

Diagnostic utility of exhaled breath condensate analysis in conjunction with specific inhalation challenge in individuals with suspected work-related asthma

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ABSTRACT

Background: Establishing the role of exhaled breath condensate (EBC) analysis in work-related asthma (WRA), and more specifically, in conjunction with specific inhalation challenge (SIC), is difficult.

Objective: To measure EBC pH, and nitrite/nitrate concentrations before and after SIC in individuals with suspected WRA exposed to either high-molecular-weight (HMW) or low-molecular-weight (LMW) agents and evaluate whether these changes are useful to distinguish between occupational asthma (OA) and work-exacerbated asthma (WEA).

Methods: One hundred twenty-five consecutive workers undergoing SIC were enrolled. Exhaled breath condensate was collected at the end of the baseline day and 24 hours after exposure to the offending agent. In all EBC samples, pH was measured, and nitrite and nitrate concentrations were determined.

Results: Specific inhalation challenge was positive in 66 individuals, who were then diagnosed with OA. Work-exacerbated asthma was diagnosed in 14, and in 45 patients establishing a direct relationship between the symptoms and work exposure was not possible. In patients with WEA, EBC pH values after SIC were significantly lower than those before SIC ($P = .0047$). Using the receiver operating characteristic (ROC) curve, we found that an EBC pH decrease of greater than 0.4 units after SIC achieved the most satisfactory sensitivity 79% (confidence interval [CI]: 49–94) and specificity of 100% (CI: 68–100), considering only patients with asthma and without OA. A decrease in EBC pH of 0.4 or more common in those exposed to HMW agents (8/19, 42%) than in those exposed to LMW agents (7/47, 15%).

Conclusions: Exhaled breath condensate pH in conjunction with SIC may be useful for diagnosing WEA.

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Introduction

An estimated 10% to 25% of all asthma cases developing in adulthood are caused by occupational exposure.^{1,2} Work-related asthma, which includes occupational asthma (OA) and work-exacerbated asthma (WEA), presents a major health challenge with significant potential for acute morbidity, long-term disability, and an adverse social and economic impact.³ Occupational asthma refers to de novo asthma caused by exposure to an agent specific to a workplace and not to stimuli outside the work environment, and WEA refers to a worsening of preexisting or concomitant asthma caused by working conditions.⁴ Specific inhalation challenge (SIC)

is considered the reference standard method for the diagnosis of OA, which is often difficult to confirm objectively. Specific inhalation challenge is intended to demonstrate a direct relationship between exposure to a test substance and an asthmatic response.⁵ However, false-positive and false-negative responses to SIC can occur.^{6–8}

Like bronchial asthma, work-related asthma is a heterogeneous chronic inflammatory disorder of the airways. Study of airway inflammation could be of great value for establishing a precise diagnosis, being a direct reflection of the disease. In this sense, induced sputum analysis preceding and following SIC has been used to support the diagnosis of OA in the laboratory⁹ or at the workplace,^{10,11} although it is currently a tool with limited availability. Another noninvasive, comprehensive view of airway inflammation can be obtained by analyzing exhaled breath condensate (EBC).^{12,13} Exhaled breath condensate collection is completely non-invasive, repeatable, and does not disturb the underlying disease process, making it a useful approach for cross-day variation studies and longitudinal studies that monitor inflammation.^{14–16}

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However, in contrast to the situation with induced sputum, few studies have evaluated the role of EBC in work-related asthma, and only 2 have done so in conjunction with SIC.^{17,18} Klusackova et al¹⁷ monitored leukotrienes and 8-isoprostane before and after SIC, but the results cannot be considered conclusive, because SIC was positive in only 6 of the 47 patients studied. Furthermore, the authors did not specify the workplace agent. The other study conducted by Ferrazzoni et al,¹⁸ performed only in 15 patients with OA due to isocyanates, found no association between asthmatic reactions and EBC acidification after SIC. From these studies, establishing the role of EBC analysis in work-related asthma, and more specifically, in conjunction with SIC, is difficult. Thus, the aims of this study were to measure EBC pH and nitrite/nitrate concentrations before and after SIC in individuals with suspected work-related asthma exposed to either high-molecular-weight (HMW) or low-molecular-weight (LMW) agents and to evaluate whether these changes are useful to distinguish between OA and WEA.

