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Difficult-to-treat asthma : mechanisms and risk factors

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Chapter 3

Deficient alpha-1-antitrypsin phenotypes and persistent airflow limitation in severe asthma

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Abstract

Background: Persistent airflow limitation is common among patients with severe asthma, but its pathogenesis has not been fully clarified. Severe alpha-1-antitrypsin (AAT) deficiency is a risk factor of chronic airflow limitation and emphysema, and partially deficient phenotypes have been associated with an accelerated decline in lung function. We hypothesized that partial deficiency of AAT (non-PiM AAT phenotype) is a risk factor of persistent airflow limitation in asthma.

Methods: In 122 patients with severe asthma (86 females; age (median (range)): 44.0 yr (18-75)) postbronchodilator FEV₁ and FEV₁/VC were measured and the AAT phenotype was determined. Persistent airflow limitation was defined as postbronchodilator FEV₁ or FEV₁/VC < 75% pred. with TLC > 75% pred.

Results: Six patients (4.9%) had a non-PiM phenotype (1 MF, 3 MS, 1 MZ and 1 SZ). Of the 58 patients with persistent airflow limitation only one patient (1.7%) had a non-PiM phenotype vs. 7.8% among the patients without persistent airflow limitation (p=0.21). Postbronchodilator FEV₁/VC (% pred.) was higher in the non-PiM patients than in the PiM patients (p=0.02), the other lung function parameters were not different. Linear regression analysis showed no association between AAT phenotype and FEV₁% predicted (p=0.26).

Conclusions: Alpha-1-antitrypsin heterozygosity does not seem to be an important risk factor of persistent airflow limitation in patients with asthma. Although confirmation by longitudinal follow-up studies with larger sample sizes is needed, these results suggest that routine assessment of the AAT phenotype is not indicated in asthmatic patients even if they exhibit fixed airflow limitation.

Introduction

Persistent airflow limitation is commonly found (up to 50%) in adult patients with severe asthma (1) and has been reported to be associated with overall mortality (2;3). Patients with asthma have a faster decline in FEV₁ than healthy subjects (4). Several factors have been suggested to contribute to the decline in lung function in asthma, including smoking, adult onset of asthma (1;5-7), severe airway hyperresponsiveness (1;8;9) and persistent eosinophilic airway inflammation (1;5;10;11). However, there might be additional factors to be taken into consideration.

Alpha-1-antitrypsin (AAT) is an inhibitor of leukocyte proteases, and deficiency of this serpin is known to predispose to the development of chronic airflow limitation and emphysema (12-14). Not only the homozygous PiZZ-AAT phenotype, but also the PiMZ phenotype has been related to a more rapid decline in FEV₁ in patients with chronic airflow limitation (15). In addition, alpha-1-antitrypsin heterozygous phenotypes have been shown to be associated with bronchial hyperresponsiveness (16;17) and asthma symptoms are common among patients with severe AAT-deficiency (18-20).

In the present study we hypothesized that a heterozygous (non-PiM) AAT phenotype is a risk factor of persistent airflow limitation in patients with asthma. To test this hypothesis we assessed the prevalence of a non-PiM phenotype in patients with severe asthma with or without persistent airflow limitation, compared lung function parameters between PiM and non-PiM phenotypes, and analysed whether a non-PiM phenotype was associated with a more impaired lung function as compared to a PiM AAT phenotype.

Methods

Subjects

A cohort of 136 patients with severe asthma who were participating in studies aimed at identifying clinical phenotypes of severe asthma (1) was recruited in 1998 and 1999. Of these, blood samples of hundred twenty-two patients were available for analysis in the current study. The patients were consecutively enrolled from the outpatient pulmonary departments of 2 teaching and 8 non-teaching hospitals in the western part of the Netherlands.

They had a history of episodic dyspnea and wheezing, a documented (recently or in the past) reversibility in FEV₁ of >12 % predicted and/or hyperresponsiveness to inhaled histamine.

