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Clinical Trial Paper

Increase in blood eosinophils during follow-up is associated with lung function decline in adult asthma

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ABSTRACT

Background: Asthma is associated with accelerated rate of lung function (FEV_1) decline.
Objective: To determine predictive factors associated with FEV_1 decline in adult asthma.
Methods: A retrospective study was conducted in 229 asthmatics recruited from the University Asthma Clinic of Liege. Subjects had at least two visits with post-bronchodilation (post-BD) FEV_1 and minimum one year between them. A multivariable linear regression analysis was conducted in order to come up with factors associated with lung function decline.
Results: Post-BD FEV_1 decline in % predicted. y^{-1} was 0.2 (95%CI -2.0 to 2.8) in the overall population. Our population was made up of mild to moderate asthmatics [1] for 58%, aged 50 (41–60) years old, 62% were female and 59% were atopic. Median ICS dose was 1000 µg beclomethasone equivalent (CFC)/day with 81% treated at baseline. Time between visits was 46.8 ± 32.1 months. The univariate linear regression analysis revealed a negative association between % predicted FEV_1 decline and baseline ACQ ($p < 0.0001$) and blood eosinophils (% and/mm^3) ($p < 0.0001$ and $p < 0.0001$). A positive association was found between % predicted FEV_1 decline and baseline pre-BD FEV1 (mL) values ($p = 0.001$), blood neutrophils (%) ($p = 0.02$), change in blood eosinophils (%) ($p < 0.0001$), time between visits (months) ($p < 0.0001$). The predictive variables for accelerated decline highlighted by the multivariable analysis ($r^2 = 0.39$) were change in blood eosinophils (%) over time ($p = 0.002$) and time between visits (months) ($p < 0.0001$).
Conclusion: These findings highlight a new value for blood eosinophil counts as their increase over time predicts greater lung function decline in asthma.

1. Introduction

Asthma is a chronic inflammatory airway disease associated with reversible airway obstruction. Asthma patients are at risk of developing structural changes (remodeling) resulting in persistent airflow limitations [2–4]. On average, asthma patients have lower lung function than healthy individuals [4] and their lung function (FEV_1) decline can be greater over time [3]. Nevertheless, not all asthma patients decline over time, some show stable lung function and some others can outgrow their asthma disease [5]. Previous studies have revealed risk factors for accelerated FEV_1 decline in asthma: low baseline lung function (FEV_1% predicted) [6,7], airway hyper-responsiveness [8], male gender [9], cigarette smoking [10,11], late asthma onset [12], duration of disease [4], frequent and severe exacerbations [13]. Moreover, the role of inflammation and eosinophilia in lung function decline has been highlighted with high eosinophil sputum numbers [5], high variability in sputum eosinophils [14], as well as higher blood eosinophil numbers [15] linked to accelerated rate of lung function decline in asthma. Since, Newby et al. [14] found that eosinophilic airway inflammation influences post-BD FEV_1, especially when high variations over time were observed, the purpose of this study was to evaluate potential risk factors for accelerated lung function decline in a secondary care asthma population, including evolution of blood and sputum eosinophils and neutrophils over time.

2. Methods

2.1. Study design, setting and participants

A retrospective longitudinal study was conducted on adult (> 18-year-old) asthmatics recruited from the University Asthma Clinic of Liege, Belgium. Eligibility for this study was defined as patients who

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had at least two visits in stable state (exacerbation-free for the last month) with post-BD FEV_1 measurements and minimum one year between them. Two hundred and twenty-nine patients qualified for the study in our database between February 2004 and January 2017.

Diagnosis of asthma was done by a Pulmonologist and based on the presence of typical symptoms such as wheezing, breathlessness, chest tightness, cough and at least one of the following: FEV_1 increase of 12% or more and 200 mL following inhalation of 400 µg salbutamol or a provocative concentration of methacholine causing a 20% fall in FEV_1 of less than 16 mg mL^{-1} [16].

First (V_1) and last (V_x) visits of each patient were used to calculate decline and evolution of blood and sputum eosinophil and neutrophil counts and ICS dosage over time.

2.2. Variables

All variables used for the analysis were collected during patient routine visits. All procedures were performed in the context of clinical practice and the retrospective data collection was conducted with approval from the ethics committee of CHU Liège (2005/181).

