

Utility of Exhaled Breath Condensate pH for Diagnosing Occupational Asthma

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Key Words

Peak expiratory flow • Specific inhalation challenge • Methacholine • Exhaled breath condensate • Occupational asthma

Abstract

Introduction: The current reference standard method for diagnosing occupational asthma (OA) is specific inhalation challenge (SIC) with the suspected agent. The alternative method is serial peak expiratory flow (PEF) monitoring. Nevertheless, PEF does not have optimal sensitivity and specificity for this purpose. The aim of this study was to evaluate the utility of exhaled breath condensate (EBC) pH for the diagnosis of OA. **Material and Methods:** A prospective study was performed in 37 subjects with suspected OA. Serial PEF monitoring was carried out for 2 weeks at work and for 2 weeks off work. At the end of each period, the EBC pH and the methacholine concentration resulting in a 20% FEV₁ decrease (PC20) were measured. SIC was subsequently performed. PEF graphs were interpreted visually by 3 experienced independent readers. **Results:** Seventeen patients tested positive with SIC. Receiver-operating characteristic curves showed that a decrease in EBC pH greater than 0.4 units during the period at work compared to the off-work period achieved the most satisfactory sensitivity (40%, CI

19.4–66.5) and specificity (90%, CI 66.9–98.2) for diagnosing OA. When EBC pH findings were added to PEF results, the diagnostic yield of PEF generally increased. Other test combinations (e.g. EBC pH plus PC20 or EBC pH plus PC20 plus PEF) did not improve diagnostic performance. **Conclusions:** Acidification of EBC pH at work and adding the EBC pH measurement to PEF monitoring during periods at work and off work may be useful for improving the diagnosis of OA.

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Introduction

Specific inhalation challenge (SIC) is considered the reference standard test for diagnosing occupational asthma (OA) and identifying possible causal agents of the disease [1]. Nonetheless, SIC is a costly, time-consuming test that is only available in highly specialized centers and can only be used in suspected OA patients with no respiratory function abnormalities. Hence, numerous strategies have been devised to enable the diagnosis of OA without using SIC [1–5]. A recent study has suggested that the combination of peak expiratory flow (PEF) monitoring and sputum eosinophil count in periods at work and off work could present the best sensitivity and specificity for this purpose [6]. However, once again, sputum induction

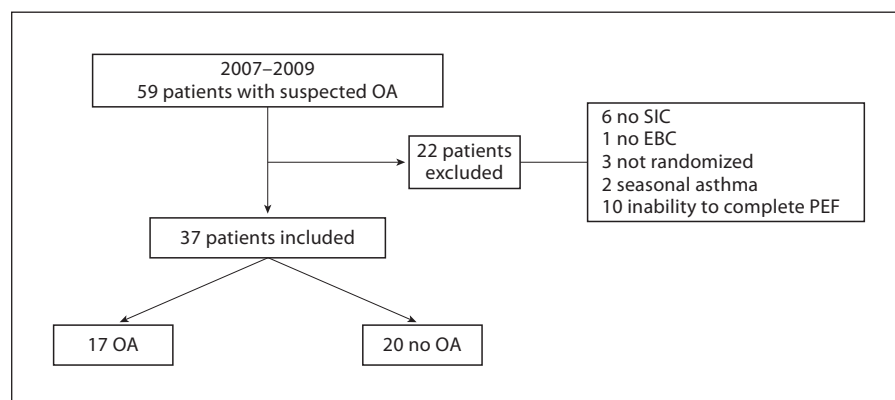


Fig. 1. Flow chart of patient enrollment.

is not possible in all patients, the sample obtained may not be adequate and must be processed immediately for the analysis, which means that a properly equipped laboratory must be available in the center where the induction is performed, a condition that is not met in all hospitals [7].

In recent years, the study of inflammatory markers in exhaled breath condensate (EBC) has acquired considerable importance in the evaluation of various diseases. In line with this, an emerging technique, pH determination in EBC [8], has proven useful as an indirect measure of the degree of bronchial inflammation in several conditions [8, 9]. It is a simple, inexpensive technique without contraindications and it can be used in all types of patients [8]. In the case of occupational airway diseases, there are few studies evaluating the role of EBC. However, two recent reviews have suggested that this technique could be useful in the diagnosis of OA [10, 11]. The main objective of this study was to evaluate the utility of EBC pH for diagnosing OA.