They were treated with inhaled corticosteroids ($\geq 1600 \mu\text{g}/\text{day}$ beclomethasone or equivalent) and long-acting bronchodilators for more than one year. Thirty-eight out of 122 patients were on daily oral prednisone $\geq 5 \text{ mg}/\text{day}$. All patients were symptomatic and had at least one severe exacerbation during the past year requiring a course of oral corticosteroids. All patients were non-smokers, and 38.5% had smoked in the past (maximum allowed smoking history of 10 packyears). At the time of study-entry patients had to be clinically stable for at least one month and not have used a course of oral steroids or antibiotics in this month previous to the study. The study was approved by the Ethics Committee of the Leiden University Medical Center.

Design

On one single day, lung function measurements were performed, blood samples were drawn, and a short questionnaire was taken. This questionnaire was used to assess patient characteristics such as severity of symptoms, medication usage and duration of asthma. The latter was estimated from the first attack of dyspnoea or wheezing ever.

Measurements

Alpha-1-antitrypsin phenotyping

AAT-phenotype was determined by analysis of serum samples using iso-electric focusing.

Lung function

FEV₁ and slow vital capacity (sVC) measurements were performed before and 30 minutes after inhalation of 400 μg salbutamol and 80 μg ipratropium bromide through a volume spacer according to standard methods, using a dry rolling seal spirometer. Functional residual capacity (FRC) and total lung capacity (TLC) were measured by the multiple breath helium equilibrium method and carbon monoxide diffusing capacity (KCO) by the single breath method. Predicted values were obtained from Quanjer and co-workers (21;22). Persistent airflow limitation was defined as postbronchodilator FEV₁ or FEV₁/VC <75% predicted with TLC >75% predicted.

Analysis

Non-parametric tests and Chi-square analysis were used to assess differences in patient characteristics between patients with a PiM-phenotype and a non-PiM phenotype. Logistic regression analysis was used to assess the association between a (non-) PiM phenotype and the presence or absence of persistent airflow limitation (dependent factor) corrected for age, gender, and duration of asthma. Multivariate linear regression analysis was used to determine the association between postbronchodilator FEV₁ and FEV₁/VC % predicted and AAT phenotype (corrected for age, sex and duration of asthma). The relationship between

asthma duration and postbronchodilator FEV₁ (%pred) and FEV₁/VC (%pred) was investigated by linear regression analyses and Pearson's correlation coefficients. Slopes of the regression lines (β : estimated regression weight) were interpreted as the estimated annual decline in lung function (23). All analyses were performed using the Statistical Package of the Social Sciences (SPSS-11.0). P-values less than 0.05 were considered statistically significant.

Results

Prevalence of AAT phenotypes

Characteristics of the participating patients are summarized in Table 1. Amongst the 122 patients with severe asthma 6 patients (4.9%, CI: 1.0-8.8%) with a non-PiM AAT phenotype were identified: 1 MF, 3 MS, 1 MZ and 1 SZ.

Persistent airflow limitation

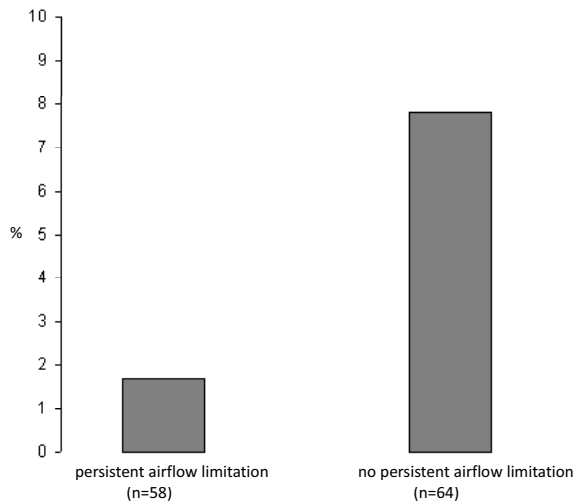
Fifty-eight patients (47.5%, CI: 39-57%) were diagnosed with persistent airflow limitation. In this group only 1 patient (1.7%) had a non-PiM phenotype, compared to 5 out of 64 patients (7.8%) in the group without persistent airflow limitation (Figure 1). There was no association between the presence of a non-PiM phenotype and the presence of persistent airflow limitation (corrected for age, gender and asthma duration), $p=0.22$ (OR 0.24 (CI:0.02-2.31)).

