FeNO analyzers, or unsuccessful sputum induction. In addition, sputum data were available in similar proportions of subjects with (53.8%) and without (49.2%) NSBH at baseline.

Conclusions

This study provides evidence that clinically quiescent asthma is the predominant factor associated with the absence of baseline NSBH in subject with ascertained OA and is a stronger predictor for the absence of NSBH than removal from work exposure. Despite limitations inherent to the retrospective design of the study, these findings have clinical implications since they indicate that a negative NSBH test does not allow for excluding OA without further investigation in subjects with quiescent asthma. On the other hand, our results further support the usefulness of NSBH measurement in subjects with active disease to rule out a diagnosis of OA with a reasonable level of confidence.

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All authors contributed to the study conception and design; as well as to the data acquisition, analysis, and interpretation. Additionally, they provided input regarding the drafting of the manuscript, critical feedback, and final approval of the manuscript. O. Vandenplas is the guarantor of the final content of the manuscript.

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585 **Table I. Clinical and functional characteristics of subjects with a positive SIC**
586 **according to the presence or absence of baseline NSBH**

Characteristic	Subjects without baseline NSBH (n=63)	Subjects with baseline NSBH (n=314)	*P* value
Causal agent, low-molecular-weight, n (%)	29 (46.0)	141 (44.9)	0.890
Age, yr	43 (35–51)	41 (34–50)	0.461
Male sex, n (%)	35 (55.6)	205 (65.3)	0.153
Current and ex-smoker, n (%)	26 (42.6)	140 (46.8)	0.549
Atopy, n (%)*	26 (41.3)	162 (51.8)	0.167
History of childhood asthma, n (%)	1 (1.7)	16 (5.2)	0.492
Work-related rhinitis, n (%)	46 (73.0)	232 (74.4)	0.875
Duration of exposure before asthma onset, mo	108 (48–198)	96 (36–204)	0.554
Duration of symptoms while at work, mo	24 (12–84)	35 (14–72)	0.794
Time interval since last workplace exposure, mo	3.5 (0.1–11.8)	2.0 (0.1–11.0)	0.290
Removal from work for ≥6 mo	29/62 (46.8)	125/313 (39.9)	0.317
Removal from work for ≥1 mo	40/62 (64.5)	180/313 (57.5)	0.306
Removal from work for ≥1 week, n (%)	44/62 (71.0)	184/313 (58.8)	0.087
Asthma medication at the time of SIC			
No treatment	24 (39.3)	61 (19.7)	**<0.001**
Inhaled short-acting β_2-agonist, n (%)	29 (46.0)	188 (60.1)	**0.050**
Inhaled corticosteroid, n (%)	26 (41.3)	186 (59.2)	**0.012**
Daily dose of inhaled corticosteroid, µg†	0 (0–500)	400 (0–500)	**0.001**
Baseline spirometry			
FEV_1, % predicted	99 (91–112)	91 (81–102)	**<0.001**
FEV_1/FVC, %	81 (77–83)	74 (67–81)	**<0.001**
FEV_1/FVC <0.70, n (%)	2 (3.2)	100 (31.8)	**<0.001**
FEV_1< 80% predicted and FEV_1/FVC <0.70, n (%)	0	48 (15.3)	**<0.001**
Quiescent asthma at the time of the SIC, n (%)‡	24 (38.1)	52 (16.6)	**<0.001**
Quiescent asthma among subjects at work, n (%)‡	5/18 (27.8)	14/129 (10.8)	**0.045**
Pattern of bronchial response to SIC, n (%)			
Isolated early reaction	22 (34.9)	135 (43.0)	0.236
Isolated late reaction	20 (31.8)	61 (19.4)	**0.030**
Dual reaction	21 (33.3)	118 (37.6)	0.523

587 Legend: *FEV_1*, forced expiratory volume in one second; *FVC*, forced vital capacity; *NSBH*, nonspecific bronchial
588 hyperresponsiveness; S*IC*, specific inhalation challenge.

