Differential Cell Counts in Sputum in Respiratory Epidemiology*

A Pilot Study

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Background: The aim of this pilot study was to determine whether measuring sputum differential cell counts, particularly eosinophils, could be a useful method of validating self-reported symptoms suggesting asthma in epidemiologic studies.

Materials and methods: In this cross-sectional study, we selected four groups of adult subjects by reported symptoms and diagnoses from among those previously randomly identified in a population study. Subjects were selected with no respiratory symptoms ever (normal group), or reporting a diagnosis of asthma (asthma group), or reporting recurrent wheezing not diagnosed as asthma (wheeze group), or reporting exposure to industrial irritants, but not asthma or wheezing (exposed group). Current respiratory symptoms, airway responsiveness to methacholine challenge, and sputum cell counts were determined. The study was completed by 107 subjects aged 20 to 44 years.

Results: There were no significant differences in FEV₁ percent predicted, total cell count, and sputum eosinophil count among the four groups. Subjects with reported asthma had greater airway responsiveness as reflected in a lower bronchial reactivity (BR) index. There was a weak correlation between BR index and sputum eosinophils.

Conclusion: In a community setting, induced sputum eosinophil cell counts in subjects reporting asthma or wheezing were most often within the normal range and not sufficiently often abnormal to be useful in validating a diagnosis of asthma in epidemiologic studies.

(CHEST 2001; 120:1107–1113)

Key words: airway hyperresponsiveness; asthma; eosinophils; epidemiology; induced sputum

Abbreviations: BR = bronchial reactivity; CI = confidence interval; ECRHS = European Community Respiratory Health Study; IQR = interquartile range; NS = not significant; PC₂₀ = provocative concentration of methacholine causing a 20% fall in FEV₁

Asthma is characterized by episodic symptoms of wheezing or chest tightness, variable airway narrowing, and heightened airway responsiveness. Differentiating with certainty between asthma and other diseases of airflow obstruction is difficult in some clinical situations, and even more difficult in epidemiologic studies. Population studies of the prevalence and characteristics of asthma have relied in large part on self-completed questionnaires regard-

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Manuscript received October 24, 2000; revision accepted February 27, 2001.

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increase in some sputum cell counts, particularly eosinophils and metachromatic cells. If sputum cell analyses could be applied in epidemiologic studies, this might yield additional information on the nature of inflammatory processes associated with respiratory symptoms in the population, and help differentiate between nonasthmatic and asthmatic causes of reported symptoms. We hypothesized that subjects reporting asthma in epidemiologic surveys would show sputum eosinophilia that might be a useful marker of asthma in the community. However, the time involved in inducing sputum and analyzing the cell counts of the expectorated material is considerable. We therefore undertook a pilot study to determine whether measuring sputum differential cell counts, particularly of eosinophils, was a useful method of validating symptoms and diagnoses suggesting asthma in subjects who had recently participated in an epidemiologic study of respiratory disease. The null hypothesis was that there would be no difference in sputum eosinophil counts between subjects stating they had asthma and those who did not.

Materials and Methods

In a recent cross-sectional epidemiologic study conducted by questionnaire as part of the European Community Respiratory Health Study (ECRHS), we recruited > 3,000 adults aged 20 to 44 years from the city of Hamilton, Ontario, using random-digit dialing to obtain a representative sampling. From this cohort, a sample of 503 subjects attended the laboratory for more detailed questionnaires and investigations, as part of the ECRHS study design. We reviewed these 503 subjects to select subjects for four groups of approximately equal size. After excluding ex-smokers (to avoid difficulties in classification of smoking status), 369 subjects were potentially eligible for this study. Of these, 147 subjects had moved from the area, 51 subjects declined participation, and 22 subjects could not be contacted, leaving 149 subjects who met the inclusion criteria. The numbers of subjects recruited in each of the four categories were not representative of their distribution in the original population, but were deliberately made approximately equal to allow comparisons among these groups with respect to symptoms, atopy, lung function, airway responsiveness, and differential cell counts in induced sputum.

The four groups were as follows: subjects with no respiratory symptoms (normal group), subjects reporting a diagnosis of asthma (asthma group), subjects reporting recurrent wheezing not diagnosed as asthma (wheeze group), and subjects reporting exposure to industrial irritants but not asthma or wheezing (exposed group). The study was approved by the Research Committee of St. Joseph’s Hospital, and each subject gave written consent.

