Airway inflammation in asthma : from concept to the clinic

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Chapter
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Bronchial CD8 cell infiltrate and lung function decline in asthma

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Abstract

Background Patients with asthma have an accelerated decline in lung function, which can lead to irreversible airway obstruction. It is generally assumed that this is related to specific aspects of airway inflammation and/or remodelling. We investigated the prognostic significance of bronchial eosinophil and CD8+ cell counts and subepithelial reticular layer thickness for the subsequent decline in lung function in patients with asthma after 7½ years of follow-up.

Methods In a prospective study, pre- and post-bronchodilator lung function (FEV_1) was measured at baseline, after 2 years and 7½ years in 32 patients with asthma. Annual decline in lung function after 7½ years of follow-up was related to type and severity of airway inflammation and remodelling in bronchial biopsies, which were taken at baseline and at year 2.

Results Annual decline in post-bronchodilator FEV_1 (mean (SD) 46.6 (53.4) ml/yr) was significantly larger than the decline in pre-bronchodilator FEV_1 (mean (SD) 27.5 (62.5) ml/yr), indicating loss in reversibility. Whereas, annual fall in post-bronchodilator FEV_1 was not related to thickness of the reticular layer or to eosinophil counts in bronchial biopsies, there was a significant correlation with CD8 positive T-cells ($r=-0.39;p=0.032$). Analyzing the biopsies taken at year 2, the significant association between annual fall in post-bronchodilator FEV_1 and CD8 cells could independently be confirmed($r=-0.39;p=0.036$).

Conclusion The outcome of asthma, as determined by the annual decline in FEV_1, can be predicted by the bronchial CD8+ cell infiltrate. This suggests that the inflammatory phenotype in asthma has prognostic relevance, which may require phenotype-specific therapeutic strategies.

Introduction

Asthma is a chronic inflammatory disease that is characterized by variable airway obstruction to various inhaled stimuli (1). Although this is largely reversible in most patients, some asthmatics develop persistent non-reversible airway obstruction despite adequate treatment (2). Longitudinal studies have shown that adult patients with asthma have an accelerated decline in lung function (FEV_1) as compared to controls (3-5). However, the rate of decline demonstrates large variability between patients, which seems to be associated with disease duration, baseline lung function and airway responsiveness (6;7). Eventually, the lung function decline may progress to irreversible airway obstruction in a subgroup of patients with asthma (8).

The current working hypothesis is that chronic inflammation promotes restructuring of the airways, which in turn results in accelerated decline in lung function in some but not all asthmatics. Airway inflammation in asthma is characterized by infiltration of lymphocytes and eosinophils in the bronchial epithelium and lamina propria (9) and of mast cells in the smooth muscle layer (10). Due to the release of growth factors and other mediators, the infiltrate is thought to induce structural changes in the bronchial wall often referred to as tissue remodelling (11). Since this process begins early in the development of asthma, remodelling may occur in parallel or could even be required for the development of persistent inflammation (12). The features of airway remodelling in asthma include thickening of the sub-epithelial reticular layer, changes of the interstitial matrix composition, increases in blood vessel area, airway smooth muscle, goblet cells in the surface epithelium and number of mucous glands (11).

The prognostic significance of airway inflammation and remodelling for the decline in lung function is still unclear. In patients with chronic obstructive pulmonary disease (COPD) fixed airway obstruction is often found to be associated with bronchial CD8+ T cell infiltration (13;14). Cross-sectional studies in severe asthma have demonstrated that sputum and tissue eosinophil counts are associated with a lower lung function (8;15). Furthermore, in some (16;17), but not all studies (15), the thickness of the sub-epithelial reticular layer was inversely associated with the level of lung function in asthma. However, it remains questionable whether these cross-sectional associations hold after longitudinal follow-up.

We postulated that the type and severity of inflammation or remodelling in bronchial biopsies are predictive of the subsequent annual decline in lung function in patients with asthma. For this reason, we performed a prospective follow-up study in a previously reported group of patients with asthma (AMPUL-cohort) (18) who underwent repeated bronchoscopies and extensive clinical measurements at baseline. We aimed to investigate the relationship of bronchial eosinophil and CD8+ cell counts and the thickness of the sub-epithelial layer as measured at baseline with the subsequent annual decline in lung function after 7½ years of follow-up.

