

Eosinophils and pulmonary function: an epidemiologic study of adolescents and young adults

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Background and Objective: In order to identify potential risk factors, other than cigarette smoking, for lung function impairment, the relationships of peripheral eosinophil count, bronchial responsiveness to inhaled histamine, skin test reactivity and respiratory symptoms to pulmonary function results in a population sample of 665 adolescents and young adults, aged 13 to 23 years.

Methods: Case history, especially concerning smoking habits and respiratory symptoms, was obtained by interview and a self administered questionnaire. Pulmonary function, peripheral blood eosinophil counts, bronchial responsiveness to inhaled histamine and skin test reactivity to common allergens were measured using standard techniques. The relationships of peripheral blood eosinophil counts, symptoms of asthma, cigarette smoking, bronchial responsiveness and skin test reactivity were compared with FEV₁, adjusted for age, sex, and height.

Results: The peripheral eosinophil count was inversely correlated to FEV₁ %predicted ($P = .001$); and subjects with eosinophilia (defined as $> .25 \times 10^9 \text{ l}^{-1}$) had significantly reduced FEV₁ %predicted compared with those without eosinophilia (95 ± 13 %pred and 102 ± 16 %predicted, respectively, $P = .001$). A close direct correlation was observed between blood eosinophils and histamine responsiveness ($r = .60$; $P < .001$). In nonatopic subjects ($n = 382$), the FEV₁ %predicted was significantly lower in those with eosinophilia compared with those without. Subjects, who had never smoked, who were nonatopic and nonasthmatic, and who had no evidence of bronchial hyperresponsiveness, were found to have a weak but statistically significant inverse relation between eosinophil count and FEV₁ %predicted ($r = -.14$, $P = .04$). In the same subgroup, subjects with eosinophilia ($n = 27$) had reduced FEV₁ %predicted compared with those without ($n = 177$) (96 and 102, respectively, $P < .05$).

Conclusions: The findings of this study support the theory that the role of the eosinophil leukocyte in obstructive pulmonary diseases extends beyond its role in allergic reactions, suggesting that an increased number of blood eosinophils reflects an inflammatory reaction in the airways, which might lead to development of obstructive airflow limitation.

Ann Allergy Asthma Immunol 1998;80:487-93.

INTRODUCTION

Cigarette smoking is clearly the major risk factor for chronic obstructive pulmonary disease (COPD), but individ-

ual differences in degree of lung function impairment cannot be fully explained by differences in smoking habits.¹ Dutch investigators have previously suggested that host factors such as increased bronchial responsiveness and atopy may predispose subjects to COPD.² Previous studies have identified environmental irritants to be associated with COPD,^{3,4} but the relationship between markers of allergy and/or airway inflammation and pulmonary function results has not

been extensively studied, especially not in adolescents and young adults.

A number of studies have demonstrated an association between the reversibility of obstructive pulmonary function impairment and the number of eosinophils in peripheral blood.^{5,6} Peripheral blood eosinophilia has generally been regarded as an allergic trait. Eosinophil leukocytes however, are now increasingly being recognized as a very important effector cell in the inflammatory reaction in the airways of patients with atopic and nonatopic asthma.^{7,8} The relationship between peripheral eosinophil counts and markers of disease activity, such as symptoms, FEV₁ and airway responsiveness, have been demonstrated in patients with asthma irrespective of their atopic status.⁹ Based on evidence from a study of Parisian policemen, Frette and coworkers¹⁰ have suggested that asthma or asthma-like symptoms do not explain the association between eosinophilia and level of FEV₁; therefore, the role of the eosinophil leukocyte in respiratory diseases extend beyond its role in allergy.

The present study analyzes the relationships between peripheral blood eosinophil counts, bronchial responsiveness, skin test reactivity, respiratory symptoms, smoking, and pulmonary function by using data collected in a cross-sectional survey of a population sample of adolescents and young adults.

