Difficult-to-treat asthma : mechanisms and risk factors

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Chapter 7

Consistency of sputum eosinophilia in difficult-to-treat asthma: a 5-year follow-up study

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Abstract

Background: Eosinophilic airway inflammation that persists despite vigorous anti-inflammatory treatment has been associated with severe asthma and fixed airflow limitation, possibly related to airway remodelling. It is unknown whether eosinophilic airway inflammation is a consistent feature over time and if so, which clinical characteristics are associated with permanent eosinophilic inflammation.

Objective: To assess consistency of sputum eosinophilia (≥ 2%) over 5 years and identify potential predictors of permanent sputum eosinophilia (sputum eosinophils ≥ 2% on two occasions).

Methods: sputum samples of 44 patients with difficult-to-treat asthma (25f; mean (sd) age 49.5 (11.7) y; 64% atopic, FEV_1 75.8 (22.2) %predicted; 30% on oral steroids) were analysed on two time points (in 1998-1999 and in 2004-2005). The association between permanent sputum eosinophilia and clinical characteristics (asthma duration and onset, atopy, number of exacerbations, PC_{20}histamine, postbronchodilator FEV_1 and sinus disease) and markers of airway inflammation (exhaled nitric oxide, blood eosinophils) were analysed.

Results: 70% of patients (14 out of 20) with initial sputum eosinophilia had eosinophils ≥ 2% on both time points, and 96% of patients (23 out of 24) had sputum eosinophils <2% on both occasions. The intraclass correlation coefficient of sputum eosinophils was 0.67. Of the investigated factors, only extensive sinus disease was independently associated with permanent sputum eosinophilia, OR(CI):9.2(1.1-75.4).

Conclusions: The presence of sputum eosinophilia despite high dose steroid treatment is a persistent phenotypic feature in the majority of patients with difficult-to-treat asthma. Severe sinus disease is strongly associated with long-lasting eosinophilic inflammation of the lower airways.

Introduction

Patients with difficult-to-treat asthma represent a heterogeneous population characterized by differences in clinical presentation, lung function impairment and type of airway inflammation (1,2). According to the type of inflammation in the airways several asthma phenotypes have been described, including the eosinophilic and non-eosinophilic phenotype (3-6). Eosinophilic airway inflammation that persists despite high doses of inhaled or oral corticosteroids, the so called "persistent eosinophilic phenotype" (5), has been associated with life threatening exacerbations, remodelling of the airways and persistent airflow limitation (5-8) in cross-sectional studies. Apparently, patients with persistent eosinophilic airway inflammation represent a specific subgroup, exhibiting asthma at the most severe end of the disease spectrum.

There remain, however, several unanswered questions regarding the patients with persistent eosinophilia. First, it is not known whether in difficult-to-treat asthma persistent eosinophilia is a consistent feature within the same subject over the years, and if so, whether patients with permanent eosinophilic airway inflammation exhibit different clinical characteristics as compared to other patients with difficult-to-treat asthma.

The answers to these questions will determine whether a single measurement of sputum eosinophilia in patients with difficult-to-treat asthma who receive high doses of corticosteroids can predict the type of inflammation over subsequent years, and whether there are any clinical characteristics that have additional predictive value. This is important for clinicians, in order to detect subtypes of asthma that are at increased risk of poor outcome at an early stage, and for researchers, to develop targeted therapies for patients with the largest unmet needs.

Therefore, the aim of this study was to assess the consistency of sputum eosinophilia in patients with difficult-to-treat asthma over a time-period of 5-6 years and to investigate whether clinical characteristics (asthma duration and onset, atopy, asthma exacerbations, airway hyperresponsiveness, postbronchodilator FEV_1 and sinus disease) or inflammatory markers (exhaled nitric oxide, blood eosinophils) are associated with permanent sputum eosinophilia. In addition, because eosinophilic airway inflammation has been reported to be associated with elevated levels of nitric oxide in exhaled air (FeNO) (9,10) we assessed the stability of this marker.

Methods

Subjects

Data were collected from patients who participated in a longitudinal follow-up study aimed at identifying clinical phenotypes of severe asthma and risk factors of accelerated decline in lung function. Characteristics of these patients are described elsewhere in detail (8,11). In short, 136 patients with "difficult-to-treat asthma" as defined by a European Respiratory Society Task Force (12) recruited from 10 pulmonary outpatient clinics were enrolled in the study in 1998 and 1999. At that time, they were treated with high doses of inhaled corticosteroids (≥1600 µg/day beclomethasone or equivalent) combined with long-acting bronchodilators for at least one year. Despite this treatment they were symptomatic and had had at least one severe exacerbation during the past year requiring a course of oral corticosteroids. In 2004-2005, all patients were approached to participate in a follow-up visit.