Methods

Subjects

75 Atopic patients with mild-moderate persistent asthma participated in the study (18). 45 Patients underwent a successful bronchoscopy at entry and 37 patients at t=2 years. At inclusion, all patients (18-50yr) were non- or ex-smokers (< 5 pack-years), all had symptoms of episodic chest tightness and wheezing, whilst 77% of patients was using regular inhaled steroids. Pre-bronchodilator forced expiratory volume in one second (FEV_1) was >50% of predicted and >1.5L, whilst post-bronchodilator was within the normal range (>80% predicted) (20). All patients were hyperresponsive to methacholine (provocative concentration causing 20% fall in FEV_1 (PC_{20}) <8mg/ml). The medical ethics committee of the Leiden University Medical Center approved the study and all participants gave written informed consent.

Design

In a prospective study design, pre- and post-bronchodilator FEV_1 and PC_{20} were measured at baseline, at years 2 and 7½. Bronchoscopies were performed at baseline and year 2. During the first two years patients were treated according to standardized guidelines, and treatment was adjusted by a chest physician every 3 months (18). In order to make this study representative for daily practice, the own physician of each patient was instructed to adjust treatment according to Dutch GINA-derived guidelines between 2 and 7½ years of follow-up.

Spirometry and airway responsiveness

Spirometry was performed according to the same procedures throughout the study (18). Patients withheld short-acting $ß_2$-agonists for 8 hours and long-acting $ß_2$-agonists for at least 24 hours prior to the measurements. Post-bronchodilator FEV_1 was measured 15 minutes following inhalation of 400 µg salbutamol (20). Airway hyperresponsiveness was determined using a methacholine challenge and was expressed as PC_{20}.

Bronchoscopy and immunohistochemistry

At baseline and after 2 years five bronchial biopsies were taken for electron and light microscopy from right lower lobe subsegments, the middle lobe and the main carina using a pair of cup forceps (Olympus FB-21C, Japan). Two biopsies were fixed immediately in Trump's fixative and ultra thin sections were processed for electron microscopy. The thickness of the sub-epithelial reticular basement membrane was determined by measuring area divided by length on electron microscopy pictures in 2-5 well oriented electron micrographs (X5700, 35x42mm), using computerized analysis(18). Three biopsies were immediately embedded in ornithyl carbamyltransferase medium and snap-frozen in isopentane. Immunohistochemistry was performed on 6mm cryostat sections. Sections were stained with monoclonal antibodies against EG2 (eosinophils) (Pharmacia, Sweden), and CD8+ cells (Becton Dickinson, USA).A validated method using computerized analysis was applied to examine the coded biopsy specimens(21). Two areas were selected and the number of positively stained cells was

determined in the lamina propria. Values were expressed as cells/0.1 mm^2. Detailed biopsy methods and cell number data, including AA1 (mast cells), CD3 and CD4+ cells, have been previously published(18).

Analysis

Post-bronchodilator FEV_1 was applied in the analysis in order to minimize the contribution of varying degrees of smooth muscle contraction to the level of airway obstruction. The decline in post-bronchodilator FEV_1 was determined between baseline and t=7½ years (FEV_1 at 7½ years – FEV_1 at baseline / 7½) and between t=2 and t=7½ years (FEV_1 at 7½ years – FEV_1 at 2 years / 5½) and was expressed as annual decline in ml/years. The declines in pre- and post-bronchodilator FEV_1 were compared using a paired t-test. Linear regression analysis was used to investigate the association between inflammation (EG2 and CD8 positive cells and reticular layer thickness) in bronchial biopsies and annual decline in post-bronchodilator FEV_1 during follow-up.

