Clinical features and airway inflammation in mild asthma versus asymptomatic airway hyperresponsiveness

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Summary

\textbf{Rationale:} We still do not know why some subjects with airway hyperresponsiveness (AHR) experience no respiratory symptoms.

\textbf{Objectives:} Our aim was to compare pulmonary function, perception of bronchoconstriction, and airway inflammation in atopic subjects with mild recently diagnosed (<5 years, \( n = 30 \)) or longer-standing (5 years or more, \( n = 30 \)) symptomatic asthma in comparison with atopic subjects with asymptomatic AHR (\( n = 27 \)).

\textbf{Methods:} All subjects had measurements of expiratory flows, PC\textsubscript{20} methacholine, perception of breathlessness and induced sputum cell differential, eosinophil cationic protein and \( \alpha \)-2-macroglobulin levels.

\textbf{Results:} Compared with the other groups, PC\textsubscript{20} was significantly lower in longer-standing asthma and perception score for breathlessness at 20\% fall in FEV\textsubscript{1} was lower in asymptomatic subjects. Markers of airway inflammation were similar in all groups. There were no significant correlations between sputum eosinophils,
Introduction

Airway hyperresponsiveness (AHR) is observed in almost all subjects with symptomatic asthma and is described as a property of the airways to respond both too much and too easily to various stimuli. In the general population, airway responsiveness follows a continuous unimodal log-normal distribution, with asthmatic subjects representing the “hyperresponsive” end of the distribution curve. A variable proportion of asymptomatic subjects, up to 50% in some studies, with no past or present history of asthma, show an increased airway response to agents such as histamine or methacholine or to stimuli such as exercise. Although the significance of such AHR in the absence of symptoms is still uncertain, it has been considered a risk factor for asthma, particularly in atopic subjects; these subjects are possibly at an early stage of a process that may lead to the classical features of symptomatic asthma. In this regard, asymptomatic AHR has been associated with airway wall inflammation and remodeling, although these features were less marked than in asthmatic subjects.

There are a number of possible explanations for the absence of symptoms in subjects with increased airway responsiveness. These include a too-high cut-off of PC_{20}, the provocative concentration of methacholine giving a 20% fall in forced expiratory volume in 1 s (FEV_{1}), that does not reflect an abnormal airway behavior; intermittent AHR; minimal variability of airway obstruction; insufficient airway inflammation/remodeling; or a defective perception of airway obstruction.

More studies are needed to look at clinical and physiological features of asymptomatic AHR, in order to better understand why some subjects with AHR have no symptoms. In the present study, our hypotheses were that subjects with asymptomatic AHR had less evidences of airway inflammation than in asthma, those with recently diagnosed asthma being intermediary between longer-standing asthma and asymptomatic AHR. We therefore assessed inflammatory cell levels, as well as markers of inflammation such as eosinophil cationic protein (ECP) and α2-macroglobulin. ECP is a cytotoxic protein released from activated eosinophil during the inflammatory process, while α2-macroglobulin has been previously validated as a marker of plasma exudation, a process that could contribute to airway obstruction and hyperresponsiveness. ECP and α2-macroglobulin have previously been successfully measured in induced sputum. Furthermore, we wanted to gather more data on perception of induced bronchoconstriction in these groups.

Methods

Subjects

We enrolled non-smoking atopic subjects currently exposed to relevant allergens with either mild asthma (n = 60) or asymptomatic AHR (n = 27) with a PC_{20} between 1 and 16 mg/ml. The asthma group included subjects with recently diagnosed (<5 years, n = 30) or longer-standing (≥5 years, n = 30) asthma. These subjects were recruited from our Asthma and Respiratory Allergy clinics or from advertisements in local media. This analysis was done on all subjects initially studied, some being thereafter included in another long-term study looking at the influence of anti-inflammatory treatment on airway inflammation in those groups. Additional inclusion criteria were, for all subjects (1) Men or women 18–45 years old; (2) at least one positive (≥3 mm wheal at 15 min) response to indoor allergens (cat, dog, house-dust mite or cockroach). Additional exclusion criteria were (1) respiratory infection within 6 weeks of the first visit of the study, (2) smoking >10 packs-years, or in the last twelve months and (3) pregnancy, breastfeeding or inadequate contraception.

