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List of Terms and A*bbreviations*

ACQ	Asthma Control Questionnaire
AQOL	Asthma Quality of Life
ATS	American Thoracic Society
CS	Corticosteroids
C-terminal	Carboxy-terminal
Eos	Eosinophils
Eos+	Eosinophilic responders
Eos-	Non eosinophilic responders
ER	Emergency room
ExS	Ex-smoker
FeNo	Fractional concentration of exhaled nitric oxide
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
HMW/LMW	High molecular weight/low molecular weight
ICS	Inhaled corticosteroids
ICTP	Carboxyterminal telopeptide of type I collagen
IFN	Interferon
IFN-G	Interferon-gamma
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-8	Interleukin-8
IL-13	Interleukin-13
LARs	Late asthmatic reactions
MAP	MultiAnalyte Profiling
MCP 3	Monocyte chemoattractant protein-3
MED-ECHO	Maintenance et exploitation des données pour l'étude de la clientèle hospitalière
M/F	Male/female
MMP	Matrix metalloproteinase
MMPs	Matrix metalloproteinases
n	Number
N	Neutrophil
Ng/ml	Nanogramme per milliter
NS	Non-smoker
OA	Occupational asthma

OR	Odds ratio
p =	p-value
pg/ml	picogram per millilitres
PICP	Procollagen type I C peptide
ppb	parts per billion
PC_{20}	Provocative concentration of methacholine causing a 20% fall in FEV_1
RAMQ	Régie de l'assurance maladie du Québec
SIC	Specific inhalation challenges
TGF-beta	Transforming growth factor-beta
t-test	Student's t-test
TIMP-1	Tissue inhibitor of metalloproteinase-1
TNF- alpha	Tumor necrosis factor-alpha
VC	Vital capacity
Y	Year

Abstract

Background: Asthma is a heterogeneous disease. Recent evidence shows that subgroups of asthmatic subjects have different responses to asthma therapy. For example, some medication was ineffective when given to a broad asthma population but highly effective when given to a group of patients with distinct clinical characteristics. The identification of those subgroups represents an important advance in the management and treatment of the disease since it allows the use of a personalized and, effective treatment.
There is a lack of studies that attempted to identify such subgroups in subjects with occupational asthma (OA). The broad aim of this study was to assess whether subgroups of subjects with a different inflammatory response (eosinophilic vs. non-eosinophilic responders) on exposure to occupational agents have different clinical characteristics.
Methods: We performed a cross sectional study of 44 subjects who had been diagnosed with OA using specific inhalation challenges at least 2 years prior to the present study. They were composed of non-eosinophilic responders (no increase in airway inflammation after exposure to the agent they were sensitized to) and eosinophilic responders (increase in eosinophilic inflammation after exposure to the sensitizer). The clinical characteristics of those two groups of subjects (eosinophilic and non-eosinophilic responders) were compared.
Results: At the time of diagnosis and during specific inhalation challenges, 15 subjects were non eosinophilic responders (change in their sputum eosinophil count less than 2% (0(2.5)%) after exposure to the offending agents) whereas 29 of them showed an increase in their sputum eosinophil count greater than 2% (14.3(22.8) (eosinophilic responders). At the time of the performance of the specific inhalation challenges, non-eosinophilic responders had a slightly greater airflow limitation but did not show a greater airway hyperresponsiveness compared with the eosinophilic responders. At the time of the present study (approximately 5 years after the performance of specific inhalation challenges), the non-eosinophilic responders had a poorer respiratory function and a poorer control of their asthma compared to the eosinophilic responders. Furthermore, they showed a greater worsening of their respiratory function over time (during the interval between the performance of specific inhalation challenge and the present study) compared to the eosinophilic responders.
Conclusion: We believe that this is the first study that shows that subgroups of subjects with occupational asthma with different inflammatory characteristics do not have the same prognosis. This results needs to be confirmed in a study of a larger scope. If we confirm that non-eosinophilic responders have a poorer prognosis than eosinophilic responders, we will need to identify the underlying pathophysiological mechanisms to prevent the deterioration of the asthma of those subjects.

Section 1
Significant (Key) findings

Asthma is a heterogeneous inflammatory disease. Although many asthmatic patients present an eosinophilic inflammation, there is a large proportion of patients who do not show such an inflammation. Recent findings show that the response to asthma treatment may differ according to the type of airway inflammation presented by the patients. Patient who do not show an eosinophilic inflammation seem to have a more severe asthma than those who present an eosinophilic inflammation. When exposed to occupational agents to which they are sensitized, the majority of patients with occupational asthma develop an eosinophilic inflammation. However, there are patients who in spite of having an asthmatic reaction on exposure to a sensitizer do not develop such an eosinophilic inflammation after exposure to this sensitizer. Whether this different inflammatory response is associated with different clinical characteristics has never been studied. We aimed to investigate whether or not the lack of sputum eosinophilic response following the exposure to their offending occupational agents is a marker of poor asthma prognosis in workers with occupational asthma (OA).

Specific objectives

The specific objectives were:

Specific Objective 1:
To compare the clinical, functional and inflammatory characteristics of the eosinophilic responders (Eos+) and the non eosinophilic responders (Eos-) after specific inhalation challenges (SIC) at the time of the diagnosis.
Key findings: At the time of diagnosis and during specific inhalation challenges, 15 subjects were non eosinophilic responders (change in their sputum eosinophil count less than 2% (0(2.5)%) after exposure to the offending agents) whereas 29 of them showed an increase in their sputum eosinophil count greater than 2% (14.3(22.8) (eosinophilic responders). Non-eosinophilic responders had a slightly greater airflow limitation but did not show a greater airway hyperresponsiveness compared with the eosinophilic responders.