MATERIAL AND METHODS

Subjects

All subjects included in the study took part in a prospective population study of asthma, allergy and bronchial hyperresponsiveness initiated in 1986 by

Department of Clinical Physiology and Nuclear Medicine, Rigshospitalet, Copenhagen, Denmark.

This study was supported by the National Union for the Fight against Lung Diseases.

Received for publication March 19, 1997.

Accepted for publication in revised form December 12, 1997.

Dr. V. Backer.¹¹ A random sample of 983 children and adolescents living in the area surrounding Rigshospitalet in the city of Copenhagen was drawn from the civil registration list. All subjects were born between 1969 and 1979.

The subjects were invited to the examination by letter and the first survey of the sample was carried out in 1986. The subjects (n = 983) were invited to a second examination in 1992, and a total of 665 (68% of those invited) participated. All data used in this cross-sectional analysis were taken from the second examination.

The question of nonresponder bias has been addressed in previous articles.^{12,13} Briefly, apart from a significantly higher number of current smokers among the subjects who only participated in the 1992 examination, no significant differences were found between the groups with respect to anthropometric data, pulmonary function, or prevalences of positive skin prick test results, bronchial hyperresponsiveness, and allergic diseases. This indicates that the subjects included in this analysis are representative of the entire sample.

Case History

The participants and, if present, their parents were interviewed by one person (CSU) about birthweight, diseases (respiratory and non-respiratory), active smoking and passive exposure to smoke, use of medication, known or suspected allergic disease, pets in the household, use of eiderdown-stuffed pillows and continental quilts, and carpets in the bedrooms.

Furthermore, all participants filled in a questionnaire about asthmatic and allergic symptoms, including rhinitis (sneezing, runny nose, or nasal congestion not associated with a cold) and eczema (an itchy dry rash on face, arms, or legs) as related to themselves, their siblings, and parents.

The questionnaire concerning respiratory symptoms and the definition of asthma were adopted from studies by the American Thoracic Society, Division of Lung Diseases of the National

Heart, Lung and Blood Institute^{14,15} and Hopp et al.^{16,17} Asthma was defined by questionnaire criteria on the basis of responses to the following questions: (1) Have you ever had asthma?, (2) Does your breathing ever sound wheezy or whistling?, (3) Do you have attacks of shortness of breath with wheezing?, (4) Do you experience wheezing, chest tightness, cough, breathlessness with any of the following: at rest, with exertion, with emotional stress, with exposure to cold air, with chest infections or a head cold?, (5) Do you experience wheezing after exposure to dust, fumes, molds, pollen, food, pets or drugs?, (6) Have you ever been hospitalized or observed and treated by a doctor for asthma?, (7) Have you ever received medication for your asthma?, (8) What was the medication that was used?, (9) Did it help?, (10) How many episodes of wheezing have you had during the last year? and (11) Have you ever had attacks of wheezing, shortness of breath or dry cough at night? Asthma was defined on the basis of positive responses to at least questions 2 and 3, and 4 and/or 5. Current asthma was defined as symptoms of asthma/episodes of wheezing within the preceding 12 months.

Furthermore, all participants reported whether they were current smokers, ex-smokers, or life-long non-smokers. For the first two categories they reported the duration of smoking. Current and ex-smokers reported their daily tobacco consumption. An estimate of their lifetime tobacco exposure was calculated as pack years (current tobacco consumption [g/day]/20) $\times$ duration of smoking [years].

Pulmonary Function Tests

The FEV₁ and forced vital capacity (FVC) were measured with a 7-L dry wedge spirometer (Vitalograph, Inc, London, UK), which was calibrated weekly. Each measurement consisted of at least three maximal expiratory maneuvers from total lung capacity to residual volume with a variation of less than 5% in FEV₁. The highest FEV₁ and FVC were used in the analyses.

