Chapter 3

THE RELATIONSHIP OF SKIN TEST POSITIVITY, HIGH SERUM TOTAL IGE LEVELS, AND PERIPHERAL BLOOD EOSINOPHILIA TO SYMPTOMATIC AND ASYMPTOMATIC AIRWAY HYPERRESPONSIVENESS

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Abstract

The relationships of skin test positivity, high serum total IgE levels (>100 kU/L), and peripheral blood eosinophilia (> 275 cells/µl) to symptomatic (either chronic cough, chronic phlegm, bronchitis episodes, dyspnea, wheeze, or asthma) and asymptomatic bronchial hyperresponsiveness (BHR) were studied cross-sectionally in 620 adult subjects who participated in the Vlagtwedde-Vlaardingen Study of 1989 and 1990. Eosinophilia (OR=2.06, 95% CI=1.28 to 3.31) and skin test positivity (OR=1.66, 95% CI=1.02 to 2.71) were both significantly associated with BHR independent of age, sex, smoking, and urban area of residence. High serum total IgE levels were not associated with BHR (OR=1.29, 95% CI=0.81 to 2.03). Separate analyses for symptomatic and asymptomatic subjects showed that the higher risk of BHR with skin test positivity applied only to symptomatic subjects (OR=5.78, 95% CI=1.63 to 20.51), independent of eosinophilia and high serum total IgE levels. The higher risk of BHR with eosinophilia was not different between symptomatic and asymptomatic subjects, and independent of skin test positivity and high serum total IgE levels. The results of this study show that, in the general adult population, eosinophilia is associated with BHR both in symptomatic and asymptomatic persons, whereas skin test positivity is associated with BHR only in symptomatic subjects.

Introduction

Bronchial hyperresponsiveness (BHR), the exaggerated airway narrowing in response to nonspecific stimuli, is a common characteristic of asthma. However, BHR is also present in 19 to 62% of subjects without respiratory symptoms in the general population (1). The mechanisms underlying asymptomatic BHR are still unclear, yet it is known that in asymptomatic subjects BHR is a risk factor for asthma and chronic obstructive pulmonary disease (COPD) (2-5). Furthermore, most subjects with BHR who develop respiratory symptoms appear to be atopic or have a positive family history of atopy (3,4). Thus, a central question is whether atopy helps to distinguish asymptomatic from symptomatic BHR.

Atopy is a process mediated by immunoglobulin E (IgE) (6). Mast cells in the bronchial wall are activated by IgE and as a consequence release mediators that may cause BHR directly (histamine, prostaglandins, and leukotrienes) or indirectly (interleukin-4 [IL-4] and tumor necrosis factor α [TNF-α]). IL-4 stimulates B cells to produce IgE, which maintains mast cell activation. Further, IL-4 and TNF-α enable eosinophils to migrate from the vessels into the bronchial mucosa by upregulation of vascular adhesion molecules (6,7). Eosinophils, once activated, release mediators, which damage the epithelium and cause BHR due to increased permeability (6,8,9). However, increased serum total IgE levels and peripheral blood eosinophil counts are neither closely related nor exclusively present in atopic individuals. Serum total IgE levels are also increased in nonatopic smokers, and peripheral blood eosinophils are also elevated in parasitic infections and in certain neoplasms (10). Thus, skin test
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responses, serum total IgE level, and number of peripheral blood eosinophils are independent features of a common underlying mechanism.

The use of different measures of atopy in different studies has complicated comparisons of the results. However, BHR is clearly more prevalent among subjects with positive skin prick tests (11,12), increased serum total or specific IgE levels (13,14), and peripheral blood eosinophilia (15-17). It is unknown which of these atopy measures most strongly correlates with BHR. To determine which atopy-related factor best predicts BHR, we performed cross-sectional analyses on the relationship between BHR and positive skin prick tests, increased serum total IgE levels, and peripheral blood eosinophilia. As we are interested in differences in mechanisms underlying asymptomatic BHR and symptomatic BHR in particular, we also determined these relationships in subjects with and without respiratory symptoms.

