Factors Associated with Persistent Airflow Limitation in Severe Asthma

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Persistent airflow limitation can develop in nonsmoking patients with asthma. However, the prevalence and risk factors for airways obstruction with incomplete reversibility in asthma are unknown. We assessed the prevalence of persistent airflow limitation (defined as postbronchodilator FEV_1 or $FEV_1/VC < 75\%$ predicted) in 132 nonsmoking outpatients with severe asthma visiting chest physicians in general hospitals in The Netherlands. They had used inhaled corticosteroids ($\geqslant 1,600$ μg/d) and/or daily oral prednisone and long-acting bronchodilators for > 1 yr. In addition, we examined whether persistent airways obstruction in these patients was associated with specific clinical characteristics (age at onset, smoking history, atopic status, bronchodilator reversibility, provocative concentration of histamine causing a 20% decrease in FEV_1 [PC_{20}histamine]) or markers of inflammation (exhaled nitric oxide [NO], blood eosinophils, total IgE; and eosinophilia or neutrophilia in induced sputum). Multiple logistic regression analyses were used to calculate adjusted odds ratios (OR). Persistent airflow limitation was observed in 49% of the patients in the study, and apart from older age and longer asthma duration, was strongly associated with a sputum eosinophils percent $\geqslant 2\%$ (OR = 7.7; confidence interval [CI]: 2.4 to 25), PC_{20}histamine $\leqslant 1.0$ mg/ml (OR = 3.9; CI: 1.2 to 13), and adult onset ($\geqslant 18$ yr) of asthma (OR = 3.3; CI: 1.2 to 9). Only sputum eosinophilia appeared to be independently associated with persistent airflow limitation (OR = 8.9; CI: 1.3 to 59). In conclusion, persistent airflow limitation is common in adult patients with severe asthma, and is associated with adult onset of the disease, airway hyperresponsiveness, and most importantly, sputum eosinophilia. These findings suggest that eosinophilic airway inflammation contributes to persistent airflow limitation in severe asthma. Whether reduction of sputum eosinophils with more vigorous treatment leads to a better prognosis in severe asthma is still an open question.

Keywords: asthma; severity of illness index; airway obstruction; sputum; eosinophilia

Asthma is a chronic inflammatory airways disease with variable but, in most patients, fully reversible airways obstruction. However, persistent airflow limitation can develop in a subgroup of patients with asthma who have no significant history of smoking, and despite optimal treatment (1–4). The etiology of persistent airflow limitation in asthma is still unknown, although most investigators assume that such loss of lung function is causally related to inflammatory processes in the airway wall (5, 6).

Persistent airways obstruction in asthma has been shown to be associated with more severe disease (7, 8) and has been reported to be a predictor of overall mortality in patients with asthma (9). However, data about the exact prevalence of and risk factors for persistent airflow limitation in patients with asthma are limited (5, 10, 11) and often contradictory. Possible risk factors, such as smoking (2, 4, 12); atopy (3, 4, 13); adult onset of asthma (14, 15); increased reversibility (4, 16); eosinophilic airway inflammation (17, 18); and increased airway hyperresponsiveness (AHR) (3, 12) have been both implicated and rejected as important for the decline in lung function in diverse asthma populations.

The present study was designed to assess the prevalence of persistent airflow limitation in a well-defined group of patients with severe asthma and to examine the clinical and pathophysiologic characteristics associated with permanent impairment of lung function. For this purpose we included age at onset, smoking history, and atopic status as clinical characteristics, bronchodilator reversibility and airway responsiveness to histamine as physiologic characteristics, and exhaled nitric oxide (NO), total IgE, and eosinophil and neutrophil percentages in peripheral blood and induced sputum as markers of airway inflammation.