Current Symptoms

Although subject selection was based on previous responses to the self-completed ECRHS questionnaire, the current status of symptoms and diagnoses was further documented for the present study using the same questions. The symptom score was based on the presence or absence of five symptoms (wheeze, cough, shortness of breath, chest tightness, sputum production) with a scale of zero (no symptoms) to 5 (all of the above). Questions to identify the likely cause of recent symptoms were included when applicable. Current treatments and occupational and smoking histories were also checked.

Skin Tests

Allergy skin prick tests to 14 common inhalant allergen extracts (Dermatophagoides pteronyssinus, Dermatophagoides farinae, olive tree, birch tree, east-western tree mixture, common ragweed, Kentucky grass, timothy grass, cat, cockroach, Cladosporium, Aspergillus, Alternaria, and Penicillium) were available for each subject from their previous participation in the ECRHS survey, or were performed during this study. Any allergen wheal > 2 mm in size than the negative control wheal was used to define atopy.

Airway Function Assessment

Subjects performed spirometry according to American Thoracic Society standards on a spirometer (Koko; Pulmonary Data Service Instruments; Louisville, KY) and then underwent a methacholine challenge test to determine current airway responsiveness. The test concluded with inhalation of salbutamol to reverse any resulting airway constriction and to prepare for sputum induction.

Methacholine Challenge

Methacholine challenge was performed according to the standardized and validated protocol of Hargrave et al, preceded by 15 min of rest. Following baseline spirometry, FEV1 was remeasured after inhalation of normal saline solution, and after doubling concentrations of methacholine from 0.03 to 16.0 mg/mL in normal saline solution, delivered using a Wright nebulizer (Aerostol Medical Limited; Colchester, Essex, United Kingdom) (output, 0.13 mL/min) and inhaled by tidal breathing for 2 min with the nose clipped. FEV1 was measured at 30 s and 90 s after each dose. If the FEV1 was lower at 90 s than at 30 s, additional measurements were made at 180 s and every 2 min thereafter until the lowest FEV1 was determined. Subsequent concentrations were given at approximately 5-min intervals until the FEV1 decreased 20% from the lowest post-saline solution FEV1, or until the highest concentration had been given. Once the FEV1 stopped falling after the last inhalation, salbutamol was administered. The provocative concentration of methacholine causing a 20% fall in FEV1 (PC20) was calculated by linear interpolation of the last two points. The dose-response slope (bronchial reactivity [BR] index) was calculated by the method of Burrows et al so that no data were censored.

Sputum Induction

Sputum was induced by the method developed and standardized at this institution, as described by Pin et al and slightly modified. An aerosol of hypertonic saline solution was inhaled in increasing concentrations (3%, 4%, and 5%) generated by an ultrasonic nebulizer (Fisoneb; Canadian Medical Products Ltd; Markham, Ontario) with an output of 0.87 mL/min and particle size of 5.58 μm aerodynamic mass median diameter. Each concentration was inhaled for 7 min.

Sputum Analysis

Sputum examination was performed as described by Pizzichini et al. The fresh expectorated sputum was poured into a petri dish, and all...
portions that macroscopically looked more opaque or dense and unlike saliva (selected portion) were placed in a 15-mL polystyrene tube and weighed. This was treated with dithiothreitol (Sputalysin 10%; Calbiochem Corporation; San Diego, CA) freshly diluted 1:10 with distilled water, in a volume equal to four times the weight of the selected sputum. The mixture was vortexed for 15 s, gently aspirated in and out of a pipette to ensure mixing, and placed on a bench rocker (Dade Tube Rocker; Baxter Diagnostics Corporation; Miami, FL) for 15 min. A further four volumes of Dulbecco's phosphate-buffered saline solution was added, and rocking was continued for another 5 min. The suspension was filtered through a 48-μm nylon mesh (BBSH; Thompson; Scarborough, Ontario) to remove cell debris and remaining mucus. A total cell count was performed in a modified Neubauer hemocytometer, and the cell viability was determined simultaneously by the trypan blue exclusion criteria. The total number of cells per milligram of processed sputum was calculated. Cytospin centrifuges were prepared by placing 60 μL of the cell suspension adjusted to obtain 1.0 × 10^6/mL into a cytocentrifuge (Shandon III; Shandon Southern Instruments; Sewickley, PA) and spinning at 450 rotations per minute for 6 min. One cytospin was dried and Wright stained, and a differential cell count was performed on 400 nonsquamous cells.