Material and Methods

Study Design

This is a prospective study performed in subjects with suspected OA. At the first visit, participants' characteristics were documented. PEF was serially monitored during a 2-week period at work and a 2-week period away from work. Subjects were seen within 24 h after completion of each period, at which time spirometry, methacholine challenge and EBC collection were performed. Lastly, SIC was carried out in the laboratory to confirm the diagnosis of OA, and was considered the reference standard for the diagnosis. Subjects who had symptoms of seasonal asthma, those who could not be included in the two study periods, those unable to undergo methacholine challenge or SIC due to contraindications and those in whom PEF could not be performed or

EBC could not be collected were excluded from the study. Patients were allowed to take any type of asthma medication during the study, but there could be no changes in the type or dose used during the two study periods. The study was approved by the ethics committee of our center and all subjects gave written consent to participate.

Study Population

Fifty-nine individuals with suspected OA were assessed between 2007 and 2009: 37 completed the study and 22 were excluded (fig. 1).

Spirometry and Methacholine Test

Spirometry was performed according to American Thoracic Society (ATS) guidelines [12], using a Datospir 200® system (Sibel, Barcelona, Spain). The reference values applied were those proposed for the Mediterranean population [13]. Bronchial challenge with methacholine was carried out using a Mefar MB3 dosimeter (Ele H2O, Medically, Brescia, Italy), following the method described by Chai et al. [14]. Increasing doses of methacholine (range 0.03–16 mg/ml) were inhaled at 3-minute intervals until forced expiratory volume in the first second (FEV₁) had fallen by 20% of the subject's baseline value or the maximum concentration of methacholine had been inhaled. The methacholine concentration resulting in a 20% FEV₁ decrease (PC20) was expressed in mg/ml. The test was considered negative when PC20 was >16 mg/ml, in keeping with the ATS guidelines [15]. To evaluate subjects in whom PC20 could not be calculated because it was greater than 16 mg/ml, the dose-response ratio (DRR) was determined, as has been proposed by some authors [16]. The DRR to methacholine was calculated according to the following formula: DRR = last FEV₁ decline (%) / last dose administered.

Monitoring Peak Expiratory Flow Rates

Serial PEF monitoring was performed during 2 weeks at work and 2 weeks off work. Subjects were provided with Mini-Wright portable peak flow meters (Clement Clarke International, UK) and diary cards, and were instructed in their use according to the indications of Moscato et al. [17]. They were asked to record PEF rates at least 4 times a day. PEF graphs were interpreted visually by 3 experienced independent readers. The recording was considered positive when qualitative assessment showed evident chang-

es between the exposure periods [18]. The recordings were classified, including and excluding the EBC pH and/or PC20 values obtained during the 2 observation periods, and visually interpreted. In addition, the serial PEF recordings were plotted and interpreted using a computerized approach with Oasys-2 software (<http://www.occupationalasthma.com>). A PEF recording was considered suggestive of OA if the score obtained applying the Oasys-2 system was ≥ 2.51 [19].

Exhaled Breath Condensate Collection and pH Measurement

EBC was collected during tidal breathing with a commercially available condenser (EcoScreen; Jaeger, Würzburg, Germany) as described [20]. To determine the ventilatory pattern, a spirometer (EcoVent; Jaeger) was connected to the equipment at the expiratory valve. Subjects were instructed to refrain from food intake during the 2 h before sample collection. A fixed volume of 150 liters of exhaled breath was collected per subject. Each EBC sample was divided into 500- μ l aliquots in 2–4 plastic tubes.

pH was measured in 1 aliquot immediately after EBC collection, and in another after deaeration with helium (350 ml/min for 10 min), using a model GLP 21 calibrated pH meter (Crison Instruments SA, Barcelona, Spain) with an accuracy of ± 0.01 pH and a probe for small volumes (Crison 50 28). The probe was calibrated daily with standard pH 7.02 and 4.00 buffers.