Mild asthma was defined as: mild intermittent asthma symptoms, less than twice a week over the last 3 months; demonstration of variable airflow obstruction according to the 1999 Canadian asthma consensus criteria; inhaled short-acting β_{2} agonist on demand as the only medication required for...
asthma within the last year. We separated the asthma group into those with a diagnosis of 5 years or less versus more than 5 years to determine if subjects with more recently diagnosed asthma had features closer to those with asymptomatic AHR.

Subjects were considered to have asymptomatic AHR if they had shown no past or present symptoms of intermittent dyspnea or wheezing, chronic cough or phlegm production as defined by negative responses to the European community respiratory health survey (ECRHS) questionnaire18 and no symptoms similar to those induced by the methacholine challenge. Each subject signed an informed consent form, approved by the Ethics Committee of the hospital.

**Study design**

Subjects had a detailed respiratory questionnaire, skin-prick tests, methacholine bronchoprovocation with measurement of perception of bronchoconstriction and an induced sputum sampling. The ECRHS questionnaire was used for screening and for evaluating associated conditions.18 Additional questions were asked about subjects’ characteristics, perceived symptoms, smoking, duration and control of asthma and its treatment, including the nature and intensity of the respiratory symptoms, asthma triggering factors (including allergens) and medication needs. Atopy was confirmed by skin-prick tests with 16 common allergens including cat, dog, horse, mixed trees, birch, mixed grasses, ragweed, Dermatophagoides farinae, Dermatophagoides pteronyssinus, Alternaria, Hormodendrum and cockroach. Normal saline and histamine were used as negative and positive controls, respectively. A positive response was defined as a skin wheal diameter of 3 mm or more at 15 min. Allergens were provided by the coordinating center for all centers.

Spirometry was done according to current American Thoracic Society (ATS) standards, using three reproducible measurements of FEV1 before inhalation of 200 μg salbutamol.19 Measurements were obtained with a calibrated spirometer meeting ATS recommendations.20

Methacholine challenges were done at the same time of day, in the morning, according to the method described by Juniper et al.21 PC20 was obtained by interpolation of the dose–response curve at 20% fall in FEV1. At the end of the methacholine test, the patient was asked if he had experienced similar symptoms in the past as those induced by methacholine inhalation. Perception of bronchoconstriction-induced breathlessness was assessed using a modified Borg Scale from 0 to 10.22

Sputum was induced by the method described by Pin et al.23 and modified by Pizzichini.24 Briefly, subjects were pre-treated with 200 μg of salbutamol before inhaling increasing concentrations of hypertonic saline (3%, 4% and 5%) for 7 min each (for a maximum of 21 min) with a Medix electronic nebulizer (Medix, Catthorp, England) without a valve or nose clip. After each inhalation, subjects were instructed to blow their nose, rinse their mouth and swallow the water to minimize postnasal drip and squamous epithelial cell contamination, respectively, before trying to expectorate in a sterile container. Mucus obtained that way was then separated from saliva using forceps, weighed, and rocked with four times its volume of dithiothreitol (Sputolysin; Calbiochem Corp., La Jolla, CA, USA) during 15 min. The reaction was stopped by adding an equal volume of Dubecco’s phosphate-buffered saline (D-PBS) 1× (Invitrogen, Burlington, Ont., Canada). The cellular suspension was centrifuged at 800 g during 4 min and the supernatant was collected and frozen at −80 °C for further analysis. The cells were then resuspended in D-PBS 1× and slides were prepared with a Cytospin 3 (Shandon Scientific Ltd., Astmoor, England) and colored with Diff-Quik solutions (Dade Diagnostics Inc., Aguada, PR, USA) for a count of 400 cells.

Sputum supernatant ECP levels were determined by the automated UniCAP method (Pharmacia Diagnostics, Mississauga, Ont., Canada) while levels of α2-macroglobulin (α2-M) were determined by ELISA. The detection limits for ECP and α2-M were 2 μg/l and 3 μg/ml, respectively.