The study was approved by the Ethics Committee of the Leiden University Medical Centre and all patients gave written informed consent.

Design and Measurements

In 1998 and 1999 (visit 1), patient characteristics (sex, age, atopic status, asthma onset and duration, medication use), prebronchodilator and postbronchodilator $FEV_{1,}$ bronchial hyperresponsiveness to histamine, eosinophils in peripheral blood and induced sputum, the fraction of nitric oxide in exhaled air (FeNO at 100 ml/sec, NOA 270B; Sievers, Boulder, Colo), and computed tomography scan of the paranasal sinuses were assessed. Five years later (visit 2), patients were re-examined with respect to medication use, lung function (prebronchodilator and postbronchodilator FEV_1), eosinophils in peripheral blood and induced sputum and FeNO. Patients had to be clinically stable without asthma exacerbations for at least one month before their visits to the clinic. The same standardized methods for the different measurements were used as those at baseline. During the 5-year interval, patients were followed and treated according to usual care by their individual chest physicians. All measurements have been described in detail in previous reports (8,11,13).

Analysis

Repeatability of sputum eosinophils as well as FeNO between 1998-1999 and 2004-2005 were assessed according to Bland and Altman (14). Percentages of sputum eosinophils and FeNO values were log transformed before the analysis. Repeatability was expressed as intra-class correlation coefficient (Ri) and coefficient of repeatability (CR). Permanent sputum eosinophilia was defined as sputum eosinophils ≥2% on both time points, based on normal values in healthy adults (15). Differences between patients with and without permanent

sputum eosinophilia were analysed by using parametric and non-parametric tests whenever appropriate. In the logistic regression analyses the following contrasts in potential associated factors were considered for patients with and without permanent sputum eosinophilia: age of asthma onset ≥18y *vs.* < 18y, atopic *vs.* non-atopic, blood eosinophilia ≥ $0.45x10^9$ *vs.* < $0.45x10^9$, FeNO ≥ 11 ppb *vs.* < 11 ppb (median value), postbronchodilator FEV_1 ≥ 75% predicted *vs.* < 75% predicted, PC_{20}histamine ≤ 1 mg/ml *vs.* ≥ 1mg/ml (16) and CT sinus score ≥ 12 *vs.* < 12 (17). Odds ratios (OR) with confidence intervals (CI) were obtained. In addition, multiple regression analysis was applied with all significant factors forced into the model. The relationship between sputum eosinophils and FeNO was analysed with Pearson correlation coefficients. Analyses were performed using SPSS version 12.0 (SPSS Inc, Chicago. IL). P-values < 0.05 were considered statistically significant.

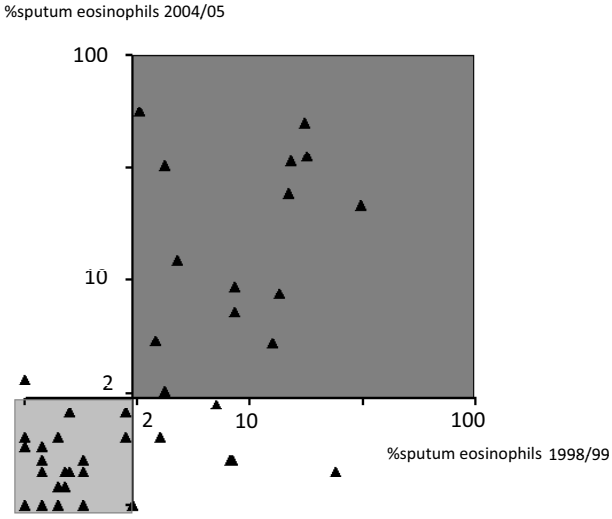
Results

Of 136 patients enrolled at baseline, 101 agreed to participate in the follow-up study 5 years later. From 66 patients, adequate baseline sputum samples were available. In 44 (67%) patients a second adequate sputum sample could be obtained 5 years later. Six patients could not produce adequate sputum samples, in 3 patients FEV_1 had become too low to perform sputum induction, 4 patients were lost to follow-up, 1 patient died, and 8 patients did not wish to participate in the follow-up study, 4 because of severe illness. Median follow-up of patients was 5.8 (range, 5.2-6.4) years.

