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## Phenotypic Characteristics of Occupational Asthma Caused by Persulfate Salts in Hairdressers: A Multicenter Cohort Study

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**Original Article**

# Phenotypic Characteristics of Occupational Asthma Caused by Persulfate Salts in Hairdressers: A Multicenter Cohort Study

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**Running head:** Persulfate-induced asthma

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60 **Keywords:** Fractional exhaled nitric oxide; occupational asthma; occupational rhinitis; persulfate salts;
61 specific inhalation challenge; low-molecular-weight agents.

62 **Total word count:** 3,595 words

63

## ABSTRACT

**BACKGROUND**: The clinical and inflammatory characteristics of occupational asthma (OA) caused by persulfate salts (PS) in hair bleaches have not yet been comprehensively characterized.

**OBJECTIVE:** This study aimed to compare the phenotypic characteristics of PS-induced OA with those of OA due to other low-molecular-weight (LMW).

**METHODS**: This study was conducted in a retrospective multicenter cohort of subjects with OA ascertained by a positive specific inhalation challenge (SIC). The clinical and inflammatory characteristics of hairdressers with PS-induced OA (n=107) were compared with those of subjects who showed a positive SIC to isocyanates (n=128) or various other LMW agents (n=164).

**RESULTS:** Subjects with PS-induced OA had a longer duration of exposure to the offending agent before the onset of asthma than those with OA caused the other LMW agents. They reported more frequently work-related rhinitis (76%) and showed a lower post-SIC level of fractional exhaled nitric oxide (FeNO) (median, 18 ppb [25$^{th}$-75$^{th}$ percentile, 13-26]) compared with OA caused by both isocyanates (36%, $P$<0.001 and 35 ppb [21-80], $P$<0.001, respectively) and the other LMW agents (53%, P<0.001 and 27 ppb [14-52], $P$<0.001). Subjects with PS-induced OA showed the highest rate of isolated late asthmatic reactions (49%), but the difference reached statistical significance only when compared to LMW agents other than isocyanates (31%, $P$<0.002).

**CONCLUSIONS:** PS-induced OA is associated with a higher prevalence of work-related rhinitis and lower levels of FeNO compared with OA caused by other LMW agents. These findings further indicate substantial phenotypic heterogeneity among this broad category of agents.


**Word count:** 248 words

89 **Keywords:** Fractional exhaled nitric oxide; induced sputum; occupational asthma; occupational rhinitis;
90 persulfate salts; specific inhalation challenge

91

## HIGHLIGHT BOX

***What is already known about this topic?***

Although persulfate salts are the predominant cause of occupational asthma among hairdressers, the clinical and inflammatory phenotypes of persulfate-induced asthma hava not been comprehensively characterized and have never been compared with those of OA caused by other low-molecular-weight agents.

***What does this article add to our knowledge?***

Persulfate-induced OA is characterized by a higher prevalence of work-related rhinitis and a lower exposure-change increase in FeNO compared with OA caused by the other low-molecular-weight agents.

***How does this study impact current management guidelines?***

The findings of this study provide some evidence against the involvement of a $T_H2$ mechanism in the development of persulfate-induced asthma and further highlight the heterogeneity of the phenotypic characteristics, and presumably of the underlying pathobiological pathways, involved in occupational asthma due to low-molecular-weight agents.

## Abbreviations used

AUC = area under the ROC curve

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

FeNO = fractional exhaled nitric oxide;

$FEV_1$ = forced expiratory volume in one second;

HMW = high-molecular-weight;

LMW = low-molecular-weight;

NSBH = nonspecific bronchial hyperresponsiveness;

OA = occupational asthma;

PS = persulfate salts

SIC = specific inhalation challenge

ROC = receiver operating characteristics

## INTRODUCTION

A number of studies have documented phenotypic differences between occupational asthma (OA) caused by high-molecular-weight (HMW) proteins and low-molecular-weight (LMW) chemicals [1, 2], although some LMW agents are associated with characteristics similar to those usually linked to HMW agents, namely a high prevalence of work-related rhinitis and a marked increase in fractional exhaled nitric oxide (FeNO) [3, 4].

Ammonium, potassium, and sodium persulfate salts (PS) are highly reactive, water-soluble LMW compounds that are widely used as oxidizing agents in hair bleaches and lightening formulations (Figure 1). PS are a leading cause of sensitizer-induced OA among hairdressers [5] and may account for 5% up to 12% of all OA cases [1, 5]. Nevertheless, the clinical and inflammatory characteristics of PS-induced OA have not yet been comprehensively investigated and have never been compared with those of OA caused by other LMW agents.

This study aimed to explore whether PS-induced OA exhibits distinct phenotypic characteristics compared with the other LMW agents in a multicenter cohort of subjects with OA ascertained by a positive specific inhalation challenge (SIC).

## METHODS

### *Population*

This retrospective observational study was conducted through the European network for the PHenotyping of OCcupational ASthma (E-PHOCAS) cohort. The network gathered the data from subjects with OA ascertained by a positive specific inhalation challenge (SIC) from 14 European tertiary centers from 2006 to 2018 [1, 6, 7]. The inclusion period was extended to April 2024 for PS-induced OA. Among this cohort, 107 hairdressers with PS-induced OA were identified and compared with subjects with OA caused by other LMW agents with available assessments of FeNO and/or sputum eosinophils, including: (1) isocyanates, the most prevalent cause of OA due to LMW agent (n=128) and (2) various other LMW agents pooled together, hereafter referred to as the "various LMW agents" group (n=198). The causal agents involved in these comparison groups are detailed in Table E1 (available in this article's Online Repository).

### *Ethics*

Approval for this retrospective anonymized study was obtained from each local institutional review board and for the central database by the "*Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé*", and the "*Commission Nationale de l'Informatique et des Libertés*".

### *Data Collection*

Anonymized data regarding demographic, occupational, and clinical characteristics of the subjects at the time of the diagnostic evaluation was entered in a standardized Excel (Microsoft, Redmond, WA) spreadsheet at each participating center [1, 6, 7]. Information on concomitant work-related disorders including rhinitis, urticaria, and contact dermatitis were collected as physician-based diagnoses. For the purpose of this study, additional questions enquired about available results of skin-prick tests and patch-tests with PS. Induced sputum

cell counts and FeNO levels measured at baseline and 24 hours after challenge exposures were also collected.

***Specific Inhalation Challenges***

The SIC methodology conformed with international recommendations [8] in terms of the performance of a control (placebo) test on a separate day before challenging the subjects with occupational agents and functional monitoring for at least 6 hours after the control and active challenges [8]. Asthma medications were withdrawn prior to the SIC procedure according to their duration of action and inhaled corticosteroids were withheld 3 to 7 days before the SIC.

Challenge exposures to occupational agents were produced using a "realistic" approach aimed at mimicking the conditions of exposure at the workplace [9]. The procedures used for delivering challenge exposure to PS are further described in Table E2 (available in this article's Online Repository).

The database collected the maximum fall in forced expiratory volume in one second ($FEV_1$) expressed as percentage change from the pre-challenge value that was recorded during the early phase of the bronchial response (i.e., the first hour after the end of the challenge exposure) and the late component (i.e., between the $60^{th}$ minute post-challenge and the end of the functional monitoring). SIC results were interpreted *a posteriori* and considered positive when a ≥15% fall in $FEV_1$ was recorded at any time point during the functional monitoring [8]. The temporal pattern of the bronchial responses was classified as an "early reaction" when a fall in $FEV_1$ of 15% or more occurred within the first hour following the end of the challenge exposure and as a "late reaction" when a ≥15% decrease in $FEV_1$ was recorded between the first hour and the end of the functional monitoring [10]. Bronchial responses were regarded as "dual reactions" when there was a ≥15% fall in $FEV_1$ during both the early and late components of the response.