Data on pulmonary function are expressed as a percentage of predicted values (%predicted), using prediction equations based on age, sex, and height.^{18–20}

The subjects were asked to abstain from smoking for at least two hours before the examination. In case they were taking medication for asthma or allergy, they were asked not to use theophylline or an antihistamine for at least 24 hours, astemizole for 6 weeks, an oral β_2 agonist for 18 hours, or an inhaled bronchodilator for six hours before the tests. They were allowed to continue use of any corticosteroids they had been taking. In the event of recent respiratory tract infection, the tests were postponed for 6 weeks.

Skin Prick Test

Skin prick test reactivity to common allergens was tested on the volar surface of the forearm with standard dilutions (100,000 BU/mL) of the allergens in 50% glycerol. The allergens used were birch, grass, mugwort, horse, dog, cat, house dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*), and two molds (*Alternaria iridis* and *Cladosporium herbarum*). Histamine HCl, 10 mg/mL in 50% glycerol was used as a positive control and a negative control (50% glycerol) was also included. The reactions were read after 15 to 20 minutes. The reaction to each test was regarded as positive if the mean wheal diameter ($[(d_1 + d_2) \times 1/2]$) was at least 3 mm.²¹ In case of reaction to the negative control, the result was read as the difference between the largest diameter of the reaction to the allergen and the largest diameter of the reaction to the negative control. A positive skin prick test was defined as a positive reaction to at least one of the allergens,²¹ and atopy was defined as a positive skin prick test.

Peripheral Blood Eosinophil Counts

Venous blood was taken (before the histamine challenge test) and put into a tube containing EDTA. Then 0.02 mL EDTA blood was mixed with the hypotonic reagent containing Na citrate, eosine, and acetone. The mixture was

left for 30 minutes and the number of eosinophilic leukocytes in 3.2 μ L was counted in a Fuchs-Rosenthal counting chamber.²² The result was calculated as (count $\times$ dilution factor)/volume = (count $\times$ 16)/3.2 $\times$ 10⁻⁶ = eosinophils $\times$ 10⁹/L blood. Countings were performed in duplicate. A variation of 5% was regarded as acceptable. For the majority of the participants, the blood eosinophilic count was determined between 1 and 6 PM. Eosinophilia was considered to be present at a count of greater than 0.25 $\times$ 10⁹/L.²³

Histamine Challenge Test

Bronchial responsiveness to inhaled histamine was measured with the method described by Cockcroft and coworkers.²⁴ Aerosols of the test solution were generated by a Wright nebulizer (Aerosol Products Ltd, London, UK) operated to provide an output of 0.14 mL/min. Each aerosol was inhaled through the mouth by tidal breathing for two minutes. The first aerosol was saline solution (0.9%), and it was followed at 4-minute intervals by 2-fold increasing concentrations of histamine (0.075 to 16.0 mg/mL). The response was measured by the FEV₁ one minute after each inhalation. The test was terminated when a decline of 20% or more in FEV₁ from the post-saline value occurred or at the end of the dose schedule if such a decline did not occur. For all subjects having a decline of at least 20% in FEV₁ by the end of the dose schedule, the concentration of histamine causing a 20% fall in FEV₁ (PC₂₀) was calculated by linear interpolation from the individual log-dose response curve as follows²⁵:

$$PC_{20} =$$

$$\frac{\text{antilog}[(\log C_1 + (\log C_2 - \log C_1)(20 - R_1)) / (R_2 - R_1)]}{(R_2 - R_1)}$$

where: C₁ = second last concentration of histamine (<20% FEV₁ fall), C₂ = last concentration of histamine (>20% FEV₁ fall), R₁ = % fall FEV₁ after C₁, R₂ = % fall FEV₁ after C₂. A positive test was defined as a PC₂₀ < 16.0 mg/mL histamine.

Histamine responsiveness was also analyzed with use of an estimate of the overall slope of the dose-response relationship.²⁶ The dose-response slope (DRS) was calculated as the decline in FEV₁ from the postsaline value (expressed as a percentage of the postsaline value) after the final dose divided by the dose of histamine administered²⁶; a constant of 3 was added to all dose-response slopes to eliminate negative and zero values.