2.3. Demographic data

Patient's age at visit, age at diagnosis (asthma onset), weight, and tobacco status were routinely recorded. Quality of Life was assessed using self-administered Asthma Quality of Life Questionnaire (AQLQ) [17] and Asthma control by the Juniper Asthma Control Questionnaire (ACQ) [18] and an Asthma Control Test (ACT) [19].

2.4. FENO measurement and spirometry

Patients underwent Fractional Exhaled Nitric Oxide (FENO) measurements at flow rate of 50 mL/s according to the ERS/ATS recommendations [20] (NIOX, Aerocrine, Sweden) followed by spirometry with bronchodilation, and sputum induction on the same day.

2.5. Sputum induction and processing

Sputum induction and processing were performed as previously described [21] using the whole expectorate.

Cell counts were estimated on samples centrifuged (Cytospin) and stained with Hemacolor® Staining set after counting 500 non-squamous cells (Merck chemical, Overijste, Belgium). Sputum cytology was analyzed and 4 phenotypes were defined: the eosinophilic phenotype with ≥3% sputum eosinophil count (and < 76% neutrophil count), the neutrophilic phenotype with ≥76% sputum neutrophil count (and < 3% eosinophil count), and the mixed granulocytic phenotype being a combination of the above [22]. The paucigranulocytic phenotype was defined as an inflammatory cell count below these thresholds.

2.6. Blood sampling

Serum total IgE were measured with the ImmunoCAP system (Phadia AB, Uppsala; Sweden). A measure of Blood eosinophil and neutrophil counts (% and/mm^3) was also performed.

2.7. Treatment characteristics

Dosages of inhaled corticosteroids (ICS) and treatment with antileukotrienes (LTRA), Long acting muscarinic antagonists (LAMA), Long acting beta-2 agonists (LABA), Anti-IgE, anti-IL5 and oral corticosteroids (OCS) were recorded and used in the analysis. ICS doses were classified as low (< 500 µg/d), moderate (> 500–1000 µg/d) or high (> 1000 µg/d beclomethasone dipropionate – chlorofluorocarbon) [23]. The evolution of ICS treatment between first and last visit was also considered with change in ICS dose over time (delta) being ICS dose at V_x minus ICS dose at V_1.

2.8. FEV_1 decline definition

Annual post-BD FEV_1 changes obtained for each patient were expressed in % predicted.y^{-1} or mL.y^{-1}. Post-BD FEV_1 change was calculated by subtracting the latest (V_x) measured value from the baseline (V_1) post-BD FEV_1, subdivided by the number of months separating the two measurements, and multiplied by 12. Post-BD FEV_1, expressed as a percentage of predicted values for age, sex and height using reference equations from Quanjer GLI 2012 [24], was selected as our marker of lung function for the linear regression model, as opposed to the absolute value (in mL) which could simply reflect the physiologic effect of aging or the influence of gender or height.

2.9. Statistical methods

Continuous variables were presented as mean and SD when normally distributed or as median and interquartile range when not normally distributed. Categorical variables were presented as frequencies and percentages. A Kruskal Wallis analysis was used to compare post-BD FEV_1 decline among sputum inflammatory phenotypes and ICS categories. T-test and Chi-2 were used to compare patients considered as "decliners" (FEV_1 decline > 0) and non-decliners (FEV_1 decline ≤0). FEV_1 declines (in %pred) between patients with or without a step-up treatment during follow-up were compared with a Mann-Whitney test. Kaplan-Meier curves were generated to assess incidence of the lung function decline. The cut-off of 150 mL of lung function decline was used.

The prediction of "decline in respiratory function per year" was analyzed by conventional linear regression using independent variables such as asthma onset, weight, tobacco status, and baseline (V_1) measurements of ACQ, FENO, pre-BD FEV_1, reversibility, IgE, blood % eosinophils and neutrophils, blood eosinophil counts (/mm^3), sputum % eosinophils and neutrophils, ICS doses, presence of antileukotrienes treatment, time between visits, duration of disease and exacerbations. Gender and height were not included in the analysis as FEV_1% predicted is already adjusted for these values.

Changes from V_1 to V_x (Deltas) for sputum and blood eosinophil and neutrophil counts, and ICS dosage were also included in the univariate analysis. Decline in lung function per year (in % predicted.y^{-1}) was used as the dependent variable.

After examining for all potential predictors, the univariate association with the outcome, a stepwise backward linear regression was conducted; initial model included variables with an association of $p < 0.20$. Then, the best predictive model was conducted deleting those variables that had the weakest association with the outcome ($p > 0.05$). A p value < 0.05 was considered statistically significant. Statistical analysis was done using STATA version 14.0 (Statistical Software, College Station, TX: StataCorp LP).