Table 1. Patient characteristics according to Pi-type.

	non-PiM (n=6)	PiM (n=116)	p-value
age (y)	37.0 (18-56)	44.0 (19-75)	0.17
gender (%female)	66.7	70.7	1.00
asthma duration (y)	13.5 (6-56)	18.5 (2-73)	0.36
pb-FEV ₁ (%pred)	96.5 (42.2-132.3)	79.9 (22.4-140.4)	0.12
pb-FEV ₁ /VC (%pred)	105.4 (56.4-111.9)	81.9 (29.3-114.6)	0.02
RV/TLC (%pred)	96.0 (66.7-114.3)	110.7 (37.3-192.5)	0.16
KCO (%pred)	98.6 (60.0-121.5)	90.2 (49.5-125.0)	0.24

Measurements in non-PiM and PiM resp.: FEV₁ in 6 vs. 116 pat., RV/TLC in 4 vs. 109 pat., KCO in 6 vs. 101 pat. Data expressed as median (range), pb=postbronchodilator

Figure 1. Prevalence of a non-PiM phenotype in patients with and without persistent airflow limitation.



PiM versus non-PiM

There was no significant difference in age, sex and duration of asthma between the 6 patients with a non-PiM phenotype and the 116 patients with a PiM phenotype (Table 1). The postbronchodilator FEV₁/VC as percentage of predicted was higher in the patients with the non-PiM phenotype than in the PiM-group (median (range):105.4% (56.4-111.9) vs. 81.9% (29.3-114.6), p=0.02). There was no significant difference between the two groups in FEV₁%predicted (non-PiM vs. PiM: 96.5% (42.2-132.3) and 79.9% (22.4-140.4) resp., p=0.15). Multiple linear regression analysis, corrected for possible confounders such as age, gender and asthma duration, showed no association between AAT phenotype and FEV₁%predicted (p=0.26) and a trend towards an association between a non-PiM phenotype and a higher FEV₁/VC%pred (p=0.07).

Estimated decline in lung function

In the group of patients with the PiM-phenotype there was a significant negative association between postbronchodilator FEV₁%pred. and the duration of asthma (r = - 0.24, p = 0.01), with similar results for FEV₁/VC %pred. (r = - 0.30, β = - 0.34, p=0.001). The estimated decline in FEV₁ in the PiM group was 0.3% predicted per year (= 17 ml in FEV₁/yr). In the 6 patients with the non-PiM phenotype there was also a significant relationship between FEV₁ and duration of asthma (r = - 0.84, p=0.04). However when disregarding one outlier, this relationship was lost. Therefore, the rates of decline between the two groups were not compared.

Discussion

This study, performed in a large cohort of patients with severe asthma shows that the prevalence of a non-PiM alpha-1-antitrypsin phenotype is low (4.9%). Persistent airflow limitation is not associated with a non-PiM phenotype in these patients. This suggests that alpha-1-antitrypsin heterozygosity is not an important contributing factor to a rapid decline in lung function in asthmatic patients.

This is the first study investigating the non-PiM AAT phenotype as a risk factor of persistent airflow limitation in non-smoking asthmatic adults. We selected a well-characterized group of patients with severe asthma because we expected that if there would be an association between alpha-1-antitrypsin deficiency and persistent airflow limitation, it was most likely to be found in this group in whom persistent airflow limitation is a common finding.