RESULTS

A total of 665 subjects (68% of the original sample) had the complete data required for this analysis. The general characteristics of the subjects are listed in Tables 1 and 2. Eosinophilia, defined as a count > 0.25 $\times$ 10⁹/L, was found in 67 (21%) male subjects and 60 (18%) female subjects.

The estimated life time active tobacco exposure (number of pack years) did not have significant impact on the number of peripheral eosinophils; however, the association between daily tobacco consumption and the eosinophil count approached the level of statistical significance ($P = .09$). Smoking habits (number of pack-years or daily tobacco consumption) did not have a statistical significant effect on pulmonary function, which was most likely due to the relatively modest tobacco exposure.

Subjects with bronchial hyperresponsiveness to inhaled histamine had higher eosinophil counts (.27 $\pm$.19 10⁹/L ver-

sus .16 $\pm$.14 10⁹/L, $P < .001$) and lower levels of FEV₁ %pred (93.9 $\pm$ 15.7 versus 102.8 $\pm$ 12.7, $P < .001$) compared with subjects without hyperresponsiveness. An increasing number of blood eosinophils was directly correlated with increasing degree of bronchial responsiveness to histamine, expressed as the dose-response slope, ($r = .60$, $P < .001$) (Fig 1).

The FEV₁ %predicted did not differ significantly between those subjects who had a positive skin prick test compared with those with a negative skin prick test ($P = .19$). Eosinophil counts were higher in atopic subjects than in nonatopic subjects (.20 $\pm$.17 10⁹/L and .15 $\pm$.13 10⁹/L, respectively, $P < .001$). Correlation analyses showed a stronger association between eosinophil count and FEV₁ %pred in atopic subjects ($r = -.27$, $P < .001$) than in nonatopic subjects ($r = -.14$, $P < .01$), although the relationship was statistically significant in both groups of subjects.

As expected, a history of past or current asthma was related both to lower FEV₁ %predicted (102.3 $\pm$ 14.6 for nonasthmatic subjects versus 94.8 $\pm$ 13.5 for ever asthmatic subjects; $P < .001$) and to higher eosinophil counts. The eosinophil counts were 0.16 $\pm$.14 10⁹/L and 0.27 $\pm$.18 10⁹/L ($P < .001$), respectively, for nonasthmatic and asthmatic subjects ($n = 85$).

There was a significant inverse correlation between the eosinophil counts

Table 1. Characteristics of the Sample (n = 665) of Adolescents and Young Adults, Stratified by Sex

	Males	Females
Characteristic	(n = 322)	(n = 343)
Age, yr [SD]	18.5 [2.8]	18.7 [2.9]
(range)	(14-23)	(13-23)
Smoking status, n		
Never smokers	212	191
Ex-smokers	3	13
Current smokers (%)	106 (33)	138 (40)
FEV ₁ [SD]*		
%predicted	95.1 [14.2]	97.9 [11.7]
1	4.5 [0.9]	3.5 [0.5]
FEV ₁ /FVC (%)	85 [7]	87 [6]

* Baseline values.

Table 2. Population Sample Comprising 665 Adolescents and Young Adults According to Asthmatic and Atopic Status, Peripheral Eosinophil Count and Bronchial Responsiveness to Inhaled Histamine

	Males (n = 322)	Females (n = 343)
Asthma, n		
Nonasthmatic	276	302
Ever asthma (%)	45 (14)	40 (12)
Current asthma (%)*	39 (12)	37 (11)
Atopy, n		
Nonatopic	166	216
Atopic (%)†	155 (48)	126 (37)
Eosinophils, * 10 ⁹ /L [SD]	0.18 [0.13]	0.17 [0.17]
(range)	(.01–.85)	(.01–.94)
<.25 * 10 ⁹ /L, n	254	282
> .25 * 10 ⁹ /L, n (%)	67 (21)	60 (18)
Histamine responsiveness		
DRS‡ [SD]	2.5 [23.7]	2.7 [25.0]
PC ₂₀ < 16.0 mg/mL, n (%)	54 (17)	49 (14)

* Symptoms of asthma within the 12 months prior to the examination.