Specific Inhalation Challenge

SIC was considered the reference standard for the diagnosis of OA, and the sensitivity and specificity of the other tests used were always determined relative to the SIC results. The SIC was carried out in subjects with no contraindications to performing this test [21], with substances suspected to be the cause of the subject's asthma according to the guidelines proposed by Pepys and Hutchcroft [22] and our group [23, 24]. For all the substances tested, prior exposure to a placebo was performed the day before the actual test. In each patient, lung function changes were monitored by measuring the FEV₁ and the forced vital capacity (FVC) every 10 min during the first hour following exposure, and thereafter, every hour up to 15 h postinhalation. Response was considered positive when FEV₁ decreased by more than 20% of the baseline value in the absence of any change with the placebo. Whenever possible, the test was performed without patients knowing to what agent they were being exposed.

Data Analysis

To compare the changes in the 2 observation periods, the Student t test for paired samples was used in variables with results following a normal distribution, and the Mann-Whitney test in those with a non-normal distribution. Differences between groups were analyzed with the Kruskal-Wallis test. Receiver-operating characteristic (ROC) curves were constructed to determine the cut-off values that best discriminated between changes in EBC pH, PC20 and DRR to methacholine between periods at work and off work to predict a positive SIC. PEF graphs were analyzed visually by 3 experienced independent readers blinded to the SIC results. The readers were asked to estimate the probability of having OA for each subject by indicating Yes or No. Agreement between the 3 evaluators was calculated using the Kappa test. The same graphs including EBC pH and/or PC20 were distributed to the evaluators to calculate the specificity and sensitivity of the 3 types of evaluation (PEF alone, PEF plus EBC pH or

PEF plus PC20). In addition, the sensitivity and specificity of the combinations of EBC pH plus PC20 and EBC pH plus DRR were calculated. All analyses were done with SPSS, version 17 (Chicago, Ill., USA).

Results

Seventeen subjects had positive SIC results and were diagnosed as having OA. SIC elicited an immediate response in 3 cases, a late response in 11 and a dual response in 3. Twenty subjects tested negative with SIC, and they were considered to not have OA. The characteristics of the overall population are shown in table 1. There were no significant differences in baseline characteristics between the 2 groups or in the type of sensitizers to which subjects were exposed. The pulmonary function and EBC pH changes after the at-work/off-work periods in patients with a positive and negative SIC are shown in table 2. Significant decreases in PC20 values at work were found in subjects with a positive SIC ($p = 0.047$). In this group, we also observed a decrease in EBC pH at work, although it did not reach statistical significance ($p = 0.08$). PC20 was lower in the periods off work in patients with a negative SIC ($p = 0.05$) versus those with a positive SIC, and the DRR was higher in periods at work in patients with positive SIC ($p = 0.048$) compared to those with a negative SIC.

The ROC curves in figure 3 show the most relevant differences in EBC pH, PC20 and the DRR between periods at work and off work for predicting positive SIC status. Using the ROC curve, we found that an EBC pH decrease greater than 0.4 units during the periods at work compared to off work achieved the most satisfactory sensitivity of 40% (CI 19.4–66.5) and specificity of 90% (CI 66.9–98.2) for the diagnosis of OA. For PC20, a 1.8-fold decrease at work versus off work resulted in a sensitivity of 50% (CI 22.3–77.7) and specificity of 87% (CI 60.4–97.8). Lastly, for the DRR, a 2-fold increase at work compared to off work achieved a sensitivity of 67% (CI 35.4–88.7) and a specificity of 69% (CI 41.5–87.9). Considering alternative cut-off values, a 3.2-fold PC20 decrease at work, a criterion similar to that reported by other authors [25], had a sensitivity of 40% (CI 11.3–64.6) and specificity of 87% (CI 60.4–97.8) in this study. The sensitivity of the PEF reading by the 3 experienced evaluators was 59% (CI 33.4–80.6), 47% (CI 23.8–71.5) and 47% (CI 23.8–71.5), whereas the specificity was 55% (CI 32.0–76.2), 50% (CI 27.8–72.1) and 75% (CI 50.6–90.4), respectively (table 3). Agreement in PEF interpretations between readers