METHODS

Patients

We consecutively recruited 152 patients with severe bronchial asthma (19) from the outpatient pulmonary departments of two teaching and eight nonteaching hospitals in the western part of The Netherlands. The patients' ages ranged from 18 to 75 yr. Sixteen patients refused to participate in the study, mainly for reasons of lack of transport and time. The participating patients had a history of episodic dyspnea and wheezing, a documented (recently or in the past) reversibility in FEV_1 of $> 12\%$ predicted (20), and/or hyperresponsiveness to inhaled histamine (21). They had been treated with inhaled corticosteroids ($\geqslant 1,600$ μg/d beclomethasone or equivalent) and long-acting bronchodilators for more than 1 yr, and all were nonsmokers ($\leqslant 10$ pack-yr). All of the patients were symptomatic and during the past year had had at least one severe exacerbation requiring a course of oral corticosteroids, and/or were receiving maintenance therapy with oral prednisone. The study was approved by the hospital medical ethics committees of the participating institutions, and all of the participating patients gave informed consent.

Procedures

At the time of patients' entry into the study, patient and disease characteristics were documented according to a detailed structured questionnaire. The age at onset of asthma was judged as possible and used to calculate the duration of asthma. In case of uncertainty, the earliest respiratory symptoms were taken into account.

Pulmonary function tests were performed at least 12 h after discontinuation of long-acting β$_2$-agonists (if possible). First, slow inspiratory VC was measured, and this was followed by measurement of FEV_1 (20). FEV_1/VC was assessed before and 30 min after the administration of 400 μg salbutamol and 80 μg ipratropium bromide (22). Reversibility of FEV_1 was defined as FEV_1 postbronchodilator $-$ FEV_1 baseline/FEV_{1pred} (20). RV and TLC were measured with the multibreath helium equilibration method (20), and carbon monoxide diffusing capacity (DLCO), expressed as the transfer coefficient (KCO), was measured with the single-breath method (23). All lung function parameters were expressed as percentages of predicted values (20). Persistent airflow limitation was defined as a postbronchodilator FEV_1 or $FEV_1/VC < 75\%$ predicted with a TLC $> 75\%$ predicted.

Airway responsiveness to histamine (expressed as the provocative dose of histamine causing a 20% decline in FEV_1 [PC_{20}histamine])

(21) and exhaled NO levels (24) were measured according to standardized procedures.

Atopic status was assessed by radioallergosorbent test (RAST) and total IgE by fluorescence enzyme immunoassay (both UniCAP; Pharmacia & Upjohn, Uppsala, Sweden). Eosinophils in blood were measured with a standard automated cell counter.

Sputum was induced and processed according to a standardized protocol (25), with some modifications for safety reasons (26). In brief, saline solutions were inhaled three times for 5 min each, with frequent monitoring of FEV_1. Depending on baseline FEV_1, the induced decrease in FEV_1, and the symptoms; isotonic saline only, or increasing saline concentrations of 0.9%, 3.0%, and 4.5%, were used.

Statistical Analysis

Adult-onset asthma was defined as asthma having an onset at ≥ 18 yr of age, with a cutoff of 18 yr being the median age at onset for the whole group. PC_{20}histamine was limited to 8.0 mg/ml if a decrease in FEV_1 of $\geq 20\%$ was not reached at the highest dose of histamine given (8 mg/ml).

In the logistic regression analyses, the following contrasts in potentially associated factors were considered for patients with and without persistent airflow limitation: age at onset of asthma ≥ 18 yr versus < 18 yr, ex-smoker versus never-smoker, atopic versus nonatopic status, reversibility of $FEV_1 \geq 9\%$ versus $< 9\%$ (27), PC_{20}histamine ≤ 1.0 mg/ml versus > 1.0 mg/ml (28), exhaled NO ≥ 10 ppb versus < 10 ppb (29), peripheral blood eosinophil count $> 450 \times 10^6$/L versus $\leq 450 \times 10^6$/L, total IgE > 100 IE/ml versus ≤ 100 IE/ml, sputum eosinophil percentage $\geq 2\%$ versus $< 2\%$ (30), and sputum neutrophil percentage $\geq 64\%$ versus $< 64\%$ (30).