† Atopy was defined as a positive skin prick test, and a positive skin prick test was defined as a positive reaction to at least one of the used allergens.

‡ DRS = dose-response slope, for further details, see text.

and the FEV₁ %predicted ($r = -0.18$, $P = .001$). Subjects with eosinophilia had significantly reduced levels of FEV₁ %predicted compared with subjects without eosinophilia ($102.1 \pm$

16.1 %predicted and 95.2 ± 13.2 %predicted, respectively, $P = .001$).

Analysis of data for life-long non-smokers only revealed a significant correlation between blood eosinophil

numbers and FEV₁ %predicted ($r = -.25$, $P = .001$). Furthermore, those who never smoked but who had eosinophilia had a reduced level of FEV₁ %predicted compared with those non-smokers without eosinophilia (94.5 ± 15.3 and 103.1 ± 14.6 , respectively, $P < .001$).

In subjects without bronchial hyper-responsiveness, that is, subjects with less than 20% decline in FEV₁ after inhalation of histamine 16 mg/mL, there was a weak but statistical significant correlation between the number of eosinophils and the level of FEV₁ %predicted, ($r = -.12$, $P = .03$).

Among nonatopic subjects, the FEV₁ %predicted was significantly lower in those with eosinophilia than in those without (96.2 versus 100.5 , $P = .03$).

By confining the analysis to subjects without a history of asthma a weak but significant correlation was found between eosinophil count and FEV₁ %predicted ($r = -0.13$, $P = .005$). There was also an association between

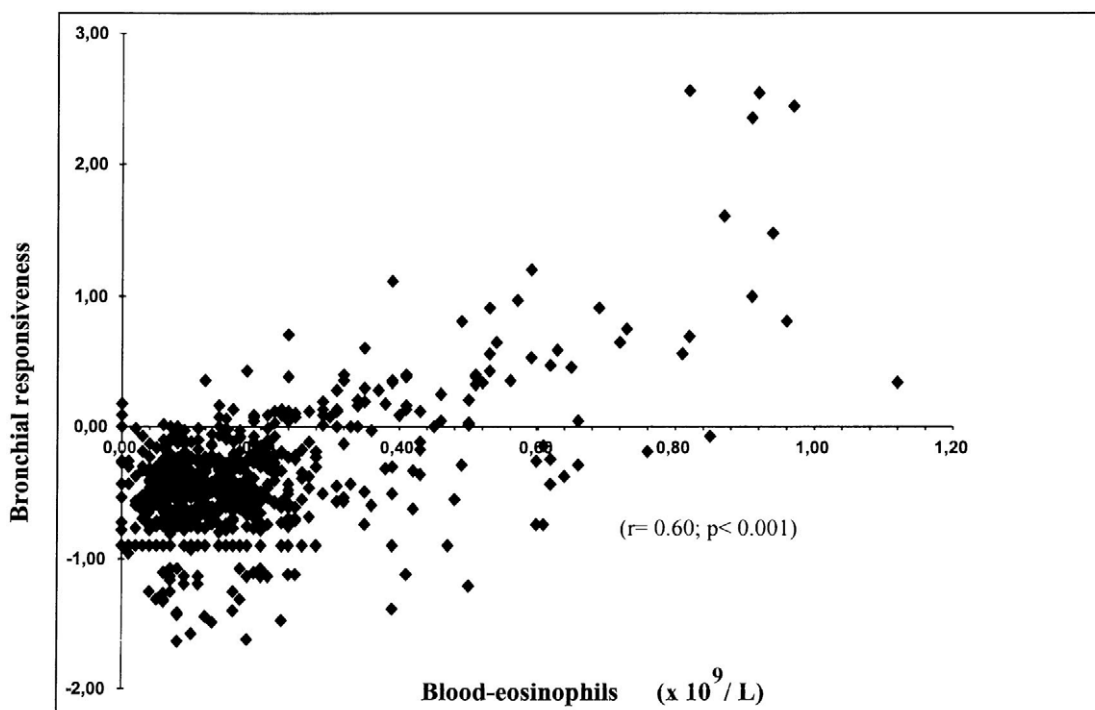


Figure 1. Blood eosinophil count versus bronchial responsiveness to inhaled histamine (dose-response slope) in a population sample comprising 665 adolescents and young adults.