Results

Patient characteristics

Thirty-two of the 45 patients who underwent the bronchoscopy at baseline, participated at follow-up after 7½ years (71% response rate) [table 1]. The participating patients were not different from the non-participants with respect to disease severity, spirometry, reticular layer thickness, EG2 and CD8+ cells ($p>0.2$). In 30 of these 32 patients biopsies were also taken at year 2. The total follow-up period was 7.6 (0.6) (mean (SD)) years. At all three time points, about 70% of the patients were using inhaled steroids [table 2]. None of the patients were using long-acting $ß_2$-agonists at t=0 and t=2, compared to six patients at t=7½ years. Seven patients stayed under regular control of a chest physician, whereas 23 patients were treated by a general practitioner. Only 2 patients stopped using any asthma medication and were free of symptoms. During the follow-up period, 1 in 5 received treatment with one or more courses of oral corticosteroids. Two patients had become current smokers after 7½ years, whereas none smoked during the first two years. PC_{20} methacholine was <8 mg/ml in all patients at baseline, and in 28 of the 32 patients at t=7½ (range 0.02 to 16.3 mg/ml) [table 2].

Lung function decline

The mean pre- and post-bronchodilator FEV_1 in % predicted stayed within the normal range at all visits with considerable scatter [table 2]. The annual decline in pre-bronchodilator FEV_1 during follow-up was (mean (SD)) 27.5 (62.5) ml/yr, whereas the annual drop in post-bronchodilator FEV_1 was 46.6 (53.4) ml/yr [figure 1]. The variability in decline in post-bronchodilator FEV_1 between individual patients was large, ranging from an annual increase by 39 ml/yr to an annual fall by 149 ml/yr. The decline in post-bronchodilator FEV_1 was significantly larger than in pre-bronchodilator FEV_1 ($p=0.022$), indicating loss in reversibility [figure 1].

Table 1. Patient recruitment: flow chart

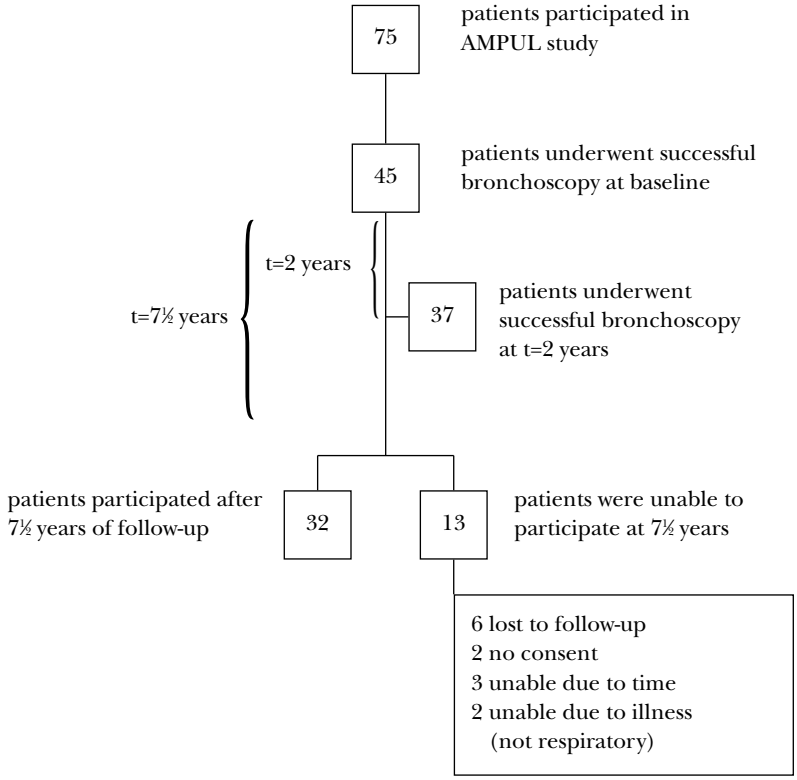

Table 2. Patient characteristics

	baseline	t=2 years	t=7½ years
age (years)	30.8 (8.9)		
follow-up (years)		2.0 (0.0)	7.6 (0.6)
inhaled steroids (% patients)	72%	75%	69%
pre-bronchodilator FEV_1 (% pred.)	87.2 (13.4)	86.3 (14.0)	84.7 (16.9)
post-bronchodilator FEV_1 (% pred.)	99.3 (11.0)	96.7 (12.6)	93.2 (15.8)
PC_{20} methacholine (mg/ml)*	0.67 (2.2)	0.88 (1.73)	0.91 (2.8)