589 Data are presented as numbers (%) for proportions and medians (25^{th}-75^{th} percentiles) for continuous variables. *P* values in
590 boldface indicate statistical significance.

591 * Atopy defined by the presence of a positive skin-prick test to at least one common allergen.

592 † Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.

593 ‡ Quiescent asthma defined by the absence of asthma medication and a FEV_1/FVC ratio ≥0.70.

TABLE II. Univariate analysis and multivariate model for the factors associated with the absence of baseline nonspecific bronchial hyperresponsiveness

Independent variables	Odds ratio (95% CI)	*P* value
Univariate associations:		
Type of causal agent, LMW vs. HMW agent	1.05 (0.61–1.80)	0.87
Duration of symptoms at work, ≥24 months vs. <24 months	0.98 (0.56–1.73)	0.943
Removal from workplace exposure ≥1 week vs. <1 week	1.71 (0.95–3.10)	0.075
Use of an inhaled short-acting β_2-agonist (SABA), no vs. yes	1.76 (1.02-3.04)	**0.041**
Treatment with an inhaled corticosteroid (ICS), no vs. yes	2.07 (1.19-3.58)	**0.010**
FEV1/FVC ratio ≥0.70 vs. <0.70	14.25 (3.42-59.45)	**<0.001**
Multivariate model:		
Type of causal agent, LMW vs. HMW agent	0.80 (0.44–1.45)	0.460
Duration of symptoms at work, ≥24 months vs. <24 months	1.40 (0.75–2.64)	0.293
Removal from workplace exposure ≥1 week vs. <1 week	1.62 (0.86–3.07)	0.134
Quiescent asthma*, yes vs. no	3.59 (1.86–6.93)	**<0.001**

Legend: *95% CI*, 95% confidence interval; *FEV_1*, forced expiratory volume in one second; *FVC*, forced vital capacity; *HMW*, high molecular weight, *LMW*, low molecular weight; *NSBH*, nonspecific bronchial hyperresponsiveness.

The multivariate logistic regression analysis included 364 subjects with a positive SIC without missing values. *P* value in boldface indicate statistical significance.

* Quiescent asthma defined by the absence of any asthma medication and a FEV_1/FVC ≥0.70.

603 **Table III. Airway inflammatory markers in subjects with and without baseline NSBH**

Characteristic	Subjects without baseline NSBH (n=63)	Subjects with baseline NSBH (n=314)	*P* value
Baseline FeNO	(n=39)	(n=205)	
Ppb	18 (13–30)	23 (13–42)	0.219
≥25 ppb, n (%)	13 (33.3)	97 (47.3)	0.117
≥50 ppb, n (%)	2 (5.1)	37 (18.0)	0.054
Post-challenge FeNO	(n=39)	(n=205)	
Ppb	29 (18–56)	39 (19–75)	0.201
Post-challenge change, ppb	7 (-2–25)* ‡	10 (2–32)* ‡	0.220
Post-challenge change ≥13 ppb, n (%)	17 (43.6)	98 (47.8)	0.727
Baseline sputum eosinophils	(n=31)	(n=169)	
% of total cell count	1.0 (1.0–2.2)	2.0 (1.0–5.0)	**0.013**
≥2% of total cell count, n (%)	13 (41.9)	95 (46.3)	0.171
Post-challenge sputum eosinophils	(n=31)	(n=169)	
% of total cell count	6.0 (2.0–13.8)	8.0 (3.0–17.2)	0.151
Post-challenge change, % of total cell count	4.0 (1.5–11.0)† §	5.0 (1.2–12.0)† §	0.808
Post-challenge change ≥2% of total cell count, n (%)	23 (74.2)	122 (72.2)	0.818

604 Legend: *FeNO*, fractional exhaled nitric oxide*; Ppb*, parts per billion. *P* value in boldface indicates statistical significance.

605 * Post-challenge change in FeNO expressed as the difference between the post-challenge FeNO value measured 24 h after
606 the last challenge exposure and the pre-challenge value expressed in ppb.