Specific Objective 2: To assess whether the non-eosinophilic phenotype shows an increase in sputum neutrophils (>60%).
Key findings: Non-eosinophilic responders had a slight increase in their neutrophil count (13.4(45.2)%), which was not significantly different from eosinophilic responders (-0.7(32.8)%) (p=0.3).

Specific Objective 3:
To compare the clinical (Asthma Control Questionnaire (ACQ), Asthma Quality of Life (AQOL)), functional (FEV_1 and PC_{20}) and inflammatory characteristics of workers Eos+ and Eos- 5 years or more after the diagnosis.
Key findings: We showed that the non-eosinophilic responders had a poorer respiratory function than the eosinophilic responders. They also had a poorer control of their asthma compared to the eosinophilic responders. We also observed a greater worsening of the respiratory function of the non eosinophilic responders over time compared to the eosinophilic responders.

Specific Objective 4: To compare markers of sensitization (total IgE), specific markers of cell activation (i.e. MPO/neutrophils and ECP/eosinophils), airway remodelling markers (PIP, ICTP, EGF, FGF-2, PDGF-AB/BB, PDGF-AA, MMP-1, -2, -3, -7, -8, -9, -12, -13 and MMP-9/TIMP-1 molar ratio), cytokines family (IL-1a, IL-1b, IL-3, -4, -5, -6, -7, -9, -10, IL-12(p40), IL-12(p70), IL-13, -15, -17, G-CSF, GM-CSF, INF-a2, TGF a, TNF-a and TNF-b), chemokines family (CXCL8/IL-8, eotaxin, Flt-3L, Fractalkine, GRO, IP-10, MCP-1, MDC, MIP-1a, MIP-1b and

RANTES) and soluble immunoregulatory receptors (IL-1ra, sCD40L and sIL-2Ra). in the Eos+ and Eos- groups.
Key findings: Levels of IL-2, IFN -gamma and MCP 3 were higher in the eosinophilic responder group than in the non-eosinophilic responder group. There was no difference in the metalloproteinases levels between groups.

Specific Objective 5: To assess the medical resource use 5 years before and after diagnosis in Eos+ and Eos- groups.
Key findings: We did not find differences in the number of outpatient's visits or emergency visits for asthma in the 5 years preceding or following the diagnosis of OA between non-eosinophilic responders and eosinophilis responders. However, there was a higher number of subjects go were hospitalized for asthma (2(15.5%) in the non eosinophilic responder group compared to the eosinophilic group (0) p=0.04.

Translation of Findings

Subjects with non-eosinophilic inflammation seem to have a poorer prognosis than eosinophilic responders. This may be due to a different pathophysiological mechanism.
At this stage it is difficult to provide recommendation, as those findings need to be confirmed in a study of a larger scope. However, it is reasonable to warn physicians to pay a special attention to non-eosinophilic responders since they seem to have a poorer prognosis than the eosinophilic responders.

Outcomes/ Impact

1) potential outcomes, i.e., findings, results, or recommendations that could impact workplace risk if used; non-eosinophilic responders should be identified and should receive special attention considering that they are at risk to have a more severe asthma and a greater decline in the pulmonary function tests.
2) intermediate outcomes, i.e., how findings, results, or recommendations have been used by others to influence practices, legislation, product design, safety management program and training and so forth; Sputum induction should be performed during the performance of specific inhalation challenge as it allows identifying eosinophilic and non-eosinophilic responders.
3) end outcomes, i.e., how findings, results, or recommendations have contributed to documented reductions in work-related morbidity, mortality, and/or exposure.
This study may help to identify and classify subjects with a poor prognosis of OA. This has the potential to modify the criteria on which the financial compensation of the workers with occupational is based. Non-eosinophilic responders may receive a higher compensation than eosinophilic responders since they are more likely to be impaired by their asthma.

Section 2

Scientific Report

Background :

Occupational Asthma and Asthma phenotypes

There is growing evidence that the identification of asthma phenotypes is crucial not only to improve our understanding of the disease but also to improve asthma treatment (1). For example Mepolizumab, an anti-IL5 medication has been first shown to be ineffective in a population of asthmatic without phenotypic differentiation but was shown highly effective later in eosinophilic asthmatics patients (2). Although several asthma phenotypes with different level of severities have been identified in asthma, there is very little information on occupational asthma phenotypes in the literature (3). We have recently shown that different inflammatory phenotypes have different makers of asthma severity in occupational asthma. For example, a neutrophilic phenotype was composed of older workers with a longer duration of exposure to the offending agent and a lower FEV_1 and a poorly controlled asthma(4).

Occupational asthma (OA) is a complex interaction between the asthmatic airways and the exposure to a variety of occupational agents with irritants and/or sensitizing properties. Airway inflammation is a characteristic of asthmatic subjects, but maybe modulated by the exposure to different types of occupational exposures.

Airway inflammation can be observed in response to the exposure to a sensitizing occupational agent(5) or after removal from this agent(6). The presence and the type of airway inflammation observed in these two occasions may have a different meaning. Eosinophilic airway inflammation only persists in approximately 20% of workers ten years after removal from exposure (6). As in asthma, the persistence of eosinophilic airway inflammation is likely to be related to a suboptimal asthma control (7).

