

## Chronic respiratory symptoms and airway responsiveness to methacholine are associated with eosinophilia in older men: The Normative Aging Study

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*Chronic respiratory symptoms and airway responsiveness to methacholine are associated with eosinophilia in older men: The Normative Aging Study. J.T. Annema, D. Sparrow, G.T. O'Connor, B. Rijcken, G.H. Koëter, D.S. Postma, S.T. Weiss. ©ERS Journals Ltd 1995.*

**ABSTRACT:** Identification of subsets of patients with chronic obstructive lung disease (COLD) in order to determine disease outcomes and, possibly, the effects of treatment is an area of clinical interest. At present, it remains unclear which patients with COLD are most likely to benefit from anti-inflammatory therapy. We investigated this question in a community-dwelling sample of men.

In this study, the relationship of chronic respiratory symptoms, airway responsiveness to methacholine, and skin test reactivity to peripheral-blood eosinophil and neutrophil counts was examined among 894 male participants in the Normative Aging Study (mean age 60 yrs; range 41–90 yrs). The symptoms considered were asthma, persistent wheeze, dyspnoea, chronic cough and phlegm. Responsiveness to methacholine was defined as a provocative dose producing a 20% fall in forced expiratory volume in one second ( $PD_{20}FEV_1$ ) of  $\leq 8.6 \mu\text{mol}$ , a positive skin test as a wheal diameter of  $\geq 5 \text{ mm}$  after subtraction of the diameter of any wheal reaction to a glycerin control, and eosinophilia as an eosinophil count of  $\geq 275 \text{ cells}\cdot\text{mm}^{-3}$  in peripheral blood.

Chronic symptoms (odds ratio (OR) 2.0; 95% confidence interval (CI) 1.4–2.7), airway responsiveness (OR 1.7; CI 1.1–2.7), and the combination of symptoms and airway responsiveness (OR 3.4; CI 2.0–5.6) were positively and significantly related to peripheral-blood eosinophil counts. These relationships remained significant after adjustment for the effects of age and smoking, and after exclusion of asthmatic subjects. Symptoms and airway responsiveness combined were not significantly related to neutrophil counts.

These data suggest that increased airway responsiveness to methacholine is associated with elevated peripheral-blood eosinophil counts in subjects with chronic respiratory symptoms. Asymptomatic subjects with increased airway responsiveness also have increases in peripheral-blood eosinophil counts.

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The use of inhaled corticosteroids for the treatment of chronic obstructive lung disease (COLD) has been a focus of recent research [1]. Clinicians are trying to identify the subsets of patients most likely to benefit from such therapy [2], the effectiveness of which remains controversial.

Dutch investigators initially suggested that increased airway responsiveness and/or atopy might be phenotypic markers of both disease susceptibility and prognosis [3]. BURROWS *et al.* [4] showed that one specific clinical phenotype, asthmatic bronchitis, is associated with better lung function and a higher rate of survival. These authors hypothesized that patients with this phenotype are usually atopic women whose smoking history is minimal, and whose forced expiratory volume in one second ( $FEV_1$ ) may decline relatively modestly in response to

treatment with steroids. However, it remains unknown exactly what pattern of factors (*e.g.* symptoms, allergy, airway responsiveness) best characterizes the patients likely to benefit most from corticosteroid therapy.

In this study, we assessed the relationship of chronic respiratory symptoms, airway responsiveness and allergy to peripheral-blood markers of inflammation in a group of community-dwelling men.

### Methods

#### Population

The Normative Aging Study is a longitudinal study of ageing established by the Veterans Administration in

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1961 [5]. The study cohort consists of 2,280 community-dwelling men from the Greater Boston area, who were 21–80 yrs of age on enrolment. Volunteers were screened at entry according to specific health criteria [5], and were found to be free of known chronic medical conditions at the outset. In particular, volunteers with asthma, chronic bronchitis, or chronic sinusitis were excluded.

Since enrolment, volunteers have reported every 3–5 yrs for a comprehensive medical examination, including medical history, physical examination, blood and urine tests, electrocardiography, chest radiography and spirometry. On the night before each visit, subjects refrain from eating or drinking after midnight and refrain from smoking after 8.0 a.m.