Methods

The current analyses made use of data from the 1989 and 1990 survey of the Vlagtwedde-Vlaardingen Study, a longitudinal population study that was started in 1965 to determine risk factors for COPD. The selection of the study population has been described previously (18,19). After baseline measurements in 1965 and 1967 in Vlagtwedde and in 1965 and 1969 in Vlaardingen, a follow-up survey was organized every 3 yr. In the first two baseline surveys a histamine provocation test was performed in a 25% random sample from the study population. From the third survey on, the strategy has been to select those subjects for histamine testing who had performed the test in prior surveys. As a result from this strategy 808 of the 2,553 participants (age 35 to 80 yr) in the surveys of 1989 and 1990 underwent a histamine provocation test. These subjects make up the group included in this cross-sectional study. Both surveys were carried out during the month of October.

Information on respiratory symptoms, smoking status, age, and sex was collected by the Dutch version of the British Medical Research Council standardized questionnaire (20). Subjects were considered symptomatic if they reported one or more of the following chronic respiratory symptoms: cough or phlegm production on most days or nights for as much as 3 consecutive months each year during winter (referred to as chronic cough/chronic phlegm), a period of at least 3 wk in the previous 3 yr with (increased) cough and phlegm (bronchitis episodes), shortness of breath when walking with other persons of the same age on level ground (dyspnea ≥ grade 3), a wheezing or whistling sound in the chest on most days or nights (persistent wheeze), or attacks of shortness of breath at any time (asthma attacks). Subjects were considered asymptomatic if they reported none of these chronic respiratory symptoms. Subjects were categorized as current smokers, ex-smokers (defined as those who had quit smoking at least 1 mo before the examination), and never-smokers.
Pulmonary function measurements were performed with a water-sealed spirometer (Lode Spirograph D53; Lode Instruments, Groningen, The Netherlands). Measurement of inspiratory vital capacity (IVC) after a deep expiration was followed by measurement of forced expiratory volume in one second (FEV₁). The higher of the values obtained in two technically satisfactory tracings was taken as the baseline measurement as long as the difference between the two IVC values was less than 150 ml and that between the two FEV₁ values was less than 100 ml.

Bronchial responsiveness to histamine was assessed by the method of Tiffeneau as modified by De Vries and coworkers (21), which meets standardization guidelines (22). After baseline measurements of pulmonary function, subjects inhaled nebulized distilled water from a Wiesbaden Doppel inhalator (Lode Instruments, Groningen, The Netherlands). If the IVC and/or the FEV₁ decreased by 10% or more, the test was terminated (n=2). If the IVC or the FEV₁ did not decrease by 10% or more, the test proceeded with the application of sequential aerosols of histamine biphosphate in concentrations of 1, 4, 8, 16, and 32 mg/ml. Each concentration was inhaled for 30 s. After each challenge, two IVC and FEV₁ maneuvers were performed. If, at a given concentration, the IVC or the FEV₁ persistently declined by 10% or more, this particular concentration was considered the threshold value (PC₁₀). The test was terminated with a persistent decrease of 10% or more or after administration of the highest concentration. BHR was defined as a PC₁₀ of ≥ 8 mg/ml histamine (including a ≥ 10% decrease after distilled water inhalation). Subjects with excessively low levels of pulmonary function (FEV₁ < 1.5 L) and those who could not perform a forced expiration were not tested. Subjects suffering from heart disease, hypertension, or acute respiratory infections were also excluded from challenge.

Skin prick tests (ALK Benelux, Woerden, The Netherlands) included six inhalant allergens: house dust mite (*Dermatophagoides pteronyssinus*), mixed grass pollen (meadow foxtail, cocksfoot, meadow fescue, rye grass [perennial], and timothy), mixed tree pollen (alder, birch, and hazel), dog epithelium, cat epithelium, and mold (*Aspergillus fumigatus*). The solvent for the allergens (50% glycerol and 50% aqueous isotone) served as a negative control, and a histamine dihydrochloride solution (3 mg/ml) served as a positive control. Skin prick tests were quantified 15 min after application as the mean value of the longest diameter of the wheal and its perpendicular and were considered positive at ≥ 3 mm. Skin test positivity was defined as one or more positive skin prick tests.