Acute exposure to occupational agents induces most of the time an eosinophilic airway inflammation in sensitized subjects (5). However, a neutrophilic inflammation has also been reported(8). The potential triggers directing the type of airway inflammation towards an eosinophilic or a non-eosinophilic response during exposure to occupational agents are unclear. It is possible that the type of asthmatic reaction induced by the exposure to occupational agents reflects an underlying immune mechanism that varies according to the type of exposure and may be associated with a different outcome of the asthmatic disease. Therefore, identifying different inflammatory phenotypes of occupational asthma may improve our management of this disease.

Prognosis of occupational asthma and airway inflammation

The vast majority of studies described the inflammatory characteristics of subjects with OA years after removal from exposure and tried to assess whether or not the current level of inflammation was correlated with the current severity of the disease(6, 9). We previously described higher levels of IL-8 in the induced sputum of patients with occupational asthma with persistent airway hyperresponsiveness years after removal from exposure compared with those without hyperresponsiveness. However, in this study there was no measure of airway inflammation at baseline (6).

Pirila et al studied the outcome of diisocyanate-induced asthma after initiation of inhaled corticosteroid treatment at a mean period of 7 months after cessation of exposure by following up lung function and bronchial inflammation(10). A bronchoscopy was performed 2 days after the specific inhalation challenges and repeated 6 months and 3 years later. Overall the subjects decreased their airway hyperresponsiveness but they also experienced a decline in their FEV_1. Subjects who remained hyperresponsive had higher levels of IL-6, IL-15 and TNF-alpha at the 3 years follow-up compared to those who were non responsive. However, whether or not the type of inflammation differed between those subjects after the specific inhalation challenge was not reported.

Some markers of airway inflammation and remodelling have been shown to persist in subjects with prior OA long after cessation of exposure even in the absence of clinical, sputum and functional abnormalities(11). Remodelling is likely to play a significant role in the pathophysiology of occupational asthma. Markers or remodelling have been described in

subjects with OA and showed a decrease after cessation of exposure(12). Matrix metalloproteinases (MMPs) play a role in matrix turnover, which may be an important factor in airway remodeling. Under pathological conditions, MMPs may be produced in excess contributing to tissue damage and activation of inappropriate repair mechanisms. MMPs have been shown to increase in sputum after exposure to isocyanates(13). An imbalance between MMP-9 and TIMP-1 has also been observed in a murine model of isocyanate-induced asthma (14). Workers who do not show eosinophilic inflammation often have an increase in sputum neutrophils. Neutrophils are the main source of MMP-9 and -8 in the lung. MMP cleaves latent TGF- beta that may explain TGF beta activation and tissue remodelling.

Specific objectives

Specific Objective 1:
To compare the clinical, functional and inflammatory characteristics of eosinophilic responders (Eos+) and non-eosinophilic responders (Eos-) after specific inhalation challenges (SIC) at the time of the diagnosis.

Specific Objective 2: To assess whether the non-eosinophilic phenotype shows an increase in sputum neutrophils (>60%).

Specific Objective 3:
To compare the clinical (Asthma Control Questionnaire (ACQ), Asthma Quality of Life (AQOL)), functional (FEV_1 and PC_{20}) and inflammatory characteristics of workers Eos+ and Eos- 5 years or more after the diagnosis.

Specific Objective 4: To compare markers of sensitization (total IgE), specific markers of cell activation (i.e. MPO/neutrophils and ECP/eosinophils), airway remodelling markers (PIP, ICTP, EGF, FGF-2, PDGF-AB/BB, PDGF-AA, MMP-1, -2, -3, -7, -8, -9, -12, -13 and MMP-9/TIMP-1 molar ratio), cytokines family (IL-1a, IL-1b, IL-3, -4, -5, -6, -7, -9, -10, IL-12(p40), IL-12(p70), IL-13, -15, -17, G-CSF, GM-CSF, INF-a2, TGF a, TNF-a and TNF-b), chemokines family (CXCL8/IL-8, eotaxin, Flt-3L, Fractalkine, GRO, IP-10, MCP-1, MDC, MIP-1a, MIP-1b and RANTES) and soluble immunoregulatory receptors (IL-1ra, sCD40L and sIL-2Ra).

) in the Eos+ and Eos- groups.

Specific Objective 5: To assess the medical resource use 5 years before and after diagnosis in Eos+ and Eos- groups.

Methods
Study Design: We performed a cross-sectional cohort study of all subjects diagnosed with OA between 2000 and 2005 at Sacre-Coeur Hospital (Quebec) Canada.
After having obtained the authorization from the Director of Professional Services from Sacre-Coeur Hospital, we identified the charts of all subjects who had a diagnosis of OA between 2000 and 2005 at Sacre-Coeur Hospital using specific inhalation challenges and who had sputum differential cell counts performed during the challenges. These subjects were invited to come to Sacre-Coeur Hospital for a clinical, functional and inflammatory assessment. Spirometry, methacholine challenge, sputum induction and measure of exhaled nitric oxide (eNO) were performed. After having obtained the authorization from the *Commission d'accès à l'information du Quebec*, we obtained the data regarding the use of medical resources (visit to the physicians, visits to the emergency and hospitalizations) from the *Régie de l'assurance maladie du Québec (RAMQ)* 5 years before the diagnosis of OA and until the follow-up visit.