Repeatability of sputum eosinophils

At baseline, 20 patients (45%) had sputum eosinophils ≥ 2%. Of these patients, 14 (70%) had sputum eosinophils ≥ 2% at the second visit. Ninety-six percent (23 of 24) without initial sputum eosinophilia (< 2%) were also noneosinophilic at follow-up (Figure 1). Figure 2 shows a Bland & Altman plot for repeatability of log sputum eosinophils between the baseline visit (1998/1999) and follow-up visit (2004/2005). The intra-class correlation coefficient Ri was 0.67 (range,0.47-0.81). The coefficient of repeatability (CR) was 0.80 for log sputum eosinophil percentage, which denotes 6.3% sputum eosinophils. 93% of the differences lie between the limits of agreement, indicating low variability of sputum eosinophils over time.

Figure 1. Persistence of the (non-) eosinophilic phenotype: sputum eosinophil percentages in 2004/2005 vs. 1998/1999.



Dark grey box represent data of patients with persistent eosinophilia in sputum. Light grey box represent data of patients with persistent non-eosinophilia. Other boxes represent discongruent data between two time points. Axes have a logarithmic scale.

Characteristics of patients with and without permanent sputum eosinophilia

Fourteen patients out of 44 patients had permanent sputum eosinophilia (sputum eosinophils ≥ 2% both in 1998-1999 and in 2004-2005). These patients had later onset of asthma, were less often atopic, had more often a history of ICU admission, had higher blood eosinophils and computed tomography sinus scores, and had a lower FEV_1 than patients without permanent sputum eosinophilia (Table 1). There was no association between changes in inhaled or oral steroid dose and changes in sputum eosinophil percentage over time ($p > 0.14$).

Odds ratios for clinical or inflammatory factors potentially associated with permanent sputum eosinophilia are shown in Table 2. Permanent sputum eosinophilia was significantly associated with high blood eosinophil counts (≥0.45 x109/l) at baseline, extensive sinus disease (CT-score ≥12), and persistent airflow limitation (FEV_1 ≤ 75% predicted). When analyzing all these factors in one model, extensive sinus disease (CT sinus score ≥12) appeared to be the only independent factor associated with permanent sputum eosinophilia (OR(CI): 9.2 (1.1-75.4).

Figure 2. Bland Altman plot for sputum eosinophil percentages in 1998/99 and 2004/05.

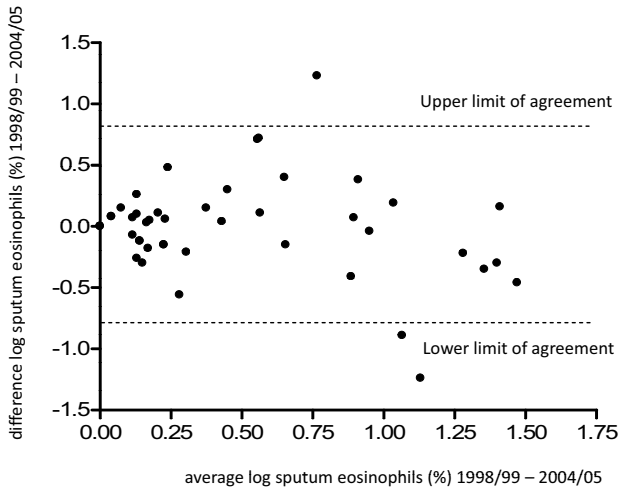


Table 1. Characteristics of patients with and without permanent sputum eosinophilia.

	no permanent eosinophilia (n=30)	permanent eosinophilia (n=14)	p-value
Age (y)	47.8 (10.7)	53.3 (13.3)	0.15
Gender (%female)	60	50	0.53
Asthma onset (y)*	16 (0.5-54)	36.5 (0.5-60)	0.03
Asthma duration (y)*	29 (6-58)	14.5 (4-53)	0.10
Atopy (%)	73%	43%	0.05
Chronic oral steroids (%)	27%	36%	0.72
ICU admission ever (%)	3%	36%	0.009
≥ 3 exacerbations/y (%)	50%	63%	0.67
FEV_1 (%pred)*	83.0 (41.7-121.7)	59.8 (35.8-111.9)	0.02
PC_{20} histamine*	1.38 (0.02-8.0)	0.18 (0.02-1.7)	0.07
CT sinus score*	3 (0-24)	12 (0-29)	0.02
FeNO (ppb)*	9.3 (2.0-72.0)	17.3 (6.3-69.7)	0.06
Blood eosinophils($x10^9$/L)*	0.14 (0.01-0.75)	0.66 (0.10-1.12)	0.000