***Nonspecific Bronchial Hyperresponsiveness***

The level of nonspecific bronchial hyperresponsiveness (NSBH) was measured at baseline before the SIC procedure (i.e., at the end of the "control day") and 24 hours after the last challenge exposure. The degree of NSBH was expressed as the concentration or dose of the pharmacological agent inducing a 15% or 20% drop in $FEV_1$ according to the bronchoprovocation method used in each center. NSBH was graded *a posteriori* as "absent", "mild", or "moderate-to-severe" according to available recommendations or a consensus Delphi approach among investigators [1]. Post-challenge change in the level of NSBH was expressed as the ratio of NSBH at baseline divided by the NSBH value measured at 24 hours after the last exposure to the offending agent. A pre/post-challenge ratio >2 was considered to reflect a significant increase in NSBH [8].

***Fractional Exhaled Nitric Oxide***

The FeNO levels were measured using different devices based on chemiluminescence or electrochemical detection and reported as parts per billion (ppb) [7]. Measurements were made at a flow rate of 50 mL/s in compliance with the recommendations of the European Respiratory Society and the American Thoracic Society [11]. The FeNO values used in this analysis were the baseline values obtained in the morning before challenge exposure to occupational agents (i.e., baseline value) and the values recorded 24 hours after the last challenge. The changes in FeNO were expressed as the absolute difference between the postchallenge and the baseline values expressed in ppb. A post challenge increase ≥13 ppb was considered a discriminant threshold based on a previous analysis of the E-PHOCAS cohort [7].

***Induced Sputum Analysis***

Sputum cell analysis was performed before and 24 hours after the SICs in 7 of the 14 participating centers [12]. Sputum was induced through slightly different methods in participating centers, including the inhalation of a single concentration of hypertonic solution (i.e., 3% or 4%) in 2 centers, and in the other 5 centers, increasing concentrations of hypertonic solutions (i.e., 3%, 4%, and 5% in 4 centers or 10% and 20% in 1 center) for a maximum cumulative duration of 15 to 30 minutes. Processing of sputum samples was carried out either

by selecting viscid portions from the expectorate (4 centers) [13] or using the whole expectorate (3 centers) [14]. Homogenization of the sample was achieved by adding dithiothreitol (0.1%). All centers applied quality criteria based on the cell viability (≥40%) and the level of contamination by squamous cells (<20% in 5 centers and <30% in 2 centers). Differential cell counts were determined by counting a minimum of 400 non-squamous cells. Changes in sputum eosinophils were computed as the difference between the post-challenge and the baseline values expressed as the percentage of non-squamous cells. A sputum eosinophil count ≥3% was categorized as “sputum eosinophilia” while a sputum neutrophil count ≥76% was regarded as reflecting “sputum neutrophilia” [15]. A post-challenge increase in sputum eosinophils ≥2% compared to the pre-challenge value was considered as an “eosinophilic response” [16].

***Data Analysis***

Continuous variables are summarized as medians and $25^{th}$-$75^{th}$ percentiles, and categorical variables by their frequencies and proportions. Comparisons between subjects were made using the chi-squared or Fisher’s exact test for categorical variables and nonparametric tests for numerical variables. The Wilcoxon signed-rank test was used for comparing variables before and after SIC in the same subjects.

To verify whether a small post-challenge FeNO increase (<13 ppb) was independently related to challenge with PS, we conducted a multivariate logistic regression analysis with a binomial generalized linear model and a stepwise procedure based on the Akaike information criterion to select the most parsimonious model. The potential confounding variables incorporated in the regression model were selected on the basis of a *P* value <0.10 in univariate analyses. Statistical analyses were performed using the R software version 4.3.2 (*www.r-project.org*, Vienna, Austria). *P*<0.05 was considered significant.

## RESULTS

### *Baseline Clinical Characteristics*

When compared with OA caused by both isocyanates and the "various LMW agents", PS-induced OA was associated with younger age, female gender, a longer duration of workplace exposure before the onset of asthma, and a longer duration of work-related symptoms prior to the diagnostic evaluation (Table I). Subjects with PS-induced OA reported concomitant work-related rhinitis more frequently (75.5%) and were more often treated with an oral $H_1$-antihistamine and/or a nasal corticosteroid (35.2%) than those with OA caused by isocyanates (35.8%, $P<0.001$ and 12.6%, $P<0.001$, respectively) and by the "various LMW agents" (53.4%, $P<0.001$ and 23.1%, $P=0.026$, respectively).

The subjects with occupational asthma induced by PS reported more often work-related dermatitis (23.6%) than did those with isocyanate-induced OA (7.0%, $P<0.001$). When the subjects with PS-induced OA were compared with subjects with OA due to the "various LMW agents", the difference did not reach statistical significance (14.9%, $P=0.063$). Patch-test results were available in only 15 of the 25 subjects with PS-induced OA who experienced work-related dermatitis. Actually, patch testing identified PS as the causal contact allergen in only 5 of these subjects while other hair products (e.g., paraphenylene diamine) were the causal agent in 7 subjects. In the remaining 10 subjects, the diagnosis was self-reported without additional information on patch-tests. Skin-prick tests with PS were positive in only 2 of 7 tested subjects; these 2 subjects experienced urticaria upon contact with PS-containing products.

### *Functional Characteristics*

Baseline spirometry showed a higher $FEV_1$/forced vital capacity ratio in PS-induced OA than in OA due to isocyanates and the "various LMW agents", but the median $FEV_1$ did not differ significantly between the groups (Table I). Subjects with PS-induced OA exhibited less frequently a baseline moderate-to-severe NSBH (14.6%) and a significant post-challenge increase in NSBH (31.4%) than did those with isocyanate-induced OA (36.8%, $P<0.001$ and

49.5%, *P*=0.014, respectively), although there were no differences when compared with the "various LMW agents" group (22.6%, *P*=0.101 and 42.0%, *P*=0.106).

Isolated late asthmatic reactions occurred more frequently in PS-induced OA (48.6%) than in OA due to the "various LMW agents" (30.8%, *P*=0.002) (Table I), whereas the difference did not reach statistical significance when PS-induced OA was compared with isocyanate-induced OA (37.5%, *P*=0.087). Among subjects with PS-induced OA (Table E3 available in this article's Online Repository), isolated late reactions (n=52) were more frequently associated with uncontrolled asthma defined by the need for an inhaled short-acting $beta_2$-agonist once a day or more (32.7%) and having experienced at least one severe asthma exacerbation in the last 12 months (32.0%) compared with the other patterns of asthmatic reactions (13.2%, *P*=0.017 and 11.5%, *P*=0.012, respectively), although the median dose of inhaled corticosteroid did not differ between the two groups.