A total of 1,413 participants in the Normative Aging Study who returned for their regularly scheduled examinations between 1 May 1984 and 30 April 1991 were considered for inclusion in the present study. Of these individuals, 109 were excluded from methacholine challenge studies because of ischaemic heart disease or cardiac arrhythmia requiring medical therapy (89 participants) and/or an FEV<sub>1</sub> below 60% of the value predicted on the basis of the regression model of MORRIS *et al.* [6] (20 participants). Of the 1,304 subjects eligible for methacholine challenge, 276 refused to participate, 41 were excluded because of their inability to perform acceptable methacholine inhalations, nine had tests that were invalid as a result of poor forced expiratory manoeuvres, and six failed to participate for various other reasons. Seven subjects did not complete the test because they found it unpleasant. The attending physician stopped testing nine additional subjects because of concern over minor side-effects (transient lightheadedness, presyncope following forced expiratory manoeuvres, unspecified discomfort, or bleeding of the gingivae due to contact with the mouthpiece of the spirometer). Data on methacholine challenge were incomplete for eight subjects because of equipment problems or technician error. A total of 54 subjects performed the methacholine challenge procedure acceptably but had incomplete covariate information. Thus, 894 subjects were included in our analysis; 294 were nonsmokers, 486 were former smokers, and 114 were current smokers.

#### Questionnaire data

Data on respiratory symptoms, respiratory illnesses, and smoking habits were obtained by means of the 1978 American Thoracic Society, Division of Lung Diseases Questionnaire [7].

Chronic symptoms were defined as follows. Cases of asthma were identified by asking subjects whether they had ever had asthma, and were subclassified according to whether this condition was still present. Persistent wheeze was defined as wheezing with colds and occasional wheeze without colds, or wheezing on most days or nights; phlegm as "usually" bringing up phlegm from the chest; chronic cough as a cough on most days for at least three consecutive months during the year; and dyspnoea as shortness of breath when hurrying on level ground or

walking up a slight hill, plus one of the following: having to walk more slowly than other people of the same age on level ground because of breathlessness, having to stop for breath when walking at your own pace on level ground, or having to stop for breath after walking about 100 yards or for a few minutes on level ground. Subjects reporting one or more of the above respiratory symptoms were classified as symptomatic (S+); subjects with none of these symptoms were classified as asymptomatic (S-).

Subjects were also categorized according to smoking history. Current smokers were defined as those men who were smoking at least one cigarette per day within 1 month before the examination; former smokers as those who had previously smoked at least one cigarette per day but who had ceased smoking more than 1 month before the examination; and lifetime nonsmokers as those who had never smoked cigarettes or had smoked fewer than 20 packs during their lifetime [3].

#### Cell count

Total blood leucocyte count (WBC) was measured by an automated cell counter (either a Hemalog 8 from Technicon Inc., Tarrytown, NY, USA, or a Coulter Counter Model S-plus 6 from Coulter Electronics, Hialeah, FL, USA). Absolute eosinophil counts were determined by a trained technician using a haemocytometer, after staining of an aliquot of blood with the Unopette reagent system (Becton-Dickinson, Rutherford, NJ, USA). We defined eosinophilia as an eosinophil count of  $\geq 275$  cells·mm<sup>-3</sup> in peripheral blood. This cut-off point was based on the study by VEENING [8] on the distribution of eosinophilic cells in the blood of subjects with asthma and in that of nonasthmatic subjects.