Data in mean (SD); *geometric mean (SD in DD)

Prognostic significance of airway inflammation

The annual decline in post-bronchodilator FEV_1 during the follow-up period was not related to thickness of the bronchial subepithelial reticular layer at t=0 (r=-0.02; p=0.92) [figure 2]. In addition, the fall in post-bronchodilator FEV_1 during follow-up showed no correlation with eosinophils at baseline (r=0.02;p=0.90). On the other hand, the annual change in post-bronchodilator FEV_1 during the follow-up period of 7½ years was significantly and inversely correlated with the bronchial CD8+ cells at t=0 (r=-0.39;p=0.032). The slope of the linear regression analysis showed that for each doubling in CD8+ cells, post-bronchodilator FEV_1 declined with an additional 13.8 ml/yr. When

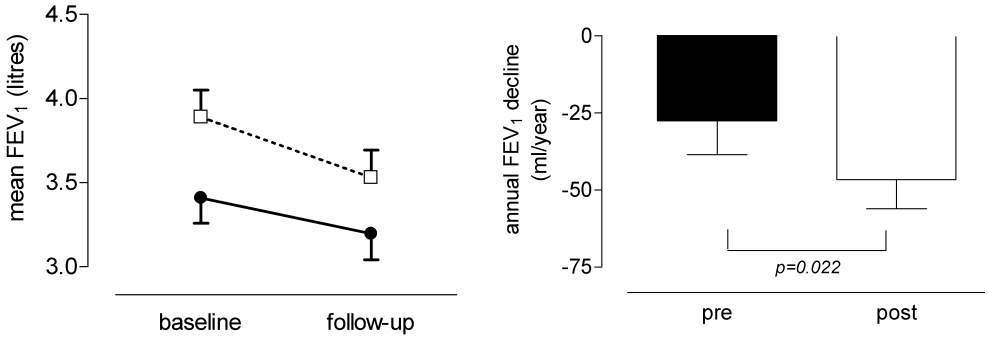

Figure 1. Left panel: mean pre-bronchodilator (closed circles) and post-bronchodilator (open squares) FEV_1 at baseline (t=0) and at follow-up (t=7½ years). Right panel: annual decline in FEV_1 from baseline to follow-up for pre-bronchodilator (black bar) and post-bronchodilator (white bar).