607 † Post-challenge change in sputum eosinophils expressed as the difference between the post-challenge sputum eosinophil
608 count assessed 24 h after the last challenge exposure and the baseline value expressed as percent of total nonsquamous
609 cells

610 ‡ *P*=0.001 when comparing pre- and post-challenge FeNO values.

611 § P<0.001 when comparing pre- and post-challenge sputum eosinophil counts.

612

613 **Table IV. Comparison of airway inflammatory markers among subjects without baseline NSBH**

Characteristic	Subjects without NSBH pre- and post-SIC (n=18)	Subjects without NSBH pre-SIC but NSBH post-SIC (n=45)	*P* value*
Baseline FeNO	(n=18)	(n=21)	
Pbb	19 (13–30)	18 (13–32)	0.855
≥25 ppb, n (%)	6 (33.3)	7 (33.3)	0.999
Post-challenge FeNO	(n=18)	(n=21)	
Ppb	28 (22–47)	32 (17–66)	0.757
Post-challenge change, ppb	8 (-1–25)*	3 (-3–25)*	0.778
Post-challenge change ≥13 ppb, n (%)	8 (44.4)	9 (42.9)	0.999
Baseline sputum eosinophils	(n=5)	(n=26)	
% of total cell count	2.0 (1.5–2.5)	1.0 (1.0–2.0)	0.321
≥2% of total cell count, n (%)	3 (60.0)	10 (38.5)	0.625
Post-challenge sputum eosinophils	(n=5)	(n=26)	
% total cell count	6.5 (3.5–15.5)	5.5 (2.0–12.0)	0.747
Post-challenge change, % of total cell count	4.0 (3.5–13.5)	4.0 (1.2–9.8)†	0.686
Post-challenge change ≥2% of total cell count, n (%)	4 (80.0)	19 (73.1)	0.999

614 Legend: *FeNO*, fractional exhaled nitric oxide*; Ppb*, parts per billion; S*IC*, specific inhalation challenge. *P* value in boldface
615 indicate statistical significance.

616 * *P*<0.05 when comparing pre- and post-challenge FeNO values using Wilcoxon signed-rank test for paired samples.

617 † *P*<0.001 when comparing pre- and post-challenge sputum eosinophil counts using Wilcoxon signed-rank test for paired
618 samples.

619

620 **Table V. Sensitivity, specificity, and predictive values of baseline NSBH in**
621 **differentiating positive from negative inhalation challenge**

Cut-off value	No. of subjects with positive/negative SIC	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All subjects (n=1068)	377/691	83 (79–87)	51 (47–54)	48 (44–52)	85 (81–88)
Subjects with quiescent asthma (n=211)*	76/135	68 (57–79)	43 (34–52)	40 (32–49)	71 (60–80)
Subjects with active asthma (n=857)	301/556	87 (83–91)	52 (48–57)	50 (45–54)	88 (84–91)
Subjects removed from work (n=623)†	228/395	81 (75–86)	52 (46–57)	49 (44–54)	82 (77–87)
Subjects still exposed at work (n=437)	147/290	88 (81–93)	50 (44–56)	47 (41–53)	89 (83–93)

622 Legend: *NPV*, negative predictive value; *PPV*, positive predictive value; *SIC*, specific inhalation challenge.

623 * Quiescent asthma defined by the absence of asthma medication and a forced expiratory volume in 1 second
624 (FEV1)/forced vital capacity >0.70.

625 † Subjects removed from workplace exposure for one week or more.

Occupational Asthma Without Nonspecific Bronchial Hyperresponsiveness

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Table E1. Causal agents involved in positive inhalation challenges