Odds ratios (OR) for patients with persistent airflow limitation versus those without persistent airflow limitation as a reference group, were obtained by multiple logistic regression analyses with one of the factors described earlier as independent at a time in the model, and corrected for possible confounders (age, gender, and asthma duration). In addition, a full multiple logistic regression analysis was applied, with all significant factors forced into the model. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Patient Characteristics

One hundred thirty-six patients with severe asthma participated in the study. Sixty-six patients (48.5%, 95% confidence interval [CI]: 40% to 57%) had persistent airflow limitation according to our definition, whereas the other 70 patients (51.5%) had postbronchodilator FEV_1 and FEV_1/VC values of $> 75\%$ predicted (Table 1). A significantly older age (mean: 49 yr versus 42 yr) and longer duration of asthma (median: 26.5 yr versus 15 yr) were found in the patients with persistent airflow limitation as compared with those without this. In addition, the predominance of female sex tended to be less pronounced in the patients with persistent airways obstruction (62.1% versus 77.1%). The level of asthma control as measured with exercise tolerance, degree of nocturnal symptoms, and use of rescue medication did not differ in the groups with and without persistent airway obstruction. Only the number of exacerbations during the past year was significantly lower in the group with persistent airflow limitation. The patients with persistent airflow limitation also had higher levels of RV/TLC than did the patients without airways obstruction.

Factors Potentially Associated with Persistent Airflow Limitation

Data for sputum cell counts, reversibility of obstruction, and airway responsiveness were not available for all patients because of a low prebronchodilator FEV_1 or the inability of patients to discontinue their bronchodilators without having severe symptoms or inability to complete the sputum induction procedure and/or to cough up an adequate sputum sample.

Sputum induction was performed in 92 patients, and an adequate sputum sample was obtained from 66 (72%) of these patients. Data on reversibility and airway responsiveness could be obtained for 95% and 45% of the patients, respectively. The number of patients who were able to produce an adequate sputum sample did not differ between the groups without persistent airways obstruction and those with persistent airflow limitation, (34 versus 32 patients, respectively; p = 0.9); however, with regard to airway responsiveness, a difference in available PC_{20} data was found (39 patients in the group without airflow limitation versus 21 patients in the group with persistent obstruction; p = 0.01).

Table 2 demonstrates data for the clinical and inflammatory factors that may have been related to persistent airflow limitation for both study groups. Sixty-seven percent of the patients with persistent airflow limitation had a PC_{20}histamine ≤ 1.0 mg/ml, as compared with 33% of the patients without persistent airflow limitation (crude OR = 4.0; CI: 1.3 to 12.3). In addition, sputum eosinophilia was found in 69% of the patients with persistent airflow limitation versus 26% of those without airflow limitation (crude OR = 6.1; CI: 2.1 to 17.8). Furthermore, 58% of the patients with irreversible airways obstruction had an increased level of total IgE, which tended to be more than the 41% observed in the control group (crude OR = 1.9; CI: 0.95 to 3.8). For the other factors, no significant differences between the groups were found.

After adjustment for age, sex, and asthma duration, it appeared that the patients with persistent airflow limitation had a greater adjusted OR for sputum eosinophils $\geq 2\%$ and for a PC_{20}histamine ≤ 1.0 mg/ml, of 7.7 and 3.9, respectively, than did those without airflow limitation (Table 3). Furthermore, the patients with adult-onset asthma had a more than threefold greater risk for the development of persistent airways obstruction for a given time and gender than did the patients with early-onset asthma. For the other factors, no significantly increased OR were found.

When we analyzed the significant factors reported above in a single model, sputum eosinophilia appeared to be the only independent factor (adjusted OR = 8.9; CI: 1.3 to 59.0) associated with persistent airflow limitation. For a PC_{20}histamine ≤ 1.0 mg/ml (adjusted OR = 3.0; CI: 0.5 to 19.0) and adult onset of asthma (adjusted OR = 2.7; CI: 0.5 to 53.0), we found no significantly increased independent OR.

When repeating the regression analysis in the small subgroup of patients receiving oral corticosteroids on a regular basis (n = 45), we observed no significantly increased OR for adult onset of asthma (adjusted OR = 7.0; CI: 0.4 to 130.3), for a PC_{20}histamine ≤ 1.0 mg/ml (adjusted OR = 0.6; CI: 0.02 to 25.9), or for sputum eosinophilia (adjusted OR = 2.4; CI: 0.3 to 18.9).