Before spirometry and skin prick tests were performed, blood samples were taken. Peripheral blood eosinophil counts were estimated with a Technicon-H1 blood cell counter (Bayer AG, Leverkusen, Germany) and were expressed as number of cells per microliter. Peripheral blood eosinophilia was defined as ≥ 275 cells/µl (23). Total serum IgE concentrations were determined with the CAP system (Pharmacia, Woerden, The Netherlands) and expressed in kU/L. Concentrations below 2 kU/L and above 2,000 kU/L cannot be detected by this system and were represented by 1.99 kU/L and 2,001 kU/L, respectively. High serum total IgE levels were defined as > 100
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Chi-square tests and t tests were used to compare the characteristics of subjects with and without respiratory symptoms (24). Logistic regression analyses were performed to determine the relationship between BHR and skin test responses, serum total IgE levels, and peripheral blood eosinophilia (independent of smoking habits, age, gender, and area of residence) (24). Each measure of atopy was dichotomized i.e., the presence or absence of skin test positivity, of a high serum total IgE level, and of peripheral blood eosinophilia. To determine how the various measures of atopy influence each other in relation to BHR, we initially included only one of the three measures of atopy in the regression analyses. We then repeated the analyses, including two measures of atopy and finally all three measures of atopy. We stratified the analyses by respiratory symptoms to determine whether the relationship was the same in symptomatic as in asymptomatic subjects. To test the difference between symptomatic and asymptomatic subjects, we added three interaction terms for each measure of atopy by respiratory symptoms to the pooled analyses (24). Because the presence of respiratory symptoms in the general population is intermittent, we also stratified by “respiratory symptoms in the past” i.e., subjects who never reported respiratory symptoms during the follow-up period from 1965 to 1990 (always asymptomatic), those who reported respiratory symptoms at least once but not at all surveys (intermittently symptomatic), and those who always reported respiratory symptoms (always symptomatic).

All analyses were repeated with bronchial responsiveness, or peripheral blood eosinophil counts, skin test positivity, and serum total IgE levels as continuous variables. Bronchial responsiveness was expressed as the values 1 to 6 for the threshold values 1, 4, 8, 16, 32, and >32 mg/ml and log-transformed. Only subjects with complete information on all variables were included in the analyses.
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Results

Of the 808 subjects available for this study, 691 (85.5%) performed technically satisfactory FEV₁ and IVC measurements. Values on peripheral blood eosinophilia, skin test positivity, or serum total IgE levels were missing for 71 subjects, leaving complete information of 620 subjects for analyses. Respiratory symptoms in the previous 3 yr were reported by 136 (22%) subjects. Subjects with respiratory symptoms were more likely than subjects without respiratory symptoms to be current smokers, to have lower levels of pulmonary function, and to be hyperresponsive to histamine (Table 1). The proportion of subjects who were male and the subjects’ mean age were not significantly different in the two groups.

Table 1. Characteristics of participants in the Vlagtwedde-Vlaardingen surveys of 1989 and 1990 by respiratory symptom status

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (% men)</td>
<td>484 (58)</td>
<td>136 (60)</td>
</tr>
<tr>
<td>Age, yr, mean ± SD</td>
<td>53 ± 10</td>
<td>53 ± 10</td>
</tr>
<tr>
<td>Current smokers, n (%)</td>
<td>149 (31)</td>
<td>60 (44)**</td>
</tr>
<tr>
<td>Ex-smokers, n (%)</td>
<td>200 (41)</td>
<td>40 (29)*</td>
</tr>
<tr>
<td>Nonsmokers, n (%)</td>
<td>135 (28)</td>
<td>36 (27)</td>
</tr>
<tr>
<td>FEV₁, %pred, mean ± SD</td>
<td>110 ± 15</td>
<td>103 ± 18***</td>
</tr>
<tr>
<td>FEV₁/VC, %, mean ± SD</td>
<td>75 ± 7</td>
<td>73 ± 9***</td>
</tr>
<tr>
<td>PC₁₀ &lt; 8 mg/ml, n (%)</td>
<td>173 (36)</td>
<td>71 (52)***</td>
</tr>
<tr>
<td>Peripheral blood eosinophilia, n (%)</td>
<td>75 (16)</td>
<td>20 (15)</td>
</tr>
<tr>
<td>Skin test positivity, n (%)</td>
<td>71 (15)</td>
<td>20 (15)</td>
</tr>
<tr>
<td>Serum total IgE level &gt; 100 kU/L, n (%)</td>
<td>83 (17)</td>
<td>22 (16)</td>
</tr>
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</table>

* p<0.05, ** p<0.01, *** p<0.001.
Peripheral blood eosinophilia was detected in 15.3% of all subjects, skin test positivity in 14.7%, and a high serum total IgE level in 16.9%. The prevalences of each atopy measure were similar in symptomatic and asymptomatic subjects. The overlap among the three measures was small, though the prevalence of atopy defined by any one of the three measures alone was almost the same (Figure 1). Nevertheless, different subjects will be considered atopic when different measures of atopy are applied. At least one positive measure of atopy was observed in 35.6% of the subjects; all three measures were observed in only 1.5%.