The present study shows no association between AAT phenotypes and lung function in these severe asthmatic patients. These findings extend the findings of two previous studies investigating the relationship between AAT-phenotypes, asthma symptoms and decline in lung function. One study in the general population showed that a non-PiM phenotype is not associated with a more rapid decline in lung function (24), whereas another study in patients with severe AAT-deficiency found no association between the presence of asthmatic features and an accelerated decline in lung function (19). A recent population study, however, suggested that asthmatic children with reduced levels of AAT might be prone to develop airway hyperresponsiveness and reduced lung function (25). The reason for the discrepancy between this study and other studies might be the difference in the selection of patients on the basis of AAT levels or AAT phenotypes. Our study shows that the prevalence of the non-PiM phenotype in patients with severe asthma is comparable to the estimated prevalence in the Dutch population (MS in Holland 4,3%, MZ 2%, SZ 0,05%) (26) and fits in with the findings in a nonselected population of asthmatic patients in Spain (27).

The results of this study might be influenced by the selection of patients. We chose to study patients with severe asthma, because we expected that an association between lung function and AAT phenotype would be easier to detect in patients with severe asthma than in a random population sample. The sample of patients with severe asthma was recruited from 10 different outpatient clinics and can therefore be considered representative of the population of such patients within the Netherlands. We believe that if the selection of patients had biased the results of our study, this would have influenced the results towards a positive association rather than towards a lack of association between these two parameters. Another point of concern is the relatively small group of patients with a non-

PiM phenotype. Given the high prevalence of asthmatic patients with persistent airflow limitation (47.5%) we expected to find a much higher prevalence of non-PiM phenotypes. However, the finding that the prevalence of a non PiM phenotype was comparable to that of the normal population, supports the conclusion of the study that a heterozygous AAT phenotype is not a risk factor for the development of persistent airflow limitation in asthma. Nevertheless, due to the limited power of this study, the results need to be confirmed by a prospective longitudinal study using a larger asthma cohort in which the AAT phenotype can be related to decline in lung function over time.

In an attempt to estimate the decline in lung function over time, we calculated the change in FEV₁ in relation to the duration of asthma for all patients by regression analysis. Unfortunately, the results of the regression analysis in the non-PiM group were not reliable because of the small sample size and uneven distribution of the data. All patients with a non-PiM phenotype had a postbronchodilator FEV₁ within the normal range except for one outlier. This patient with a postbronchodilator FEV₁ of 46% predicted had a duration of asthma of 56 years. In view of the negative association between duration of asthma and postbronchodilator FEV₁ in the group as a whole this impaired lung function is not surprising. Still, we cannot exclude that partial AAT deficiency becomes a contributing factor to persistent airflow limitation if asthma persists for several decades. One interesting mechanism might be related to the elastase/ alpha-1-antitrypsin balance. Vignola et al. have demonstrated that the levels of elastase in induced sputum increase with the duration of asthma, resulting in a disturbance of the elastase/ alpha-1-antitrypsin balance (28). This might contribute to airway remodelling, in particular in patients with AAT deficiency. Taken together, it is most likely that the combination of genetic, environmental and intrinsic factors determines whether the decline in lung function exceeds normal physiological levels.

This study has both pathophysiological and clinical implications. The results suggest that persistent airflow limitation in asthma is not related to alpha-1-antitrypsin deficiency and subsequent development of emphysema. This is supported by the lack of any difference in CO-transfer factor between the patients with a PiM or non-PiM phenotype. Although the results clearly need confirmation in longitudinal studies using larger cohorts, the present study suggests that routine assessment of the AAT phenotype is not indicated in patients with asthma because it most likely does not provide additional insight into the phenotypic expression of the disease nor into the long-term prognosis.

In conclusion we have shown that a heterozygous (non-PiM) AAT phenotype is not likely to be a risk factor of persistent airflow limitation in patients with asthma. The prevalence of an abnormal AAT phenotype in patients with severe asthma is low, even in those with fixed airflow limitation. Since the aetiology of fixed airflow limitation in asthma is still far from clarified, the search for risk factors must continue.

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