Methods

Study Design and Subjects

This is a cross-sectional observational study to know the utility of EBC analysis in conjunction with SIC in individuals with suspected work-related asthma (WRA). The study included all individuals older than 18 years of age referred to our center to investigate possible OA by SIC testing between 2004 and 2010. All patients had a medical history consistent with OA, and a workplace agent was considered the probable cause of their respiratory symptoms. Patients with a positive SIC result received a diagnosis of OA, and those with a negative SIC were divided into 2 groups, according to clinical criteria.¹⁹ Patients who presented symptoms and abnormal findings on pulmonary function study in relation to work received a diagnosis of WEA, whereas those who did not fulfill the criteria for asthma or who had asthma that was unrelated to work were grouped as *no work-related asthma* (NWRA). All patients signed informed consent documents for participation. The local Ethics Committee approved the study.

Inhalation Challenge

Specific inhalation challenge was performed with the substance suspected to be the causal agent in each patient, only when there was no contraindication to this testing.²⁰ The test was mainly carried out according to the method proposed by Pepys et al²¹ and by our group.^{22,23} Briefly, patients were examined on 5 consecutive days. On day 1 (baseline), clinical and occupational histories were collected, skin-prick tests with common allergens, radiography study, pulmonary function tests, and methacholine challenge were performed, and EBC was collected. On day 2, a first placebo inhalation challenge was performed. On days 3 and 4, patients underwent SIC with the appropriate workplace agent. On day 5 after SIC, pulmonary function tests and methacholine challenge were performed, and EBC was collected again. Pulmonary function changes were monitored in each patient by measuring the forced expiratory volume in 1 second (FEV₁) every 10 minutes during the first hour after exposure and every hour thereafter up to 15 hours after inhalation. The response was considered positive when FEV₁ decreased by more than 20% the baseline value in the absence of any change with the placebo test.

EBC Collection

Exhaled breath condensate was collected during tidal breathing with a commercially available condenser (EcoScreen; Jaeger, Würzburg, Germany), as described.¹⁶ Exhaled air entered and left the chamber through 1-way valves at the inlet and outlet, thus maintaining the chamber closed. Patients breathed tidally through a mouthpiece connected to the condenser while wearing nose clips.

The low temperature within the condensing chamber cooled the sample throughout the collection time. Samples were collected in sampling tubes that before use, had been disinfected for 30 minutes using sodium dichloroisocyanurate (Inibsa Lab, Barcelona, Spain) and rinsed for 24 hours with distilled water and 2 hours with ultrapure water (Fresenius Kabi, Barcelona, Spain). To determine the ventilatory pattern, a spirometer (EcoVent; Jaeger) was connected to the equipment at the expiratory valve. The spirometer recorded the total exhaled breath, time of collection, tidal volume, minute ventilation, and breathing frequency. Patients were instructed to refrain from food intake during the 2 hours before sample collection. A fixed volume of 150 L exhaled breath was collected per patient. Each EBC sample was divided into 500- μ L aliquots in 2 to 4 plastic tubes. The aliquots, used for measuring nitrite and nitrate, were immediately stored at -70°C and analyzed within 1 month of collection. Other aliquots were used to measure the pH after deaeration.

EBC pH Measurement

The pH was measured in 1 of the aliquots after EBC deaeration with helium (350 mL/min for 10 min), using a calibrated pH meter (Model GLP 21; Crison Instruments SA; Barcelona, Spain) with an accuracy of ± 0.01 pH, and a probe for small volumes (Crison 50 28; Crison Instruments SA). The probe was calibrated daily with standard pH 7.02 and 4.00 buffers.²⁴ Evaluation of the reproducibility of EBC pH measurements from 5 consecutive measurements revealed a mean coefficient of variation of 2% (range, 0.5–14%). The mean intraday coefficient of variation was 4% (range, 1–20%).