DISCUSSION

In the present study, about half of the nonsmoking patients with severe asthma appeared to have persistent airflow limitation despite extensive antiasthma treatment. Apart from being associated with older age and a longer duration of asthma, persistent airflow limitation was strongly associated with sputum eosinophilia and AHR, with OR of 7.7 and 3.9, respectively. Furthermore, the patients with adult-onset asthma had more than a threefold greater risk of persistent airflow limitation for asthma of a given duration than did the patients with severe asthma that began before the age of 18 yr. Sputum eosinophilia was the only independent factor associated with persistent airflow limitation, with an adjusted OR of 8.9. These findings emphasize the high prevalence of persistent

TABLE 1. CHARACTERISTICS OF 136 SEVERELY ASTHMATIC PATIENTS WITH AND WITHOUT PERSISTENT AIRFLOW LIMITATION

	Persistent Airflow Limitation ($n = 66$)		No Persistent Airflow Limitation ($n = 70$)		p Value
Male gender, %	37.9		22.9		0.056
Age, yr*	49.3	± 13.7	41.7	± 14.0	0.002
Asthma duration, yr[†]	26.5	(2–73)	15.0	(2–57)	0.002
Ex-smoker, %	43.9		34.3		0.25
Dose of inhaled corticosteroids, $\mu g/d$[†]	1,600	(1,600–6,400)	1,600	(1,600–4,800)	0.09
Maintenance oral steroids, %	34.8		30.4		0.58
Exercise tolerance, WHO class[†]	2	(0–5)	2	(0–5)	0.87
Nocturnal symptoms, nights/wk[†]	1	(0–7)	2	(0–7)	0.37
Rescue β_2-agonist use, puffs/d[†]	2	(0–8)	5	(0–10)	0.10
Exacerbations in last yr, number ($n = 91$)[†]	1	(1–7)	3	(1–7)	0.01
Baseline FEV_1, %pred*	46.5	± 14.5	84.9	± 17.6	< 0.001
Postbronchodilator FEV_1, %pred*	57.7	± 14.8	96.4	± 14.4	< 0.001
Baseline FEV_1/VC, %pred*	57.6	± 13.0	88.6	± 13.7	< 0.001
Postbronchodilator FEV_1/VC, %pred*	64.6	± 14.7	94.7	± 10.3	< 0.001
RV/TLC, %pred*	130.2	± 29.3	101.6	± 22.9	< 0.001
Kco, %pred*	90.2	± 16.4	90.9	± 15.6	0.82

Definition of abbreviations: Kco = diffusion constant for carbon monoxide; WHO = World Health Organization.
* : mean $\pm$ SD; [†] : median (range).

airflow limitation in severe asthma, and suggest that ongoing airway inflammation underlies the development of persistent airway narrowing in patients with this condition.

To our knowledge, this is the first study exploring the prevalence of persistent airflow limitation in nonsmoking patients with severe asthma, and of the factors that might be associated with its development. Although airway obstruction with incomplete reversibility in asthma has been observed by many investigators (1, 3), its exact prevalence is unknown. From our data it appeared that in a population of severely asthmatic patients regularly supervised by pulmonologists and treated with high doses of corticosteroids and long-acting bronchodilators, the prevalence of persistent airflow limitation was as high as 49%. In another study, in moderate to severe asthma, only 23% of the patients exhibited incomplete reversibility of airways obstruction (31). The difference in prevalence of incompletely reversible airway obstruction in this study as opposed to our study is likely to have been due to the inclusion of patients with milder disease in this other study and fits with the hypothesis that persistent airflow limitation in asthma is related to more severe disease.

In the present study, the risk of persistent airway obstruction for a given asthma duration was about threefold greater in patients with adult-onset asthma, a clinical subclass of patients suggested to have more severe asthma (32). Moreover, persistent airflow limitation was associated with increased

AHR and sputum eosinophilia. The severity of AHR has been shown to be an independent predictor of the annual decline in lung function in the general population (33), but in asthma, this is still a controversial issue. In mild and moderate asthma, a positive correlation has been found between the degree of AHR and the rate of decline in FEV_1 (3), whereas in another longitudinal study of young adults, no such association could be found (12). Since increased AHR might be related to airway geometric factors (34), the question remains of whether AHR in severe asthma is not also a consequence of impaired lung function.