Subjects :
All the subjects who had a diagnosis of OA proven by specific-inhalation challenge tests at

Sacre-Coeur Hospital between 2000 and 2005 and fulfill the inclusion/exclusion criteria were enrolled.

Inclusion criteria

- Occupational asthma proven by specific inhalation challenge between 2000 and 2005
- Sputum differential cell counts at the time of diagnosis after exposure to the offending agent. This test has been included in our routine investigation of OA during the past years.

Exclusion criteria :

- Upper or lower airway infection in the month preceding the follow-up visit.
- Inability to sign the consent form
- Recent or uncontrolled coronary disease (unstable angina).
- Recent cerebral vascular accident

Procedures :

1. Questionnaires. Clinical characteristics of the patients were collected in a case record form. Asthma symptoms were assessed using the validated Asthma Control Questionnaire (15). Quality of life was assessed using the Juniper asthma quality of life questionnaire. (16).

2. Spirometry and methacholine challenge. Forced expiratory volume in one second (FEV_1) and vital capacity (VC) were performed according to the standards of the ATS(17). Methacholine Inhalation test was performed using the method described by Juniper and coworkers and the results were expressed as the PC_{20} in non-cumulative units (18).

3. Skin-prick tests with 12 common inhalant allergen extracts and a negative (diluent) and positive (histamine 10 mg/ml) control were performed by the modified prick method as described by Pepys (12) if not performed at the time of diagnosis. A result was documented as positive if the wheal is 3 mm in diameter.

4. Sputum Induction and Processing. Sputum induction with normal or hypertonic saline was adapted from the technique previously described by Pin et al(19). Sputum was processed as described by Pizzichini et al(15) for total and differential cell counts(20).

5. Measures of remodeling markers

Because up or down regulation of specific MMPs have consequences on structural changes in airways, MMP-1, 2, 3, 7, 8, 9, 12 and 13 will be quantified in sputum supernatants using the Bio-Plex workstation and the MultiAnalyte Profiling Fluorokine MAP assay based on the Luminex technology. The tissue inhibitor of matrix metalloprotease-1 (TIMP-1) and TGF-beta was also assayed in sputum supernatants. Procollagen type I C Peptide (PICP) and C-terminal telopeptide of the collagen type I (ICTP) will be also quantified as specific markers of collagen synthesis and degradation, respectively(21).

6. Measure of Exhaled Nitric Oxide: Exhaled NO was collected using a Sievers chemiluminescent NO analyzer according to the ATS recommendations(22).

7. Assessment of medical resource use: This study uses data from two administrative databases from Québec; the *Régie de l'assurance maladie du Québec* (RAMQ) and the MED-ECHO databases. The RAMQ provides medical coverage to all residents of Québec and pharmaceutical coverage to the elderly (≥ 65 years), persons receiving social assistance, persons who do not have access to a private insurance plan. The RAMQ database provides information related to the type & date of the dispensed medical services as well as where they were dispensed – clinics, emergency department or hospitals. The RAMQ database also provides information on prescription claims including the drug code which identifies the product name, the unit dose, the form and other product information, the type of prescription (new or refill), the number of prescribed refills (refills allowed by the prescribing physician on the prescription), the duration of the prescription and, the dispensing date. The MED-ECHO database provides information on acute care hospital admissions including data on the patient unique identifier (encrypted), the discharge diagnoses, and the duration of the hospitalization for all residents of Québec. The patient's encrypted unique identifier will be used to link the RAMQ

database with the MED-ECHO database. We estimated the medical visits for asthma (visits to the clinics, to the emergency ward or hospitalizations) one year prior and after their diagnosis of OA and one year prior their follow-up visit.

Outcome

Primary Outcome:

- Change in non-specific airway responsiveness between diagnosis and follow-up visit in Eos+ and Eos- groups.

Secondary Outcomes:

- Sputum neutrophil counts in Eos+ and Eos- groups.
- Change in FEV_1 between diagnosis and follow-up visit in Eos+ and Eos- groups
- Asthma control and quality of life at follow-up visit in Eos+ and Eos- groups.
- Exhaled NO levels at follow-up visit in Eos+ and Eos- groups.
- Markers of sensitization (total IgE), specific markers of cell activation (i.e. MPO/neutrophils and ECP/eosinophils), airway remodelling markers (PIP, ICTP, EGF, FGF-2, PDGF-AB/BB, PDGF-AA, MMP-1, -2, -3, -7, -8, -9, -12, -13 and MMP-9/TIMP-1 molar ratio), cytokines family (IL-1a, IL-1b, IL-3, -4, -5, -6, -7, -9, -10, IL-12(p40), IL-12(p70), IL-13, -15, -17, G-CSF, GM-CSF, INF-a2, TGF a, TNF-a and TNF-b), chemokines family (CXCL8/IL-8, eotaxin, Flt-3L, Fractalkine, GRO, IP-10, MCP-1, MDC, MIP-1a, MIP-1b and RANTES) and soluble immunoregulatory receptors (IL-1ra, sCD40L and sIL-2Ra).
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- Utilization of medical resources and asthma treatment one year prior and after their diagnosis of OA and one year prior their follow-up visit in Eos+ and Eos- groups.

Statistical analysis

Statistical analysis: Continuous variables were reported as mean and standard deviation, except for PC_{20}, which was reported as geometric mean and 95% confidence intervals, and sputum cell counts which were reported as median and interquartile ranges. A Chi-square test was used to compare the categorical variables between groups.