EBC Nitrite and Nitrate

Nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were determined with a colorimetric assay based on the Griess reaction. Briefly, NO_3^- was measured as NO_2^- after enzymatic conversion by nitrate reductase, and the total $\text{NO}_2^-/\text{NO}_3^-$ (converted NO_3^- plus NO_2^-) was measured in sample duplicates using Griess reagent (Cayman Chemical Company; Ann Arbor, Michigan). Concentrations were measured with a microplate reader at 540 nm absorbance. Assay sensitivity was 1 $\mu\text{mol/L}$ for nitrite and 2.5 $\mu\text{mol/L}$ for nitrate. Within-run coefficients of variation were 6%, and 4%, and between-run coefficients of variation were 9% and 5% for nitrate and nitrite, respectively. The authors decided to measure the end products of NO metabolism instead of exhaled NO to compare compounds generated from the same specimen.

Data Analysis

A 1-sample Kolmogorov-Smirnov test was calculated to assess whether the assumption that variables analyzed had normal distributions was tenable. The results of the test refuted the assumption; hence, the Kruskal-Wallis test and the adjusted Mann-Whitney *U* test were used to compare the age, duration of exposure and symptoms, interval from last exposure, lung function data, EBC pH, and nitrite/nitrate concentrations between the OA, WEA, and NWRA groups. Wilcoxon signed rank test was used to compare lung function data, EBC, pH, and nitrite/nitrate concentrations within groups. Spearman's rank correlation test was applied to determine correlations between EBC pH and nitrite or nitrate concentrations. A *P* value of $<.05$ was considered significant. Nondetectable nitrite and nitrate concentrations were assigned the value of the detection limit of the method (1 $\mu\text{mol/L}$ and 2.5 $\mu\text{mol/L}$, respectively). Receiver operating characteristic (ROC) curves were constructed to determine the most discriminating cutoff value of changes in EBC pH before and after SIC for predicting WEA. SPSS 17.0 for Windows (SPSS Inc, Chicago, Illinois) was used for the statistical analyses.

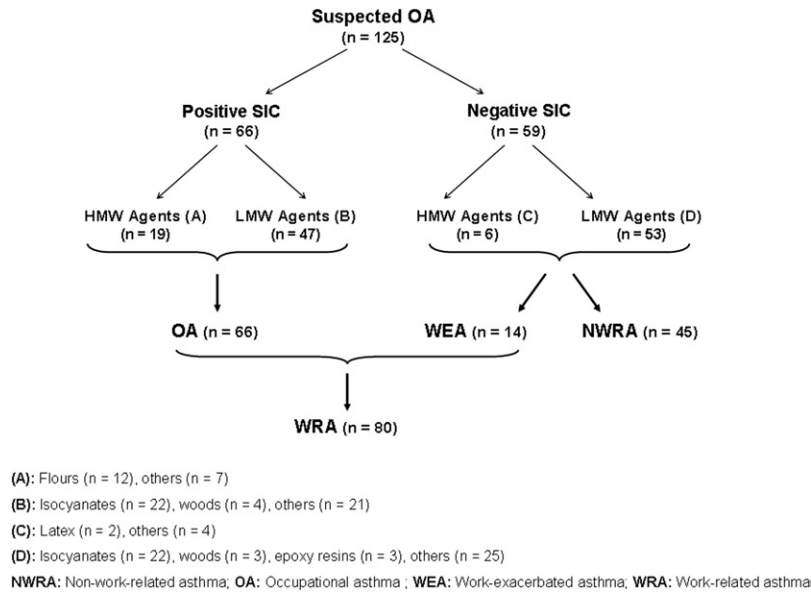


Figure 1. Study population and agents tested.

Results

Study Population

One hundred twenty-five consecutive patients referred for possible OA were assessed. Sixty-six patients had a positive SIC and were diagnosed with OA (31 patients had an immediate asthmatic reaction, 23 a late asthmatic reaction, 10 a dual asthmatic reaction, and 2 patients had other types of asthmatic reactions), and 59 patients had a negative SIC to the workplace agents tested (Fig 1). Among the 59 SIC-negative patients, 14 were diagnosed with WEA according to clinical criteria, and 45 were considered NWRA, either

because they did not meet the criteria for asthma ($n = 34$) or because they had asthma in which the symptoms were unrelated to the workplace ($n = 11$). Patients in the NWRA group who did not have asthma were ultimately diagnosed with eosinophilic bronchitis ($n = 2$), chronic obstructive pulmonary disease (COPD) ($n = 2$), chronic bronchitis ($n = 2$), bronchiolitis ($n = 1$), smoker's bronchiolitis ($n = 1$), pulmonary tuberculosis sequelae ($n = 1$), and bronchiectasis ($n = 1$); in 24 patients, the respiratory disease could not be identified.