#### Spirometry and methacholine challenge protocol

Spirometry and methacholine challenge were performed as described previously [9, 10]. The methacholine challenge protocol was adapted from that of CHATHAM *et al.* [11]. Saline and methacholine solutions were aerosolized with a DeVilbiss 646 nebulizer attached to a DeVilbiss air compressor (DeVilbiss, Somerset, PA, USA). All inhalations were 6 s vital capacity inhalations followed by 2 s of breath-holding. Incremental doses of methacholine were inhaled at 5 min intervals, according to the following schedule: five inhalations of 0 mg·ml<sup>-1</sup> (phenol-buffered saline alone); one inhalation of 1 mg·ml<sup>-1</sup>; one inhalation of 5 mg·ml<sup>-1</sup>; four inhalations of 5 mg·ml<sup>-1</sup>; one inhalation of 25 mg·ml<sup>-1</sup>; and four inhalations of 25 mg·ml<sup>-1</sup>. Determination of nebulizer output by weight [9] indicated that the methacholine inhalation schedule corresponded to the following cumulative doses of methacholine (in micromoles): 0, 0.33, 1.98, 8.58, 16.8 and 48.8. Spirometry was performed 30, 90 and 180 s after each inhalation level. If the first two spirometrys at each level were consistent (FEV<sub>1</sub> within 5%), then the higher of these two values was chosen for analysis. Otherwise, the higher FEV<sub>1</sub> from the two most

consistent acceptable spirometry was used. The test was terminated when the FEV<sub>1</sub> had declined by 20% from the postsaline value or (if there was no such decline) at the end of the dose schedule. Subjects with a provocative dose producing a 20% fall in FEV<sub>1</sub> (from the postline value) (PD<sub>20</sub>FEV<sub>1</sub>) at or before four inhalations of 5 mg·ml<sup>-1</sup> ≤8.6 μmol) were classified as responders (R+); all others were classified as nonresponders (R-).

#### Skin testing

Skin testing was performed as described previously [9] with the prick method of PEPYS [12]. Subjects were tested in double-blind fashion with four common aero-allergen preparations preserved in glycerin (ragweed 1:20; mixed trees 1:20; mixed grasses 1:20; and house dust 1:10) and with a glycerin control. Wheal reactions were measured at 20 min, as the sum of the largest wheal diameter plus the diameter perpendicular to it. Results are expressed as half of this sum, representing the mean wheal diameter. For the current analysis, a positive skin test was defined as a mean wheal diameter of ≥5 mm after subtraction of any wheal reaction to the glycerin control.

#### Subject subgroups

We created several subgroups of subjects prior to the analyses: 1) skin test positive/skin test negative (ST+/ST-); 2) responders (R) to methacholine/nonresponders (R+/R-); 3) symptomatic/asymptomatic (S+/S-); and 4) asthmatic (asthma, persistent wheeze, or dyspnoea)/chronic bronchitic (chronic cough or phlegm) (SA+/SA-).

#### Data analysis

Because of their skewed distributions, peripheral-blood eosinophil and neutrophil counts were logarithmically transformed (log<sub>10</sub>) for all analyses. To permit analysis in the log scale, a small constant (0.01) was added to each value of the eosinophil and neutrophil count to eliminate 0 values. The neutrophil count was considered as a continuous variable. The eosinophil count was considered as both a continuous and a categorical variable, with eosinophilia (yes/no) defined as ≥275 cells·mm<sup>-3</sup> in peripheral blood. We also utilized eosinophils as a percentage of total leucocytes (top 5%) of the distribution. Since this definition gave identical results to the categorized analysis using ≥275 cells·mm<sup>-3</sup>, we have only presented these latter results. Age and cigarette smoking status were included as covariates in the analyses. Independent t-tests were used for comparisons of continuous variables and Chi-squared tests for comparisons of categorical variables. Relationships were explored by analysis of variance and covariance, logistic regression, and graphics. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated in standard fashion [13]. Statistical significance was defined as a p value of <0.05. The SAS statistical software package (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

## Results

The 894 subjects with complete methacholine challenge, skin test, questionnaire, and peripheral-blood cell count data, and the 519 subjects with incomplete data, were compared with regard to several characteristics (table 1). The mean age of the study subjects was 60 yrs (range 41–90 yrs). Subjects with incomplete data were more likely to be current smokers, to have lower values for spirometric indices, and to have phlegm and dyspnoea. These differences reflect the exclusion from the methacholine challenge protocol of subjects with a prechallenge FEV<sub>1</sub> below 60% of the predicted value. Overall, the prevalence of asthma in the study sample was low relative to that in the general population, a difference probably reflecting the initial health screening of the Normative Aging Study cohort. Furthermore, the proportion of subjects who were current cigarette smokers was low in both groups; this observation