A Student t-test was used to compare the characteristics of continuous variables: between non-eosinophilic and eosinophilic responders. Paired analyses were conducted to compare the data at baseline and follow-up. Non-parametric tests were performed for data that were not normally distributed. A linear regression analysis was conducted to assess the predictors of the decline in the FEV1/FVC ratio over time between diagnosis and the current study.

The statistical analysis was performed with the IBM SPSS statistical software (version 19.0.0), IBM Corporation (Somers, NY). Significance was accepted when $p \leq 0.05$.

Results:

Characteristics of the study population at diagnosis

One hundred and fourteen patients were identified. Forty two (37.2%) subjects were non-eosinophilic responders. Twenty-four subjects refused to participate, 40 subjects could not be reached. Fifty subjects were enrolled for further evaluation at follow-up, but six of them were excluded due to missing values of sputum cell counts before challenge. The data of 44 subjects were analyzed. They had been diagnosed 5.4±2.3 years the present evaluation. At the time of diagnosis and during specific inhalation challenges, 15 of them had a change in their sputum eosinophil count less than 2% (0(2.5)%) after exposure to the offending agents (non-eosinophilic responders) whereas 29 of them showed an increase in their sputum eosinophil count greater than 2% (14.3(22.8) (eosinophilic responders). Non-eosinophilic responders had a slight increase in their neutrophil count (13.4(45.2)%), which was not significantly different from eosinophilic responders (-0.7(32.8)%) (p=0.3). (Table 1).

Non-eosinophilic responders had a slightly greater airflow limitation but did not show a greater airway hyperresponsiveness compared with the eosinophilic responders.

Characteristics of the eosinophilic and non-eosinophilic responders at the time of the study

At the time of the study, non-eosinophilic responders showed a lower FEV_1, a greater airflow limitation and, greater airway responsiveness. They also had a poorer control of their asthma as shown by an ACQ score greater than 0.5 compared to the eosinophilic responders, which indicates an important clinical difference and was close to statistical significance. (Table 2). Airflow limitation worsened in the non-eosinophilic group (difference between FEV_1/FVC ratio at follow-up and at baseline = -0.03±0.08) from diagnosis to the current study whereas it remained stable in the eosinophilic group (0.01±0.03) (p=0.01). (Figure 1). There was also a greater decline in FEV_1 in the non-eosinophilic group (- 8.4±12.1 % predicted) compared to the eosinophilic group (-3.1± 6.85 predicted).

Determinants of the eosinophilic response

We assessed potential determinants (sex, atopy, type of occupational agent (high- vs. low-molecular-weight agent), treatment with inhaled corticosteroids, smoking habits and, FEV_1) associated with an eosinophilic vs non eosinophilic response. A FEV_1 lower than 80% was associated with a non-eosinophilic response (OR (95%CI): 4.0 (1.0-16.5)), whereas atopy almost reached significance for an association with an eosinophilic phenotype (OR 5.9(0.8-41.1).

Variables associated the progression of airflow obstruction

We assessed whether different potential determinants (age, duration of asthma, airflow limitation at diagnosis. smoking habits, treatment with inhaled corticosteroids and type of eosinophilic response (eosinophilic vs non eosinophilic response) after exposure to the offending agents were associated with the progression of airflow limitation (difference between the FEV_1/FVC ratio at follow-up and at diagnosis). A non-eosinophilic response (p= 0.01) and a high inhaled-corticosteroid dose were significantly associated with a greater progression of airflow limitation (p=0.02) (Table 3).

Difference in cytokines levels betweens groups

Levels of IL-2, IFN -gamma and MCP 3 were higher in the eosinophilic responder group than in the non-eosinophilic group. There was no difference in the metalloproteinases levels between groups (Table 4).

Healthcare utilization

We did not find differences in the number of outpatient's visits or emergency visits for asthma in the 5 years preceding or following the diagnosis of OA between non-eosinophilic responders and eosinophilis responders. However, there was a higher number of subjects go were hospitalized for asthma (2(15.5%) in the non eosinophilic responder group compared to the eosinophilic group (0) p=0.04. (Table 5).

Discussion and Conclusions

We believe that this study is the first to suggest that the type of airway inflammation induced by the exposure to an occupational agent maybe associated with a different outcome of the disease. Not only the non-eosinophilic responders with OA appeared to have a more severe asthma than the eosinophilic responders at the time of diagnosis but they also had a poorer prognosis than the eosinophilic responders as shown by a greater airflow obstruction, a greater airway hyperresponsiveness, poorer asthma control in spite of a higher dose of inhaled corticosteroids five years after diagnosis.

Identifying inflammatory phenotypes has been shown essential for an optimal management of asthma. Although the response to treatment and the severity of the disease seem to be different according to the inflammatory phenotype (eosinophilic vs. non eosinophilic airway inflammation), these phenotypes were usually characterized cross-sectionnally without taking into account the

potential environmental exposures of the asthmatic subjects. These phenotypes may represent the current state of the subjects' asthma control rather than an intrinsic characteristic of the disease as suggested by the variation of sputum cell counts over time (23, 24). The originality of our study was to assess the inflammatory phenotype in response to the exposure to a specific agent. The inflammatory phenotypes described in our study are likely to reflect the underlying pathophysiological mechanism of the asthmatic reaction.

