Sputum neutrophilia can mask eosinophilic bronchitis during exacerbations
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BACKGROUND: Exacerbations of airway disease are eosinophilic, neutrophilic, both or neither. The primary objective of the present study was to identify whether the treatment of a neutrophilic bronchitis can unmask an associated eosinophilia.

METHODS: A retrospective survey of 2160 consecutive sputum cell counts from 1343 patients with airway disease was conducted to identify patients with an isolated neutrophilic bronchitis, which was defined as a sputum total cell count of greater than or equal to 12×10^6 cells/g of sputum and a proportion of neutrophils of 80% or greater. The characteristics of the patients who subsequently demonstrated sputum eosinophilia (3% or greater) within eight weeks of resolving the neutrophilia were compared with the patients who subsequently did not have sputum eosinophilia.

RESULTS: Two hundred thirty-seven patients had 273 neutrophilic exacerbations. The sputum was re-examined within eight weeks in 65 patients (27.4%), of whom 38 (58.5%) had resolution of the neutrophilic bronchitis after treatment with an antibiotic. Of these 38 patients, 13 (34%) showed eosinophilia.

CONCLUSIONS: A neutrophilic exacerbation of airway disease was observed to mask sputum eosinophilia in one-third of patients who had sputum cell counts available before and after antibiotic therapy. Hence, the absence of sputum eosinophilia during an infective exacerbation should not be used as an indication to reduce the dose of corticosteroids. To optimize therapy, repeat sputum cell count measurements are recommended after antibiotic treatment before changing corticosteroid treatment.

Key Words: Eosinophilic bronchitis; Neutrophilic bronchitis; Sputum cell counts

Exacerbations of airway disease are common in tertiary care clinics (1-3). They are a major cause of morbidity and mortality, and are an economic burden to the patient and society (1,2). Exacerbations are heterogeneous in nature, and can be associated with a neutrophilic, eosinophilic, or combined neutrophilic and eosinophilic bronchitis (4,5). Neutrophilic exacerbations are common, and are usually associated with bacterial or nonbacterial infections (6). Noninfective exacerbations are usually eosinophilic (7). When exacerbations are noneosinophilic, the condition is unlikely to benefit from added corticosteroid treatment (8-10). However, when there is an infection with an increase in both total cell count and neutrophil differential, it is possible that the airway neutrophilia may mask an eosinophilic bronchitis and lead to an inappropriate reduction of corticosteroid treatment. We examined the frequency and clinical predictors of such masking in a retrospective survey of exacerbations over a two-year period.

PATIENTS AND METHODS

Subjects
Patients referred for sputum cell counts between January 2004 and January 2006 from the clinics of eight respiratory physicians at the Firestone Institute for Respiratory Health, Hamilton, Ontario, were enrolled in the present study. Patients were 30 to 81 years of age and had an exacerbation of physician-diagnosed asthma, chronic airflow limitation, bronchiectasis or chronic cough.
TABLE 1
Characteristics of patients at the time of a neutrophilic exacerbation

| Characteristics                  | Masking effect (n=13) | No masking effect (n=25) | P     |
|----------------------------------|-----------------------|-------------------------|-------|
| Male, n (%)                      | 4 (30.8)              | 14 (56)                 | NS    |
| Age, years, mean ± SD            | 60.2±15.3             | 60.5±14.4               | NS    |
| Current or former smokers, n (%) | 2 (15.4)              | 5 (20)                  | NS    |
| Asthma, n (%)                    | 10 (76.9)             | 5 (20)                  |       |
| Chronic airflow limitation, n (%)| 2 (15.4)              | 14 (56)                 |       |
| Bronchiectasis, n (%)            | 0                     | 1 (4)                   |       |
| Chronic cough, n (%)             | 1 (7.7)               | 5 (20)                  |       |
| FEV1, L, mean ± SD               | 1.9±0.5               | 2.0±1.1                 | NS    |
| FEV1 % predicted, mean ± SD      | 72.0±17.5             | 65.5±22.9               | NS    |
| Atopy, %                         | 61.5                  | 41.7                    | NS    |

FEV1, Forced expiratory volume in 1 s

Design
A retrospective survey of a computerized database of spontaneous or induced sputum cell counts was designed. The database was used to identify those patients with neutrophilic bronchitis who were treated with an antibiotic for seven to 10 days and whose sputum cell counts were re-examined within eight weeks. The patient’s clinical characteristics, including atopy and spirometry, were documented at baseline, as well as their medications on both occasions. The patients were divided into two groups: those who subsequently demonstrated sputum eosinophilia and those who did not. The study was approved by the Research Ethics Board of St Joseph’s Healthcare, Hamilton, Ontario.

Study definitions
Neutrophilic bronchitis, which is suspected to mask an eosinophilic reaction, was defined as a cell count of more than or equal to 12×10^6 cells/l of sputum and a proportion of neutrophils of 80% or greater (11). Eosinophilic bronchitis, which is not usually associated with a raised total cell count, was defined as a percentage of sputum eosinophils of 3% or greater (12,13). Asthma was defined as variable airflow limitation as described by Scadding (14). The presence of moderate or many macrophage smokers’ inclusions was used to indicate that the patient was a current or former smoker. Chronic airflow limitation included patients who were diagnosed with chronic obstructive pulmonary disease as indicated by a postbronchodilator forced expiratory volume in 1 s slow vital capacity of less than 70% (15). The masking effect of neutrophilic bronchitis was identified by the presence of sputum eosinophilia after the sputum neutrophilia had resolved with antibiotic treatment.