Agents	Subjects without NSBH pre- and post-SIC (n=18)	Subjects without NSBH pre-SIC but NSBH post-SIC (n=45)	Subjects with baseline NSBH (n=314)
Low-molecular-weight agents:	**(n=10)**	**(n=19)**	**(n=141)**
Isocyanates	2 (11.1)	6 (13.3)	40 (12.7)
Cleaning products/disinfectants	0	1 (2.2)	30 (9.6)
Persulfate salts	0	1 (2.2)	22 (7.0)
Acrylate compounds	1 (5.6)	3 (6.7)	8 (2.6)
Metals	1 (5.6)	2 (4.4)	7 (2.2)
Drugs	2 (11.1)	1 (2.2)	7 (2.2)
Wood dusts	0	1 (2.2)	8 (2.6)
Aldehydes	1 (5.6)	1 (2.2)	4 (1.3)
Epoxy resins	2 (11.1)	0	2 (0.6)
Metal working fluid	1 (5.6)	0	1 (0.3)
Styrene	0	1 (2.2)	2 (0.6)
Eugenol	0	1 (2.2)	0
Permethrin	0	1 (2.2)	0
Various low-molecular-weight agents	0	0	10 (3.2)
High-molecular-weight agents:	**(n=8)**	**(n=26)**	**(n=173)**
Flour/grains	7 (38.9)	18 (40.0)	135 (43.0)
Latex	0	4 (8.9)	17 (5.4)
Enzymes	0	1 (2.2)	10 (3.2)
Fish/seafood	0	1 (2.2)	2 (0.6)
Insects (*Spodoptera sp.*)	1 (5.6)	0	0
Turmeric (*Curcuma longa*)	0	1 (2.2)	0
Almorta flour (*Lathyrus sativus*)	0	1 (2.2)	1 (0.3)
Various animals and derived products	0	0	3 (0.9)
Various plant-derived agents	0	0	5 (1.6)

Table E2. Clinical, functional, and inflammatory characteristics of subjects with quiescent vs. active asthma among those with a positive SIC

Characteristic	Subjects with quiescent asthma* (n=76)	Subjects with active asthma (n=301)	*P* value
Causal agent, low-molecular-weight, n (%)	48 (63.2)	122 (40.5)	**<0.001**
Age, yr	40 (31–47)	42 (35–50)	**0.043**
Male sex, n (%)	46 (60.5)	194 (64.4)	0.525
Current and ex-smoker, n (%)	32/73 (43.8)	134/287 (46.7)	0.662
Atopy, n (%)†	29 (38.2)	159/300 (53.0)	**0.021**
Duration of exposure before asthma onset, mo	63 (12–124)	120 (48–218)	**<0.001**
Duration of symptoms while at work, mo	14 (8–35)	36 (18–79)	**<0.001**
Time interval since last workplace exposure, mo	3 (0.4–12)	2 (0.03–11)	0.084
Removal from work for ≥1 wk, n (%)	57 (75.0)	171/299 (57.2)	**0.004**
Asthma medication at the time of SIC			
No treatment, n (%)	76	12 (4.0)	**<0.001**
Inhaled SABA, n (%)	0	218 (72.4)	**<0.001**
ICS, n (%)	0	212 (70.4)	**<0.001**
Baseline spirometry			
FEV_1, % predicted	104 (93–111)	91 (81–100)	**<0.001**
FEV_1/FVC, %	80 (76–83)	74 (67–81)	**<0.001**
FEV_1/FVC <0.70, n (%)	0	102 (33.9)	**<0.001**
FEV_1< 80% predicted and FEV_1/FVC <0.70, n (%)	0	48 (15.9)	**<0.001**
Histamine/methacholine PC_{20} <16 mg/mL, n (%)	52 (68.4)	262 (87.0)	**<0.001**
Pattern of bronchial response to SIC, n (%)			0.114
Isolated early reaction	28 (36.8)	129 (42.9)	0.342
Isolated late reaction	23 (30.3)	58 (19.3)	**0.037**
Dual reaction	25 (32.9)	114 (37.9)	0.421
Baseline blood eosinophil count	(n=34)	(n=212)	
Cells/µl	202 (154–300)	266 (166–400)	0.139
Baseline FeNO	(n=63)	(n=181)	
Pbb	17 (10–30)	24 (13–42)	**0.018**
≥25 ppb, n (%)	22 (34.9)	88 (48.6)	0.085
Post-challenge FeNO	(n=63)	(n=181)	
Ppb	26 (14–71)	41 (22–73)	0.070
Post-challenge change, ppb	6 (0–27)	12 (2–31)	0.231
Post-challenge change ≥13 ppb, n (%)	25 (39.7)	90 (50.3)	0.169
Baseline sputum eosinophils	(n=23)	(n=177)	
% of total cell count	2.0 (1.0–4.5)	2.0 (1.0–4.2)	0.768
≥2% of total cell count, n (%)	14 (60.9)	94 (53.1)	0.482
Post-challenge sputum eosinophils	(n=23)	(n=177)	
% of total cell count	13.0 (2.8–19.0)	7.0 (3.0–17.0)	0.971
Post-challenge change, % of total cell count	8.0 (1.5–13.0)	4.5 (1.2–10.8)	0.625
Post-challenge change ≥2% of total cell count, (n%)	17 (73.9)	128 (70.7)	0.872

