Original Article

# Diagnostic Accuracy of Inflammatory Markers for Diagnosing Occupational Asthma

**Geneviève Racine, Roberto Castano, MD, PhD, André Cartier, MD, and Catherine Lemiere, MD, MSc** *Montréal, Quebec, Canada*

***What is already known about this topic?*** The lack of airway hyperresponsiveness makes the diagnosis of occupational asthma (OA) very unlikely in workers still exposed to the offending agent. Sputum eosinophil count is an effective aid to the diagnosis of OA.

***What does this article add to our knowledge?*** In contrast to its utility in the management of asthma, blood eosinophil counts do not appear to be a reliable surrogate marker of airway inflammation to be used in the investigation of OA.

***How does this study impact current management guidelines?*** Performing both sputum induction and methacholine challenge before and after exposure to the offending agent may represent an effective alternative to a specific inhalation challenge for diagnosing OA when this test is unavailable.

**BACKGROUND: The assessment of airway responsiveness and inflammation is key to the investigation of occupational asthma (OA).**
**OBJECTIVE: We sought to assess and compare the diagnostic accuracies of the blood and sputum eosinophil counts and the methacholine challenge for the diagnosis of OA.**
**METHODS: We conducted a retrospective study assessing 618 patients who underwent specific inhalation challenges (SICs) for symptoms suggestive of OA between 2000 and 2015. A sputum induction and a methacholine challenge were performed before and after SICs. Blood samples were collected in all subjects before the SICs and in 100 subjects before and after SICs. The diagnostic accuracies of blood and sputum eosinophil counts and methacholine challenge were calculated for diagnosing OA.**
**RESULTS: The change in blood eosinophil count failed to differentiate workers with positive and negative SICs. The change in sputum eosinophil counts induced by the exposure to the offending agent had the highest diagnostic accuracy (receiver operating characteristic area under the curve: 86% [95% confidence interval: 0.8-0.9, $P < .001$]) for diagnosing OA compared with changes in concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second ($PC_{20}$) and blood eosinophils. Combining a 2-fold or greater decrease in $PC_{20}$ or a 3% or greater increase in sputum eosinophil count achieved a sensitivity of 84% and a specificity of 74% with a negative predictive value of 91% for the diagnosis of OA.**
**CONCLUSIONS: Blood eosinophil counts do not appear to be an effective aid for diagnosing OA. The performance of both sputum cell count analysis and a methacholine challenge before and after exposure to the offending agent may represent an effective alternative in diagnosing OA when SICs are unavailable.**  **(J Allergy Clin Immunol Pract 2017;■:■-■)**

***Key words:*** *Occupational asthma; Blood eosinophil count; Sputum eosinophil count; Airway responsiveness*

Occupational asthma has been defined as a type of asthma caused by conditions attributable to the workplace and not to causes outside the workplace.[1] Occupational asthma can be caused by the sensitization to a specific agent at work ("allergic occupational asthma" or "sensitizer-induced occupational asthma")—hereafter referred to as occupational asthma (OA)—or by the acute or chronic inhalation of high to moderate concentration of irritant agents ("irritant-induced asthma").[2] The diagnosis of allergic OA requires a comprehensive investigation involving the performance of different and complementary tests. Assessing airway responsiveness and airway inflammation is key to the investigation of OA. The lack of airway hyperresponsiveness makes the diagnosis of OA very unlikely in workers who are still exposed to the offending agent.[3] Sputum eosinophil counts have been shown as an effective aid to the investigation of OA during specific inhalation challenges

Centre intégré universitaire de santé et des services sociaux du Nord-de-l'Île-de-Montréal, Hôpital du Sacré-Cœur de Montréal, Université de Montréal, Montréal, Quebec, Canada
No funding was received for this work.
Conflicts of interest: A. Cartier is on the boards for Teva Canada and AstraZeneca Canada; has received lecture fees from Merck-Frosst Canada; receives royalties from UpToDate; and has received travel support from AstraZeneca. C. Lemiere has received consultancy fees from AstraZeneca, GlaxoSmithKline, and Teva; has received research support and payment for developing educational presentations from Teva and AstraZeneca; has received lecture fees from Teva; receives royalties from UpToDate; and has received travel support from AstraZeneca. The rest of the authors declare that they have no relevant conflicts of interest.
Received for publication October 12, 2016; revised January 1, 2017; accepted for publication February 3, 2017.
Available online ■■
Corresponding author: Catherine Lemiere, MD, MSc, Department of Chest Medicine, Hôpital du Sacré-Cœur de Montréal, 5400 Gouin West, Montreal H4J 1C5, Quebec, Canada. E-mail: catherine.lemiere@umontreal.ca.
2213-2198

http://dx.doi.org/10.1016/j.jaip.2017.02.001

*Abbreviations used*
*AUC- Area under the curve*
*CI- Confidence interval*
*HMW- High-molecular weight agent*
*ICS- Inhaled corticosteroids*
*IQR- Interquartile ranges*
*LMW- Low-molecular weight agent*
*OA- Occupational asthma*
*$PC_{20}$- Concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second*
*ROC- Receiver operating characteristic*
*SIC- Specific inhalation challenge*