Figure 1. Prevalence of atopy defined by eosinophilia, skin test positivity, and high serum total IgE levels. EO+ = eosinophilia; IgE+ = serum total IgE level >100 kU/L; ST+ = skin test positivity.

Figure 2. Prevalence of BHR by quartiles of eosinophil counts and respiratory symptoms.
* p<0.05, (*) p=0.07 compared with asymptomatic.
Figure 2 shows that the percentage of subjects with BHR increases with higher numbers of peripheral blood eosinophils both among subjects with and without symptoms. The prevalences of BHR without respiratory symptoms were similar among skin test-positive and skin test-negative subjects, whereas the prevalence of BHR with respiratory symptoms was higher among skin test-positive subjects (Figure 3). The prevalences of BHR were similar across quartiles of serum total IgE levels among both asymptomatic and symptomatic subjects, although symptomatic subjects had higher serum total IgE levels (Figure 4).

** Figure 3. Prevalence of BHR by skin test positivity and respiratory symptoms. **

\[ p<0.01 \text{ compared with asymptomatic} \]

** Figure 4. Prevalence of BHR by quartiles of serum total IgE levels and respiratory symptoms. **

\[ * p<0.05 \text{ compared with asymptomatic} \]
Logistic regression for each measure of atopy

Logistic regression analyses for each of the three measures of atopy considered separately with age, sex, smoking status, and area of residence taken into account showed that subjects with peripheral blood eosinophilia or with skin test positivity were more likely to have BHR than subjects in the corresponding negative groups (Table 2). High serum total IgE levels were not associated with BHR. Stratified analyses showed a higher risk of BHR with skin test positivity in symptomatic subjects than in asymptomatic subjects; the difference was of borderline significance (p=0.08). The associations of BHR with peripheral blood eosinophilia and high serum total IgE levels were not significantly different for symptomatic versus asymptomatic subjects. Exclusion of the 35 subjects with asthma-like symptoms (e.g., wheeze most days and/or nights or attacks of shortness of breath with wheeze) did not change the observed relationships between measures of atopy and BHR in symptomatic subjects.

Table 2. Odds ratios (95% CI) for BHR calculated in three logistic regression models for each atopy parameter separately†

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total group (n=620)</th>
<th>Asymptomatic (n=484)</th>
<th>Symptomatic (n=136)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>Odds ratio (95% CI)</td>
<td>Odds ratio (95% CI)</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>2.06** (1.28-3.31)</td>
<td>2.16** (1.25-3.70)</td>
<td>1.76 (0.64-4.89)</td>
</tr>
<tr>
<td>Positive skin tests</td>
<td>1.66* (1.02-2.71)</td>
<td>1.31 (0.74-2.30)</td>
<td>4.19* (1.36-12.89)</td>
</tr>
<tr>
<td>Serum total IgE &gt; 100 kU/L</td>
<td>1.29 (0.81-2.03)</td>
<td>1.47 (0.88-2.47)</td>
<td>0.91 (0.35-2.37)</td>
</tr>
</tbody>
</table>

† All odds ratios are adjusted for age, sex, smoking status, and area of residence. The risks of BHR for the three measures of atopy were not significantly different for symptomatic versus asymptomatic subjects. *p<0.05, **p<0.01.

Logistic regression including different combinations of two measures of atopy

To determine whether the various measures of atopy are independently associated with BHR, the analyses were repeated with the inclusion of combinations of two measures. In the total group, the overall risks of BHR assessed in terms of the presence of peripheral blood eosinophilia, skin test positivity, or high serum total IgE level did not change when adjusted for one of the other measures of atopy. The risks of BHR in symptomatic subjects with skin test positivity or high serum total IgE levels changed when these two measures of atopy were adjusted for each other: the risk with skin test positivity increased from 4.19 (Table 2) to 5.82 (95% confidence interval [CI]=1.66 to 20.46), whereas the risk with high serum total IgE levels decreased from 0.91 (Table 2) to 0.48 (95% CI=0.16 to 1.44). In asymptomatic
subjects, the risk estimates remained similar after these adjustments.