3. Results

3.1. Patients' characteristics at baseline

Two hundred and twenty-nine patients, seen between February 2004 and January 2017, had at least 2 visits with post-BD FEV_1 measurements, minimum one year apart.

Baseline demographic and clinical data of these patients are presented in Table 1.

Patients were mild to moderate asthmatics [1] for 58% of them and 42% severe asthmatics, aged 50 (41–60) years old, with 62% of female and 59% of atopics.

Sputum induction was successful in 82% of the patients. Eosinophilic inflammatory phenotype represented 52% of the population, along with 14% neutrophilic, 5% mixed granulocytic and 29%

Table 1
Demographic and clinical data at first visit (baseline) and evolution over time (deltas) for sputum and blood eosinophil and neutrophil counts.

N.	229
Gender (F) (%)	142 (62)
Age (years)	50 (41–61)
Age asthma onset (y)	35 (16–50)
Asthma onset category (%)	
- Early (< 12)	46 (20)
- Intermediate (12–40)	73 (32)
- Late (> 40)	109 (48)
Time between First and last visit (months)	46.8 ± 32.1
Height (cm)	166.7 ± 8.8
Weight (Kg)	72.8 ± 15.9
BMI (kg/m^2)	26.1 ± 5.2
Obesity (BMI > 30) (%)	51 (22)
Smoking status (%)Ex-smokersEx-smokers	
Non-smokers	126 (55)
Current smokers	38 (17)
Ex-smokers	65 (28)
Atopy (Y) (%) (n = 220)	129 (59)
Pre FEV1 (mL)	2171 ± 768
Pre-BD FEV_1 (% predicted)	74.2 ± 20.4
Post-BD FEV1 (% predicted)	81.9 ± 20.4
Post FEV1/FVC (%)	69.3 ± 14.6
Reversibility (%)	8.4 (3–17)
PC20 M (mg/mL) (n = 74)	2.12 (0.05–16.00)
FENO (ppb) (n = 198)	28 (16–62)
Sputum eosinophils (%) (n = 187)	4.4 (0.7–32.0)
Sputum neutrophils (%) (n = 187)	44.2 (18.4–69.6)
Sputum inflammatory phenotypes: (%)	
- Eosinophilic	97 (52)
- Neutrophilic	25 (13)
- Mixte	10 (5)
- Paucigranulocytic	55 (29)
Serum IgE. (kU/L)	165 (53–407)
Blood eosinophils (%) (n = 207)	3.2 (1.6–5.8)
Blood eosinophils (/μL) (n = 206)	188 (69–399)
Blood neutrophils (%) (n = 221)	55.7 (49.8–63.7)
Blood neutrophils (/μL) (n = 203)	4150 (1300–5170)
ACT score	13.1 ± 5.8
ACQ score	2.4 ± 1.3
AQLQ score	4.2 ± 1.6
ICS (Y) (%)	186 (81)
ICS dose	1000 (400–2000)
ICS category: (%)	
- Steroid naïve	43 (19)
- Low dose	26 (11)
- Moderate dose	64 (28)
- High dose	96 (42)
OCS (Y) (%)	20 (9)
LTRA (Y) (%)	25 (11)
Decline mL.y^{-1}	27.5 (−39.4–113.8) or 45.7 (± 210.6)
Decline FEV1. %pred.y^{-1}	0.20 (−2.0-2.8) or 0.17 ± 7.8
Delta Sputum eosinophils (%) (n = 135)	−0.8 (−15.7–0.8)
Delta Sputum neutrophils (%) (n = 134)	12.6 (−6.2 - 31.2)
Delta Blood eosinophils (%) (n = 161)	0 (−1.4 - 0.9)
Delta Blood neutrophils (%) (n = 138)	0.6 (−5.5 - 7)

Data are presented as mean ± SD or median and IQR. PC20 M is presented as geometric mean (min-max).
Low-dose ICS: < 500 μg/d; moderate-dose ICS: > 500–1000 μg/d; high-dose ICS: > 1000 μg/d beclomethasone dipropionate – chlorofluorocarbon.
ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; CRP, C reactive protein; FENO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1s; ICS, inhaled corticosteroid; LTRA, Leucotriene Receptor Antagonist; PC20 M, provocative concentration of metacholine causing a 20% fall in FEV_1; ppb, parts per billion; Delta, V_x value minus V_1 value.

paucigranulocytic phenotypes at baseline.