***Inflammatory Biomarkers***

Subjects with PS-induced OA exhibited a significantly smaller change in post-SIC FeNO compared with OA caused by both isocyanates and the "various LMW agents" group (Table II). Univariate associations with a small post-challenge FeNO change <13 ppb are detailed in Table E4 (available in this article's Online Repository). The multivariable logistic regression analysis confirmed that PS-induced OA was significantly and independently associated with a post-challenge change in FeNO <13 ppb (odds ratio [OR], 4.74; 95% confidence interval [CI], 2.16-12.0; *P*<0.001) (Table III). Obesity (OR, 2.31; 95% CI, 1.26-4.41; *P*=0.008) and a low baseline FeNO level <25 ppb (OR, 2.51; 95% CI, 1.50-4.27; *P*<0.001) were also identified as significant determinants of a change in FeNO <13 ppb.

Only a small fraction of subjects with PS-induced OA (14 of 107, 13.1%) had available sputum cell counts before and 24 hours after the SIC with PS. These subjects were more often current smokers (50%) than those without sputum assessment (13%, *P*=0.004), but there were no differences between the two subsets of subjects pertaining to other relevant demographic, clinical, and functional characteristics (Table E5 in this article's Online Repository). Subjects

with PS-induced OA showed a trend toward a lower baseline sputum eosinophil count, but the difference reached statistical significance only when compared with the “various LMW agents” group (Table II). There was a significant post-challenge increase in sputum eosinophil count in each group and there was no significant difference between the groups in the magnitude of the post-challenge increase in sputum eosinophils.

As a complementary analysis, inflammatory biomarkers were compared in subjects with positive and negative SIC with PS. This comparison is detailed in Table E6 in this article’s Online Repository. The post-challenge increase in FeNO was not statistically significant in subjects with a positive SIC with PS (n=81) neither in those with a negative SIC (n=62). In contrast, subjects with a positive SIC showed a significant increase in post-challenge sputum eosinophil counts, whereas those with a negative SIC had no significant changes. A receiver operating characteristics (ROC) analysis showed that the post-challenge changes in FeNO were unable to differentiate positive from negative SICs with PS, with an area under the curve (AUC) of 0.44 (95% CI, 0.34-0.53). This analysis did not allow for identifying a relevant threshold change in FeNO that would be useful for diagnosing PS-induced OA.

## DISCUSSION

To our knowledge, this study is the first to investigate the clinical and inflammatory characteristics of PS-induced OA in comparison with OA caused by other LMW sensitizers. The findings indicate that PS-induced OA is characterized by a higher prevalence of work-related rhinitis and a lower post-exposure increase in FeNO as compared with OA caused by all other LMW agents. Furthermore, PS-induced OA is associated with a high rate of isolated late reactions.

A marked post-challenge increase in FeNO has been documented as a feature of OA caused by HMW agents acting through the production of specific IgE antibodies and a $T_H2$ mechanism [1, 2, 17]. Nevertheless, marked increases in FeNO levels have also been recently demonstrated after asthmatic reactions elicited by LMW platinum salts presumably operating through an IgE-mediated mechanism [4] and acrylate compounds for which specific IgE antibodies have never been substantiated [3]. As for most LMW agents, the immunological mechanisms leading to the development of PS-induced OA remain largely unknown [18]. An experimental mouse model of PS-induced asthma documented a mixed $T_H1/T_H2$ immune response based on cytokine profiles in local draining lymph nodes and bronchoalveolar lavage fluid. However, attempts at identifying specific IgE against PS conjugated to human serum albumin have mostly failed in subjects with PS-induced OA [5]. A positive SPT response to PS solutions has been documented in only a small fraction (approximately 7%) of subjects with PS-induced OA/rhinitis [5, 19-25]. The low FeNO concentrations recorded in our cohort,both before and after positive SICs with PS argue against the involvement of a $T_H2$ mechanism, although this would require confirmation through evaluation of $T_H2$-related cytokines in sputum samples or bronchial biopsies. Alternatively, the strongly oxidizing PS could interact with inducible nitric oxide synthase in epithelial cells and inhibit the production of FeNO in the lower airways. Several studies have investigated FeNO levels related to oxidative air pollutants with inconclusive results. Controlled exposure to ozone at 300 ppb in healthy volunteers did not affect FeNO levels [26]. Some studies found elevated FeNO values in spray painters,

shoemakers, and welders [27]. Only one study investigated FeNO variations during exposure in hair salons [27]. Hildre et al. [27] failed to detect significant diurnal variations in FeNO related to occupational exposure among hairdressers investigated over 2 consecutive work weeks. However, hairdressers performing bleaching and dyeing had the lowest FeNO levels. A better understanding of the effects of PS on nitric oxide synthase would require further animal or *in vitro* experiments.

Whatever the underlying pathophysiological mechanisms, the low post-challenge FeNO values observed after PS-induced asthmatic reactions have relevant diagnostic implications. A recent analysis conducted among 321 subjects (156 with a positive SIC) recruited from the E-PHOCAS cohort showed a high accuracy of the post-challenge changes in FeNO for identifying asthmatic reactions induced by various occupational agents, with an area under the receiver operating characteristics (ROC) curve of 0.78 (95% CI, 0.72-0.83) [7]. A post-challenge FeNO increase of ≥13 ppb was the optimal threshold value for identifying an asthmatic reaction during the SIC, yielding a sensitivity of 55% (95% CI, 46%-62%), and a high specificity (95%; 95% CI, 90%-98%) and positive predictive value (90%; 95% CI, 82%-96%). In contrast, among this subset of subjects challenged with PS, a post-challenge increase in FeNO ≥13 ppb provided a much lower sensitivity (10%) while the specificity remained high (95%). These findings indicate that the assessment of changes in FeNO after exposure to PS may not be a useful tool in the investigation of OA among hairdressers exposed to PS.

Concomitant work-related rhinitis has been previously reported as a distinct clinical feature of OA due to HMW agents [1, 2], but this association has also been reported in OA due to LMW platinum salts [4] and acrylates [3]. The high prevalence of work-related rhinitis in subjects with PS-induced OA could be due to an irritant effect of the various hairdressing products used at work [23]. However, nasal eosinophilic inflammation has been described in subjects with OA and rhinitis due to PS [22], suggesting an underlying immunological mechanism. Identifying the inflammatory pathways involved in PS-related rhinitis warrants further prospective investigation.

Remarkably, PS-induced OA was associated with a higher rate of isolated late asthmatic reactions (48.6%) than was OA caused by either isocyanates (37.5%) or the “various LMW agents” (30.8%), but the difference reached statistical significance only when PS-induced OA was compared with OA caused by LMW agents other than isocyanates. This finding is consistent with previous challenge studies that reported a high rate of late asthmatic responses (73% of 33 subjects) among positive SICs with PS [20, 21, 24]. The development of isolated late asthmatic reactions has been consistently associated with LMW agents when these compounds are considered as a single group compared with HMW agents [1, 2]. Our findings indicate that this pattern of reactions among LMW agents is predominantly observed during SICs with PS and isocyanates.

There is only scarce information on the determinants of isolated late reactions induced by occupational sensitizers. In our cohort of subjects with PS-induced OA, this temporal pattern of reaction was more frequent in subjects with worse asthma control —based on the frequency of inhaled bronchodilator use and the occurrence of severe asthma exacerbations— than in those who developed other patterns of bronchial responses, although the dose of inhaled corticosteroids did not differ. Among subjects challenged with LMW occupational agents, Prince et al. [28] found a greater increase in sputum eosinophils after isolated late reactions compared with early and dual bronchial responses. In our cohort, there were no differences between isolated late reactions and the other patterns of reaction as regards sputum inflammatory cells at baseline and 24 hours after the SIC, but the small number of subjects with available sputum data prevented definitive conclusions. To date, the pathophysiological mechanism underlying isolated late reactions induced by common allergens has never been specifically investigated because this pattern of bronchial response is uncommon after exposure to protein allergens [29]. For instance, an isolated late asthmatic response was observed in only 13% of the subjects challenged with HMW occupational agents in the E-PHOCAS cohort [1]. Further prospective studies should focus on comparing the physiological

and inflammatory responses during isolated late reactions with those associated with other patterns of reactions.