Outcomes

The primary outcome was the percentage of eosinophils in sputum in the different self-reporting symptom groups. The secondary outcome was the level of airway hyperresponsiveness in the different groups and its relationship with sputum eosinophil count and symptoms.

Analysis

Results were expressed as mean and SDs, except for data with nonnormal distribution (sputum cell count), which were expressed as median and interquartile range (IQR). Airway reponsiveness was expressed both as PC_{20} and as an index of bronchial responsiveness calculated by a continuous index of the decline of FEV1 during the methacholine test (BR index).^7^ Despite usual transformations, the distribution of the sputum eosinophil count could not be normalized. The mean normal sputum eosinophil count has been reported as being 0.4 ± 0.9%.^9^ Most of the healthy subjects (normal and exposed) of our population showed a sputum eosinophil count of < 1% (Fig 1). We have used 1% as an arbitrary cut point to express sputum eosinophils as a binary variable 0 if < 1%, and 1 if ≥ 1%. Blood eosinophil count was also expressed as a binary variable, coding 0 if < 0.2 × 10^9/L and 1 if ≥ 0.2 × 10^9/L. Symptoms of cough, wheezing, dyspnea, chest tightness, and sputum production were recorded. The current number of symptoms reported by the subjects was coded from 0 (no symptoms) to 5 (all five symptoms reported).

One-way analysis of variance with Bonferroni corrections for multiple comparisons was used to compare FEV1 percent predicted and airway responsiveness (BR index) among the four groups. The Mann-Whitney test was used to compare sputum eosinophil counts between atopic and nonatopic subjects. Kruskal-Wallis one-way analysis of variance was used to compare the total and differential cell counts in induced sputum among the different groups. Correlations were examined using Spearman rank correlation test. Logistic regression with sputum eosinophils as the dependent variable was performed to examine the relationship between sputum eosinophil counts and clinical parameters. Airway responsiveness using the PC_{20} value was expressed as a binary variable, coded 0 if PC_{20} was > 8 mg/mL, and 1 if PC_{20} was ≤ 8 mg/mL. Separate multiple regression analyses of clinical and biological factors potentially associated with airway responsiveness were performed. The four subgroups were expressed as dummy variables using the normal group as the reference group. Significance was accepted at a level of 95%. The analysis was performed using statistical software (SPSS version 7.5; SPSS; Chicago, IL).

![Figure 1. Distribution of sputum eosinophils among the four subgroups in atopic and nonatopic subjects.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21968/)
RESULTS

Of 149 subjects recruited, 107 subjects (71.8%) produced sputum suitable for analysis. We did not observe any adverse event during sputum inductions. Characteristics of the 107 subjects who completed the study are shown in Table 1. Approximately one third of subjects reporting asthma received inhaled corticosteroids.

There was no difference in mean FEV$_1$ and FEV$_1$ percent predicted among the four subgroups, but there was greater airway responsiveness (expressed as BR index) among subjects reporting asthma compared to the other three groups. The distribution of sputum eosinophil counts in the four groups is shown in Figure 1. Although some subjects with wheeze or reported asthma had sputum eosinophil counts > 1%, the majority had eosinophil counts of 0.0 as did the normal subjects. Only 13 of 67 subjects reporting respiratory symptoms had sputum eosinophil counts > 1%. There were no significant differences in the total or differential cell counts in sputum among the groups (Table 1). Hence, we cannot reject the null hypothesis. The difference in median (IQR) sputum eosinophil count between subjects with PC$_{20}$ of ≥ 8 mg/mL and 26 subjects with PC$_{20}$ of < 8 mg/mL was not significant (NS): 0.00 (0.4) vs 0.3 (1.3) [p = 0.08]. Likewise, there was no difference in the median sputum eosinophil count among non-smokers in the four groups (p = 0.3).

Correlations among airway responsiveness, smoking habits and sputum and blood eosinophils, atopy, and symptoms are shown as Spearman’s correlation coefficients in Table 2. Airway responsiveness was weakly but significantly correlated with both sputum eosinophils and atopy, while smoking habits were weakly but significantly correlated with both FEV$_1$ and symptoms. Blood and sputum eosinophil counts were also correlated.

Results of multiple logistic regression analyzing the potential determinants of sputum eosinophilia are summarized in Table 3. Controlling for atopy, smoking, airway responsiveness, blood eosinophils, exposure to irritants, self-reported asthma, and self-reported wheeze, we found that blood eosinophils, self-reported asthma, and symptoms of wheezing explain some of the variation in sputum eosinophilia.