Table 3. Odds ratios (95% CI) for BHR of three different atopy parameters combined in one logistic regression model†

<table>
<thead>
<tr>
<th>Atopy measures</th>
<th>Odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophilia</td>
<td>2.07** (1.28-3.37)</td>
</tr>
<tr>
<td>Skin test positivity</td>
<td>1.13 (0.63-2.03)</td>
</tr>
<tr>
<td>Serum total IgE&gt;100 kU/L</td>
<td>1.40 (0.81-2.41)</td>
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</table>

<table>
<thead>
<tr>
<th>Variables adjusted for</th>
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<tbody>
<tr>
<td>Respiratory symptoms</td>
<td>1.25 (0.63-2.48)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>1.04** (1.01-1.06)</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.48** (0.31-0.75)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>0.12 (0.01-1.10)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>1.56 (0.96-2.53)</td>
</tr>
<tr>
<td>Urban area of residence</td>
<td>0.59** (0.41-0.85)</td>
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<table>
<thead>
<tr>
<th>Interaction terms</th>
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<tbody>
<tr>
<td>Current smoker x age</td>
<td>1.07** (1.02-1.11)</td>
</tr>
<tr>
<td>Symptoms x skin test positivity</td>
<td>4.90* (1.30-18.51)</td>
</tr>
<tr>
<td>Symptoms x serum total IgE&gt;100 kU/L</td>
<td>0.34 (0.10-1.17)</td>
</tr>
<tr>
<td>Symptoms x male sex</td>
<td>2.45* (1.04-5.78)</td>
</tr>
</tbody>
</table>

† All odds ratios are adjusted for age, sex, smoking status, and area of residence.
* P<0.05, ** P<0.01, *** P<0.001. § The risk of BHR with skin test positivity is significantly higher among symptomatic than asymptomatic subjects.
Logistic regression including all three measures of atopy
The changes previously described persisted when all three measures of atopy were combined in the same regression model (Table 3). In symptomatic subjects, skin test positivity was a stronger predictor of BHR than peripheral blood eosinophilia. Serum total IgE levels were not associated with BHR, though levels were almost significantly different between symptomatic and asymptomatic subjects (p=0.09). A further difference with respect to respiratory symptoms is that among asymptomatic subjects females are more likely to have BHR than males, whereas among symptomatic subjects no difference was observed between the sexes. This difference between the symptomatic and asymptomatic groups was no longer significant after adjustment for FEV1. Furthermore, smoking at an older age had a synergistic effect on the risk of BHR, as indicated by a significant positive interaction between smoking and age (Table 3).
Finally, we stratified the analyses not only by the presence or absence of respiratory symptoms in the surveys of 1989 and 1990 but also with reference to “respiratory symptoms in the past.” As the relations among the three measures of atopy and BHR were not significantly different for subjects who had been intermittently symptomatic in the past but were asymptomatic in the last survey (1989/1990) versus subjects who had been intermittently symptomatic in the past and were symptomatic in the last survey, these groups were pooled and designated as “intermittently symptomatic.” Analyses showed that peripheral blood eosinophilia is a risk factor for BHR in subjects who have been intermittently symptomatic but not in subjects who have always been either asymptomatic or symptomatic (Table 4). No changes were observed in the relationships between skin test positivity and BHR and between high serum total IgE levels and BHR when “respiratory symptoms in the past” were taken into account.

Table 4. Odds ratios (95% CI) for BHR according to symptom history, based on symptom status in all surveys†

<table>
<thead>
<tr>
<th></th>
<th>Always Asymptomatic (n=287)</th>
<th>Intermittently Symptomatic (n=295)</th>
<th>Always Symptomatic (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophilia</td>
<td>1.04 (0.47-2.30)</td>
<td><strong>3.45</strong>* (1.74-6.86)§</td>
<td>0.58 (0.06-5.69)</td>
</tr>
<tr>
<td>Skin test positivity</td>
<td>0.98 (0.41-2.35)</td>
<td>1.61 (0.80-3.23)</td>
<td>22.09 (0.84-580)</td>
</tr>
<tr>
<td>Serum total IgE &gt;100 kU/L</td>
<td>1.55 (0.70-3.45)</td>
<td>0.87 (0.45-1.69)</td>
<td>1.12 (0.11-11.48)</td>
</tr>
</tbody>
</table>