Table 1. — Characteristics of subjects with and without complete data on methacholine challenge and respiratory symptoms

Variable	Complete data (n=894)	Incomplete data (n=519)
Age† yrs	60 (41–90)	61 (40–89)
Smoking status n (%)		
Current	114 (12.8)	100 (19.3)**
Former	486 (54.4)	285 (54.9)
Never	294 (32.9)	134 (25.8)
FVC % pred‡	97 (13.9)	91 (17.4)***
FEV <sub>1</sub> % pred‡	95 (14.8)	88 (19.5)***
Response (≤8.6 μmol) n (%)		
Yes	113 (12.6)	5 (9.3)
No	781 (87.4)	49 (90.7)
Missing data	-	465
Asthma n (%)		
Current	13 (1.5)	6 (1.2)
Past	20 (2.2)	18 (3.5)
Never	860 (96.3)	492 (95.3)
Missing data	1	3
Persistent wheeze n (%)		
Yes	69 (7.7)	47 (9.2)
No	824 (92.3)	466 (90.8)
Missing data	1	6
Phlegm n (%)		
Yes	217 (24.3)	154 (30.4)*
No	677 (75.7)	353 (69.6)
Missing data		12
Dyspnoea n (%)		
Yes	14 (1.6)	39 (7.6)**
No	880 (98.4)	473 (92.4)
Missing data		7
Chronic cough n (%)		
Yes	105 (11.8)	69 (13.5)
No	787 (88.2)	443 (86.5)
Missing data	2	7

†: data are presented as mean, and range in parenthesis; §: predicted values are from regression equations based on asymptomatic nonsmokers in the present sample, data are presented as mean, and sd in parenthesis; \*: p=0.02; \*\*: p≤0.001; \*\*\*: p=0.0001.

suggested an increased level of health consciousness among these men, who have participated in the Normative Aging Study for more than 20 yrs.

*Relationship of airway responsiveness and symptoms to peripheral-blood neutrophil and eosinophil counts*

Symptomatic responders (S+R+) had higher mean eosinophil counts (240.9 cells·mm<sup>-3</sup> (geometric mean)) than asymptomatic nonresponders (S-R- 143.3 cells·mm<sup>-3</sup>; p=0.001), symptomatic nonresponders (S+R- 169.0 cells·mm<sup>-3</sup>; p=0.03), or asymptomatic responders (S-R+ 194.4 cells·mm<sup>-3</sup>; p=0.29) after adjustment for age and smoking status. The linear trend for the association of peripheral blood eosinophil count with airway responsiveness and respiratory symptoms in the different groups was statistically significant (p=0.0004) (fig. 1). The neutrophil count differed significantly among these groups (p=0.02). No significant linear trend was documented for the different symptom/responsiveness groups with the neutrophil count as the dependent variable (linear trend, p=0.22) (fig. 2).

Subjects with respiratory symptoms (S+) were further divided into two groups: an asthma/persistent wheeze/dyspnoea group (SA+) and a chronic cough/phlegm group (SA-). The SA- group had higher neutrophil counts than the SA+ group (p=0.34). No relationship of responsiveness to neutrophils was noted (data not shown). The SA+R+ group had significantly higher mean eosinophil counts (286.9 cells·mm<sup>-3</sup>) than the three groups of nonresponders (S-R- 143.4 cells·mm<sup>-3</sup>; p=0.002; SA-R- 174.7 cells·mm<sup>-3</sup>; p=0.03; and SA+R- 153.1 cells·mm<sup>-3</sup>; p=0.02). The association of the peripheral-blood eosinophil count with the different airway responsiveness and respiratory symptoms groups was statistically significant (p=0.04). The test for a linear trend was also significant (p=0.001).