Values in mean(sd) or median*(range). Permanent sputum eosinophilia is defined as sputum eosinophils ≥ 2% on both time points. PC_{20} histamine: provocative concentration of histamine causing a 20% reduction in FEV_1. CT: computed tomography

Correlation between sputum eosinophils and FeNO levels, and repeatability of FeNO

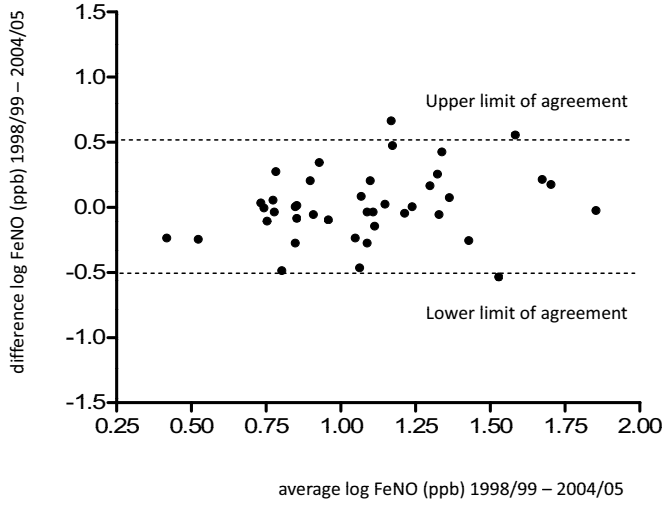
The percentage of eosinophils in induced sputum was weakly correlated with FeNO at baseline and 5 years later (r= 0.38, p= 0.01 and r= 0.41, p=0.007, respectively). Compared with eosinophils, FeNO was equally reproducible between 1998/1999 and 2004/2005, with a Ri of 0.71 (range, 0.52-0.84) and a coefficient of repeatability of 0.26 (Figure 3).

Table 2. Odds ratios for factors potentially associated with permanent sputum eosinophilia.

	Odds ratio	(95% CI)	Adjusted Odds ratio	(95% CI)
Asthma onset ≥ 18y	3.8	(0.95-14.8)		
Atopic	0.3	(0.1-1.0)		
FEV_1 ≤ 75% predicted	4.3	(1.1-17.1)	3.6	(0.4-31.5)
PC_{20}histamine ≤ 1mg/ml	2.2	(0.3-15.2)		
CT sinus score ≥ 12	11.2	(1.7-72.3)	9.2	(1.1-75.4)
FeNO ≥ 11 ppb	1.8	(0.5-6.9)		
Blood eosinophils ≥ $0.45.10^9$/L	13.3	(2.6-68.6)	2.8	(0.3-28.7)

Permanent sputum eosinophilia is defined as sputum eosinophils ≥ 2% on both time points. PC_{20} histamine: provocative concentration of histamine causing a 20% reduction in FEV_1. CT: computed tomography

Figure 3. Bland Altman plot for FeNO in 1998/99 and 2004/05.



Discussion

This study shows that the presence of sputum eosinophilia in patients with difficult-to-treat asthma is a consistent feature over a period of 5 years in the vast majority of patients and that the percentage of sputum eosinophils is highly reproducible. Adult onset asthma, extensive sinus disease, high peripheral blood eosinophil counts, and persistent airflow limitation are associated with permanent sputum eosinophilia, but extensive sinus disease is the only independent factor.
FeNO levels were only weakly correlated with sputum eosinophils, but showed similar consistency over time. This implies that patients with eosinophilic airway inflammation despite vigorous steroid treatment do represent a separate phenotype and that FeNO may provide additional information on the inflammatory process in the airways.