Legend: *FeNO*, fractional exhaled nitric oxide*; FEV_1*, forced expiratory volume in one second; *FVC*, forced vital capacity; ICS, inhaled corticosteroid; *PC_{20}*, provocative concentration of pharmacologic agent inducing a 20% fall in FEV_1; *Ppb*, parts per billion; *SABA*, inhaled short-acting β_2-agonist; S*IC*, specific inhalation challenge.

Data are presented as numbers (%) of available data for proportions and medians (25^{th}-75^{th} percentiles) for continuous variables. *P* values in boldface indicate statistical significance.

* Quiescent asthma defined by the absence of any asthma medication and a FEV_1/FVC ratio ≥0.70 at the time of the SIC procedure

Table E3. Characteristics of subjects without NSBH both before and after a positive SIC

Subject no.	Causal agent	Age (yr)	Sex	Smoking status	Atopy*	WRR	SABA†	ICS†	Duration of symptoms at work (mo)	Delay since last work exposure (mo)	Pattern of SIC reaction‡ §	FeNO (ppb)		Sputum eosinophil count (%)	
												Pre-SIC	Post-SIC	Pre-SIC	Post-SIC
1	Isocyanate (HDI)	31	M	Never	No	No	No†	No†	22	0.5	Late (26%)	19.7	23.8	NA	NA
2	Flour	52	M	Ex	Yes	Yes	No	Yes	79	9	Dual (23%)§	30.0	77.5	2.0	15.5
3	Flour	41	M	Current	Yes	Yes	No†	No†	35	0.1	Early (24%)	10.0	24.2	1.5	0.5
4	Flour	52	M	Current	Yes	Yes	Yes	Yes	39	0.1	Early (36%)	8.9	26.1	2.5	6.5
5	Flour	28	M	Never	Yes	Yes	Yes	No	8	11	Early (33%)§	15.0	91.6	7.7	48.5
6	Platinum salt	59	F	Never	No	Yes	No	No	NA	180	Early (39%)	13.0	45.0	0	3.5
7	Flour	43	M	Current	No	Yes	No†	No†	25	7	Early (26%)	5.0	7.0	NA	NA
8	*Spodoptera* moth	25	M	Ex	Yes	Yes	Yes	Yes	48	0.1	Late (17%)	30.1	29.4	NA	NA
9	Epoxy resin	40	M	Never	Yes	Yes	No	No	48	NA	Early (18%)	23.0	21.0	NA	NA
10	Glutaraldehyde	48	F	Never	No	Yes	No	No	24	11	Dual (35%)	18.0	14.0	NA	NA
11	Flour	48	M	Ex	No	Yes	Yes	No	252	0.1	Late (24%)	33.0	58.0	NA	NA
12	Epoxy resin	43	M	Never	No	No	No†	No†	12	1.5	Late (27%)	14.9	23.0	NA	NA
13	Metal working fluid	31	M	Never	Yes	Yes	No	Yes	108	1	Early (19%)§	17.7	17.0	NA	NA
14	Ranitidine	41	F	Current	Yes	Yes	No†	No†	12	0.1	Late (15%)	11.8	37.4	NA	NA
15	Acrylate compound	44	M	Never	No	No	No†	No†	24	3	Early (20%)§	57.0	48.0	NA	NA
16	Clarithromycine	35	M	Current	Yes	No	No†	No†	1	1	Dual (20%)	20.0	14.0	NA	NA
17	Isocyanate (HDI)	46	F	Ex	No	No	No†	No†	12	12	Late (27%)	30.0	38.0	NA	NA
18	Flour	37	M	Never	No	Yes	No	No	30	3	Late (20%)	30.0	55.0	NA	NA