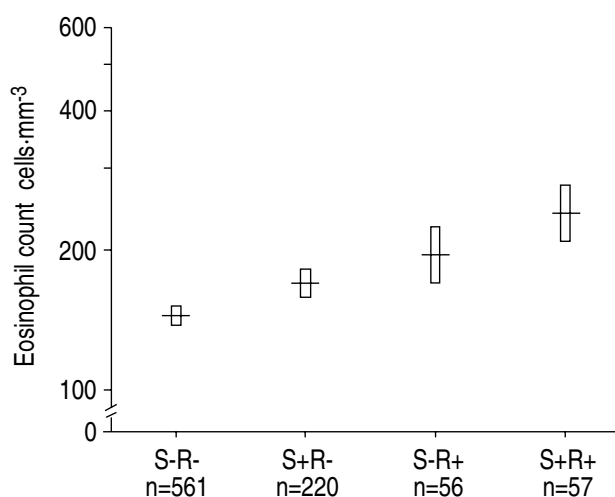


Fig. 1. - Eosinophil count (mean and SD) by symptomatic and nonsymptomatic (S+/S-), airway responsive and nonresponsive (R+/R-) groups, with adjustment for age and smoking status. Linear trend (p=0.0004), analysis of covariance. Note that the vertical axis is cut off from zero.

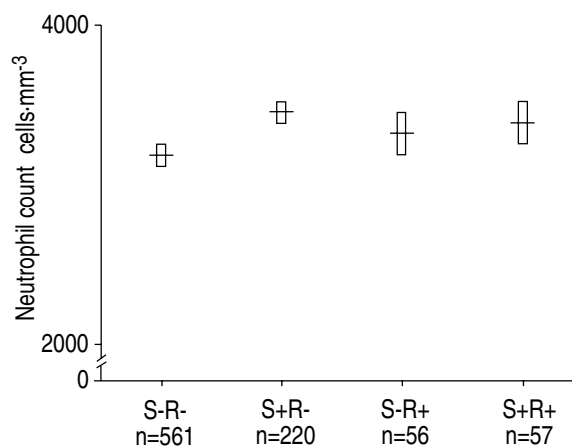


Fig. 2. - Neutrophil count (mean and SD) by symptomatic and nonsymptomatic (S+/S-), airway responsive and nonresponsive (R+/R-) groups, with adjustment for age and smoking status. Linear trend (p=0.22), analysis of covariance. Note that the vertical axis is cut off from zero.

*Relationship of skin test reactivity and respiratory symptoms to peripheral-blood neutrophil and eosinophil counts*

Subjects with both symptoms and a positive skin test (S+ST+) had a higher mean eosinophil count (228.8 cells·mm<sup>-3</sup>) than subjects who had only one of these characteristics (S+ST- 169.7 cells·mm<sup>-3</sup>; S-ST+ 142.3 cells·mm<sup>-3</sup>) or neither (S-ST- 149.1 cells·mm<sup>-3</sup>). The mean eosinophil count of the S+ST+ group was significantly higher than that of the S+ST- group (p=0.05), that of the S-ST+ group (p=0.004), and that of the S-ST- group (p=0.004). The linear trend for the relationship of peripheral-blood eosinophil count to skin test reactivity and respiratory symptoms in the different groups was statistically significant (p=0.02) (fig. 3). No linear trend was found for the different symptom/skin test reactivity groups with the neutrophil count as the dependent variable (linear trend, p=0.27) (fig. 4).

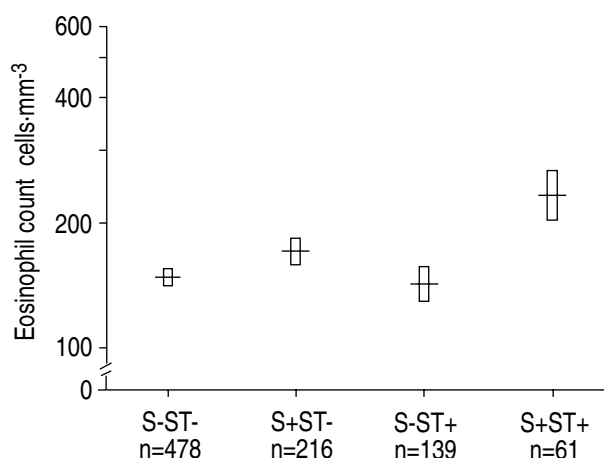


Fig. 3. - Eosinophil count (mean and SD) by symptomatic and nonsymptomatic (S+/S-), skin test reactive and nonreactive (ST+/ST-) groups, with adjustment for age and smoking status. Linear trend (p=0.02), analysis of covariance. Note that the vertical axis is cut off from zero.