Most of these patients (81%) were treated with ICS at baseline with a median dose of 1000 (400–2000) mcg beclomethasone equivalent (CFC)/day. Nineteen percent of patients were not receiving treatment at presentation, they had been sent to the asthma clinic by their

Table 2
Comparison: "Declining" (FEV_1 decline %pred > 0) sub-population (n = 120) to "not declining" (FEV_1 decline %pred ≤0) sub-population (n = 109). Demographic and clinical data at first visit (baseline).

Variables	Decline %pred/year		P value
	"No" (< =0) n = 109	"Yes" (> 0) n = 120	
Gender (F) (%)	74 (68)	68 (57)	0.081
Age (years)	51 (38–61)	51 (45–61)	0.6036
Age asthma onset (y)	32 (15–50)	41 (19–53)	0.1111
Disease duration	13 (1 − 26)	8.5 (1 − 22)	0.0671
Time between First and last visit (months)	45.3 ± 33.4	48.2 ± 31.1	0.4970
Height (cm)	166.2 ± 8.8	167.2 ± 8.7	0.4082
Weight (Kg)	73.0 ± 15.8	72.7 ± 16.0	0.8926
BMI (kg/m^2)	26.4 ± 5.6	25.9 ± 5.0	0.4654
Living area: (n = 167)			
Countryside	30 (36.5)	54 (63.5)	0.001
Suburban	18 (21.9)	15 (17.6)	
City	34 (41.6)	16 (18.8)	
Smoking status (%)			
Non-smokers	70 (64)	56 (47)	0.028
Current smokers	14 (13)	24 (20)	
Ex-smokers	25 (23)	40 (33)	
Atopy (Y) (%)	54 (50)	58 (48)	0.855
Pre-BD FEV_1 (% predicted)	70.5 ± 20.2	74.2 ± 20.4	0.0076
Pre FEV1/FVC (%)	67.7 ± 10.8	69.0 ± 11.1	0.2727
Pre FEV1 (ml)	2037 ± 755	2171 ± 768	0.0115
Post-BD FEV1 (% predicted)	78.6 ± 19.9	81.9 ± 20.4	0.0193
Post FEV1/FVC (%)	68.9 ± 11.1	69.6 ± 11.6	0.6772
Post-BD FEV1 post (ml)	2282 ± 791	2479 ± 797	0.0615
Reversibility (%)	9.7 (3–20)	8 (3–14)	0.0382
PC20 M (mg/ml)	2 .12 (0.05–16)	2.13 (0.05–16)	0.8477
FENO (ppb) (n = 198)	37.3 (18.5–73)	22.5 (14.4–49)	0.0056
Sputum eosinophils (%) (n = 187)	8.5 (0.8–43.6)	3.1 (0.7–17)	0.0044
Sputum neutrophils (%) (n = 187)	31.1 (14.9–64.2)	52.3 (28.7–73.3)	0.0052
Sputum inflammatory phenotypes: (%)			
- Eosinophilic	54 (58)	43 (46)	0.301
- Neutrophilic	9 (10)	16 (17)	
- Mixte	5 (5)	5 (5)	
- Paucigranulocytic	25 (27)	30 (32)	
Serum IgE. (kU/L)	178 (58–416)	138 (48–407)	0.5339
Blood eosinophils (%) (n = 207)	4.2 (2.1–7.8)	2.7 (1.3–4.3)	0.0011
Blood eosinophils (/μL) (n = 206)	262 (139–616)	126 (26–298)	0.0027
Blood neutrophils (%) (n = 221)	53.5 (48.8–60.3)	59.2 (51.0–65.6)	0.0084
Blood neutrophils (/μL) (n = 203)	3993 (3186–5094)	4251 (2585–5294)	0.7162
ACT score	12.8 ± 5.8	13.5 ± 5.9	0.5408
ACQ score	2.7 ± 1.4	2.1 ± 1.3	0.0032
AQLQ score	4.1 ± 1.6	4.3 ± 1.5	0.2339
ICS (Y) (%)	87 (80)	100 (83)	0.5000
ICS dose	1000 (400–2000)	1000 (400–2000)	0.8675
ICS category: (%)			
- Steroid naïve	22 (20)	21 (18)	0.759
- Low dose	10 (9)	16 (13)	
- Moderate dose	30 (28)	34 (28)	
- High dose	47 (43)	49 (41)	
OCS (Y) (%)	11 (10)	9 (8)	0.640
LTRA (Y) (%)	9 (8)	16 (13)	0.219