Although a positive asthmatic reaction is usually associated with an eosinophilic inflammation in subjects with OA, a non-eosinophilic response is not uncommon since 40% of our subjects with an asthmatic reaction had a non-eosinophilic response at diagnosis. The type of inflammatory phenotype has been shown to be reproducible in allergen challenges (25)but has never been tested with occupational agents. The exposure to some specific agents such as isocyanates has been reported to result in both an eosinophilic(26) and a neutrophilic inflammation(27, 28), but the reproducibility of the inflammatory phenotype has never been tested within the same subjects.

A non-eosinophilic response is uncommon with inhalation challenges using common allergens in which an eosinophilic reaction is identified in the majority of cases (25). However, there is evidence that eosinophilic or mast cells are not always involved in late asthmatic reactions (LARs). Larché et al. have demonstrated that direct activation of allergen specific airway T cells, independent of the mast cells, can increase airway hyperresponsiveness in humans(29). Furthermore he showed that late asthmatic reaction induced by inhalation challenge of allergen-derived T cell peptides induced increases in airway hyperresponsiveness with a dominant CD3+ and CD4+ bronchial mucosal inflammatory cell response without evidence of an involvement of eosinophils, mast cells, or basophils in these peptide-induced LARs (30).

Distinct populations of T cells have different functions that drive different airway inflammatory responses (e.g. eosinophilic versus neutrophilic inflammation). The determination of the expression of multiple immunoregulatory and remodelling mediators allow proposing immune response signatures (IRS) in response to sensitization and exposure to the offending agent (e.g. diisocyanates or flour). On a quantitative basis, IRS is defined, in the same (i.e. baseline vs exposed) or a different population (e.g. two different groups) of subjects, as the specific, predominant and significant expression of markers over others quantified. These IRS are useful for a better understanding of the underlying molecular mechanisms of respective types of airway immune responses. For instance, the development of the Th17 lineage required the IRS: $TGFb^{+}$, $IL\text{-}6^{+}$, $IL\text{-}1^{+}$ and $IL\text{-}23^{+}$ (31). Furthermore, identified (not exclusively referring to the ones for T-cell lineages development) and undergoing discovered IRS may also constitute a useful and powerful tool to identify subgroups in subjects with OA for a better management and treatment of the disease, and to predict positive or negative clinical outcomes (e.g. prognosis). Therefore, a specific identified IRS should be only suitable and relevant for the group of comparison for which it has been defined. It is postulated that shorter is IRS emerging from an analysis of multiple biomarkers higher is the specificity and relevance of this newly identified IRS.

In the present study, analyses of sputum samples for the determination of inflammatory and remodelling mediators indicated no statistical differences between eosinophilic and non-eosinophilic responders for markers of sensitization (total IgE), specific markers of cell activation (i.e. MPO/neutrophils and ECP/eosinophils), airway remodelling markers (PIP, ICTP, EGF, FGF-2, PDGF-AB/BB, PDGF-AA, MMP-1, -2, -3, -7, -8, -9, -12, -13 and MMP-9/TIMP-1 molar ratio), cytokines family (IL-1a, IL-1b, IL-3, -4, -5, -6, -7, -9, -10, IL-12(p40), IL-12(p70), IL-13, -15, -17, G-CSF, GM-CSF, INF-a2, TGF a, TNF-a and TNF-b), chemokines family (CXCL8/IL-8, eotaxin, Flt-3L, Fractalkine, GRO, IP-10, MCP-1, MDC, MIP-1a, MIP-1b and RANTES) and soluble immunoregulatory receptors (IL-1ra, sCD40L and sIL-2Ra).

Statistical significant differences in sputum markers between eosinophilic and non-eosinophilic responders allow proposing the following IRS for eosinophilic responders: $IL\text{-}2^{+}$ $INF\text{-}g^{+}$

MCP3/CCL7^{+}.

To the best of our knowledge there are no previous published reports on the expression of IL-2 and MCP-3 in the airways (biopsy, bronchoalveolar lavage or induced-sputum) of subjects with occupational asthma. The significance of the presence of IL-2 and IFN-g in the IRS of eosinophilic responders is currently unknown, but its relevance may be explained. IL-2 inhalation in human subjects has been shown to induce airway eosinophilia(32). Furthermore, in contrast to IFN-a (33), eosinophil-derived IFN-g induces allergic airway responses (34). Interestingly, ligation of CD28 molecules expressed in human eosinophils results in the secretion of both IL-2 and IFN-g, whereas IL-4, IL-5, and IL-10 were not detected (35). Whether the activation of CD28 in airway eosinophils is responsible for the specific expression of IL-2 and IFN-g in the IRS of eosinophilic responders is presently unknown, but it is an interesting issue to be addressed. There is no evidence for a role of MCP3/CCL7 in the recruitment of eosinophils in allergic conditions. However, MCP3/CCL7 has been identified as one of the most effective chemokines to activate eosinophils (36).

Therefore, we have identified in the subgroup of subjects with OA named eosinophilic responders the following IRS: IL-2^{+} INF-g^{+} MCP3/CCL7^{+} when this subject population was compared to the non-eosinophilic responders. The pathological and clinical significances of this IRS are not presently fully understood and will require further research investigations.

This study has limitations that should be noted. First, our sample size was limited. Although we have access to a large population of subjects with OA tested in the past diagnosed with specific inhalation challenges, the systematic collection of sputum cell counts was implemented in the early 2000s resulting in fewer numbers of subjects to be assessed. This small sample prevented us to study the effect of the type of agent on airway inflammation. We did not collected supernatant at the time of investigation, therefore the mediators measured in the study were not the reflect of the airway inflammation induced by the exposure to occupational agents preventing us to explore the mechanism at the time of exposure.