(SICs)[4,5] because exposure to the offending agent induces an increase in sputum eosinophil counts in the majority of workers with OA.[6] Furthermore, changes in airway inflammation precede changes in airway caliber after exposure to the offending agent.[7] However, sputum induction and processing remains a cumbersome procedure that is not widely available. In contrast, blood eosinophil counts are not only widely available but also easy to perform. They have been shown to be reasonably well correlated with sputum eosinophil counts.[8,9] Blood eosinophil counts have been increasingly used as a surrogate marker of airway inflammation over the past 10 years. Blood eosinophil counts can identify eosinophilic phenotypes of asthma quite accurately when blood eosinophil counts are higher than $0.45 \times 10^9$/L.[10] Blood eosinophil counts higher than $0.15 \times 10^9$/L seem to predict a good clinical response to some of the new biological agents.[11] However, to our knowledge, the diagnostic accuracy of blood eosinophil counts has never been assessed and compared with the current tests used in the investigation of OA.

The aim of this study was to assess and compare the respective diagnostic accuracies of blood eosinophil counts, sputum eosinophil counts, and airway responsiveness to methacholine for diagnosing OA using an SIC as the standard of reference.

## MATERIALS AND METHODS

### Definitions

OA was defined as a positive SIC in the laboratory or at the workplace.

Non-OA was defined as a diagnosis of asthma based on a reversible airflow limitation or a concentration of methacholine inducing a 20% fall in forced expiratory volume in 1 second ($PC_{20}$) equal to or lower than 16 with a history suggestive of asthma with negative SICs.

No functional evidence of asthma referred to subjects without airflow limitation and with $PC_{20} > 16$ mg/mL.

### Subjects

This retrospective study was conducted using a database that includes 618 subjects investigated at the Hôpital du Sacré-Coeur de Montréal (Montreal, Quebec, Canada) where they had undergone SICs between 2000 and 2015 for symptoms suggestive of OA. The Hôpital du Sacré-Coeur de Montréal is a tertiary care center, which is one of the 2 referral centers for OA in the province of Quebec. A sputum induction and a methacholine inhalation challenge were performed before and after the SICs. Blood samples were collected in all subjects before the SICs and in a subgroup of subjects before and after the SICs. The study was approved by the Research Ethics Committee of Sacré-Coeur Hospital.

### Procedures

- Spirometry was assessed in accordance with the standards of the American Thoracic Society.[12] A methacholine inhalation challenge was performed as previously described with a Wright nebulizer (output 0.14 mL/min) at tidal volume breathing for 2 minutes.[13]
- Sputum was induced using inhalations of increasing concentrations (3%, 4%, 5%) of hypertonic saline and processed as previously described.[14]
- Blood was drawn in every worker before the investigation to obtain a blood cell count. The procedure was repeated only in subjects who were likely to have a positive SIC response.
- Subjects underwent skin prick tests with 14 common inhalants. Atopy was defined by the presence of at least one positive skin test with a wheal diameter $\geq$3 mm.
- *SICs*: As previously described, SICs were performed in the laboratory or at the workplace.[15] On the first day, the subjects were either not exposed to any substance or exposed to a sham substance (eg, lactose or thinner) for 30 minutes to ensure that their asthma was stable. On subsequent days, the subjects were progressively exposed to the occupational agent suspected of causing their asthma-like symptoms. When the SIC was negative in the laboratory or when the workplace exposure could not be reproduced in the laboratory, the subjects were returned to their workplace under the supervision of a respiratory technologist, who assessed their respiratory function hourly over 7 hours on 2 consecutive days. SICs were considered positive if there was a fall in forced expiratory volume in 1 second of 20% or greater after exposure. The diagnosis of OA was retained if an SIC was positive. Methacholine challenge and sputum induction were performed at the end of the control day and at the end of the last day of exposure. Long-acting $\beta_2$-agonists were interrupted 72 hours before the challenge. Inhaled corticosteroids (ICS) were continued during the challenge, but the total daily dose was administered 12 hours before the challenge or in the evenings of the challenge days.

### Statistical analysis

Descriptive statistics were used to summarize clinical and demographic characteristics of the sample. Results were expressed as means and standard deviation, except for data with an abnormal distribution, which were expressed as median and interquartile ranges (IQR) (which are the difference between the 75th and 25th percentiles). Correlations between blood, sputum eosinophil count, and methacholine $PC_{20}$ were examined by using a Spearman rank-order test. Changes in blood and sputum eosinophil counts after exposure to the offending agent were expressed as differences, whereas changes in $PC_{20}$ were expressed as ratios ($PC_{20}$ before exposure/$PC_{20}$ after exposure to the offending agent). Receiver operating characteristic (ROC) curves were built to calculate the respective diagnosis accuracies of blood and sputum eosinophil counts and $PC_{20}$ methacholine for differentiating subjects with positive and negative SIC. ROC curves give information on the discriminatory ability of a test. The closer the apex of the curve is to the upper-left corner, the better the discriminatory property of the test will be. This ability is also measured quantitatively by the area under the curve (AUC). Although an AUC value of greater than 96% indicates excellent discriminatory ability, a value of 50% indicates no discriminative value. AUC $\leq$ 75% are usually not clinically useful.[16]