Table 1. Patients' clinical characteristics

	SIC+ (n = 17)	SIC- (n = 20)	p
Age, years	42 (22–62)	44 (23–64)	0.184
Sex, male/female	9/8	10/10	0.860
Atopy, %	40	68	0.066
Smoking habit: smoker/non-smoker/ex-smoker	2/9/7	3/9/8	0.942
Pack-years ^a	12 (1–30)	5 (1–70)	0.837
Agent: HMW/LMW	1/16 ^b	2/18 ^c	0.352
Total duration of exposure, years	17 (4–43)	18 (3–46)	0.945
Asthma duration, years	4 (2–32)	3 (1–20)	0.073
Subjects treated with ICS, n (%)	11 (65)	12 (60)	0.732
Subjects treated with LABA, n (%)	9 (53)	10 (50)	0.833

Data expressed as median (range), unless indicated otherwise.

HMW = High molecular weight; ICS = inhaled corticosteroids; LABA = long-acting beta2 agonist; LMW = low molecular weight.

^a Smokers and ex-smokers.

^b Isocyanates (n = 8), persulfate salts (n = 5), cork dust (n = 2), welding fumes (n = 1) and wheat (n = 1).

^c Persulfate salts (n = 9), isocyanates (n = 6), styrene (n = 1), chromium (n = 1), pine wood (n = 1), mouse proteins (n = 1) and animal food (n = 1).

Table 2. At-work and off-work pulmonary function and EBC pH data

	Positive SIC (n = 17)			Negative SIC (n = 20)		
	at work	off work	p	at work	off work	p
FEV ₁ , % predicted	81.7 (17.4)	82.1 (14.3)	0.801	81.5 (15.7)	82.8 (14.6)	0.274
Methacholine test						
Positive, n (%)	15 (88)	12 (71)	–	11 (55)	6 (30)	–
PC20, mg/ml ^a	5.02 (4.61)	7.29 (4.88)*	0.047	7.05 (4.46)	6.08 (5.44)*	0.09
DRR ^b	11.86 (19.52)**	8.32 (20.79)	0.126	7.2 (21.17)**	5.9 (18.08)	0.214
EBC pH	7.01 (1.25)	7.6 (0.82)	0.08	7.6 (0.98)	7.43 (0.69)	0.362

Results presented as mean (SD), unless indicated otherwise. * p = 0.05 and ** p = 0.048.

^a Only positive values.

^b All subjects.

was high, with a Kappa index of 0.7 ($p < 0.0001$). Analysis of PEF monitoring by the Oasys-2 computerized approach achieved a sensitivity of 19% (CI 4.7–44.2) and a specificity of 95% (CI 73.0–99.7).

When EBC pH (considering a decrease of 0.4 units at work clinically significant) was assessed in combination with PEF and/or PC20 (considering a 1.8-fold decrease at work clinically significant), the sensitivity and specificity obtained by the 3 readers generally increased, although

the improvement was similar regardless of the combination (table 3). Adding EBC pH to PEF monitoring by the computerized Oasys-2 approach increased the sensitivity to 38% (CI 16.3–64.1) while specificity remained at a similar level of 90% (CI 58.4–97.6). Evaluation of changes greater than 1.8-fold in PC20 and/or greater than 0.4 in EBC pH, without considering PEF results, yielded a sensitivity of 50% (CI 25.5–74.5) and a specificity of 81% (CI 57.4–93.7) for diagnosing OA. The combination of a

Table 3. Sensitivity and specificity of PEF and EBC pH combined with PEF and/or PC20