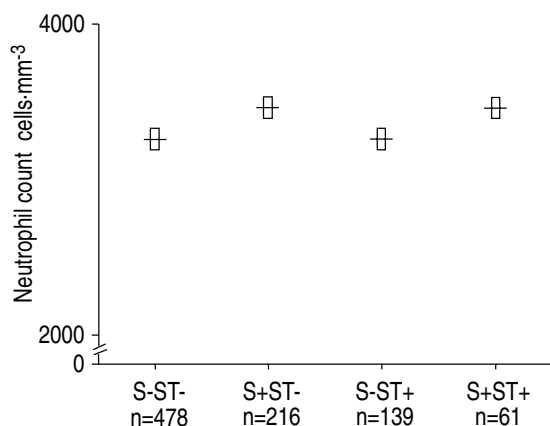


Fig. 4. – Neutrophil count (mean and sd) by symptomatic and nonsymptomatic (S+/S-), skin test reactive and (ST+/ST-) groups, with adjustment for age and smoking status. Linear trend ( $p=0.27$ ), analysis of covariance. Note that the vertical axis is cut off from zero.

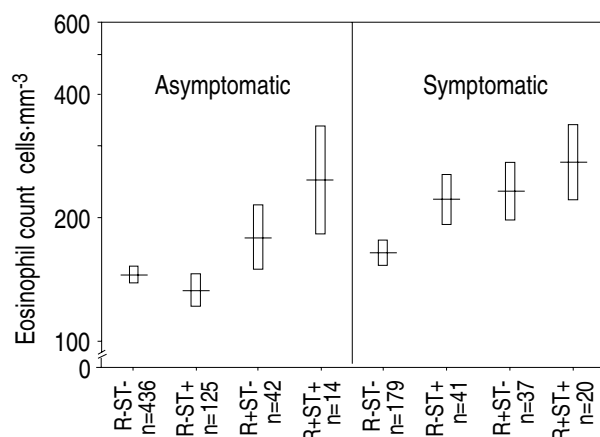


Fig. 5. – Eosinophil count (mean and sd) by symptomatic and nonsymptomatic (S+/S-), airway responsive and nonresponsive (R+/R-), skin test reactive and nonreactive (ST+/ST-) groups, with adjustment for age and smoking status. Linear trend for asymptomatic subjects ( $p=0.04$ ); linear trend for symptomatic subjects ( $p=0.03$ ), analysis of covariance. Note that the vertical axis is cut off from zero.

#### Relationship of symptoms, airway responsiveness, and skin test reactivity to the peripheral-blood eosinophil and neutrophil counts

Finally, we simultaneously examined the relationship of symptoms, airway responsiveness, and skin test reactivity to peripheral-blood eosinophilia (fig. 5). The eosinophil count was increased for all R+ subjects. Skin test-positive responders had the highest counts. The linear trend for the association of peripheral-blood eosinophil count with airway responsiveness and skin test reactivity was tested separately in asymptomatic and symptomatic subjects. For both groups the linear trend was statistically significant ( $p=0.04$  for S-;  $p=0.03$  for S+).

We repeated all analyses as described above measuring eosinophils and neutrophils as a percentage of the total WBC count and found similar results. We also repeated all analyses with the exclusion of subjects with current asthma. The relationships described did not change and remained statistically significant.