In conclusion, non-eosinophilic responders seems to be a phenotype of OA associated with a poor outcome. Performing sputum cell counts during the investigation of OA allows the identification of this phenotype. Studies of a larger scope will be needed to confirm those results and provide alternative of management/treatment for non-eosinophilic responders.

Tables

Table 1. Characteristics of the eosinophilic and non-eosinophilic responders at diagnosis

	Change in eosinophil counts during SIC <2%	Change in eosinophil counts during SIC ≥2%	p
n	15	29	
Sex, M/F	13/2	21/8	0.3
Age, y	48±12.3	45.1±9.4	0.5
Atopy, n,%	11(73.3%)	27(93.1%)	0.07
Smoking habits, NS, CS/ exS	4 (26.6)/4(26.6)/7(46.7)	16(55.1)/4(13.8)/9(31.0)	0.2
Pack-year	24.3±22.6 (n=11)	19.1±18.9 (n=13)	0.4
Agents (HMW/LMW), n(%)	4(26.7)/11(73.3) Isocyanates (5), wood dust (3), sanitizer (2), colophony (1), pork (1), chicken (1), insecticide(1), flour (1)	13(44.8)/16(55.2) Flour (10), isocyanates (9), acrylates (1), formaldehyde (2), red cedar (2), latex (1), enzymes (1), beaver fur (1), ammonium (1)	0.2
Asthma duration, y	9.8±3.3	13.1±7.9	0.1
Duration of follow-up, y	5.6 ±2.9	5.3±2.0	0.7
Duration of exposure, y	11.9±11.8	9.3±6.9	0.4
Treatment with ICS, n (%)	10(66.7)	14(48.3)	0.3
FEV_1 at diagnosis,% pred	81.0±20.7	92.1±16.7	0.06
FEV_1/FVC	0.69±0.1	0.76±0.05	0.01
PC_{20} pre SIC, mg/ml	3.0±15.4	8.1±7.7	0.1
PC_{20} post SIC, mg/ml	0.9±6.9	3.4±5.8	0.04
Asthmatic reaction (I,L,D)	8/7/0	19/7/3	0.2
Eos,% at baseline	1.0(2.7)	1.0(2.5)	0.8
Neu,% at baseline	42.7(50.5)	43.0(39.2)	0.7
Eos after SIC	0.5(2.0)	16.3(26.7)	<001
Neu after SIC	55.0(36.9)	42.5(39.8)	0.1

Table 2: Characteristics of the eosinophilic and non-eosinophilic responders at the time of the study

	Change in eosinophil counts during SIC <2%	Change in eosinophil counts during SIC ≥2%	p
n	15	29	
Duration of follow-up, y	5.6±3.0	5.3±2.1	0.7
FEV_1 at follow-up, % pred	72.6±23.0	89.0±18.5	0.04
FEV_1/FVC	0.66±0.1	0.77±0.1	0.001
PC_{20} at follow-up, mg/ml	3.3±5.5	11.9±5.6	0.04
Eos at follow-up %	0.7(3.0)	1.0(3.5)	0.8
Neu at follow-up,%	53.2(46.5)	45.7(47.8)	0.4
FeNO, ppb	13.8±7.5	15.9±6.3	0.3
ACQ at follow-up	1.6±1.3	1.01±0.8	0.07
AQLQ at follow-up	5.5±1.1	5.8±1.1	0.3
Treatment with ICS, n(%)	11(73.3)	9(31)	0.01

Table 3: Linear regression determining the predictors of the decline of FEV1/FVC between time of diagnosis and time of the study.

Dependent Variables	β	SE (β)	t	P value
Age	0.0	0.001	-0.9	0.4
Pack/year	0.0	0.001	-0.7	0.4
Duration of asthma, y	0.0	0.0	-1.2	0.2
FEV_1/FVC at diagnosis	-0.13	0.9	-1.5	0.1
Eosinophilic response	0.04	0.02	0.4	0.02
Inhaled corticosteroid dose	$-4.4\ 10^{-5}$	0.0	-2.5	0.01

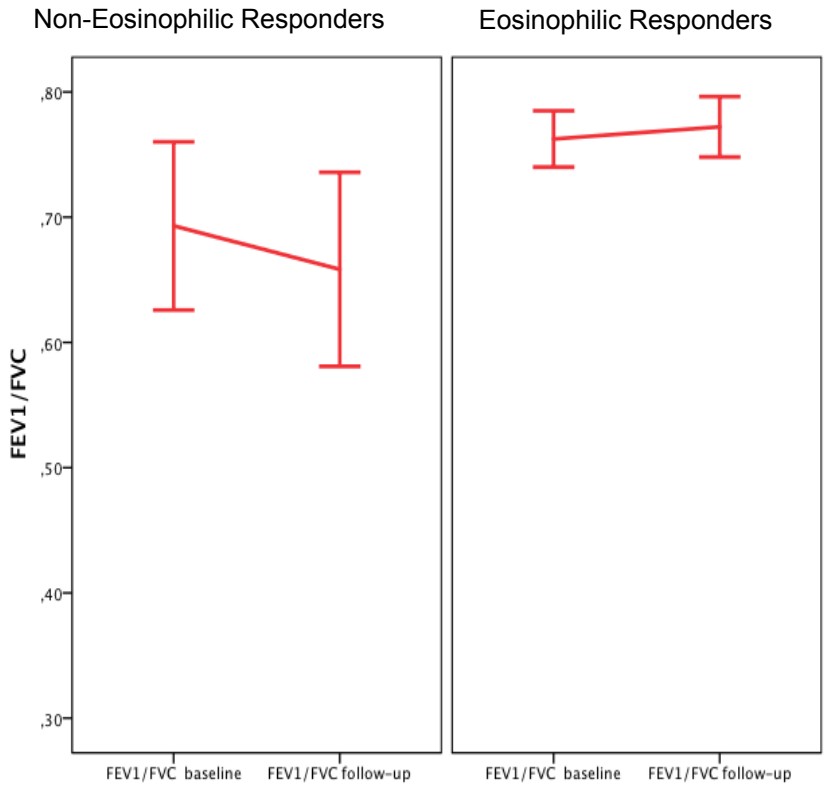
Table 4: Cytokines and metalloproteinases levels at the time of the study