Demographic data and clinical characteristics of the patients included are presented in Table 1. The FEV₁ values (% predicted)

Table 1
Characteristics of the Subjects Studied

Characteristics	WRA Group		NWRA Group (n = 45)
	OA Group (n = 66)	WEA Group (n = 14)	
Sex, M/F	50/16	6/8	30/15
Age, years	40 (22–57)	43.5 (24–62)	44 (18–64)
Smoking habits, NS/exS/CS	30/17/19	5/6/3	14/17/14
Atopy, %	48	62	50
Duration of exposure, months	115 (3–533)	153.5 (23–365)	143 (5–532)
Duration of symptoms, months	48 (1–365) ^b	36 (5–197)	23 (2–177) ^b
Interval from last exposure, months	1.25 (0–65)	3 (0–21)	2 (0–27)
FEV ₁ % pred.			
Before exposure	88.5 (53–124)	82 (69–99) ^c	92.5 (53–125) ^c
24 h after exposure	86 (39–125)	82 (48–95) ^d	90 (53–125) ^d
Methacholine before exposure, n	66	14	45
% Positive	72	75	44
PC ₂₀ , mg/mL ^a	2 (0.19–16) ^e	4 (0.22–13)	4.5 (0.21–12.7) ^e
Methacholine 24 h after exposure, n	44	12	43
% positive	75	67	42
PC ₂₀ , mg/mL ^a	2.4 (0.05–16) ^f	8 (0.85–12)	6.8 (0.52–16) ^f
≥2-fold % PC ₂₀ decrease	36	0	0
Occupational agents, HMW/LMW	19/47	2/12	4/41
Type of asthmatic reactions, immediate/late/dual/others	31/23/10/2	...	...

Abbreviations: CS, current smoker; exS, ex-smoker; F, female; HMW, high-molecular-weight agent; LMW, low-molecular-weight agent; M, male; NS, never smoker; NWRA, no work-related asthma; OA, occupational asthma; PC₂₀, methacholine concentration inducing a 20% fall in FEV₁; SIC, specific inhalation challenge; WEA, work-exacerbated asthma; WRA, work-related asthma.

Data are given as the number or median (range), unless otherwise noted.

^aOnly positive tests.

^b $p = .009$.

^c $p = .034$.

^d $p = .039$.

^e $p = .028$.

^f $p = .004$.

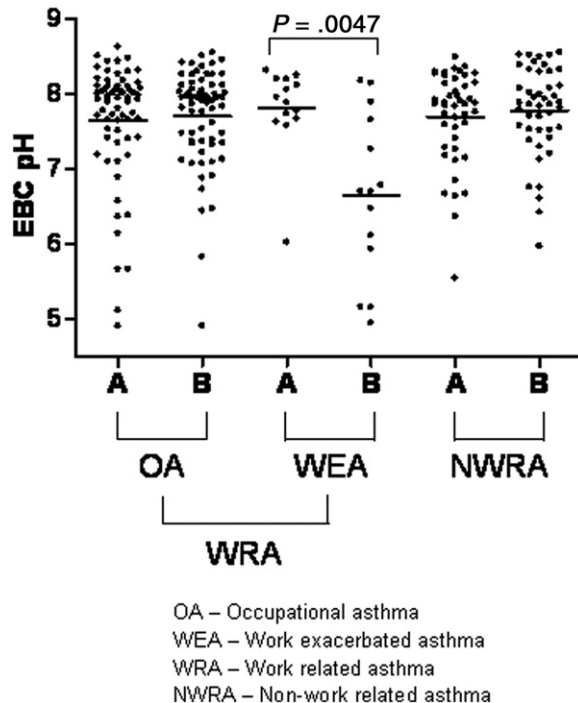


Figure 2. EBC pH in the 3 groups studied. A, Before SIC; B, After SIC.

before exposure and 24 hours after exposure were lower in the WEA group compared with the NWRA group ($P = .034$ and $.039$, respectively). Methacholine challenge 24 hours after exposure could not be performed in 22 patients in the OA group because their FEV_1 was less than 60% of the theoretical value after SIC. Two patients in the WEA group and 2 in the NWRA group did not agree to repeat methacholine challenge testing at 24 hours after SIC. The PC_{20} was significantly lower in the OA group compared with the NWRA before exposure ($P = .028$) and at 24 hours after exposure ($P = .004$). A PC_{20} decrease of at least 2-fold at 24 hours occurred in 36% of patients in the OA group and none of the patients in the WEA or NWRA groups. Symptom duration was also greater in

OA than in NWRA ($P = .009$). No significant differences were seen between the groups in any of the remaining variables analyzed.