In subjects with PS-induced OA, the duration of exposure before the onset of work-related asthma and the duration of work-related symptoms before seeking diagnostic evaluation were longer than those reported in subjects with OA due to all other LMW agents. These findings are likely related to the fact that hairdressers are only intermittently exposed to PS-containing materials.

### Limitations of the Study

The strength of our study was its multicenter design, which allowed for gathering a large cohort of subjects with OA induced by PS and other LMW agents ascertained by a positive SIC. The major limitation resulted from the unavailability of sputum samples in a large proportion (87%) of the subjects with PS-induced OA. However, the subjects with available sputum cell counts did not differ from those without sputum assessment pertaining to the demographic and clinical characteristics that could have led to a bias toward a greater eosinophilic response (i.e., duration of symptoms, time elapsed since last workplace exposure, severity of asthma, treatment with inhaled corticosteroids, baseline level of NSBH) [12]. Despite the small number of subjects with available sputum cell counts both before and 24 hours after the SIC, asthmatic reactions elicited by PS were associated with a significant increase in sputum eosinophils whereas there was no post-challenge increase in FeNO. These data further highlight the possible dissociation between changes in FeNO and sputum eosinophils during asthmatic reactions induced by occupational agents [7]. Nevertheless, the small number of sputum data limited our ability to compare the changes in sputum inflammatory cells between the three groups of LMW agents and to assess reliably the inflammatory events associated with isolated late reactions. Another limitation arises from the lack of standardized assessment of rhinitis during the SICs, which across centers was evaluated in different ways.

## Conclusion

Together, the findings of this study further highlight the heterogeneity of the phenotypic characteristics of OA caused by LMW sensitizers and suggest that PS may induce OA through pathobiological pathways that are different from those driven by other LMW agents. In addition, our data indicate that exposure-related changes in FeNO have a low sensitivity for diagnosing PS-induced OA.

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506 **TABLE I. Clinical and functional characteristics of subjects with occupational asthma induced by persulfate salts compared to**
507 **isocyanates and various low-molecular-weight agents**

| Characteristic | Missing values | Persulfate salts (n=107) | Isocyanates (n=128) | Persulfate salts vs. isocyanates *P* value | Various LMW agents (n=198) | Persulfate salts vs. various LMW agents *P* value |
|---|---|---|---|---|---|---|
| Age, y* | 0/0/0 | 40 (31–50) | 44 (37–54) | **0.006** | 45 (36–52) | **0.003** |
| Female gender, n (%) | 0/0/0 | 103 (96.3) | 27 (21.1) | **<0.001** | 86 (43.4) | **<0.001** |
| Body mass index ≥30 kg/m$^2$, n (%) | 0/1/2 | 21 (19.6) | 31 (24.4) | 0.381 | 52 (26.5) | 0.179 |
| Smoking status | 11/0/0 | | | 0.398 | | 0.822 |
| Ex-and never-smoker, n (%) | | 78 (81.3) | 98 (76.6) | | 163 (82.3) | |
| Current smoker, n (%) | | 18 (18.8) | 30 (23.4) | | 35 (17.7) | |
| Atopy, n (%)† | 1/0/1 | 49 (46.2) | 44 (34.4) | 0.065 | 88 (44.7) | 0.795 |
| Self-reported work-related rhinitis, n (%) | 1/5/5 | 80 (75.5) | 44 (35.8) | **<0.001** | 103 (53.4) | **<0.001** |
| Treatment with oral $H_1$-antihistamine and/or NCS, n (%) | 2/17/12 | 37 (35.2) | 14 (12.6) | **<0.001** | 43 (23.1) | **0.026** |
| Self-reported work-related urticaria, n (%) | 2/14/6 | 9 (8.6) | 4 (3.5) | 0.113 | 19 (9.9) | 0.709 |
| Self-reported work-related contact dermatitis, n (%) | 1/13/4 | 25 (23.6) | 8 (7.0) | **<0.001** | 29 (14.9) | 0.063 |
| Duration of exposure before asthma onset, mo* | 2/5/9 | 108 (36–228) | 54 (16–132) | **0.001** | 70 (18–156) | **0.007** |
| Duration of symptomatic exposure, mo* | 1/15/5 | 36 (14–87) | 22 (12–50) | **0.003** | 25 (12–53) | **0.009** |
| Removal from exposure for ≥1 mo, n (%) | 0/0/1 | 48 (44.9) | 77 (60.2) | **0.019** | 107 (54.3) | 0.115 |
| Inhaled short-acting $\beta_2$-agonist at work, n (%) | 4/18/7 | 58 (56.3) | 69 (62.7) | 0.340 | 129 (67.5) | 0.056 |
| Inhaled corticosteroid at work, n (%) | 1/18/6 | 57 (53.8) | 63 (57.3) | 0.605 | 118 (61.5) | 0.197 |
| Daily dose of inhaled corticosteroid at work, µg*‡ | 2/24/6 | 200 (0–800) | 360 (0–500) | 0.721 | 500 (0–1000) | 0.224 |
| ≥1 severe exacerbation last 12 mo, n (%) | 5/28/20 | 22 (21.6) | 18 (18.0) | 0.526 | 50 (28.1) | 0.230 |
| Baseline spirometry | 0/0/0 | | | | | |
| FVC, % predicted* | | 98 (89–105) | 100 (88–112) | 0.194 | 101 (92–111) | **0.046** |
| $FEV_1$, % predicted* | | 93 (84–100) | 91 (81–101) | 0.868 | 91 (80–101) | 0.598 |
| $FEV_1$ <80% predicted, n (%) | | 19 (17.8) | 27 (21.1) | 0.521 | 45 (22.7) | 0.309 |
| $FEV_1$/FVC, %* | | 80 (74–84) | 75 (69–80) | **<0.001** | 75 (69–81) | **<0.001** |
| $FEV_1$/FVC, <70%, n (%) | | 12 (11.2) | 35 (27.3) | **0.002** | 56 (28.3) | **<0.001** |
| Baseline NSBH | 4/3/12 | | | | | |
| Absent, n (%) | | 38 (36.9) | 31 (24.8) | **0.048** | 62 (33.3) | 0.542 |
| Mild, n (%) | | 50 (48.5) | 48 (38.4) | 0.124 | 82 (44.1) | 0.466 |
| Moderate-to-severe, n (%) | | 15 (14.6) | 46 (36.8) | **<0.001** | 42 (22.6) | 0.101 |
| Change in post-challenge NSBH | 22/35/48 | | | | | |
| Pre/post-challenge ratio* | | 1.0 (1.0–2.3) | 2.0 (1.0–3.7) | 0.052 | 1.2 (1.0–3.8) | 0.347 |
| Pre/post-challenge ratio >2, n (%) | | 27 (31.4) | 46 (49.5) | **0.014** | 63 (42.0) | 0.106 |
| Pattern of bronchial response to SIC | 0/0/0 | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Isolated early reaction, n (%) | | 36 (33.6) | 33 (25.8) | 0.187 | 77 (38.9) | 0.366 |
| Isolated late reaction, n (%) | | 52 (48.6) | 48 (37.5) | 0.087 | 61 (30.8) | **0.002** |
| Dual reaction, n (%) | | 19 (17.8) | 47 (36.7) | **0.001** | 60 (30.3) | **0.017** |

Legend: *FEV₁*, forced expiratory volume in one second; *FVC*, forced vital capacity; *LMW*, low-molecular-weight; *NCS*, nasal corticosteroid; *NSBH*, nonspecific bronchial hyperresponsiveness; S*IC*, specific inhalation challenge. Values in boldface are statistically significant.