Data are presented as mean ± SD or median and IQR. PC20 M is presented as geometric mean (min-max).
Low-dose ICS: < 500 μg/d; moderate-dose ICS: > 500–1000 μg/d; high-dose ICS: > 1000 μg/d beclomethasone dipropionate – chlorofluorocarbon.
ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; CRP, C reactive protein; FENO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; ICS, inhaled corticosteroid; LTRA, Leucotriene Receptor Antagonist; PC20 M, provocative concentration of metacholine causing a 20% fall in FEV_1; ppb, parts per billion.

physicians in order to confirm asthma diagnosis and start the appropriate treatment based on their phenotype. Proportions of subjects in low, moderate and high ICS treatment categories were 11%, 28%, and 42% respectively. Subjects receiving antileukotrienes, LAMA and LABA treatments represented 11%, 11%, and 74% of the population respectively. Mean (± SD) time between visits was 46,8 ± 32,1 months. Ten percent of these patients were receiving chronic OCS while none were receiving anti-IgE and antiI-L5 at baseline.

3.2. Lung function decline

In the overall population, the post-BD FEV_1 decline was 0.2% predicted. y^{-1} (95%IC -2.0 to 2.8) or 27.5 mL. y^{-1} (95%IC -39.4 to 113.8).

Fifty two percent of the population had slow (< 30 mL. y^{-1}), 11.8% intermediate (30–60 mL y^{-1}), and 35.8% fast (> 60 mL.Yr^{-1}) post-BD FEV_1 decline, ranges previously defined by Sposato [25].

A hundred and nine patients (48%), however, did not decline (≤0% predicted.y^{-1}).

A sub-analysis comparing the clinical characteristics of patients declining (decline % predicted.y^{-1} > 0) and those not declining (Table 2) was made. Smoking status was statistically different between groups, with “decliners” having a larger proportion of smokers and ex-smokers. Their lung function (pre-BD and post-BD FEV_1) values were higher at baseline. Reversibility and FENO values at baseline were smaller in the declining group. Declining patients had also more neutrophils and less eosinophils in both compartments (airway and systemic) than non-declining patients. Asthma control (ACQ) was significantly better in the declining group.

Compared to baseline, a LAMA was added in 14 patients, a LABA in 43 patients, and a biologic treatment (14 Anti-IL5 and 4 Anti-IgE) in 18 patients. However, there is no significant difference in FEV_1 decline (% predicted.y^{-1}) between patients with or without added treatment during the follow-up (p = 0.718).

Lung function decline was similar among inflammatory phenotypes or ICS treatment categories. Moreover, when considering ICS treatment change, there was no influence of stable, increased or decreased treatment over time on post-BD FEV_1 decline.

Kaplan-Meier survival curve is presented in Fig. 2. Sixty-seven months are needed for half the patients to experience a loss of at least 150 mL of their lung function. It is also apparent that no lung function decline (of at least 150 mL) can be seen before about 40 months (see Fig. 3).

3.3. Factors associated with lung function decline

The univariate linear regression analysis (Table 3) revealed a negative association between % predicted post-BD FEV_1 decline and baseline ACQ (−1.2 (IC95% −1.9; −0.5), p < 0.001), exacerbations (−1.1 (−2.2; −0.1), p = 0.039), and blood eosinophils (%) (−0.43 (−0.6; −0.2), p < 0.0001) or (/mm^3) (−0.003 (−0.005; −0.001), p < 0.0001). A positive association was observed between % predicted post-BD FEV_1 decline and baseline pre-BD FEV_1 (mL) values (0.002 (0.000; 0.003), p = 0.001), blood neutrophils (%) (0.11 (0.01; 0.22), p = 0.02), delta blood eosinophils (%) (0.42 (0.21; 0.63), p < 0.0001), time between visits (months) (12.6 (10.4; 14.7), p < 0.0001).

The multivariable analysis (r^2 = 0.39) (Table 4) showed that variables in favor of post-BD FEV_1 decline were an increase in blood % eosinophils over time (0.29 (0.11; 0.46), p = 0.002) (Fig. 1) and longer time between visits (months) (12.1 (9.3; 14.8), p < 0.0001) (Fig. 2).

No additional association was observed between post-BD FEV_1 decline and asthma onset, weight, tobacco status, total IgE levels, FENO levels, reversibility (% predicted), ICS dose or antileukotrienes treatment.