Table 2. – Odds ratios (ORs) and 95% confidence intervals (95% CIs) for the presence of eosinophilia ( $\geq 275$  cells·mm<sup>-3</sup>), estimated from multiple logistic regression analysis ( $n=894$ )

Independent variable	OR	95% CI	p-value
Skin test reactivity*	1.1	0.8–1.7	0.47
Airway hyperresponsiveness†	1.7	1.1–2.7	0.012
Symptoms#	2.0	1.4–2.7	0.0001
Age	1.0	1.0–1.0	0.034
Current smokers§	1.3	0.8–2.2	0.29
Former smoker**	1.1	0.8–1.6	0.54

\*: positive skin test is defined as a wheal diameter of  $\geq 5$  mm after subtraction of the diameter of any wheal reaction to the glycerin control - effect is relative to subjects with negative skin tests; †: responsiveness is defined as a  $PD_{20}FEV_1$  of  $\leq 8.6$   $\mu\text{mol}$  - effect is relative to nonresponders; #: symptoms are defined as asthma, persistent wheeze, dyspnoea, chronic cough, and phlegm - effect is relative to subjects with no symptoms; §: effects are relative to lifetime nonsmokers (1=current smoker; 0=otherwise); \*\*: effects are relative to lifetime nonsmokers (1=former smoker, 0=otherwise).  $PD_{20}FEV_1$ : provocative dose of methacholine producing a 20% fall in forced expiratory volume in one second.

In a multiple logistic regression model, we examined the relationship of symptoms, airway responsiveness, and skin test reactivity to the likelihood of eosinophilia, simultaneously adjusting for age and cigarette smoking status (table 2). The OR for the occurrence of eosinophilia for a subject with symptoms was 2.0 (95% CI 1.4–2.7); thus, we predicted that symptomatic subjects would be approximately 2.0 times more likely than asymptomatic subjects to have eosinophilia, with adjustment for airway responsiveness, skin test reactivity, age, and smoking status. The OR for the occurrence of eosinophilia for a responder was 1.7 (CI 1.1–2.7); and that for its occurrence for a skin test-positive subject was 1.1 (CI 0.8–1.7). With the results from this logistic regression model (table 2), we calculated the ORs for the occurrence of peripheral-blood eosinophilia for different combinations of symptoms, airway responsiveness, and skin test reactivity, adjusting for the other covariates in the model. This OR was 2.0 (CI 1.2–3.4) for an R+ST+ individual, 2.2 (CI 1.4–3.7) for an S+ST+ individual, 3.4 (CI 2.0–5.6) for an S+R+ individual, and 3.9 (CI 2.1–7.1) for an S+R+ST+ individual.

#### Discussion

In the present study the relationship of chronic respiratory symptoms, airway responsiveness to methacholine, and skin test reactivity to eosinophil and neutrophil counts in peripheral blood was examined in 894 middle-aged and elderly men. Symptoms, airway responsiveness, and the combination of symptoms and airway responsiveness were significantly related to peripheral-blood eosinophil counts, whereas skin test reactivity alone was not. In addition, asymptomatic subjects with increased airway responsiveness had increased numbers of peripheral-blood eosinophils. The demonstrated relationships remained significant after adjustment for the effects of age and

smoking, and after the exclusion of asthmatic subjects. The combination of symptoms and airway responsiveness was not significantly related to peripheral-blood neutrophil counts.

These data suggest an important association of the number of peripheral-blood eosinophils with airway symptoms. The peripheral-blood eosinophil count may not accurately reflect events occurring in lung tissue and/or airway walls: 24 h after allergen challenge, the eosinophil count is decreased in peripheral blood [14], whereas it is increased in bronchoalveolar lavage fluid [15] and airway walls [16]. However, KIRBY *et al.* [17] demonstrated a significant relationship between the number of eosinophils and neutrophils in bronchoalveolar lavage fluid and the number in peripheral blood in both asthmatic and normal subjects. The inflammatory cell content of bronchoalveolar lavage fluid appears to reflect the severity of the inflammatory process in the bronchial mucosa [18]. The peripheral-blood eosinophil count is a convenient (albeit indirect) marker for airway inflammation, because it is easy information for clinicians to obtain.