* Median and 25$^{th}$-75$^{th}$ percentiles between parentheses.

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

‡ Dose of inhaled corticosteroid expressed as µg of beclomethasone dipropionate equivalent.

**TABLE II. Inflammatory biomarkers in subjects with occupational asthma induced by persulfate salts compared to isocyanates and various other low-molecular-weight agents**

| Characteristic | Persulfates (n=107) | Isocyanates (n=128) | Persulfates vs. isocyanates *P* value | Various LMW agents (n=198) | Persulfates vs. various LMW agents *P* value |
|---|---|---|---|---|---|
| Blood eosinophil count, baseline | (n=67) | (n=66) | | (n=118) | |
| Cells/µl* | 170 (100–300) | 200 (110–368) | 0.160 | 220 (132–336) | **0.036** |
| ≥300/µL, n (%) | 20 (29.9) | 22 (33.3) | 0.666 | 39 (33.1) | 0.653 |
| Baseline FeNO | (n=86) | (n=115) | | (n=164) | |
| Ppb* | 16 (10–26) | 21 (12–45) | **0.032** | 20 (10–36) | 0.112 |
| Post-challenge FeNO | (n=81) | (n=115) | | (n=164) | |
| Ppb* | 18 (13–26) | 35 (21–80) | **<0.001** | 27 (14–52) | **<0.001** |
| Post-challenge change in FeNO, ppb*† | 0 (-3–4) | 10 (2–32)‖ | **<0.001** | 4 (0–17)‖ | **<0.001** |
| Post-challenge change in FeNO ≥13 ppb, n (%)† | 8 (9.9) | 54 (47.0) | **<0.001** | 50 (30.5) | **<0.001** |
| Baseline sputum eosinophil count, | (n=14) | (n=32) | | (n=65) | |
| %* | 1.0 (0-2.4) | 1.3 (0.9–5.3) | 0.129 | 3.0 (1.0–7.0) | **0.033** |
| ≥3%, n (%)‡ | 3 (21.4) | 12 (37.5) | 0.331 | 33 (50.8) | **0.046** |
| Post-challenge sputum eosinophil count | (n=14) | (n=32) | | (n=65) | |
| %* | 2.0 (0.6–8.9) | 5.1 (1.6–11.0) | 0.315 | 7.0 (2.0–19.5) | 0.072 |
| ≥3%, n (%)‡ | 7 (50.0) | 21 (65.6) | 0.318 | 45 (69.2) | 0.217 |
| Post-challenge change, %* § | 1.3 (0–4.9)‖ | 2.9 (0–6.7)‖ | 0.876 | 3.0 (1.0–12.3)‖ | 0.313 |
| Post-challenge change ≥2%, n (%)§ | 6 (42.9) | 17 (53.1) | 0.522 | 40 (61.5) | 0.199 |
| Baseline sputum neutrophil count, | (n=14) | (n=32) | | (n=65) | |
| %* | 35 (26–47) | 46 (32–73) | 0.073 | 50 (37–67) | **0.010** |
| ≥76%, n (%)‡ | 1 (7.1) | 7 (21.9) | 0.403 | 9 (13.8) | 0.681 |
| Post-challenge sputum neutrophil count, § | (n=14) | (n=32) | | (n=65) | |
| %* | 33 (30–48) | 56 (32–74) | 0.066 | 54 (44–69) | **0.004** |
| ≥76%, n (%)§‡ | 0 | 6 (18.8) | 0.312 | 6 (9.2) | 0.584 |

Legend: *FeNO*, fractional exhaled nitric oxide*; LMW*, low–molecular–weight; *PS,* persulfate salt(s)

Data are presented as numbers (% of available data) for proportions and medians ($25^{th}$-$75^{th}$ percentiles) for continuous variables. Values in boldface are statistically significant.

* Median and $25^{th}$-$75^{th}$ percentiles between parentheses.

† Difference between the post-challenge value measured 24 hours after the last challenge exposure and the pre-challenge value expressed in ppb.

‡ A sputum eosinophil count ≥3% was considered as reflecting “sputum eosinophilia” while a sputum neutrophil count ≥76% was regarded as reflecting “sputum neutrophilia” [15]. A post-challenge increase in sputum eosinophils ≥2% compared to the pre-challenge value was considered as reflecting an “eosinophilic response” [16].

§ Difference between the post-challenge assessment completed 24 hours after the last challenge exposure and the baseline value expressed as percent of nonsquamous cells.

‖ P value <0.01 using the paired Wilcoxon signed-rank test.