the number of blood eosinophils and histamine responsiveness (DRS) ($r = .34$, $P < .001$).

The observed association between the level of FEV₁ %predicted and eosinophil counts in life-long nonsmokers was not accounted for by a history of asthma, bronchial hyperresponsiveness, or atopy. Considering the subgroup comprising the 204 life-long nonsmokers without a history of asthma and no evidence of atopy and bronchial hyperresponsiveness to inhaled histamine, those with eosinophilia ($n = 27$) had a significantly lower FEV₁ %predicted than those without ($n = 177$) (96.1 and 101.9, respectively, $P < .05$). In the same subgroup, there was a weak but statistically significant inverse correlation between the eosinophil counts and the levels of FEV₁ ($r = -.14$, $P = .04$) (Fig 2).

DISCUSSION

In this population sample of adolescents and young adults, a significant association was observed between higher peripheral blood eosinophil count and lower FEV₁. This associa-

tion was not fully explained by smoking, atopy, asthma, and bronchial hyperresponsiveness to inhaled histamine.

The finding relating eosinophilia to respiratory symptoms and lower levels of pulmonary function is in agreement with the theory termed the "Dutch hypothesis" by Fletcher and coworkers³ which was originally formulated by Orie and coworkers.² This hypothesis ascribes both reversible and nonreversible obstructive pulmonary function impairment to a constitutional "asthmatic predisposition," manifested by eosinophilia, airway hyperresponsiveness, and possibly also IgE-mediated allergy. This "asthmatic predisposition" may be the host factor that determines whether or not a smoker will develop nonreversible airflow obstruction.^{2,3} The assumption of eosinophilia as an independent risk factor for chronic obstructive pulmonary disease remains controversial. From studies of adults, van der Lende et al⁴ and Kauffmann et al²⁷ have previously reported an inverse association between blood eosinophil counts and the level of pulmonary function, which in the latter

study was not accounted for by atopy, asthma, and bronchial hyperresponsiveness. It might be inferred from these studies that eosinophilia is more important for pulmonary function impairment than smoking; however, it can not be refuted that the observed association might be partly due to smoking. Cigarette smoking is generally accepted to be associated with higher eosinophil counts, although this relationship did not reach the level of statistical significance in this study. The present study showed that the inverse association between eosinophils and FEV₁ %predicted persisted after exclusion of smokers, and, furthermore, also after exclusion of asthmatic subjects and subjects with a positive skin prick test or histamine challenge test. These findings suggest that eosinophilia might be a marker for a host factor related to an increased risk for obstructive lung function impairment, also in nonsmokers without evidence of reversible obstructive lung disease and IgE-mediated allergy.

The inflammatory changes in the airways of subjects suffering from asthma, even in mild cases, are characterized by a persistent accumulation of eosinophils.²⁸ Previous findings indicate that activated eosinophils can induce tissue damage.²⁹ Peripheral eosinophilia in nonatopic, nonasthmatic subjects may reflect a subclinical inflammatory reaction in the airways. Comparable to findings concerning asymptomatic bronchial hyperresponsiveness, peripheral blood eosinophilia may occur in some of the asymptomatic subjects as a transient phenomenon. In subjects with persistent eosinophilia, however, release of mediators such as eosinophil peroxidase and eosinophil cationic protein can be toxic to the airway epithelium and might lead to permanent changes in the airways and a consequent decline in pulmonary function. The damage to the airways may include thickening of the lamina reticularis below the basement membrane, as found in bronchial biopsies from asthmatic subjects.³⁰