Table 3
Results of the linear regression - univariate analysis. N = 229*.

Lung function decline (post-BD FEV_1% pred)	Total population*		
	UNIVARIATE		
	Coeff.	IC 95%	P value
Onset (years)	0.02	−0.02; 0.07	0.268
Weight (Kg)	−0.02	−0.08; 0.04	0.516
Smoking status (%)			
Non-smokers	0		
Current smokers	2.4	−0.3; 5.3	0.091
Ex-smokers	1.7	−0.6; 4.0	0.153
ACQ	−1.2	−1.9; −0.5	< 0.0001
FENO (ppb)	−0.01	−0.03; 0.01	0.435
Pre-bronchodilator FEV_1 (mL)	0.002	0.000; 0.003	0.001
Reversibility	−0.01	−0.07; 0.05	0.765
IgE	−0.000	−0.00006; 0.00005	0.830
Blood % Eosino	−0.43	−0.6; −0.2	< 0.0001
Blood % neutro	0.11	0.01; 0.22	0.020
Delta Blood % Eosino	0.42	0.21; 0.63	< 0.0001
Delta Blood % Neutro	−0.03	−0.15; 0.07	0.505
Blood Eosino/mm3	- 0.003	- 0.005; - 0.001	< 0.0001
Sputum % Eosino	−0.04	−0.08; −0.0001	0.149
Sputum % Neutro	0.02	−0.01; 0.06	0.226
Delta Sputum % Eosino	0.04	−0.007; 0.08	0.099
Delta Sputum % Neutro	0.002	−0.03; 0.04	0.929
ICS dose	−0.0005	−0.001; 0.0005	0.339
Delta ICS dose	0.0001	−0.0007; 0.0009	0.788
LTRA	2.0	−1.2; 5.2	0.230
Duration of disease	−0.037	−0.099; 0.025	0.241
Exacerbations	−1.148	−2.235; - 0.0617	0.039
Time between visits (months)			
< 24	0		< 0.001
≥24; < 36	5.6	3.2; 7.9	
≥36; < 60	6.6	4.3; 8.9	
≥60	12.6	10.4; 14.7	

ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; CRP, C reactive protein; FENO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; ICS, inhaled corticosteroid; LTRA, Leucotriene Receptor Antagonist; PC20 M, provocative concentration of metacholine causing a 20% fall in FEV_1; ppb, parts per billion; Delta, V_x value minus V_1 value.

Table 4
Factors associated with lung function decline. Results of the linear regression – multivariable analysis – Backward predictive model. N = 161*.

Lung function decline (pb FEV1% pred)	Total population*					
	UNIVARIATE			MULTIVARIATE		
	Coeff.	IC95%	P value	Coeff.	IC95%	P value
Delta blood % eosino	0.42	0.21; 0.63	< 0.0001	0.29	0.11; 0.46	0.002
Time between visits (months)						
< 24	0		< 0.001	0		< 0.001
≥24; < 36	5.6	3.2; 7.9		5.1	2.1; 8.0	
≥36; < 60	6.6	4.3; 8.9		5.7	2.9; 8.5	
≥60	12.6	10.4; 14.7		12.1	9.3; 14.8	

4. Discussion

In this retrospective study, we have shown that in a university secondary care asthma clinic, patients with an increase in blood eosinophil (%) counts over time or a longer follow-up duration are more at risk of accelerated annual lung function decline than others.

A large proportion (42%) of patients were severe asthmatics, which represents a proportion much higher than expected in the general population (with 10% of severe asthmatics) [26]. These patients were indeed uncontrolled despite high doses of ICS/LABA treatment. Most of

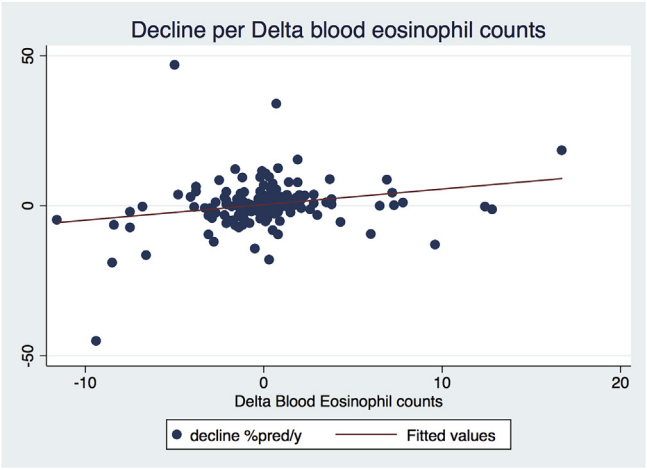

Fig. 1. Decline (% predicted.y^{-1}) per Delta blood eosinophil counts (scatter plot).