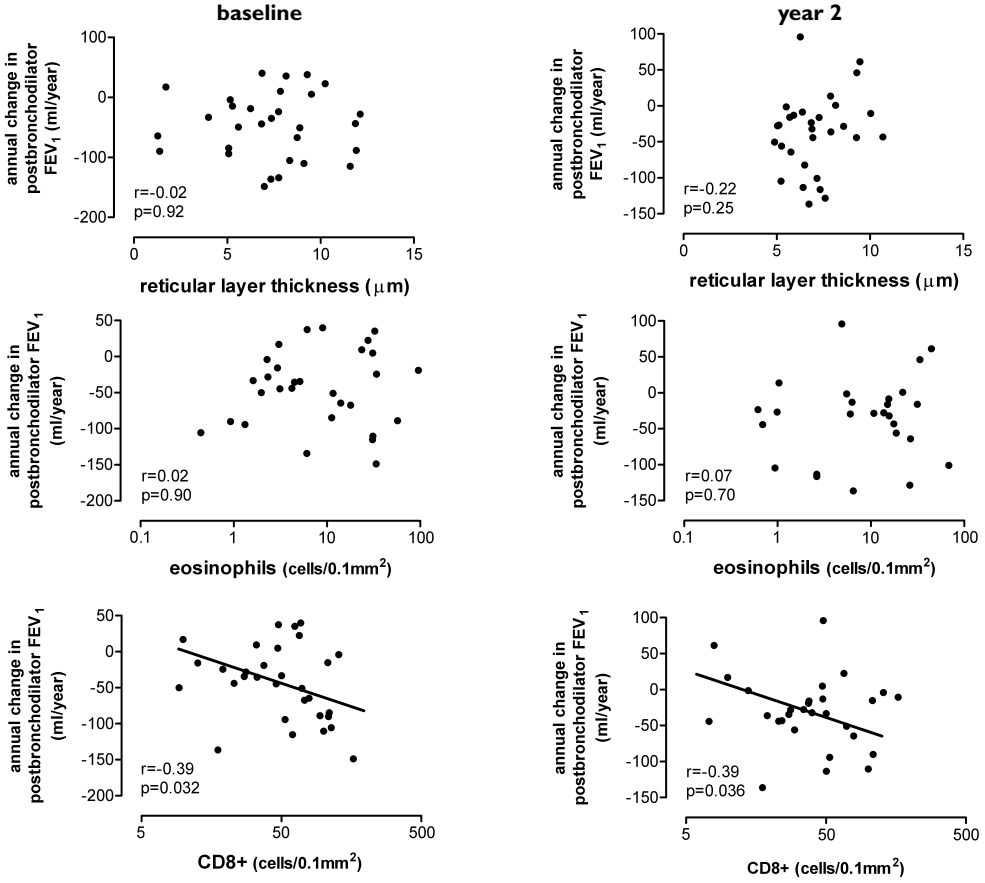

Figure 2. Left panel: associations of annual change (year 0 to 7½) in post-bronchodilator FEV_1 with reticular layer thickness, eosinophils and CD8+ at baseline. Right panel: associations of annual change (year 2 to 7½) in post-bronchodilator FEV_1 with reticular layer thickness, eosinophils and CD8+ at t=2 years.

repeating the analysis using the bronchial biopsies taken at 2 years these findings were entirely confirmed. There was a consistent, significant correlation of annual fall in post-bronchodilator FEV_1 with the number of CD8+ cells at t=2 years (r=-0.39;p=0.036), but not with bronchial eosinophils or reticular layer thickness [figure 2]. All other cell types (AA1, CD3 and CD4) demonstrated no significant associations with the annual change in FEV_1 (r<-0.20;p>0.28).

Discussion

The results of this study show that the number of CD8+ cells in bronchial biopsies in patients with asthma is associated with disease outcome, as determined by loss of lung function. Other markers of inflammation or remodeling were not related to the decline in lung function during follow-up. Furthermore, the loss in post-bronchodilator FEV_1 was significantly larger than the decline in pre-bronchodilator FEV_1. These findings indicate that CD8 cells can be predictive of disease outcome in asthma and therefore suggest that targeting specific elements of inflammation may be required when aiming to prevent the accelerated decline in lung function in patients with asthma.To our knowledge, this is the first longitudinal study showing the prognostic significance of type and severity of inflammation on the outcome of asthma. A cross-sectional relationship between CD8+ cells and the outcome of asthma has been observed in patients with fatal asthma (22). Recently, increased cytokine production of sputum CD8+ cells has been shown in patients with asthma that was related with disease severity (23). Interestingly, the association between lung function and CD8 cells has also been demonstrated in other diseases: not only by cross-sectional analysis in COPD patients (13), but also regarding decline in lung function in patients with systemic sclerosis (24). This shows that our longitudinal findings in asthma are in line with those in other inflammatory lung disorders.

The magnitude of the annual decline in lung function is in keeping with other longitudinal studies in patients with asthma and is higher as compared to the figures previously published for normals (normals: 22 ml/year and asthma: 38 ml/year) (5). Our results extent previous findings by demonstrating that the decline in post-bronchodilator FEV_1 is larger than the decline in pre-bronchodilator FEV_1. This puts emphasis on measuring post-bronchodilator FEV_1, as a ceiling of lung function, in prospective studies in asthma.