**Statistical analysis**

Results of representative measures were expressed using mean ± SD. Analyses of all parameters were performed using one-way ANOVA. For some variables, the graphical analyses of residuals with predicted values revealed a relationship between the variances of the observations and the means for these variables. The logarithm was the estimate form of the required transformation associated with these variables to stabilize the variance.25 Statistical results from these parameters were expressed with transformed values. The normality assumption was verified with the Shapiro–Wilk test and the Brown and Forsythe’s variation of Levene’s test statistic was used to verify the homogeneity of variances. All assumptions were fulfilled. A similar
An approach was performed to analyze data expressed in percentage using the square root arcsinus transformation. Relationships between parameters were expressed with the Pearson’s correlation coefficients. The results were considered significant with \( P \)-values \( < 0.05 \). The data were analyzed using the statistical package program SAS v8.2 (SAS Institute Inc., Cary, NC).

### Results

A total of 87 subjects took part to this study. They included 60 subjects with mild asthma, either with a recently diagnosed \(( n = 30 \) or with longer-standing asthma \(( n = 30 \) and 27 subjects with asymptomatic AHR, with a respective mean age of 26.0 ± 6.5, 26.0 ± 6.9 and 28 ± 7.9 years. Mean ± SD duration of asthma was 0.8 ± 1.5 years for subjects with recently diagnosed asthma and 12.8 ± 8.1 years for those with longer-standing asthma. Subjects’ characteristics are summarized in Table 1.

### Expiratory flows

Mean FEV\(_1\) and mean predicted forced vital capacity (FVC) were similar in the three groups except for the percent predicted FEV\(_1\), which was lower in longer-standing asthma than in asymptomatic AHR \(( P < 0.05 \), Table 1).  

### Methacholine responsiveness and perception of bronchoconstriction

Mean ± SD PC\(_{20}\) was 4.9 ± 2.3 in recently diagnosed asthma, 2.9 ± 2.2 in longer-standing asthma and 5.7 ± 2.3 in asymptomatic subjects. PC\(_{20}\) values were significantly different between all groups except for recently diagnosed asthma versus asymptomatic subjects. Mean perception scores at

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Asymptomatic airway hyperresponsiveness</th>
<th>Recently diagnosed asthma (&lt;5 years)</th>
<th>Longer-standing asthma (≥5 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (30)</td>
<td>11 (36.7)</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>Female</td>
<td>20 (70)</td>
<td>19 (63.3)</td>
<td>14 (46.7)</td>
</tr>
<tr>
<td>Age (years), mean (SD)</td>
<td>28 (7.9)</td>
<td>26 (6.5)</td>
<td>26 (6.9)</td>
</tr>
<tr>
<td>Duration of asthma (years), mean (SD)</td>
<td>N/A</td>
<td>0.8 (1.5)</td>
<td>12.8 (8.1)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>21 (77.8)</td>
<td>24 (80)</td>
<td>22 (77.8)</td>
</tr>
<tr>
<td>Ex-smoker*</td>
<td>6 (22.2)</td>
<td>6 (20)</td>
<td>8 (22.2)</td>
</tr>
<tr>
<td>FEV(_1) (l), Mean (SD)</td>
<td>3.41 (0.65)</td>
<td>3.59 (0.67)</td>
<td>3.38 (0.59)</td>
</tr>
<tr>
<td>(% predicted), mean (SD)</td>
<td>101 (15)</td>
<td>100 (12)</td>
<td>93 (10)†</td>
</tr>
<tr>
<td>FVC (l), Mean (SD)</td>
<td>4.06 (0.82)</td>
<td>4.27 (0.86)</td>
<td>4.19 (0.87)</td>
</tr>
<tr>
<td>(% predicted), mean (SD)</td>
<td>102 (16)</td>
<td>101 (13)</td>
<td>97 (12)</td>
</tr>
<tr>
<td>Atopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal, n (%)</td>
<td>16 (59)</td>
<td>26 (87)</td>
<td>23 (77)</td>
</tr>
<tr>
<td>Pollen, n (%)</td>
<td>16 (59)</td>
<td>26 (87)</td>
<td>23 (77)</td>
</tr>
<tr>
<td>House-dust mite, n (%)</td>
<td>23 (85)</td>
<td>24 (80)</td>
<td>25 (83)</td>
</tr>
<tr>
<td>Others, n (%)</td>
<td>18 (67)</td>
<td>28 (93)</td>
<td>28 (93)</td>
</tr>
<tr>
<td>PC(_{20}) (mg/ml), geometric mean (SD)</td>
<td>5.7 (2.3)</td>
<td>4.9 (2.3)</td>
<td>2.9 (2.2)†</td>
</tr>
<tr>
<td>Borg score at 20% fall in FEV(_1) (0–10) mean (SD)</td>
<td>1.5 (1.8)</td>
<td>2.1 (1.8)</td>
<td>2.1 (1.3)†</td>
</tr>
<tr>
<td>Maximum Borg score (0–10) mean (SD)</td>
<td>3.3 (1.3)</td>
<td>2.75 (2.4)</td>
<td>2.4 (1.5)</td>
</tr>
<tr>
<td>% Fall in FEV(_1) at max Borg score mean (SD)</td>
<td>26.1 (8.2)</td>
<td>28.4 (8.2)</td>
<td>28.8 (12.1)</td>
</tr>
</tbody>
</table>