Legend: *FeNO*, fractional exhaled nitric oxide; *FEV_1*, forced expiratory volume in one second; *HDI*, hexamethylene diisocyanate; *ICS*, inhaled corticosteroid; *NA*, not available; *SABA*, inhaled short-acting β_2-agonist; *SIC*, specific inhalation challenge; *WRR*, work-related rhinitis.

* Atopy defined by the presence of a positive skin-prick test to at least one common allergen

† Subjects with quiescent asthma defined by the absence of any asthma medication and a FEV_1/forced vital capacity ratio ≥0.70. Four subjects who did not use a SABA and were not treated with ICS were not categorized as having quiescent asthma because they were either treated with an inhaled long-acting β_2-agonist alone (subjects no. 10 and 18) or had a FEV_1/forced vital capacity ratio <0.70 (subjects no. 6 and 9). All subjects showed a FEV_1 ≥80% of predicted value.

‡ Maximum fall in FEV_1 during the SIC between parentheses expressed as % change from pre-challenge value.

§ Documentation of nonspecific bronchial hyperresponsiveness while exposed at work prior to the SIC procedure.

Table E4. Comparison of clinical and functional characteristics among subjects without baseline NSBH

Characteristic	Subjects without NSBH pre- and post-SIC (n=18)	Subjects without NSBH pre-SIC but NSBH post-SIC (n=45)	*P* value*
Causal agent, low-molecular-weight, n (%)	10 (55.6)	19 (42.2)	0.407
Age, yr	42 (36–48)	44 (35–52)	0.513
Male sex, n (%)	14 (77.8)	21 (46.7)	**0.029**
Body mass index, kg/m^2	25.5 (23.2–27.4)	27.2 (24.2–30.2)	0.136
Current and ex-smoker, n (%)	9 (50.0)	17 (39.5)	0.451
Atopy, n (%)*	9 (50.0)	17 (37.8)	0.408
Work-related rhinitis, n (%)	13 (72.2)	33 (73.3)	1.000
Duration of exposure before asthma onset, mo	108 (48–210)	114 (58–195)	0.721
Duration of symptoms at work, mo	25 (12–48)	24 (12–86)	0.717
Interval since last workplace exposure, mo	2 (0.1–9)	6 (0.1–17)	0.451
Removal from work ≥1 week, n (%)	12 (70.6)	32 (71.1)	1.000
Asthma medication at the time of SIC			
No treatment, n (%)	9 (52.9)	15 (34.1)	0.177
SABA, n (%)	4 (22.2)	25 (55.6)	**0.025**
ICS, n (%)	4 (22.2)	22 (48.9)	0.088
Baseline spirometry			
FEV_1, % predicted	99 (92–108)	99 (91–114)	0.676
FEV_1/FVC, %	82 (76–85)	80 (77–82)	0.277
FEV_1/FVC <0.70, n (%)	2 (11.1)	0	0.078
FEV_1 <80% predicted and FEV_1/FVC <0,70, n (%)	0	0	1.000
Quiescent asthma, n (%)†	8 (44.4)	16 (35.6)	0.512
Pattern of bronchial response to SIC, n (%)			
Isolated early reaction	8 (44.4)	14 (31.1)	0.316
Isolated late reaction	7 (38.9)	13 (28.9)	0.441
Dual reaction	3 (16.7)	18 (40.0)	0.138

Legend: *FEV_1*, forced expiratory volume in one second; *FVC*, forced vital capacity; *ICS*, inhaled corticosteroid; *NSBH*, nonspecific bronchial hyperresponsiveness; *Ppb*. parts per billion; *SABA*, inhaled short-acting β_2-agonist; S*IC*, specific inhalation challenge.