**TABLE III. Multivariate analysis of factors associated with a post-challenge change in FeNO <13 ppb**

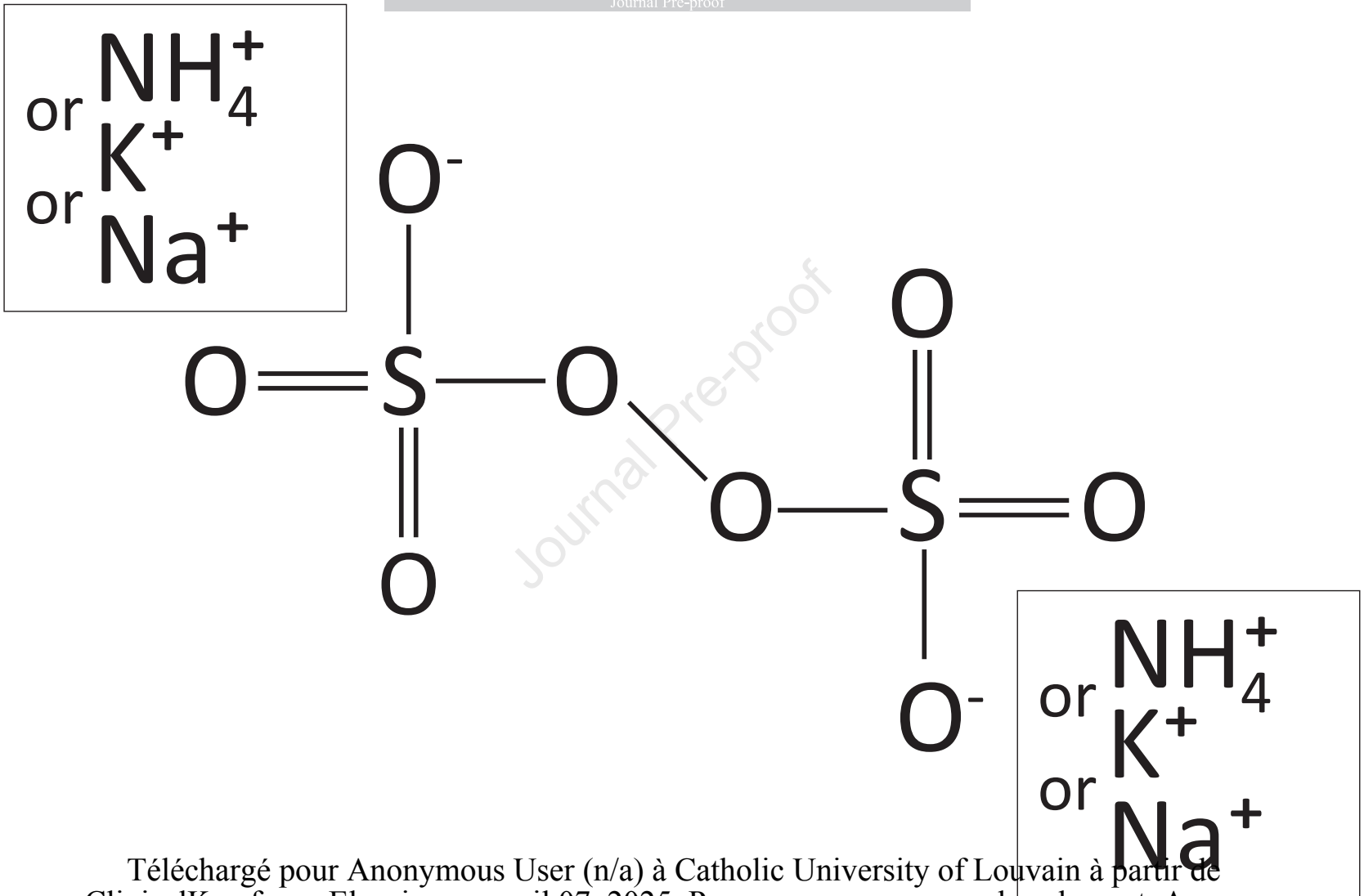
| Independent variables | Odds ratio (95% CI) | *P* value |
|---|---|---|
| **Initial model:** | | |
| Positive SIC with persulfate salts vs. all other LMW agents | 4.50 (1.88–12.1) | **0.001** |
| Gender, male vs. female | 0.93 (0.53–1.62) | 0.794 |
| Current vs. ex-and never smokers | 0.61 (0.32–1.15) | 0.123 |
| Body mass index ≥30 kg/m$^2$ vs. <30 kg/m$^2$ | 2.32 (1.26–4.43) | **0.008** |
| Treatment with an inhaled corticosteroid, yes vs. no | 0.97 (0.58–1.62) | 0.912 |
| Duration of exposure at work before asthma onset | 1.00 (1.00–1.00) | 0.146 |
| Baseline FeNO <25 ppb vs. ≥25 ppb | 2.51 (1.49–4.26) | **<0.001** |
| **Final model:** | | |
| Positive SIC with persulfate salts vs. all other LMW agents | 4.74 (2.16–11.99) | **<0.001** |
| Baseline FeNO <25 ppb vs. ≥25 ppb | 2.51 (1.50–4.27) | **<0.001** |
| Body mass index ≥30 kg/m$^2$ | 2.31 (1.26–4.41) | **0.008** |

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

The multivariate logistic regression analysis included 312 subjects. The independent variables incorporated into the initial regression model were selected on the basis of *P* <0.10 in univariate analysis (Table E3). *P* values in boldface indicate statistical significance.

## LEGEND TO FIGURES

Figure 1 - *Chemical structure of persulfate salts, including ammonium ($[NH_4]_2S_2O_8$, CAS 7727-54-0), potassium ($K_2S_2O_8$, CAS 7727-21-1), and sodium ($Na_2S_2O_8$, CAS 7775-27-1) persulfate salts.*



# Phenotypic Characteristics of Occupational Asthma Caused by Persulfate Salts in Hairdressers: A Multicenter Cohort Study

## ONLINE REPOSITORY

### APPENDIX E1. List of investigators participating to the European network for the PHenotyping of OCcupational ASthma (E–PHOCAS)

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- Iben Brock Jacobsen. Department of Pulmonary Medicine and Occupational Medicine, Odense University Hospital, Odense, Denmark.
- Hille Suojalehto, Irmeli Lindström, and Katri Suuronen. Occupational Medicine, Finnish Institute of Occupational Health, Helsinki, Finland.
- Nicolas Migueres (Chair) and Frédéric de Blay. Service de Pneumologie et Allergologie, Pôle de Pathologie Thoracique, University Hospital of Strasbourg, Strasbourg, France.
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- Gareth Walters and Vicky Moore. Occupational Lung Disease Unit, Birmingham Heartlands Hospital, Birmingham, UK.

7

**Table E1. Low-molecular-weight causal agents other than persulfate salts**

| Isocanates | N | Various low-molecular-weight agents | N |
|---|---|---|---|
| Methylene diisocyanate (MDI) | 64 | Cleaning products/disinfectants (NOS) | 32 |
| Toluene diisocyanate (TDI) | 32 | Metals | 28 |
| Hexamethylene diisocyanate (HDI) | 30 | Acrylate compounds | 22 |
| Isophorone diisocyanate (IPDI) | 1 | Wood dusts | 20 |
| Mixture of isocyanates | 1 | Quaternary ammonium compounds | 12 |
| | | Welding | 12 |
| | | Epoxy resins | 11 |
| | | Drugs | 11 |
| | | Aldehydes | 7 |
| | | Amines | 5 |
| | | Acid anhydrides | 5 |
| | | Metal working fluids | 4 |
| | | Resins/glues/paints (NOS) | 4 |
| | | Styrene | 3 |
| | | Colophony | 3 |
| | | Reactive dyes | 2 |
| | | Chloramine-T | 2 |
| | | Various low-molecular-weight agents (NOS) | 15 |
| **Total:** | **128** | **Total:** | **198** |

Legend: NOS = not otherwise specified.

**Table E2. Procedures used for delivering persulfate salts during inhalation challenges**

| Source of persulfate salts | Mode of challenge exposure | No. of positive SICs |
|---|---|---|
| Commercial bleaching product used at work | Tipping powder (~200 g) from one tray to another for gradually increasing periods of time | 38 |
| | Mixing powder with liquid oxidizing agent (hydrogen peroxide) as done at work | 23 |
| Pure potassium persulfate | Tipping gradually increasing quantities (i.e., 5, 10, 15, and 30 g) of potassium persulphate powder mixed with 150 g lactose powder for 10-min periods | 21 |
| | Tipping 15 g potassium persulfate in 150 g lactose powder for 30 min followed by 30 g potassium persulfate in 150 g lactose powder up to 90 min | 23 |
| Pure ammonium persulfate | Nebulizing (DeVilbiss 646 dosimeter) fourfold increasing doses of ammonium persulfate diluted in phosphate buffer from 0.4 up to a cumulative dose of 600 µg | 2 |

Legend: SIC = specific inhalation challenge.