	Sensitivity	Specificity
EBC pH	40 (19.4–66.5)	90 (66.9–98.2)
PEF		
Observer 1	59 (33.4–80.6)	55 (32.0–76.2)
Observer 2	47 (23.8–71.5)	50 (27.8–72.1)
Observer 3	47 (23.8–71.5)	75 (50.6–90.4)
Oasys	19 (4.7–44.2)	95 (73.0–99.7)
EBC pH + PEF		
Observer 1	65 (38.6–84.7)	60 (36.4–80.0)
Observer 2	59 (33.4–80.6)	65 (40.9–83.7)
Observer 3	65 (38.6–84.7)	60 (36.4–80.0)
Oasys	38 (16.3–64.1)	90 (58.4–97.6)
EBC pH + methacholine		
EBC pH + PC20	50 (25.5–74.5)	81 (57.4–93.7)
EBC pH + DRR	61 (32.3–84.9)	73 (44.8–91.1)
EBC pH + PEF + PC20		
Observer 1	50 (25.5–74.5)	67 (43.1–84.5)
Observer 2	75 (47.4–91.7)	52 (30.3–73.6)
Observer 3	69 (41.4–87.8)	67 (43.1–84.5)

Data expressed as % (CI).

change greater than 2-fold in the DRR and/or a change of more than 0.4 in the EBC provided a sensitivity of 61% (CI 32.3–84.9) and a specificity of 73% (CI 44.8–91.1) (table 3). Lastly, the sensitivity of the PEF reading plus PC20 by the 3 experienced evaluators was 56% (CI 30.5–79.2), 62% (CI 35.9–83.7) and 83% (CI 57.7–95.6), whereas the specificity was 57% (CI 34.4–77.4), 67% (CI 43.1–84.5) and 89% (CI 65.4–98.1), respectively.

Discussion

The most important finding of this study is the fact that a decrease of 0.4 EBC pH units in individuals with suspected OA between periods at work and off work had a specificity of 90% for the definite diagnosis. Despite the low sensitivity observed, this is a useful finding as it indicates that this parameter can be incorporated in the diagnostic work-up of OA when other more specific tests, such as SIC, cannot be applied. Furthermore, we found that adding the EBC pH generally improves the performance of serial PEF monitoring for this purpose.

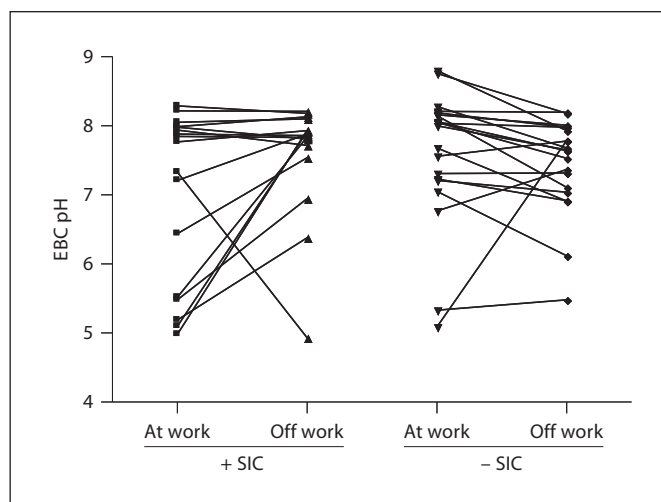


Fig. 2. EBC pH changes after the on-work and off-work periods in patients with positive and negative SIC.

As is well recognized, SIC is the reference standard for diagnosing OA, but it is costly and is only available in highly specialized centers. One alternative method is serial, self-recorded PEF measurement during periods when patients are working and when they are away from work [17, 26]. The sensitivity and specificity of PEF have been evaluated in several studies [6, 26–28], most of them performed in the 1990s in England and the USA. In these studies, which were focused on evaluating the diagnostic performance of the technique, PEF findings were interpreted by various observers and the diagnosis of OA was made by consensus. However, in daily clinical practice, the test is interpreted by a single physician, and sensitivity and specificity may differ in this situation. Furthermore, as other authors have suggested, the number of patients taking corticosteroids at the time those studies were performed was lower than now; hence, the differences in PEF at work versus off work might have been more marked [6]. These factors could be implicated in the low sensitivity and specificity of the PEF measurement observed in this study in comparison to previous efforts.