EBC pH Measurement

The EBC pH values before and after exposure are shown in Figure 2. After SIC, a significant decrease in EBC pH was seen in the WEA group ($P = .0047$). Most patients with WEA (12/14) had been exposed to LMW agents.

The ROC curves showed the most relevant differences in EBC pH before and after SIC for predicting WEA. Considering only patients with negative SIC and a reliable diagnosis of asthma (all 14 patients with WEA group and 11 patients of the NWRA group), we found that an EBC pH decrease greater than 0.4 units after SIC achieved the most satisfactory sensitivity: 79% (49–94) and specificity of 100% (68–100) (Fig 3A). Considering all the patients included in the study, and the same decrease of 0.4 units in the EBC pH, we obtained a sensitivity of 78% (CI: 49–94) and specificity: 82% (CI: 73–88) for the diagnosis of WEA (Fig 3B).

In the group of patients with OA, a decrease in EBC pH of 0.4 or more was more common in those exposed to HMW agents (8/19, 42%) than in those exposed to LMW agents (7/47, 15%). In patients with WEA, this significant pH decrease was observed in 2 patients in contact with HMW agents and in 9 of 12 (75%) exposed to LMW agents. In the NWRA group, a significant pH decrease occurred in only 5 (11%) patients, all of whom had been in contact with LMW agents. The final diagnoses in these patients were eosinophilic bronchitis in 2 and bronchiectasis in 1; a definite diagnosis could not be established in the remaining 2 patients.

Nitrite and Nitrate Measurement

In the group of OA patients exposed to HMW agents, a significant increase in NO_3^- values occurred after SIC ($P = .0002$) (Fig 4A). In these same patients, the pH decrease after SIC was greater in those with more elevated baseline NO_2^- levels ($r = -0.685$, $P = .001$) (Fig 4B). No significant differences were seen in NO_2^- or NO_3^- concentrations before and after SIC in the remaining groups analyzed.

Discussion

The most important result of this study is that a decrease of more than 0.4 units in the EBC pH after SIC, in patients with asthma