† The allergy parameters are simultaneously included in the model and adjusted for age, sex, smoking status, and area of residence. **p<0.001. § The risk of BHR for subjects with peripheral blood eosinophilia is significantly higher in the presence of intermittent respiratory symptoms than in the presence of continuous symptoms.
Similar relationships were found between the three atopy measures when bronchial responsiveness was analyzed as a continuous variable. Analyses with the continuous measures of the number of peripheral blood eosinophils, the sum of the wheal sizes of the positive skin tests, and the level of serum total IgE yielded results comparable to those from the analyses with the dichotomized measures. Similarly, additional adjustment for pulmonary function (FEV1) did not change the results. To be sure that the exclusion of subjects who performed technically unsatisfactory lung function measurements (FEV1 and IVC) caused no selection, we reanalyzed the relationships including the information of this group. The results were similar.

**Discussion**

The results of this study conducted in a general adult population with a mean age of 53 years show that peripheral blood eosinophilia and skin test positivity significantly predict BHR, independent of each other and of a high serum total IgE level. The relationship between peripheral blood eosinophilia and BHR was the same in asymptomatic subjects and symptomatic subjects, whereas skin test positivity was predictive of BHR in symptomatic subjects only. In contrast, high serum total IgE levels were not associated with BHR in this middle-aged population.

Positive skin test responses, an increased level of serum total IgE, and increased numbers of peripheral blood eosinophils are generally considered to reflect atopy. Earlier results in the Vlagtwedde-Vlaardingen Study showed positive associations among these three measures of atopy (25). Therefore, their relationships with BHR were expected to become weaker when the measures of atopy were adjusted for one another. However, peripheral blood eosinophilia, skin test positivity, and a high serum total IgE level did not influence one another’s relationships with BHR, apart from a higher risk of BHR with skin test positivity in symptomatic subjects when adjusted for serum total IgE levels. This result is explained to a large extent by the small overlap between the three measures of atopy (Figure 2). The implication is that skin test positivity, a high serum total IgE level, and peripheral blood eosinophilia are different expressions of the atopic phenotype. This conclusion is in accord with other studies in children (26) and young adults (27), in which many young adults without a positive skin test have had high serum total IgE levels.

One of the important messages of this study is that peripheral blood eosinophilia is associated with an independent increased risk of BHR in asymptomatic and symptomatic individuals. Only two previous studies have determined the relationships between different atopy measures and BHR independent of each other (28,29). In an adult population including only males, all three measures were significantly, though weakly, associated with increased bronchial responsiveness (28). In another study conducted in a general population, both skin test positivity to cat dander (odds ratio [OR]=5.5, p<0.05) and high serum total IgE levels (OR=2.2 per log unit, p<0.05) were strong predictors of BHR, whereas peripheral blood
eosinophilia was not related to BHR (29). A lower number of peripheral blood eosinophils (median, 110 cells/µl versus 154 cells/µl in our population) might explain this lack of relation between BHR and peripheral blood eosinophilia (personal communication). Our study showed a higher risk of BHR in symptomatic and asymptomatic subjects with peripheral blood eosinophilia, confirming the observations by Annema and coworkers (16) and Ulrik (17) of higher counts in both symptomatic and asymptomatic hyperresponsive men and young adults.

This study does not elucidate why peripheral blood eosinophils are related to BHR. Eosinophils play an important role in the inflammatory process of the airways of asthmatics (6). Activated eosinophils in the bronchial wall of asthmatics release arginine-rich products such as major basic protein (MBP), eosinophil cationic protein (ECP), and eosinophil peroxidase (EPO), causing BHR due to epithelial damage and increased permeability (8,9). Whether increased numbers of peripheral blood eosinophils reflect an inflammatory process in the airways of asymptomatic subjects that is responsible for BHR is unknown. So far, there are only two studies available showing no difference in the number of eosinophils in sputum (30) or airway wall (31) from asymptomatic children and adults with and without BHR. Therefore, it may well be that another common underlying mechanism is responsible for both peripheral blood eosinophilia and BHR. Increased numbers of eosinophils in peripheral blood may suggest a signal to the bone marrow (e.g., interleukin-5 released by T lymphocytes in the blood) that recruits eosinophils to the circulation and allows recruitment of eosinophils into the airways wall via endothelial adhesion and chemotactic factors, yet only after certain stimuli. Another possibility is that eosinophils are residing in peripheral airways, as has been observed in asthma (32). Eosinophils in either the peripheral or the central airways might create a local basic environment that easily permits the eventual development and persistence of symptoms. This possibility is supported by our finding that peripheral blood eosinophilia in asymptomatic subjects is predictive of BHR, particularly in those subjects who have been intermittently symptomatic in the past. Moreover, recent longitudinal analyses of the Vlagtwedde-Vlaardingin Study by our group have shown that BHR in asymptomatic subjects is a risk factor for subsequent development of respiratory symptoms (2).