Asymptomatic AHR: Asymptomatic airway hyperresponsiveness.
*Ex-smoker: Smoking <10 pack-years and no smoking in the last year.
†Significantly different from asymptomatic AHR \(( P < 0.05 \).
Airway inflammation and correlation with other parameters

Induced sputum analysis showed a higher number of total cell counts in recently diagnosed asthma than in the two other groups \((P<0.05, \text{ Table 2})\). However, median (25%, 75% interquartile) eosinophil percentages were similar in the three groups \((P>0.05)\); 33% of recently diagnosed and 24% of longer-standing asthmatic subjects had more than 2% eosinophils on induced sputum compared with 28% in asymptomatic AHR (data not shown). Mean total eosinophil counts were not significantly different between the three groups (Table 2). Macrophages and neutrophils total absolute numbers were higher in recently diagnosed asthma although this was not different in percentage of cells. Total number of lymphocytes was lower in longer-standing asthma. Bronchial epithelial cells were similar in the three groups.

There were no correlations between induced sputum eosinophils, other types of white blood cells or total cell counts, with either FEV\(_1\), FVC, PC\(_{20}\), perception score at 20% fall in FEV\(_1\) or \(\Delta\) perception score/\(\Delta\) FEV\(_1\) in either group. There was a strong overall correlation between ECP levels and both total counts and percentage of eosinophils \((r = 0.83\) and \(0.63,\) respectively, \(P<0.0001)\) for the totality of subjects. There were no differences in ECP or \(\alpha\)2-macroglobulin levels between the three groups \((P>0.05, \text{ Table 2})\).

Discussion

This study showed that subjects with asymptomatic AHR have airway inflammatory features such as induced sputum eosinophil counts, ECP and \(\alpha\)2-macroglobulin levels similar to mild asthmatic subjects either with a recently diagnosed asthma or a longer-standing asthma. However, asthmatic patients with recently diagnosed asthma had an increased total numbers of cells and a trend towards an increased ECP, in sputum, although this last was not significant. Mean PC\(_{20}\) was slightly lower in longer-standing asthma than in asymptomatic AHR, in keeping with one of our previous studies.\(^{26}\) There were no significant correlations in either group between airway inflammation and breathlessness perception scores at 20% fall in FEV\(_1\).