Other possible confounding factors in the interpretation of PEF are respiratory tract infections and possible falsification of the recordings [29]. Accurate PEF monitoring depends on the patient's compliance and honesty, and this fact may contribute to differences in sensitivity and specificity levels according to the country studied. This is the first study performed in Spain to indirectly evaluate the diagnostic performance of PEF. The fact that

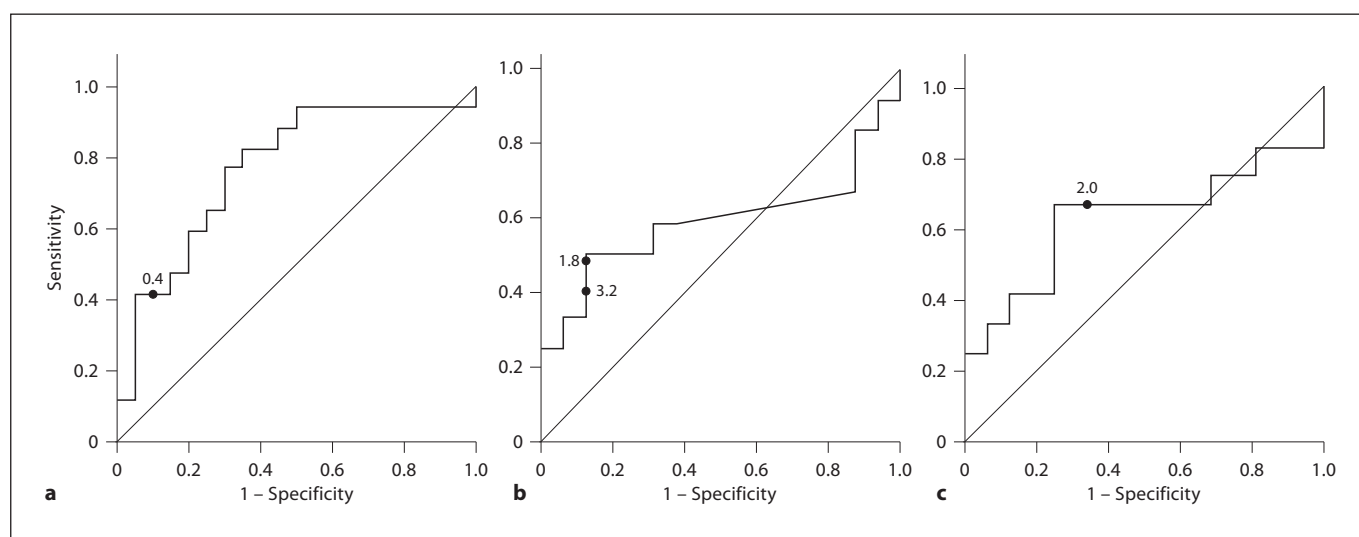


Fig. 3. ROC curves constructed to determine the most relevant difference in EBC pH (a), PC20 (b) and the DRR (c) between periods at work and off work for predicting positive status by SIC. The dots represent the optimal cut-off points.

the 3 PEF readers in our study obtained a Kappa index of agreement of 0.7 and that the sensitivity reached by Oasys-2 was only 19% indicates that the low sensitivity and specificity observed is not due to poor interpretation of the recordings, and suggests that there may be a technical problem in our setting. It is relevant that 17% of the patients in the study were excluded because they did not correctly perform PEF recording.

Another test that has proven useful for diagnosing OA is methacholine challenge. Some authors believe that a negative methacholine result when a patient is working virtually rules out OA [30]. Nevertheless, there is no defined criterion to establish positive status for the test. The European Respiratory Society considers the test negative when PC20 is greater than 8 mg/ml [21], whereas the ATS has established the cut-off at 16 mg/ml [15]. Because of these discrepancies, some authors advocate using the DRR [16]. In this study, among the 17 patients with a positive SIC who were ultimately diagnosed with OA, 3 had a PC20 of >8 mg/ml at work and 2 showed a value greater than 16 mg/ml, whereas the DRR ranged from 0.5 to 66.7. When the on- and off-work PC20 values were compared, as in Girard et al. [6], we observed a significant improvement in the off-work PC20 in patients with a positive SIC. Furthermore, in contrast to other authors who have reported that PC20 does not provide a higher diagnostic yield than PEF results [6, 26], we found that a 1.8-fold PC20 change or a 2-fold DRR change at work com-

pared to off work show a slightly higher sensitivity and specificity than PEF monitoring.