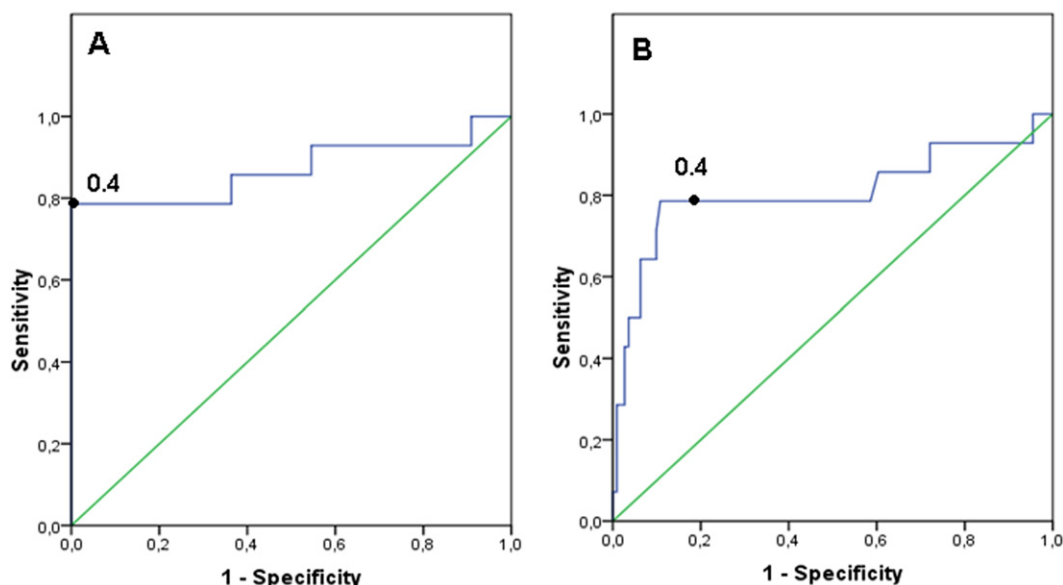


Figure 3. Receiver operating characteristic curves constructed to determine the most relevant difference in EBC pH before and after SIC for predicting WEA in (A) patients with negative SIC and reliable diagnosis of asthma and (B) all the study population. The dots represent the optimal cutoff points.

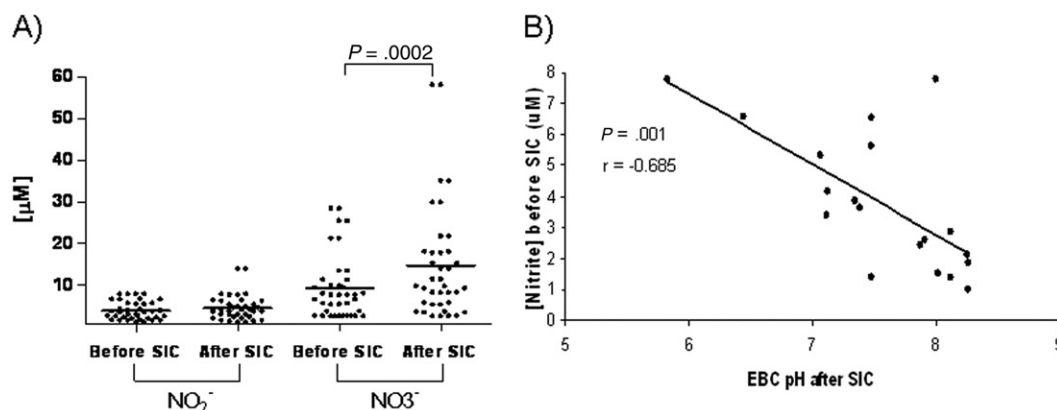


Figure 4. OA group exposed to HMW agents. (A) EBC NO_2^- and NO_3^- concentrations; (B) correlation between EBC pH after SIC and EBC NO_2^- concentration before SIC.

in which we exclude the diagnosis of OA, has a sensitivity of 79% and a specificity of 100% for the diagnosis of WEA. Most of patients with WEA had been exposed to LMW agents. In OA patients, a decrease of more than 0.4 units was only found in 15% of those exposed to LMW agents and in 42% of those exposed to HMW agents. In addition to the potential value of these findings for diagnostic purposes, they suggest that the mechanism of action of LMW agents may differ according to whether they provoke OA or WEA.

In studies on work-related asthma, the percentage of patients with WEA ranges from 13% to 58%.⁴ Studies investigating socioeconomic outcomes in work-related asthma have generally shown that OA and WEA patients report similar income loss, rates of unemployment, and partial work disability.^{25,26} Nevertheless, some authors suggest that patients with WEA, unlike those with OA, can usually return to their workplace with adjustments to reduce exposure to likely airway irritants or with optimized asthma medication.²⁷ As compared with OA, studies on WEA are limited, and this leads to relative uncertainty regarding the definition, prevalence, diagnosis, and management of these conditions. The diagnosis of WEA is usually based on the existence of asthma before occupational exposure and to changes occurring in the symptoms, medication use, or lung function temporally related to work, whereas the diagnosis of OA is based on the fact that asthma onset occurs after starting a job, with similar changes in symptoms, medication use, or lung function temporally related to work.^{3,4} Nonetheless, according to the American College Of Chest Physicians Consensus Statement,⁵ the possibility that an individual with previous asthma will present with OA cannot be excluded, nor can it be ruled out that a patient will develop nonoccupational asthma during his or her working life and that this will be exacerbated by work. In fact, only 10% to 25% of asthma cases initiating in adults are of occupational origin.¹ In this context, a simple differentiation between OA and WEA based on a temporal relationship between the onset of asthma and the start of an occupation can be erroneous. Thus, it would be useful to have diagnostic methods that would enable differentiation between these 2 conditions. Peak expiratory flow recording cannot reliably distinguish OA from WEA, because both may be associated with work-related changes in airway caliber.^{5,19} Some authors have suggested that SIC could be useful because a positive result confirms the diagnosis of OA. Nonetheless, when SIC is negative, the situation is more complex. In a study population comprising patients with work-related asthma, one can conclude that individuals with a negative SIC have WEA.²⁸ However, in clinical practice, SIC is often performed in patients with asthma unrelated to the workplace and in patients who do not have asthma.²⁹ In these situations, it would be of use to