<table>
<thead>
<tr>
<th>Table 1—Clinical, Functional, and Biological Characteristics of Subjects</th>
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<tbody>
<tr>
<td><strong>Groups</strong></td>
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<tr>
<td>Subjects, No.</td>
</tr>
<tr>
<td>Male gender, No. (%)</td>
</tr>
<tr>
<td>Atopic, No. (%)</td>
</tr>
<tr>
<td>Smokers, No. (%)</td>
</tr>
<tr>
<td>Treatment, No.</td>
</tr>
<tr>
<td>Nasal corticosteroids</td>
</tr>
<tr>
<td>Inhaled β₂-agonists</td>
</tr>
<tr>
<td>Oral antihistamines</td>
</tr>
<tr>
<td>No therapy</td>
</tr>
<tr>
<td>FEV$_1$, % predicted*</td>
</tr>
<tr>
<td>PC$_{20}$, mg/mL†</td>
</tr>
<tr>
<td>PC$_{20}$ &lt; 8 mg/mL, No. (%)</td>
</tr>
<tr>
<td>BR index§</td>
</tr>
<tr>
<td>TCC, × 10$^6$/mL¶</td>
</tr>
<tr>
<td>Sputum eosinophils, %‖</td>
</tr>
<tr>
<td>Sputum eosinophils, × 10$^6$/mL‖</td>
</tr>
<tr>
<td>Sputum neutrophils, %‖</td>
</tr>
<tr>
<td>Blood eosinophils, × 10$^9$/L¶</td>
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</table>

*Data are presented as mean (SD) unless otherwise indicated.
†Data presented as geometric mean (SD).
‡Data presented as median (IQR).
§p = < 0.001 for comparison with normal group.
||p = 0.02 for comparison with wheeze group.
¶p = 0.03 for comparison with exposed group.
However, the relatively low number of subjects in each group results in a wide range in the confidence intervals (CIs) for the odds ratios.

Results of multivariate analyses of potential determinants of airway responsiveness and the final regression model are shown in Table 4. The variables included in the final model explained 22% of the variation in airway responsiveness. Sputum eosinophilia and a diagnosis of asthma made by a physician were positively associated with airway responsiveness. The odds ratio for sputum eosinophilia included in the final model explained 22% of the regressors. The variables of airway responsiveness and the final regression model are shown in Table 4. The variables included in the final model explained 22% of the variation in airway responsiveness. Sputum eosinophilia and a diagnosis of asthma made by a physician were positively associated with airway responsiveness independently of other variables in the model.

Table 2—Spearman’s Correlation Coefficients (ρ) Between Variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>BR Index</th>
<th>Sputum Eosinophils</th>
<th>Smoking Habits*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood eosinophils†</td>
<td>0.12</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Atopy†</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1, L</td>
<td>−0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptoms‡</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Smoking habits coded as 0 = nonsmokers and 1 = smokers.
†Blood eosinophils coded as 0 = < 0.2 x 109/L and 1 = ≥ 0.2 x 109/L.
‡Atopy coded as 0 = nonatopic and 1 = atopic.
§Symptoms coded as 0 = no symptoms to 5 = maximal symptoms.
||p < 0.05.

### Discussion

The purpose of this study was to determine the usefulness of differential cell counts in induced sputum as an epidemiologic tool to discriminate asthma from other causes of respiratory symptoms reported in population studies. We have previously shown an increase in sputum eosinophils to a median of 4.5% in subjects presenting with uncontrolled asthma and airway hyperresponsiveness with PC20 of < 4 mg/mL.2 However, in a community setting, sputum examination did not differentiate subjects with asthma from those reporting wheezing, or from those exposed to industrial irritants, or from normal subjects, as the great majority of all subjects had sputum eosinophil counts within the normal range. Subjects with reported asthma did however demonstrate greater airway responsiveness compared to other subgroups.

In our study, subject selection was from a community population self-reporting asthma or respiratory symptoms by questionnaire. In this setting, subjects did not require demonstration of airway hyperresponsiveness or FEV1 reversibility to be classified as having self-reported asthma. Indeed, 16 of the 31 self-reported asthmatic subjects (52%) had a PC20 of > 8 mg/mL, and only 11 subjects (35%) had a PC20 of < 4 mg/mL. It is therefore possible that a proportion of subjects classified as asthmatics by questionnaire did not have current asthma. Moreover, Turner et al10 found almost half the adult subjects with mildly uncontrolled asthma recruited for a drug trial did not have increased sputum eosinophils. Considering that not all asthmatic subjects with symptoms show increased eosinophil counts even when recruited in an asthma clinic, and that the subjects classified as asthmatics by questionnaire had mild asthma or did not have current asthma with increased airway responsiveness, it is not surprising that our asthmatic group did not have a significant increase in their sputum eosinophil count.