	Non eosinophilic responder N=15	Eosinophilic responder N= 29	p
MMP 1 pg/ml	78.2±81.2	71.22±75.3	0.8
MMP2 pg/ml	1042.4±1246.7	832.9±596.9	0.5
MMP3 pg/ml	197.1±136.1	269.5±292.9	0.4
MMP 8 pg/ml	59824.4±2.1 10^4	58346.6±5.7 10^4	0.9
MMP9 pg/ml	1.4.10^5 ±1.9 10^5	1.8 10^5±2.6 10^5	0.6
MMP12 pg/ml	66.4±33.9	91.9±111.5	0.4
TIMP 1 ng/ml	119.9±65.6	124.3±69.8	0.8
MMP9/TIMP1	0.3±0.4	0.5±0.6	0.4
IL-4 pg/ml	0±0	0.3±1.2	0.3
IL-5 pg/ml	1.7±3.6	4.0±7.6	0.3
IL-13 pg/ml	0±0	0.02±0.1	0.4
IL-2 pg/ml	0.4±0.4	1.0±0.9	0.02
IFN-G pg/ml	0.2±0.3	0.5±0.5	0.03
IL-8 pg/ml	415.9±113.3	475.8±435.2	0.6
MCP3 pg/ml	6.9±3.7	10.4±5.7	0.04
ICTP ng/ml	0,04±0.05	0.04±0.04	0.7
PICP ng/ml	16.2±32.2	5.7±7.3	0.1

Table 5: Healthcare utilization of eosinophilic and non-eosinophilic responders

	Non eosinophilic responders	Eosinophilic responders	p value
Subjects admitted for asthma in the 5 years prior to diagnosis of OA, n(%)	2 (15.5)	0	0.04
Subjects admitted for asthma 5 years after diagnosis	0	0	-
Number of hospitalizations for asthma in 5 years preceding the diagnosis	0.1 ± 0.4	0	0.2
Subjects with at least 1 emergency visit for asthma in the 5 years preceding the diagnosis of OA	3(20%)	8(27.6)	0.6
Number of ER visits for asthma in the 5 years preceding the diagnosis of OA	0.9 ± 2.1	0.6 ± 1.1	0.6
Subjects with at least 1 emergency visit for asthma in the 5 years following the diagnosis of OA	1 (6.7)	3(10.3)	0.7
Number of ER visits for asthma in the 5 years following the diagnosis of OA	0.1 ± 0.3	0.2 ± 0.5	0.4
Number of out patient visits for asthma in the 5 years preceding the diagnosis of OA ±	14.7 ± 5.7	12.5 ± 6.7	0.3
Number of out patient visits for asthma in the 5 years following the diagnosis of OA	12.0 ± 4.8	11.1 ± 5.7	0.6

Figure 1: Outcome of the FEV_1/FVC ratio between diagnosis and follow-up in eosinophilic and non-eosinophilic responders



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Program Director/Principal Investigator (Last, First, Middle): LEMIERE, Catherine

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Is sputum eosinophilia a prognosis factor for occupational asthma

Total Enrollment: 50 **Protocol Number:**

Grant Number: 5R03OH009734

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not	Total
Hispanic or Latino	,0	,1	,0	,1 **
Not Hispanic or Latino	1,10	3,8	,0	49
Unknown (individuals not reporting ethnicity)	,0	,0	,0	,0
Ethnic Category: Total of All Subjects*	1,1	3,9	,0	5,0 *
Racial Categories				
American Indian/Alaska Native	,0	,0	,0	,0
Asian	,0	,0	,0	,0
Native Hawaiian or Other Pacific Islander	,0	,0	,0	,0
Black or African American	,0	,0	,0	,0
White	1,1	3,9	,0	5,0
More Than One Race	,0	,0	,0	,0
Unknown or Not Reported	,0	,0	**,0**	,0
Racial Categories: Total of All Subjects*	1,1	3,9	,0	5,0 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Femelles	Males	Sex/Gender Unknown or Not	Total
American Indian or Alaska Native	,0	,0	,0	,0
Asian	**,0**	,0	,0	,0
Native Hawaiian or Other Pacific Islander	,0	,0	,0	**,0**
Black or African American	,0	,0	,0	,0
White	,0	,1	,0	,0
More Than One Race	,0	,0	,0	,1
Unknown or Not Reported	,0	,0	,0	,0
Racial Categories: Total of Hispanics or	,0	,1	,0	,1 **

* These totals must agree.
** These totals must agree.