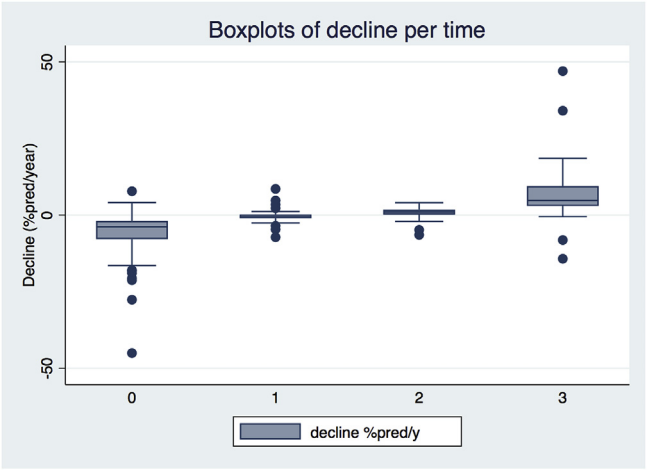

Fig. 2. Decline (% predicted.y^{-1}) per time between visits. Categories: 0 < 24 months; 1 ≥ 24 and < 36 months; 2 ≥ 36 and < 60 months and 3 ≥ 60 months.

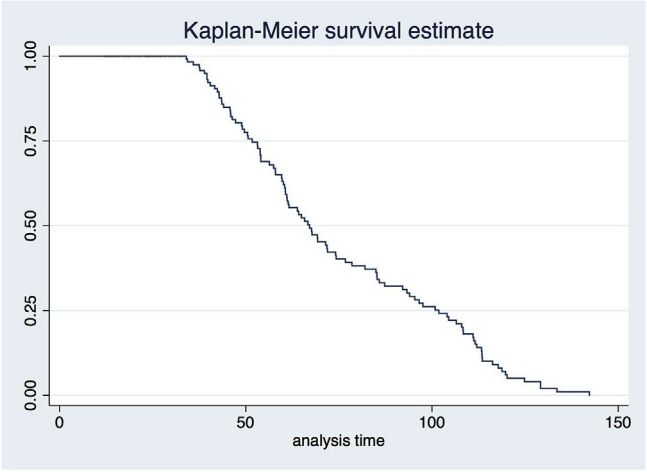

Fig. 3. Kaplan-Meier survival curve of end point (lung function decline of 150 mL).

them also presented with an eosinophilic inflammatory phenotype (52%) which is in line with what was previously reported [27,28]. These patients are indeed much in need of a frequent visit follow-up and can be expected to decline faster than the general population of asthmatics.

In previous studies, rates of post-BD FEV_1 decline have varied from 38 mL y^{-1} for asthmatics compared to 22 mL y^{-1} in healthy subjects, 25.7 mL y^{-1} and 27.1 mL y^{-1} in severe asthma [14,29], 31.5 mL y^{-1} in asthmatic patients with frequent exacerbations vs 14.6 mL y^{-1} in those with infrequent exacerbations [13], 28.4–39.7 mL y^{-1} in adult non-smoker asthmatic patients in the Busselton cohort [11], to 16.1–21.5 mL y^{-1} in ICS treated asthmatic patients [30,31]. Decline in our secondary care population, with 27.5 mL y^{-1}, is consistent with the observations reported in other populations.

Prognosis with regard to decline in lung function is worse for patients with eosinophilic blood counts increasing over time. Hancox [32] recently showed that persistent blood eosinophilia was linked to accelerated lung function decline in young adults with or without self-reported asthma. They found that patients with a mean blood eosinophilia > 0.4 × 10^9/L across four assessments had lung function decline of a similar magnitude to ones observed among those who smoked 10 pack-years or more over the same time. Our observations go further and show that an evolution towards an increase in blood eosinophils over time in asthmatics predicts accelerated FEV_1 decline. We found a linear relationship between blood eosinophilia increasing over time and FEV_1 decline.

Until now, several groups including ours have shown pretty consistently that high blood eosinophils are associated with low FEV_1 [33–36]. However, confirming the results of most previous longitudinal studies, we show no association between blood eosinophils at baseline and FEV_1 decline [15,35,37–39]. This is therefore not surprising since it has also been shown that high initial FEV_1 are associated with greater lung function decline, due most probably to a "regression toward the mean" [40,41].