Gibson et al11 did find a significant increase in sputum eosinophils in an epidemiologic study of children, especially in those with airway hyperres-

Table 3—Logistic Regression With Dependent Variable as Sputum Eosinophils Expressed as a Binary Variable > 1% or < 1%*

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood eosinophils</td>
<td>11.68</td>
<td>2.48 – 55.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–13.31 – 131</td>
</tr>
<tr>
<td>Airway responsiveness†</td>
<td>2.93</td>
<td>0.65 – NS</td>
</tr>
<tr>
<td>Self-reported asthma</td>
<td>17.40</td>
<td>1.20 – 251.50</td>
</tr>
<tr>
<td>Self-reported wheeze</td>
<td>13.56</td>
<td>1.08 – 170.55</td>
</tr>
<tr>
<td>Exposed to irritants</td>
<td>11.52</td>
<td>0.68 – NS</td>
</tr>
<tr>
<td>Atopy</td>
<td>0.33</td>
<td>0.07–1.49 NS</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>0.60</td>
<td>0.14–2.62 NS</td>
</tr>
</tbody>
</table>

*NS = not significant.
†Airway responsiveness coded as 0 = PC20 > 8 mg/mL and 1 = PC20 ≤ 8 mg/mL. Homes-Lemeshow goodness of fit, χ² = 4.13, p = 0.65.
sputum as an epidemiologic tool. In their study,\textsuperscript{11} way inflammation, not to determine the usefulness of whether childhood asthma was associated with airway wheezing in an epidemiologic survey did not allow counts in adult subjects self-reporting asthma or habits.

sponsiveness, but symptoms were correlated to smoking symptoms and sputum eosinophils or airway responsiveness. We likewise found a higher sputum eosinophil count in subjects with airway hyperresponsiveness (PC_{20} < 8 mg/mL), but this difference did not achieve statistical significance and most eosinophil counts were within the normal range. Gibson et al\textsuperscript{11} undertook their study to determine whether childhood asthma was associated with airway inflammation, not to determine the usefulness of sputum cell measurements routinely in epidemiologic studies.

The use of inhaled corticosteroid by almost one third of our subjects reporting asthma may have reduced sputum eosinophil counts in this group, but the relatively low use of inhaled corticosteroid also reflects the mild nature of the disease reported in this study. In contrast to the ease with which an inhaled \( \beta \)-agonist can be withheld in an epidemiologic study to allow measurement of baseline spirometry or airway responsiveness, inhaled corticosteroid withdrawal is not practicable given that the treatment effect may last several weeks and there are risks of exacerbating asthma by such withdrawal. This is a further reason for not advocating sputum cell measurements routinely in epidemiologic studies.

The relationship between the level of airway responsiveness and the magnitude of the airway inflammation is variable. Although some studies\textsuperscript{11,12} found a relationship between airway responsiveness and airway inflammation represented by the percentage of mast cells and eosinophils, the correlation between airway responsiveness and airway inflammation is not straightforward.\textsuperscript{13-18} We found a weak correlation between sputum eosinophil count and airway responsiveness, but sputum eosinophilia explained little of the variation in airway responsiveness. Airway responsiveness probably reflects many factors, one of which is airway inflammation. Cellular analysis of induced sputum appears to provide less positive information than measurement of airway responsiveness in epidemiologic studies. We did not find a significant correlation between respiratory symptoms and sputum eosinophils or airway responsiveness, but symptoms were correlated to smoking habits.

In summary, the spectrum of sputum eosinophil counts in adult subjects self-reporting asthma or wheezing in an epidemiologic survey did not allow the use of this test for confirmation of a diagnosis of asthma, whereas the degree of airway responsiveness was increased in subjects reporting asthma. Hence, this pilot study suggests that the analysis of differential cell counts in induced sputum is not appropriate in epidemiologic studies to validate reported diagnoses or symptoms suggesting asthma. Toelle et al\textsuperscript{19} have suggested that current symptoms (wheezing within the last 12 months) together with documented increased airway responsiveness is a useful definition for current asthma in epidemiologic studies. While this definition will exclude some with mild asthma, sputum cell counts do not add to the precision of the diagnosis.

\textbf{References}