The method used for counting eosinophils has previously been shown to be

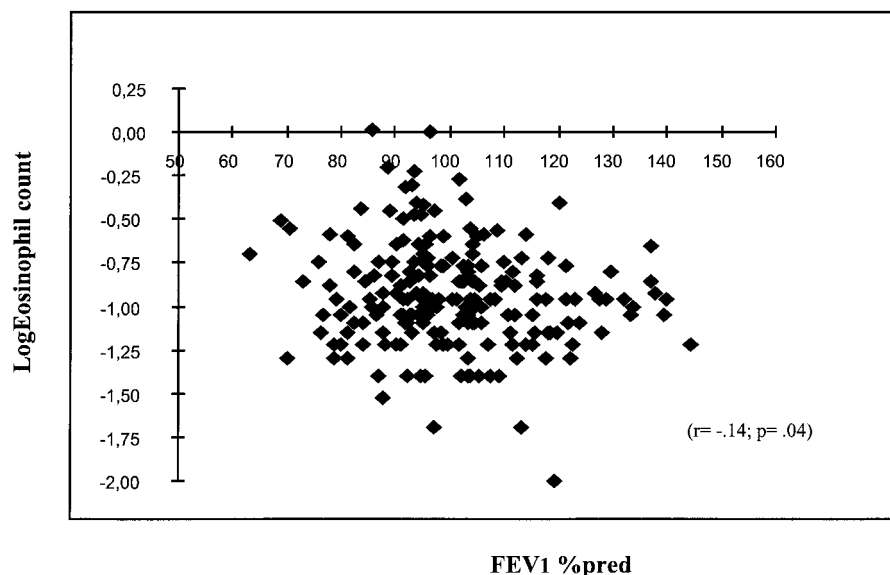


Figure 2. Scatter plot of the relationship between eosinophil count and level of FEV₁ %pred in nonatopic, nonasthmatic life-long nonsmokers without bronchial hyperresponsiveness to inhaled histamine ($n = 204$). The correlation between eosinophil count and FEV₁ %pred is statistically significant, but there is a wide scatter of individual data.

relatively precise.^{22,30} Introduction of substantial bias due to measurement error therefore seems less likely; however, the use of an arbitrary definition of eosinophilia²³ in some analyses obviously has its limitations. Furthermore, the possible confounding effects of medication,⁵ especially corticosteroids; allergen exposure³⁰; seasonal variations³¹; and diurnal variations in circulating eosinophils²² were not taken into account. None of the participants was on oral corticosteroids, and to reduce the influence of circadian variation,³² blood samples for eosinophil counts were obtained for most participants at approximately the same time of day.

It may be argued that the magnitude of the observed differences in the level of FEV₁ between subjects with and without eosinophilia are small. But considering the young age of the subjects studied, it seems reasonable to believe that the observations reflect differences of potential clinical importance later in life. Despite the mentioned limitations, this study has provided interesting insight into the cross-sectional relationship of the eosinophil leukocyte to pulmonary function results, taking smoking habits, respiratory symptoms suggesting asthma, skin test reactivity, and bronchial responsiveness into account.

In conclusion, the findings in this study may suggest that the blood eosinophil counts are related to the level of pulmonary function in nonsmokers, and, furthermore, that this association cannot be explained by asthma, histamine hyperresponsiveness, or IgE-mediated allergy. These results seem to support the hypothesis that the eosinophil leukocyte plays a role in obstructive lung function impairment that extends beyond its role in allergic reactions. Future follow-up studies of this and other population samples will likely bring an answer to the question of whether eosinophilia is an independent risk factor for development of nonreversible obstructive lung function impairment.

ACKNOWLEDGMENT

I thank Dr. V. Backer for having initiated the study in 1986 and thus forming the basis for this investigation.

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