**TABLE E3. Clinical characteristics associated with isolated late reactions compared to those with other patterns of reactions among subjects with persulfate-induced asthma**

| Characteristic | Missing values | Isolated late asthmatic reactions (n=52) | Isolated early and dual asthmatic reactions (n=55) | P value |
|---|---|---|---|---|
| Age, y* | 0/0 | 41 (31–48) | 40 (32–50) | 0.998 |
| Female gender, n (%) | 0/0 | 52 (100) | 51 (92.7) | 0.118 |
| Body mass index, kg/m$^2$ * | 0/0 | 24.4 (22.0–27.9) | 26.4 (23.1–29.2) | 0.132 |
| Smoking status | 4/7 | | | 0.872 |
| Ex- and never-smoker, n (%) | | 38 (89.1) | 40 (83.3) | 0.601 |
| Current smokers, n (%) | | 10 (20.8) | 8 (16.7) | 0.601 |
| Atopy, n (%)† | 1/0 | 54 (47.1) | 25 (45.5) | 0.868 |
| Self-reported work-related rhinitis, n (%) | 1/0 | 40 (78.4) | 40 (71.7) | 0.495 |
| Self-reported work-related urticaria, n (%) | 2/0 | 5 (10.0) | 4 (7.3) | 0.733 |
| Work–related contact dermatitis, n (%) | 1/0 | 11 (21.6) | 14 (25.5) | 0.638 |
| Duration of exposure before asthma onset, mo* | 1/1 | 96 (26–222) | 114 (38–237) | 0.521 |
| Duration of symptomatic exposure, mo* | 1/0 | 36 (13–67) | 39 (14–93) | 0.510 |
| Interval since last exposure ≥1 month* | 0/0 | 22 (42.3) | 26 (47.3) | 0.606 |
| Inhaled SABA use at the time of SIC, n (%) | 0/0 | 33 (63.5) | 26 (47.3) | 0.092 |
| Inhaled SABA ≥1/day at the time of SIC, n (%) | 0/2 | 17 (32.7) | 7 (13.2) | **0.017** |
| Inhaled corticosteroid use, n (%) | 0/1 | 25 (48.1) | 23 (42.6) | 0.571 |
| Daily dose of inhaled corticosteroid, µg*‡ | 0/1 | 500 (0–800) | 0 (0–788) | 0.623 |
| ≥1 severe exacerbation (last 12 mo), n (%) | 2/3 | 16 (32.0) | 6 (11.5) | **0.012** |
| Baseline spirometry | 0/0 | | | |
| FVC, % pred* | | 98 (89–104) | 98 (91–105) | 0.990 |
| $FEV_1$, % pred* | | 93 (83–101) | 92 (84–99) | 0.958 |
| $FEV_1$/FVC, %* | | 80 (75–82) | 80 (74–84) | 0.629 |
| Baseline NSBH | 3/1 | | | 0.650 |
| Absent, n (%) | | 17 (34.7) | 21 (38.9) | 0.659 |
| Mild, n (%) | | 26 (53.1) | 24 (44.4) | 0.382 |
| Moderate–to–severe, n (%) | | 6 (12.2) | 9 (16.7) | 0.525 |
| Change in post-SIC NSBH | 9/13 | | | |
| Pre/post-challenge ratio* | | 1.2 (1.0–2.4) | 1.0 (1.0–2.2) | 0.745 |
| Pre/post-challenge ratio >2, n (%) | | 14 (32.6) | 13 (30.2) | 0.816 |
| Baseline blood eosinophils, cells/µl* | 18/22 | 154 (100–300) | 200 (100–300) | 0.920 |
| Baseline FeNO, ppb* | 14/7 | 15 (9–31) | 18 (10–25) | 0.764 |
| Post–challenge FeNO | 15/11 | | | |
| Ppb* | | 18 (12–23) | 18 (13–28) | 0.985 |
| Post-challenge change in FeNO, ppb*§ | | 0 (-4–3) | 1 (-2–5) | 0.270 |
| Baseline sputum eosinophils, %* | 46/47 | 0.8 (0.1–2.1) | 1.0 (0–2.4) | 0.842 |
| Post-challenge sputum eosinophils | 46/47 | | | |
| %* | | 2.0 (1.0–4.1) | 5.0 (0.5–13.8) | 0.603 |
| Post-challenge change, %*‖ | | 1.3 (0.3–2.3) | 1.9 (0–8.6) | 0.845 |
| Baseline sputum neutrophils, %* | 46/47 | 28 (26–38) | 45 (21–49) | 0.605 |
| Post-challenge sputum neutrophils, %* | 46/47 | 32 (31–40) | 39 (27–53) | 0.477 |

Legend: *$FEV_1$*, forced expiratory volume in one second; *FVC*, forced vital capacity; *ICS*, inhaled corticosteroid; *NSBH*, nonspecific bronchial hyperresponsiveness; *SABA*, short–acting $\beta_2$–agonist; *SIC*, specific inhalation challenge.

* Median and 25$^{th}$-75$^{th}$ percentiles between parentheses.

† 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.

[§] Difference between the post-challenge value measured 24 hours after the last challenge exposure and the baseline value expressed in ppb.

[‖] Difference between the post-challenge assessment performed 24 hours after the last challenge exposure and the baseline value expressed as percent of nonsquamous cells.

## TABLE E4. Univariate associations with a post-challenge change in FeNO <13 ppb

| Characteristic | Odds ratio | 95% CI | P value |
|---|---|---|---|
| Positive SIC with persulfate salts vs. all other LMW agents | 5.42 | 2.66–12.6 | **<0.001** |
| Age, y | 1.00 | 0.98–1.02 | 0.674 |
| Gender, male vs. female | 0.51 | 0.32–0.80 | **0.004** |
| Body mass index ≥30 kg/m$^2$, yes vs. no | 2.08 | 1.19–3.78 | **0.009** |
| Current smoker vs. ex- and never-smoker | 0.89 | 0.52–1.56 | 0.682 |
| Atopy, yes vs. no * | 1.11 | 0.71–1.76 | 0.638 |
| Work-related rhinitis, yes vs. no | 0.88 | 0.56–1.38 | 0.568 |
| Duration of symptomatic exposure ≥ 30 mo vs. < 30 mo | 1.28 | 0.79-2.07 | 0.312 |
| Interval since last exposure ≥1 month vs <1 mo | 1.33 | 0.85-2.08 | 0.212 |
| Inhaled short-acting $\beta_2$-agonist use at the time of SIC, yes vs. no | 0.76 | 0.48–1.20 | 0.246 |
| Inhaled short-acting $\beta_2$-agonist ≥1/day | 1.18 | 0.66-2.19 | 0.588 |
| At least 1 exacerbation last 12 mo prior to the SIC | 0.86 | 0.49-1.56 | 0.618 |
| Treatment with an inhaled corticosteroid at the time of SIC, yes vs. no | 1.03 | 0.66–1.63 | 0.890 |
| Baseline airway obstruction, yes vs. no‡ | 1.14 | 0.54–2.58 | 0.731 |
| Baseline nonspecific bronchial hyperresponsiveness, present vs. absent | 0.70 | 0.43–1.15 | 0.158 |
| Baseline FeNO <25 ppb vs. ≥25 ppb | 2.33 | 1.49–3.70 | **<0.001** |
| Isolated late reaction during SIC vs. early and dual reactions | 1.22 | 0.69–2.15 | 0.793 |

Legend: *CI*, confidence interval; 95%*FeNO*, fractional exhaled nitric oxide; *FEV$_1$*, forced expiratory volume in one second; *FVC*, forced vital capacity; *LMW*, low–molecular weight; *SIC*, specific inhalation challenge.

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

† Airway obstruction defined by an FEV$_1$ <80% predicted value and an FEV$_1$/FVC ratio <70%.