In our study, the only independent factor associated with persistent airflow limitation was sputum eosinophilia. In one other study, it was shown that asthmatic patients with persistent eosinophilic airway inflammation despite treatment with oral corticosteroids also had an increased thickness of the subbasement membrane associated with increased concentrations of transforming growth factor (TGF)-β in bronchial biopsy specimens (35). Taken together, these findings support the hypothesis that uncontrolled inflammation in asthma of a specific kind causes structural changes in the airway wall and eventually leads to persistent airflow limitation.

Remarkably, the patients with persistent airflow limitation, although clearly distinct with respect to sputum eosinophil percentages, were not different with regard to symptoms, and even had less asthma exacerbations than did those without

TABLE 2. COMPARISON OF CLINICAL AND INFLAMMATORY FACTORS BETWEEN SEVERELY ASTHMATIC PATIENTS WITH AND WITHOUT PERSISTENT AIRFLOW LIMITATION

	Persistent Airflow Limitation ($n = 66$)		No Persistent Airflow Limitation ($n = 70$)		p Value
Age at onset asthma, yr[†]	14.5	(0.5–60)	17.5	(0.5–68)	0.41
Pack-yr,[†]	0	(0–10)	0	(0–10)	0.45
Atopic, %	57.6		58.6		0.99
Reversibility FEV_1, % change[†]	10.6	(2.1–32.7)	9.9	(−1.1–35.9)	0.83
PC_{20}histamine, mg/ml[†]	0.19	(0.02–8.0)	2.50	(0.02–8.0)	0.002
NO, ppb[†]	11.1	(2–76)	8.4	(2–202)	0.35
Total IgE, IE/L[†]	109	(2–4,448)	78.5	(2–3,544)	0.21
Blood eosinophil count, $\times 10^6$/L[†]	158	(0–1,350)	200	(10–1,290)	0.34
Sputum eosinophils, %[†]	3.2	(0–54.5)	0.5	(0–59.4)	0.005
Sputum neutrophils, %[†]	66.3	(23.0–97.4)	64.3	(11.6–95.2)	0.39

Definition of abbreviation: PC_{20}histamine = provocative concentration of histamine causing a 20% decrease in FEV_1.
* : mean $\pm$ SD; [†] : median (range).

TABLE 3. ODDS RATIOS FOR FACTORS POTENTIALLY ASSOCIATED WITH PERSISTENT AIRFLOW LIMITATION IN SEVERE ASTHMA

	Adjusted OR*	95% CI
Sputum eosinophils $\geqslant$ 2%	7.7	(2.4–25.1)
PC_{20}histamine $\leqslant$ 1.0 mg/ml	3.9	(1.2–13.0)
Adult onset of asthma	3.3	(1.2–9.0)
Exhaled NO $\geqslant$ 10 ppb	1.9	(0.8–4.8)
Reversibility of FEV_1 $\geqslant$ 9%	1.7	(0.8–3.6)
Total IgE > 100 IE/ml	1.7	(0.8–3.7)
Blood eosinophil count > 450 $\times$ 10^6/L	1.5	(0.6–3.6)
Sputum neutrophils $\geqslant$ 64%	1.4	(0.5–4.0)
Ex-smoker	1.3	(0.6–2.9)
Atopic	0.8	(0.4–1.9)

Definition of abbreviations: CI = confidence interval; OR = odds ratio; PC_{20}histamine = provocative concentration of histamine causing a 20% decrease in FEV_1.

* OR adjusted for age, sex, and asthma duration.

permanent airway obstruction. This suggests that there is little association between symptoms on the one hand and lung function or airway inflammation on the other. These features probably represent different aspects of the same disease.