This study is the first follow-up study in patients with difficult-to-treat asthma, showing that the eosinophilic phenotype is consistent over time. One earlier observational study in patients with asthma of varying severity showed that 10 out of 11 patients classified as eosinophilic asthma remained in that classification on the second visit after 5 years (18). A previous study from our department focused on the question whether the measurement itself was reproducible, in order to evaluate the validity of the method. It showed that sputum eosinophil percentages were reproducible in patients with varying asthma severity when measurements were performed with a median interval of four days (19).
Interestingly, in the current study, the absence of sputum eosinophilia seemed to be even more consistent. One explanation for this consistency might be that in these patients the eosinophilic component of airway inflammation was adequately suppressed by inhaled corticosteroids, but that other, steroid unresponsive factors were still causing asthma symptoms. An alternative explanation might be that these patients never had eosinophilic airway inflammation, but predominantly neutrophilic inflammation, which has been shown to be insensitive to corticosteroids (20,21) Although it was not the purpose of our study, in retrospect we did not observe a difference in neutrophilic inflammation between patients with and without sputum eosinophilia in our cohort. Taken together, although the precise mechanisms underlying the eosinophilic as well as the non-eosinophilic phenotypes remain unresolved, both phenotypes seem to be clinically distinct and stable over time.

More frequent measurements of sputum eosinophils over time might have strengthened our finding that sputum eosinophils are highly reproducible; however, the strength of our study is that measurements were performed during the natural course of the disease, without planned interventions, and despite variation in exposure to asthma triggers in between and preceding the two measurements. There was no correlation between changes in sputum

eosinophils and inhaled or oral corticosteroid dose, indicating that anti-inflammatory treatment was not an explanatory factor.
The number of patients in whom adequate sputum could be obtained on two occasions was limited. Still, we do not think this has influenced the results, because the distribution of eosinophilic and non-eosinophilic phenotypes among the 44 patients was equal to the 66 patients who could produce adequate sputum at baseline. This suggests that the sample of 44 patients was not biased by the presence or absence of eosinophilic inflammation.

Remarkably, exhaled nitric oxide levels were as reproducible as sputum eosinophil percentages, but the association between sputum eosinophils and exhaled nitric oxide in our study was weak. In contrast to previous studies (9,10) we could not identify a FeNO level that determined sputum eosinophilia with an acceptable degree of sensitivity or specificity (data not shown). Our findings confirm earlier observations in adults (6,22) and children (23) with severe asthma, showing that FeNO and sputum eosinophil levels are not simply interchangeable. Apart from reflecting eosinophilic inflammation, exhaled nitric oxide levels represent a composite picture of airway nitrogen redox chemistry, such as the metabolic regulation of GSNO (*S*-nitrosoglutathione) which is an endogenous bronchodilator (24). Whilst permanent eosinophilic inflammation seems to be related to chronic rhinosinusitis, persistent airflow limitation and uncontrolled disease, elevated levels of FeNO may provide additional information about chronic injuring processes in the airways that ultimately lead to loss of lung function (13).
The mechanisms of persistent eosinophilic airway inflammation are not clear. Intense inflammatory processes with relative steroid resistance or inflammation in parts of the body that cannot be easily reached by inhaled corticosteroids such as the paranasal sinuses (25,26) and the peripheral airways (27) have been postulated. In this study, severe sinus disease was the only independent factor associated with permanent sputum eosinophilia. This fits in with the assumption that both conditions are manifestations of a common underlying disease. The finding that nasal provocation with allergen can induce airway inflammation (28) and bronchial allergen challenge can induce mucosal inflammation in the nose (29) supports a causal relationship between the two conditions, although the precise mechanisms of this interaction are still obscure. Treatment with high doses of parenteral steroids (30), as well as treatment of chronic sinusitis and nasal polyps (31) has indeed resulted in improvement of lung function. However, in view of the many unwanted side effects of systemic corticosteroids, other anti-inflammatory treatments are needed in this subgroup of difficult-to-treat asthma patients.
Recently, specific therapy targeted at reducing circulating and tissue eosinophils has shown promising results for patients with asthma with persistent eosinophilia. It has been demonstrated that the novel humanized monoclonal antibody against interleukin-5,

mepolizumab, reduces exacerbations, improves asthma related quality- of-life scores, and allows prednisone sparing in patients with refractory asthma and sputum eosinophilia (32,33). The next step will be to demonstrate that this new drug also improves chronic rhinosinusitis and prevents progressive loss of lung function in severe refractory asthma.

In conclusion, sputum eosinophilia is a consistent feature over time in patients with difficult-to-treat asthma despite treatment with high doses of corticosteroids. Persistent sputum eosinophilia should be considered the key characteristic of a specific asthma phenotype characterized by adult-onset of the disease, absence of atopy, extensive sinus disease, and persistent airflow obstruction. Patients with this asthma phenotype have severe exacerbations and loss of lung function and are therefore the ideal candidates for targeted therapy with new biologicals aimed at reducing eosinophil recruitment and activation.

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