32 **TABLE E5. Comparison of subjects with and without sputum cell counts**

| Characteristic | Missing values | Subjects with sputum sample (n=14) | Subjects without sputum sample (n=93) | P value |
|---|---|---|---|---|
| Age, y* | 0/0 | 36 (29-51) | 40 (31-50) | 0.518 |
| Female gender, n (%) | 0/0 | 14 (100.0) | 89 (95.7) | 1.000 |
| Body mass index, kg/m$^2$ * | 0/0 | 26.0 (21.1-29.7) | 25.8 (22.8-28.6) | 0.857 |
| Smoking status | 0/11 | | | **0.004** |
| Ex- and never-smoker, n (%) | | 7 (50.0) | 71 (86.6) | |
| Current smokers, n (%) | | 7 (50.0) | 11 (13.4) | |
| Atopy, n (%)† | 0/1 | 6 (42.9) | 43 (46.7) | 0.786 |
| Self-reported work-related rhinitis, n (%) | 0/1 | 11 (78.6) | 69 (75.0) | 1.000 |
| Self-reported work-related urticaria, n (%) | 0/2 | 2 (14.3) | 7 (7.7) | 0.343 |
| Work–related contact dermatitis, n (%) | 0/1 | 2 (14.3) | 23 (25.0) | 0.511 |
| Duration of exposure before asthma onset, mo* | 0/2 | 82 (30-216) | 108 (36-234) | 0.865 |
| Duration of symptomatic exposure, mo* | 0/1 | 34 (12-48) | 38 (16-102) | 0.102 |
| Interval since last exposure ≥1 month* | 0/0 | 9 (64.3) | 39 (41.9) | 0.117 |
| Inhaled SABA use at the time of SIC, n (%) | 0/0 | 9 (64.3) | 50 (53.8) | 0.460 |
| Inhaled SABA ≥1/day at the time of SIC, n (%) | 0/2 | 5 (35.7) | 19 (20.9) | 0.302 |
| Inhaled corticosteroid use, n (%) | 0/1 | 8 (57.1) | 40 (43.5) | 0.339 |
| Daily dose of inhaled corticosteroid, µg*‡ | 0/1 | 275 (0-1000) | 0 (0-800) | 0.334 |
| ≥1 severe exacerbation (last 12 mo), n (%) | 0/5 | 2 (14.3) | 20 (22.7) | 0.729 |
| Baseline spirometry | 0/0 | | | |
| FVC, % pred* | | 102 (93-109) | 98 (89-104) | 0.274 |
| $FEV_1$, % pred* | | 96 (83-101) | 92 (84-100) | 0.582 |
| $FEV_1$/FVC, %* | | 80 (73-82) | 80 (75-84) | 0.722 |
| Baseline NSBH | 1/3 | | | |
| Absent, n (%) | | 5 (38.5) | 33 (36.7) | |
| Mild, n (%) | | 8 (61.5) | 42 (46.7) | |
| Moderate–to–severe, n (%) | | 0 | 15 (16.7) | |
| Change in post-SIC NSBH | 1/22 | | | |
| Pre/post-challenge ratio* | | 1.2 (1.0-2.7) | 1.0 (1.0-2.3) | 0.824 |
| Pre/post-challenge ratio >2, n (%) | | 4 (28.6) | 23 (31.9) | 1.000 |
| Baseline blood eosinophils, cells/µl* | 0/40 | 130 (100-212) | 200 (100-300) | 0.402 |
| Baseline FeNO, ppb* | 1/20 | 12 (10-18) | 18 (10-27) | 0.392 |
| Post–challenge FeNO | 1/25 | | | |
| Ppb* | | 19 (13-28) | 18 (13-26) | 0.648 |
| Post-challenge change in FeNO, ppb*§ | | 4 (-1-10) | 0 (-3-4) | 0.070 |
| Post-challenge change in FeNO ≥13 ppb | | 3 (23.1) | 5 (7.4) | 0.113 |
| Pattern of bronchial response to SIC | 0/0 | | | 0.318 |
| Isolated early reaction, n (%) | | 7 (50.0) | 29 (31.2) | |
| Isolated late reaction, n (%) | | 6 (42.9) | 46 (49.5) | |
| Dual reaction, n (%) | | 1 (7.1) | 18 (19.) | |

33 Legend: *$FEV_1$*, forced expiratory volume in one second; *FVC*, forced vital capacity; *ICS*, inhaled corticosteroid; *NSBH*,
34 nonspecific bronchial hyperresponsiveness; *SABA*, short–acting $\beta_2$–agonist; *SIC*, specific inhalation challenge.

35 * Median and 25$^{th}$-75$^{th}$ percentiles between parentheses.

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

37 ‡ Daily dose of inhaled corticosteroid expressed as beclomethasone dipropionate equivalent.

38 [§] Difference between the post-challenge value measured 24 hours after the last challenge exposure and the baseline value
39 expressed in ppb.
40 [‖] Difference between the post-challenge assessment performed 24 hours after the last challenge exposure and the baseline
41 value expressed as percent of nonsquamous cells.
42

**TABLE E6. Comparison of inflammatory biomarkers in subjects with positive and negative SIC with persulfate salts**

| Characteristic | Negative SIC with PS (n=72) | Positive SIC with PS (n=107) | *P* value |
|---|---|---|---|
| Blood eosinophil count, baseline | (n=60) | (n=67) | |
| Cells/µl* | 125 (65–224) | 170 (100–300) | 0.218 |
| Baseline FeNO | (n=63) | (n=86) | |
| Ppb* | 14 (9–20) | 16 (10–26) | 0.059[2] |
| Post-challenge FeNO | (n=62) | (n=81) | |
| Ppb* | 15 (11–22) | 18 (13–26) | 0.099 |
| Post-challenge change in FeNO, ppb*† | 1 (0–5) | 0 (-3–4) | 0.209[2] |
| Post-challenge change in FeNO ≥13 ppb, n (%)† | 3 (4.8) | 8 (9.9) | 0.350 |
| Baseline sputum eosinophil count, | (n=14) | (n=14) | |
| %* | 0.7 (0–2.0) | 1.0 (0– 2.5) | 0.741 |
| ≥3%, n (%)‡ | 2 (14.3) | 3 (21.4) | 0.999 |
| Post-challenge sputum eosinophil count | (n=14) | (n=14) | |
| %* | 0.5 (0–1.0) | 2 (0.5–9.0) | **0.032** |
| ≥3%, n (%)‡ | 1 (7.1) | 7 (50.0) | **0.033** |
| Post-challenge change, %*§ | 0 (-1.0–0) | 1.3 (0–5.5) ‖ | **0.003** |
| Post-challenge change ≥2%, n (%)§ | 0 | 6 (42.9) | **0.016** |

Legend: *FeNO*, fractional exhaled nitric oxide*; PS,* persulfate salt(s); SIC, specific inhalation challenge with persulfate salts.

Data are presented as numbers (% of available data) for proportions and medians ($25^{th}$-$75^{th}$ percentiles) for continuous variables. Values in boldface are statistically significant.

* Median and $25^{th}$-$75^{th}$ percentiles between parentheses.

† Difference between the post-challenge value measured 24 hours after the last challenge exposure and the pre-challenge value expressed in ppb.

‡ A sputum eosinophil count ≥3% was considered as reflecting "sputum eosinophilia" and a post-challenge increase in sputum eosinophils ≥2% compared to the pre-challenge value was considered as reflecting an "eosinophilic response".

§ Difference between the post-challenge assessment completed 24 hours after the last challenge exposure and the baseline value expressed as percent of nonsquamous cells.

‖ *P* value <0.01 using the paired Wilcoxon signed-rank test