Data are presented as numbers (%) for proportions and medians (25^{th}-75^{th} percentiles) for continuous variables. *P* values in boldface indicate statistical significance.

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

† Quiescent asthma defined by the absence of any asthma medication and a FEV_1/FVC ratio ≥0.70.

Table E5. Baseline clinical characteristics and airway inflammatory markers in subjects with negative vs. positive SIC

Charcteristic	Positive SIC (n=377)	Negative SIC (n=691)	*P* value*
Causal agent, low-molecular-weight, n (%)	170 (45.1)	479 (69.3)	**<0.001**
Age, yr	42 (34–50)	43 (35–52)	0.109
Male sex, n (%)	240 (63.7)	372 (53.8)	**0.002**
Body mass index, kg/m^2	26.5 (23.4–30.1)	26.5 (23.7–30.1)	0.935
Current and ex-smoker, n (%)	166 (46.1)	335 (50.4)	0.185
Atopy, n (%)*	188 (50.0)	298 (44.0)	0.071
Work-related rhinitis, n (%)	278 (74.1)	373 (61.4)	**<0.001**
Duration of exposure before asthma onset, mo	98 (36–204)	96 (36–236)	0.673
Duration of symptoms at work, mo	32 (12–72)	26 (12–60)	
Interval since last workplace exposure, mo	2 (0.1–11)	2 (0.1–14)	0.282
Removal from work ≥1 week, n (%)	228 (60.8)	395 (57.7)	0.328
Asthma medication at the time of SIC			
No treatment, n (%)	85 (22.9)	162 (24.4)	0.582
SABA, n (%)	217 (57.7)	371 (54.8)	0.365
ICS, n (%)	212 (56.2)	421 (62.1)	0.066
Daily dose of ICS, µg†	400 (0–500)	500 (0–800)	**0.001**
Baseline spirometry			
FEV_1, % predicted	92 (83–102)	93 (84–104)	0.468
FEV_1/FVC, %	76 (69–81)	77 (72–82)	**0.002**
FEV_1/FVC <0,70, n (%)	102 (27.1)	130 (18.8)	**0.002**
FEV_1 <80% predicted and FEV_1/FVC <0.70, n (%)	48 (12.7)	60 (8.7)	**0.043**
Baseline histamine/methacholine PC_{20} <16 mg/mL, n (%)	314 (83.3)	341 (49.3)	**<0.001**
Quiescent asthma at the time of the SIC, n (%),‡	76 (20.2)	135 (19.5)	0.807
Quiescent asthma among subjects at work, n (%)‡	19/147 (12.9)	40/290 (13.8)	0.802
Baseline blood eosinophils	(n=246)	(n=403)	
Cells/µl	250 (163–400)	182 (80–290)	**<0.001**
Baseline FeNO	(n=244)	(n=334)	
Pbb	23 (13–39)	15 (11–25)	**<0.001**
≥25 ppb, n (%)	110 (45.1)	87 (26.0)	**<0.001**
Baseline sputum eosinophils	(n=200)	(n=295)	
% of total cell count	2.0 (1.0–4.4)	1.0 (0.5–2.4)	**<0.001**
≥2% of total cell count, n (%)	108 (54.0)	110 (37.3)	**<0.001**

Legend: *FeNO*, fractional exhaled nitric oxide*; FEV$_1$*, forced expiratory volume in one second; *FVC*, forced vital capacity; *ICS*, inhaled corticosteroid; *NSBH*, nonspecific bronchial hyperresponsiveness; *PC$_{20}$*, concentration of histamine/methacholine inducing a 20% fall in FEV_1; *Ppb*, parts per billion; *SABA*, inhaled short-acting β_2-agonist; S*IC*, specific inhalation challenge. Data are presented as numbers (%) for proportions and medians (25th-75th percentiles) for continuous variables. *P* values in boldface indicate statistical significance.

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

† Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.

‡ Quiescent asthma defined by the absence of any asthma medication and a FEV_1/FVC ratio ≥0.70.