The present study design may have potential limitations. During the follow-up period of 7½ years, the patients were treated by their own physician as opposed to controlled standardized therapy. This may have introduced variability in asthma control, since some patients were seeing a chest physician regularly (22% of patients), whereas others had not been visiting their doctor for asthma symptoms at all (6% of patients). We consider this strategy to be representative for the daily practice. Prior to the baseline bronchoscopy the patients were also treated by their own physician, or were newly

diagnosed (23% of patients). Moreover, any variability in therapy may have led to a broader disease outcome, which is likely to be represented by the large range in annual decline in FEV_1. For that reason, we chose common asthma management as opposed to protocolized therapy during follow-up of this cohort.

It is unlikely that the present association between decline in FEV_1 and CD8+ cells, is due to chance. It was a consistent finding when using bronchial biopsies of two separate bronchoscopies two years apart. The first and second bronchoscopy differed in such way that the second biopsy was taken following 2 years optimal treatment according to the GINA guideline or management additionally based on airway hyperresponsiveness (18). This suggests that treatment level is not affecting the association between airway inflammation and lung function decline in asthma.

How can CD8 cells contribute to the accelerated, irreversible airway obstruction in asthma? In vitro studies have characterized CD8+ cells in regard to their cytokine production (Tc1 vs Tc2) and populations (effector vs memory) (25). Interestingly, a subset of antigen specific "non-lymphoid" memory CD8 T cell population, which can be isolated from several organs including the lungs, demonstrate a high lyctic activity and proliferate rapidly (26). Various antigens, like allergens and viruses can rapidly activate specific effector/memory T cells (27). In mice models, CD8 cells are required for the development of airway hyperresponsiveness following allergic sensitization (28) leading to increased inflammation (29). During respiratory virus infections, CD8 cells appear to be essential for the influx of eosinophils into the lung and the development of airway hyperresponsiveness in mouse models (30). Indeed, we have recently demonstrated that rhinovirus infection in asthmatic subjects is associated with accumulation of CD8 cells (31). Interestingly, antigen specific CD8 cells can persist in the lung for several months (32) and may also activate resident cells such as epithelial cells (33). Therefore, CD8 cells can induce potential conditions that are required for changes in airway structure, which eventually may lead to changes in airway structure. However, we cannot exclude the possibility that the association of CD8 cells with lung function decline is just an epiphenomenon and a marker of a complex immunopathologic pathway.

Eosinophils were not predictive for the decline in lung function in our study. Increased numbers of sputum and tissue eosinophils have been associated with persistent airway obstruction in patients with severe asthma (8;15). However, these conclusions were derived from cross-sectional data. Interestingly, it has been shown that elevated sputum eosinophil numbers may predict the short-term worsening of asthma as reflected by exacerbations (34). This suggests that the inflammatory profile may have distinct effects on short- and long-term disease outcome.

Remarkably, the thickness of the sub-epithelial reticular layer was not related to lung function decline either. This probably illustrates that restructuring of the airways as measured in large airway biopsies is not sufficient to represent other aspects of (small) airways remodeling (35). When sampling the latter in patients with COPD, Hogg et al.

recently did show an association between airway structure and lung function level (36). However, comparable data in asthma will not be readily available.

Our findings can have implications for clinical management and drug development. First, the consistent association between FEV_1 decline and CD8 cells even after 2 years of optimal standardized therapy in our study suggests that the current treatment strategies for asthma may not be effective in preventing or reversing the accelerated fall in lung function in patients with asthma. Second, even though the CD8 cell may just be a marker of another causative mechanism, the possibility to manipulate the presence and/or phenotype of CD8 cells should be considered. Glucocorticoids are able to induce a CD8 cell phenotype that is producing high levels of IL-10 and reduced levels of IL-4 and IL-5 (37). However, the effect of glucocorticoids modulation of CD8 cell cytokine production is much smaller as compared to CD4 cells (37). Therefore, the development of new interventions, specifically targeting CD8+ T cells, may need to be explored when aiming to prevent the persistent airway obstruction in asthma.

In conclusion, we have shown that outcome of asthma, as determined by the annual decline in FEV_1, can be predicted by bronchial CD8 cell infiltrate. CD8+ cells may have, as previously suggested in patients with COPD, a significant role in the clinical course of asthma. We could speculate that this requires phenotype-specific therapeutic strategies in order to prevent the accelerated decline of lung function in asthma.

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