In recently diagnosed asthma, an important increase in total, neutrophil and macrophage cell counts was observed compared with asymptomatic AHR and longer-standing asthma. It may suggest that over time, airway inflammation decreases and that the changes in airway function could mostly be due to airway remodeling, as in subjects with asymptomatic AHR. A previous study by our group\(^{26}\) did not suggest that there was such a difference in baseline airway inflammation according to the duration of asthma but in the present study, the definition of long-standing asthma was different and we had a larger number of subjects. This raises the possibility that an increase in the airway inflammatory process in a predisposed individual leads to the development of asthma. This is supported by our previous observations on asymptomatic AHR showing that the development of asthma in this group is associated with an increased
airway inflammation and a reversal of the CD4+ over CD8+ ratio, although it is also associated with an increased in airway remodeling.

This study also shows that perception of bronchoconstriction was slightly lower in asymptomatic AHR than in longer-standing asthma, although it is unlikely that this explains why these subjects do not experience any symptoms. In this regard, it was previously suggested that subjects with asymptomatic AHR were less likely to report an increase in dyspnea score during histamine bronchoprovocation compared to subjects with symptomatic AHR.27 Other reports on children and adults with asymptomatic AHR, however, including some studies from our laboratory, showed that these subjects were generally able to perceive bronchoconstriction adequately.6,28,29 We nevertheless cannot exclude that a lower perception of induced symptoms could be a contributing factor to their asymptomatic status.

Most of our subjects had mild AHR, although PC_{20} was slightly lower in asthmatic subjects with a longer-standing asthma. We previously observed that asymptomatic subjects with AHR had increases in airway responsiveness when they developed symptoms of asthma over a period of 2 years.30 In another study on mild asthmatic patients, in keeping with the present study, those with a long duration of asthma had a significantly higher degree of AHR compared to recently diagnosed asthma.26

Nevertheless, there was no significant correlation between perception of bronchoconstriction-induced breathlessness and PC_{20}. This may suggest that there is a threshold of airway responsiveness in a given individual at which symptoms begin to be perceived. This may be related to associated increased variations in expiratory flows following environmental triggers or the development of other physiological abnormalities such as lung hyperinflation, which has been thought to contribute to respiratory symptomatology.31,32 Previous studies had also shown a slight increase in diurnal variation of peak expiratory flows in asymptomatic subjects with AHR compared to normal subjects but lower than in asthmatic subjects.29,30

Other possible causes of this absence of symptoms in the presence of AHR could have been the absence of airway inflammation in the asymptomatic AHR group. However, we found that there was a similar degree of airway inflammation in all groups, at least for eosinophils and markers of eosinophil activation or plasma transudation such as ECP and a_{2}-macroglobulin.33 Nevertheless,
induced sputum only assesses intraluminal inflammatory cells at the level of medium to large airways and it may not always reflect airway wall inflammation. As bronchial biopsies and induced sputum sampling assess different airway compartments and aspects of the inflammatory process, it is not surprising that there are only weak correlations between these measures. In this regard, our results differ from some of our previous findings on bronchial biopsies, in which the number and state of activation of inflammatory cells were higher in asymptomatic AHR than in normal controls but lower than in mild asthma. However, the way in which airway eosinophilia may contribute to the development of symptoms is unclear. Airway edema is another inflammatory change that could influence airway function and increase changes in airway caliber. However, we found no significant difference in a marker of plasma transudation, α2-macroglobulin. There may be other components of the inflammatory process that differ in the two groups, however. It is possible that differences in airway remodeling between asthma and asymptomatic AHR, as we previously reported, are involved in the expression of symptoms, and this should be further explored.

Finally, it may be possible that some subjects do not report symptoms because they are not exposed enough to triggers or they do not interpret those symptoms as due to asthma. This did not seem the case in our subjects as they had no previous symptoms such as those experienced during bronchoprovocation. In the context of this study, we did not include a group of atopic subjects without AHR, our focus being only in subjects with AHR, either symptomatic or not, as the question was to help determine why some subjects with AHR had no symptoms.

In conclusion, we found no significant differences in markers of airway inflammation in mild asthma compared with asymptomatic AHR. Although perception of bronchoconstriction was slightly lower in asymptomatic subjects, the significance of such mild reduction in perception is uncertain and further studies should explore why these patients have no symptoms.

**Acknowledgments**

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**References**