The eosinophil count has appeared to be inversely related to FEV<sub>1</sub> in several population studies [19–21]. In addition, an increased peripheral-blood eosinophil count is significantly associated with nonspecific airway hyperresponsiveness in asthmatic subjects [21]. Several studies have shown that high peripheral-blood eosinophil counts are associated with chronic respiratory symptoms (asthma, persistent wheeze, dyspnoea, chronic cough and phlegm) [19, 22–26]. The identification of eosinophilia as an independent risk factor for symptoms of cough and phlegm [23] and asthma [26] also suggests a role for eosinophils in the pathogenesis of COLD [26, 27]. In our data, the relationship of symptoms to peripheral-blood eosinophilia was strongest for asthma symptoms. Between 60–80% of patients with COLD have increased airway responsiveness [28, 29]. Airway responsiveness is related both to reduced level [30, 31], and accelerated decline of lung function [32–35], and, thus, is a potential risk factor for the development of COLD. Moreover, severe airway responsiveness has been related to accelerated decline in lung function in patients with established COLD [35]. A decrease in eosinophil count coincided with an improvement in lung function and airway responsiveness in allergic asthmatics [36]. Our results suggest that chronic respiratory symptoms and increased methacholine airway responsiveness are associated with eosinophilic airway inflammation in a population of middle-aged and elderly men. Although these men exhibited some symptoms, their pulmonary function was normal (table 1). The lack of association between neutrophils and symptoms or airway responsiveness is consistent with the analyses in this and other populations that have suggested a more prominent role for eosinophils than neutrophils in airway inflammation.

Inhaled corticosteroids appear to have a beneficial effect on the FEV<sub>1</sub> [37, 38] and on symptoms [39] in patients with asthma. The use of inhaled corticosteroids is also related to a decrease in eosinophil count [1, 38] and in the severity of airway hyperresponsiveness [38, 39–41] in asthmatics. In some studies of COLD, inhaled corticosteroids have had a beneficial effect on FEV<sub>1</sub> [2, 42],

symptoms [1, 2], and severity of airway responsiveness [2]. However, in at least two studies in which subjects were selected for evaluation because they lacked either asthma symptoms or eosinophils and had negative skin tests [1, 43], no effect of corticosteroids was observed. These reports suggest the importance of eosinophil count, and perhaps of skin test reactivity, as a marker of steroid responsiveness in COLD; this association has been suggested previously by studies in which sputum eosinophilia was related to prednisone responsiveness [43].

Certain limitations apply to our study. Participants who had a FEV<sub>1</sub> that was  $\leq 60\%$  of the predicted value were not eligible for methacholine challenge and were excluded from analysis. Therefore, subjects with symptoms in our study had only mild chronic respiratory symptoms. This situation is actually advantageous, because clinicians need to identify the subset of patients who might benefit most from corticosteroid therapy and whose disease is at an early stage. If corticosteroid therapy is to be successful, early intervention in COLD would be helpful. All participants in this analysis were screened for health problems at the outset, and those with asthma were excluded. Thus, the asthma in this population was adult-onset disease, which may differ in prognosis from childhood-onset asthma. Again, this situation may be advantageous, as we are most interested in identifying that subset of symptomatic COLD patients with active airway inflammation. Exclusion of adult-onset asthmatics from our analyses did not influence our results. As the Normative Aging Study includes only middle-aged and elderly men who were all healthy at enrolment, our results may not be generalized to women [5]. Prior studies have suggested that cigarette smoking can independently influence peripheral blood eosinophil count. Our population is older, has a low percentage of smokers, and cigarette smoking was controlled for in the analysis.

In conclusion, these data suggest that increased methacholine airway responsiveness is associated with elevated peripheral-blood eosinophil counts in patients with chronic respiratory symptoms and in asymptomatic subjects. Skin test reactivity was not as good a marker as airway responsiveness for identifying patients with peripheral-blood eosinophilia. Screening for airway responsiveness and eosinophilia - both clinical markers of potential susceptibility to corticosteroid therapy - may help select symptomatic patients most likely to benefit from this treatment. Treatment of carefully selected subgroups of symptomatic patients with corticosteroids may mitigate the peripheral-blood eosinophilia and, possibly, airway eosinophilia in such patients, and may reduce or reverse increased airway responsiveness. Prospective studies will determine whether this hypothesis is correct.

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