Previous studies have shown that subjects with BHR are more likely than those without BHR to have positive skin tests (11,12). In our study, skin test positivity was associated with BHR, yet only in symptomatic subjects: symptomatic skin test-positive subjects had an almost sixfold higher risk of BHR than symptomatic skin test-negative subjects. Earlier studies demonstrated that allergen exposure results in BHR and respiratory symptoms in atopic asthmatics (21,33). Thus allergen exposure might account for respiratory symptoms in skin test-positive individuals.

In contrast to the findings of most other studies (27-29), we observed no relationship between high serum total IgE levels and BHR in our middle-aged population. Higher serum total IgE levels are known to be associated with smoking, male gender, and
younger age. Smoking is associated with both increased serum total IgE levels and BHR. Without adjustment for smoking, a high serum total IgE level was also not associated with BHR in our study. Thus, smoking is unlikely to explain differences between our study and others. Serum total IgE levels in females are reported to be 26% lower than those in males (34). Use of separate definitions for high IgE levels in males and females (as the highest quartiles, e.g., ≥ 57 kU/L for females and ≥ 80 kU/L for males) did not change our results (OR= 1.30, 95% CI=0.88 to 1.93). Compared with the two previously reported studies on the independent relationships of the three measures of atopy to BHR (28,29), our study concerned not only males but both males and females, and our population was older than that studied by Boezen and coworkers (29). Because serum total IgE levels in females are lower than those in males and because IgE levels decline with age (34), the range of levels in our population is probably smaller than that in previous studies and perhaps too small to detect an association with BHR.

The definition of BHR used in our study was more stringent than the commonly used definition i.e., a PC_{20} of ≤ 8 mg/ml (2 min of inhalation), which is comparable to a PC_{10} of ≤ 16 mg/ml (30 s of inhalation) (35). The threshold value was based on a 10% decrease in FEV\textsubscript{1}, or IVC. Of all subjects considered to have BHR, more than 30% were so categorized solely on the basis of a decrease in IVC. To reduce the likelihood of misclassification, we lowered the threshold value. If some subjects were nevertheless misclassified as having BHR, the observed relationships would have been weakened. However, repetition of the analyses with a definition of BHR as only a 10% decrease in FEV\textsubscript{1}, (excluding subjects with a PC_{10}[IVC] of ≤ 8 mg/ml histamine) did not change the results.

Finally, we found a higher risk of BHR in females than males only among asymptomatic individuals. The difference in the relationship between gender and BHR for symptomatic and asymptomatic subjects disappeared after the level of FEV\textsubscript{1} was taken into account. This confirms other study results (36,37). Leynaert and colleagues (36) suggested adjusting for FEV\textsubscript{1} as the percentage predicted in order to control for potential bias when comparing BHR in males and females with similar FEV\textsubscript{1} values. These investigators observed a higher risk of BHR in females when FEV\textsubscript{1} percentage predicted was taken into account. Our results confirmed these findings, indicating that females are more likely to have BHR than males. However, it is difficult to separate the effects of smoking and gender in these data because more males than females smoke. Although we adjusted in our analyses for smoking habits and gender, we cannot determine whether the different relation to BHR by symptom status is driven by smoking or gender.

In conclusion, the results of our study in a general adult population show that peripheral blood eosinophilia is associated with BHR in symptomatic as well as in asymptomatic subjects, whereas skin test positivity is strongly associated with BHR in symptomatic subjects only. Peripheral blood eosinophilia in subjects with BHR may facilitate recruitment and activation of eosinophils in the airway wall, giving rise
to inflammatory changes resulting in intermittent respiratory symptoms upon inhalation of appropriate stimuli. This requires further study.

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**References**