perform other tests in conjunction with SIC to help establish the definite diagnosis.

In the current study, in the WEA group, 79% (11/14) experienced an EBC pH decrease 0.4 units or more after SIC. Moreover, most patients who experienced this significant pH decrease had been exposed to LMW agents (9/11). Work-exacerbated asthma can result from a variety of occupational triggers, including physical factors (eg, extreme temperatures, exercise), behavioral states (eg, strong emotions, stress), odors (eg, perfume), general irritants and dust, and second-hand cigarette smoke.⁴ Because of this large variety of potential triggering factors, we cannot exclude a nonspecific mechanism that would lead to a decrease in EBC pH. In fact, LMW substances may more easily cause nonspecific airway irritation with symptoms in patients with WEA or other respiratory diseases. This could be the case, for example, of 3 patients in our NWRA group, who also showed a pH decrease after SIC and were affected with eosinophilic bronchitis or bronchiectasis. It is important to highlight that there was no decrease in EBC pH after SIC in 22 of the 24 patients in whom no disease was documented or in any of the patients with asthma unrelated to work.

In patients with OA, no significant decrease was seen in EBC pH after SIC regardless of whether they had been exposed to HMW or LMW agents. Nonetheless, 42% of OA patients exposed to HMW agents and only 15% of those exposed to LMW agents presented an EBC pH decrease of 0.4 units or more after SIC. In the case of HMW agents, the decrease was related to the fact that baseline EBC NO_2^- levels were more elevated. Furthermore, an increase in NO_3^- levels after SIC was found in OA patients in contact with HMW agents, but not in those exposed to LMW agents. No other studies have been done in this area, and only a few studies have focused on NO determination in OA. In general, in these studies, the authors observed an increase in the levels of NO in response to HMW agents, as this increase was not observed in response to LMW agents.^{18,30–32} These findings might be attributable to differences in the pathophysiology of asthma induced by these 2 types of agents. The airway changes produced by LMW and HMW agents differ, and these differences could be reflected in the EBC pH. Nevertheless, no clear evidence exists that LMW agent-induced asthma is associated with acidification of EBC pH, because the available studies report conflicting findings. The EBC pH results in the current study are consistent with those of Ferrazzoni et al,¹⁸ who found no evidence that isocyanate-induced asthma is associated with EBC acidification. However, Boyce et al³³ reported increased pH values in workers exposed to welding fumes, and Fireman et al³⁴ found significantly lower levels in welders exposed to cadmium/chromium/iron/lead/nickel, and no significant changes in those exposed to aluminum/iron. The discrepancies existing between studies sug-

gest a different mechanism of action in the 2 types of work-related asthma and would explain the differences found in the current study regarding EBC pH and NO_2^- and NO_3^- levels in patients with OA exposed to HMW and LMW agents.

This study has some limitations. First, it is an observational study with no follow-up data. Thus, we do not know whether the diagnoses established by SIC have been maintained over time. Another limitation is derived from the diagnosis of WEA. In the current study, we chose to use a combination of clinical data and SIC findings, such that WEA was diagnosed when the patient had asthma, the asthma was exacerbated by work, and SIC was negative. Despite these limitations, the current study is of value, being the first in a large number of patients in which the role of EBC pH in conjunction with SIC is analyzed. Moreover, the results demonstrate that EBC pH in conjunction with SIC may be useful for diagnosing WEA and suggest that mechanism of action of LMW agents seems to differ according to whether they cause OA or induced WEA. We can conclude that these EBC inflammatory markers should be carefully evaluated to investigate their potential capability to establish differences between OA caused by LMW or HMW agents and to distinguish between patients with OA, WEA, and asthma unrelated to work.

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