Procedures
Sputum induction and examination for total and differential cell counts were performed by the methods described by Pizzichini et al (16). Spontaneous sputum was considered appropriate if the viability of the sample was more than 50% (17). Spirometry was performed according to the standards of the American Thoracic Society, before or 10 min after the administration of 200 μg of salbutamol (18). Reference values were taken from Crapo et al (19). An exacerbation was defined by an increase in cough, dyspnea, sputum volume or purulence, or a fall in forced expiratory volume in 1 s by at least 20% that, in the opinion of the physician, required an adjustment to therapy (4). Allergy skin tests were performed by the modified skin prick technique with 14 common allergen extracts (20), and atopy was defined as one or more wheals of larger than 3 mm in diameter.

Analysis
Descriptive statistics were used to identify the demographic characteristics of the patients. Normally distributed data were summarized by the arithmetic mean ± SD. Variables with non-normal distributions, such as the dose of corticosteroid, percentage of eosinophils and absolute eosinophil count, were summarized using the median and interquartile range. Student’s t tests were used for comparisons between normally distributed groups. Mann-Whitney U tests were used to compare non-normally distributed data. A multiple logistic regression model was used to determine the predictive value of clinical features in patients who showed the masking effect. The statistical software used was SPSS Graduate Pack 13.0 (SPSS Inc, USA).

RESULTS
The sputum database consisted of 2160 cell counts from 1343 patients. Two hundred thirty-seven patients had 273 neutrophilic exacerbations, of whom only 65 (27.4%) had sputum cell count measurements repeated within eight weeks. Of these 65 patients, 38 (58.5%) had a resolution of the neutrophilic bronchitis after seven to 10 days of antibiotic treatment, and of these, 13 (34%) had eosinophilia, suggesting a masking effect of the previous neutrophilia (Tables 1 and 2).

The predictive values of the disease diagnosis, atopy and decrease in total steroid dose between the two visits were assessed for a masking effect. Only diagnosis was a significant predictor (P=0.009); the odds of demonstrating the masking effect was lower in patients with chronic airflow limitation than in those with asthma (OR 0.07, 95% CI 0.01 to 0.44; P=0.005). Atopy and decrease in total steroid dose between the two visits were not predictors of the masking effect.

DISCUSSION
The results show that among patients with an exacerbation of neutrophilic bronchitis (presumed to be infective) and a normal percentage of sputum eosinophils, approximately one-third had sputum neutrophilia after the neutrophilia was treated. This demonstrates that the previous neutrophilia had masked the eosinophilia. These observations are relevant in clinical practice when having to choose the appropriate therapy to treat neutrophilic exacerbations.

The strengths of the present study are the excellent reliability, validity and responsiveness of quantitative sputum cell count measurements. The major weaknesses are the retrospective design and failure to ensure that corticosteroid dose was left unchanged between the two measurements. Furthermore, as sputum measurements were not repeated in all patients who had neutrophilic bronchitis, the results may not be representative of the entire sample of patients with isolated neutrophilic bronchitis. The mean length of time between the end of antibiotic treatment and the second sputum examination in both groups of patients was between 23 and 26 days, close to the required time period of four to six weeks for symptomatic and physiological recovery to occur (21). The present study did not include patients whose exacerbations were severe enough to warrant hospitalization, and therefore, the findings are limited to mild to moderate exacerbations of airway disease. Also, while
TABLE 2
Sputum cell counts and concomitant corticosteroid treatment at the time of the exacerbation and after antibiotic treatment

|                          | At the time of the exacerbation | After antibiotic treatment |
|--------------------------|---------------------------------|-----------------------------|
|                          | Masking effect (n=13)            | No masking effect (n=25)    | P              | Masking effect (n=13) | No masking effect (n=25) | P              |
| Sputum total cell count, ×106/g, mean ± SD | 53.6 (49.5)                   | 44.1 (37.8)                | NS             | 7.2 (5.1)              | 5.1 (3.2)                | NS             |
| Neutrophils, %, mean ± SD | 93.3 (5.2)                     | 91.2 (4.4)                 | NS             | 51.8 (23.3)            | 62.8 (26.7)              | NS             |
| Neutrophils, ×106/g, mean ± SD | 51.3 (48.8)                   | 40.4 (35.5)                | NS             | 4.1 (3.5)              | 4.0 (3.0)                | NS             |
| Eosinophils, %, median (interquartile range) | 0.5 (0.3 to 1.3) | 0 (0 to 0.3)              | 0.006          | 17.8 (6.5 to 30.4)     | 0.3 (0 to 1.2)            | <0.001         |
| Eosinophils, ×106/g, median (interquartile range) | 0.2 (0.1 to 0.4) | 0 (0 to 0.2)              | 0.011          | 1.0 (0.3 to 2.7)       | 0 (0)                    | <0.001         |

|                          | Inhaled steroid, n (%)          | 13 (100)                   | 20 (80)        | NS             | 13 (100)                   | 20 (80)        | NS             |
| Dose of Inhaled steroid, μg, median (interquartile range) | 1000 (900 to 1800) | 1000 (500 to 1300) | NS | 1000 (1000 to 1800) | 1000 (500 to 1300) | NS |
| Prednisone, n (%)        | 8 (61.5)                       | 9 (36)                     | NS             | 7 (53.8)              | 9 (36)                    | NS             |
| Dose of prednisone, mg, median (interquartile range) | 10 (0 to 27.5) | 0 (0 to 8.8)              | NS             | 5 (0 to 15)           | 0 (0 to 6)                | NS             |

The masking effect of neutrophilic bronchitis was identified by sputum eosinophilia after the sputum neutrophilia had resolved with antibiotic treatment.

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