We are the first to show that a significant increase in systemic eosinophilic inflammation, even in patients with normal baseline value, can predict FEV_1 decline while a unique measurement of blood eosinophil counts fails to inform us on the likely evolution of the lung function.

Perhaps surprisingly, the change in sputum eosinophils was not associated with lung function decline. This may be explained by the fact that it represents a phenotype shown to be responsive to increase ICS both in controlled clinical studies and in real life [42,43]. Therefore, sputum eosinophils are often associated with improvement in FEV_1 when change in ICS dosing is appropriate.

Despite correction for annual decline, we also discovered that time between visits had also a major impact on lung function decline. We can observe a faster decline as time category increases with much higher declines in the latest category (> 60 months period between first and last visits). This could be related to the efficacy of ICS treatment over time. Sin et al. [44] showed previously that ICS are mainly effective on lung function over the first 3–4 months of treatment; after that period of time, lung function decline seems to resume as observed in patients receiving placebo. This being said, the compliance to treatment in this study was not measured, which could have an impact on lung function decline, especially for those having a long period of time between first and last visit. It is also known that in healthy subjects, lung function declines with ageing in a non-linear fashion; annual decline accelerates with age, so as follow-up increases it can be expected that decline increases [45,46].

Considering a cut-off of 30 mL/year [5] for fast decline, we can expect that it would take about 60 months to observe a 150 mL decline and this is confirmed by the KM curve. Indeed, on this curve we can see that it takes about 67 months for 50% of our population to decline significantly.

Many risk factors for accelerated lung function decline have already been featured. Late onset of asthma, longer disease duration, frequent asthma symptoms and severe exacerbations [47], airway hyperresponsiveness (AHR), smoking, blood and sputum eosinophilia and

genetic predisposition could influence lung function decline in asthma [48]. Also, FENO was found to be linked to accelerated declines in asthma [49]. Late onset, highlighted as a risk factor for accelerated decline by some authors, was not confirmed by our analysis. These inconsistencies can be explained by the difference in late onset definition varying from 18 years old to an onset during the time of study [9,12,50] or the possible misclassification of asthmatics in these studies due to self-reported diagnostic [9,12].

Disease duration, usually associated with decline [51], was not statistically associated with lung function decline in our model. Increased exacerbation rate at baseline was associated with lower lung function decline in our population. A possible explanation being that patients with high rate of exacerbation have a lower FEV_1 to start with. Indeed there is a significant negative correlation between FEV_1 and exacerbation rate at baseline in our population. Also, no information regarding genetics was routinely collected in our database.

Neither could we confirm the results of Lange [3] or Ulrik [10] showing that smoking is associated with greater lung function decline which could be attributed to the small proportion (17%) of smokers in our population, compared to more than 50% in theirs, leading to a lack of significance. Coumou [49] showed that increased FENO levels were associated with accelerated FEV_1 decline, which was not confirmed by our results. This could be explained by the differences in the population studied with a population with less severe asthmatics, less atopic, and with lower pack-year for Coumou as compared to ours, or the difference in treatment regimens. Indeed FENO value has been shown to be greatly influenced by atopy, smoking habit or ICS treatment [52].

This study presents some limitations. First, lung function decline calculation was based on 2 measurements only. This is mainly due to the retrospective nature of this study. Indeed, only visits with full spirometry (post-BD FEV_1) measurements and sputum collection were considered for the study. All disease severities were present in our population. Asthma, however, was diagnosed by specialists as opposed to self-reported [11,12] and this population is representative of a university secondary care population. We did not record adherence to treatment. Time between first and last visits was also much variable but decline was annualized to correct for that possible bias. Another limitation of our study is its cross-sectional design with clinical and demographic variable mostly considered at baseline with only a limited number of parameters (sputum and blood cells, and time between visits) being looked at longitudinally together with change in FEV_1.

The usefulness of blood eosinophil counts has already been demonstrated as high blood eosinophils are associated with poor asthma control and risk of exacerbations [36,53,54]. Moreover, blood eosinophils are able to predict the response to anti-IL5 and anti-IgE [55] therapy [56]. Our findings highlight a new value for blood eosinophil counts as their increase over time predicts greater lung function decline in asthma. Moreover, it has a clinical implication as blood collection is easily measured, more widely available, less time consuming and with a higher success rate than sputum collection.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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