EBC contains numerous mediators that are influenced by lung disease. Similarly, EBC pH changes in relation to eosinophilic or neutrophilic airway inflammation in respiratory disease [9]. Determination of EBC acidity is a noninvasive, inexpensive method to estimate airway inflammation in various inflammatory lung diseases, including asthma [8]. There is evidence that individuals with poorer asthma control have lower EBC pH values and that the pH decrease is even more marked during exacerbations. EBC pH is highly reproducible and informative with regard to disease severity, and it is inversely associated with airway inflammatory cells/inflammatory cell counts and directly associated with FEV₁ values [9]. Nevertheless, some authors have suggested that EBC pH may vary in certain circumstances, and this can affect the acidification/inflammation relationship. This variability may be related to the sample collection equipment or the temperature at which samples are collected, and it is likely that smoking plays a large role [31, 32].

Owing to the fact that asthmatic reactions induced by exposure to occupational agents in sensitized individuals are similar to natural worsening of the disease, such exposure can produce airway acidification. However, there is little information on the degree to which EBC acidifies in patients with OA. Ferrazzoni et al. [33] investigated the usefulness of EBC pH for monitoring airway response

after performing SIC with isocyanates in sensitized workers. The authors found no association between the disease and EBC acidification. However, when EBC pH is monitored in situations of chronic exposure at work, not in an acute context as occurs during SIC, a more acid pH has been reported. In a study of 75 grain workers, Do et al. [34] observed a correlation between pH decrease and the duration of work on the study day. In parallel, these authors found that acute exposure has a smaller effect on pH and is more closely associated with oxidative stress. In line with this, acute exposure to grain dust and endotoxin can initially produce oxidative stress in the airways, but this response would decrease over time as the airways adapt to the constant exposure and EBC pH acidification could then develop. Thus, the differences observed in EBC pH by both authors [33, 34] more likely reflect the differences between a natural asthma exacerbation and laboratory-induced asthmatic reactions.

To our knowledge, this is the first study attempting to establish the usefulness of EBC pH in the diagnosis of OA. We found that a pH decrease at work of 0.4 units has a lower sensitivity than PEF or methacholine challenge findings, but a much higher specificity. Combining the different tests did not substantially improve the diagnostic yield. The combination of EBC pH with PC20 or with PEF raised the sensitivity, but decreased the specificity. Combining EBC pH, PC20 and PEF was also ineffective for improving the diagnostic performance, likely because the discordance between these parameters observed in some patients leads to confusion, which, as other authors have noted, may result in a decrease in the specificity of the tests [6]. It is important to point out, however, that the combination of EBC pH and PEF generally improved the diagnostic yield of the latter. This fact, together with the

high specificity observed with EBC pH measurement could, in itself, justify its use, particularly taking into consideration that EBC pH determination is an easily performed, inexpensive and noninvasive method.

One of the limitations of this study might be that induced sputum analysis was not carried out in the patients studied. Determination of eosinophil count in induced sputum has proven to be useful in the diagnosis of OA both in the context of SIC [35] and at-work/off-work monitoring [6]. An eosinophil count increase of 1 or 2% in periods at work with respect to periods off work has a sensitivity of 65 and 52% and a specificity of 76 and 80%, respectively. Addition of sputum cell count to PEF monitoring increases the specificity and sensitivity of this test by 27 and 12%, respectively [6], and it is now probably the best strategy for diagnosing OA when SIC cannot be performed. Comparison of the diagnostic yield of induced sputum and EBC pH would have enabled definitive establishment of the role of EBC in diagnosing OA.

In conclusion, although the best strategy for the diagnosis of OA when SIC cannot be performed is probably a combination of PEF and induced sputum eosinophil count at work and off work, the results obtained in this study demonstrate that EBC pH alone or in combination with PEF may also be a good diagnostic option, particularly in centers where induced sputum testing is not available.

Acknowledgments

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