The results of this study might have been influenced by our selection of patients with severe asthma. We investigated outpatients with severe asthma who were visiting chest physicians in general hospitals in a Western European country. It cannot be excluded that in other patients with severe asthma (e.g., those with a history of near-fatal asthma) or patients selected from hospital admissions for asthma, the prevalence of persistent airflow limitation may be different. Neither can it be excluded that patients with severe asthma who reside in other parts of the world, with different environmental trigger factors, might show different lung function characteristics. The diagnosis of "severe asthma" in our study was based on the judgement of a pulmonologist who had supervised the patient for more than 1 year. We cannot fully exclude that some patients might have been misdiagnosed as having severe asthma and had other (associated) conditions with asthmalike symptoms, such as vocal-cord dysfunction. However, the incorporation in the study inclusion criteria of a reversibility in FEV_1 of > 12% and/or a PC_{20}histamine of < 8 mg/ml, documented either recently or in the past, strongly supports the diagnosis of asthma. We defined persistent airflow limitation as an FEV_1 or FEV_1/VC < 75% predicted after bronchodilation with high doses of salbutamol and ipratropium bromide. It can be argued that this postbronchodilator FEV_1 is not the maximal attainable FEV_1, since we did not include an oral steroid trial in our study. However, the patients in our study were already taking high doses of inhaled and/or oral corticosteroids and had had at least one course of oral steroids in the year preceding the study. Furthermore, lung function was measured during a clinically stable period and was very similar to lung function data that were available from previous investigations. Therefore, we believe that the postbronchodilator FEV_1 for our patients closely approached the maximal attainable FEV_1. Still, we cannot exclude the possibility that a longer course or higher doses of systemic corticosteroids might have further improved our patients' FEV_1. Furthermore, the results of our study might have been biased by missing data on airway responsiveness or sputum cell counts for some of the patients. Data on airway responsiveness were missing for the patients with the lowest FEV_1 values. We therefore believe that such bias, if any, would have led to an underestimation of the strength of the association of persistent airflow limitation with sputum eosinophilia and AHR. Missing sputum data are inevitable in a group of patients with severe asthma and might have influenced our results. However, there were no differ-

ences with respect to lung function measurements between the patients who did and those who did not produce an adequate sputum sample.

We found that persistent airflow limitation occurred about eight times more often in severely asthmatic patients with increased percentages of sputum eosinophils than in those with less than 2% eosinophils in their sputum. The concept that eosinophils may be active contributors to the development of fibrosis has been suggested previously in the literature. Eosinophils are capable of stimulating fibroblast replication *in vitro* (36, 37) and have been shown to be a major source *in vivo* of TGF-β_1 (38), a cytokine assumed to play a role in fibrotic changes in asthmatic airways. Our results fit with such a link between chronic eosinophilic inflammation and structural changes in the airways.

Several possible mechanisms can be used to explain the persistence of sputum eosinophilia despite treatment with high doses of inhaled and/or oral steroids. The eosinophilia might be due to relative undertreatment of the asthmatic inflammatory process or to more severe or active airway inflammation (39), with subsequent development of edema of the airway wall and airway closure. Sputum eosinophilia might also reflect ongoing inflammation in small airways (40) that are inaccessible or barely accessible to inhaled therapy. Favoring this latter hypothesis is the finding in our study that the association between sputum eosinophilia and persistent airflow obstruction was not apparent in the subgroup of patients taking oral corticosteroids. The persistence of sputum eosinophilia might also be a feature of glucocorticoid resistance or reduced glucocorticoid receptor binding affinity (41). On the other hand, ongoing eosinophilic inflammation despite extensive antiasthma treatment might be caused by poor inhalation technique or noncompliance with treatment and might also be attributed to unrecognized aggravating factors such as nasal polyposis (42).

The hypothesis that airway remodeling and the resulting airflow limitation are driven by ongoing inflammatory processes might have important implications for the prognosis and treatment of patients with asthma. Although persistent airflow limitation occurs in only a minority of asthmatic patients, its unfavorable influence on asthma prognosis (9) makes it essential to identify patients at increased risk for such airflow limitation. In view of the strong association between sputum eosinophilia and loss of lung function in our patients, there is the need to establish whether treatment adjustments based on monitoring of eosinophilic inflammation might prevent the development of persistent airflow limitation in patients with asthma.

In conclusion, we have shown that persistent airflow limitation is a frequent phenomenon in nonsmoking patients with severe asthma, despite extensive antiasthma treatment. Factors associated with persistent airflow obstruction are adult onset of asthma, increased AHR, and, most importantly, sputum eosinophilia. These findings suggest that persistent airflow limitation in severe asthma is a result of ongoing airway inflammation, thereby identifying an area for potential intervention in this disease.

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