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RESEARCH

ASTHMA

Increase in Blood Eosinophil Count Over Time and Sputum IL8 are Associated with FEV_1 Decline in Asthma

S. Graff[1] · C. Moermans[1] · S. Gerday[1] · M. Henket[1] · V. Paulus[1] · F. Guissard[1] · R. Louis[1] · F. Schleich[1]

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Abstract

Background Asthma is associated with accelerated rate of FEV_1 decline.

Objective To determine predictive factors associated with accelerated FEV_1 decline in adult asthma and evaluate sputum cytokines as potential biomarkers for airflow decline.

Methods We recruited 125 asthmatics evaluated at the asthma clinic of Liège and reevaluated them at least 5 years later. Clinical, functional and inflammatory characteristics were compared between patients with accelerated decline (FEV_1 decline > 0.85% pred.y^{-1}) and others. Predictive factors were highlighted with linear regression analysis. Sputum EGF, VEGF, FGF, IL5, IL8, TGF-β, and IgE levels were measured in 58 of these patients at both visits by Human XL cytokine Luminex Performance assay and Elisa.

Results Post-BD FEV_1 decline was 0.06 ± 2.44% pred.y^{-1} in the overall population. Median (IQR) time between visits was 66 (62 – 86) months. The multivariable analysis showed that an increase in blood eosinophils over time (Δ BEC) (Reg. Coef. (95%CI): 0.002 (0.001 to 0.004), p = 0.005)) and onset of asthma (0.04 (0.003 to 0.07), p = 0.036) were independently associated with FEV_1 decline. IL8 levels measured at baseline were higher (499 (408—603) pg/ml, p = 0.0040) in patients with accelerated decline compared to others (143 (88—308) pg/ml).

Conclusion In this study, we have confirmed that an increase in blood eosinophil counts over a follow-up of at least 5 years and later onset of asthma are associated with accelerated annual FEV_1 decline. Moreover, high sputum IL8 levels could be a risk factor for accelerated decline in asthma patients.

Keywords Asthma · Airway inflammation · Decline · Eosinophils · Lung Diseases

Introduction

Asthma patients may develop structural changes, called remodeling, that, along with inflammation, may lead to accelerated lung function (FEV_1) decline [1]. The role of eosinophilic inflammation in lung function decline has been demonstrated with high sputum eosinophil count [2–5], high variability in sputum eosinophils [6], as well as increased blood eosinophil count over time [7] being linked to accelerated rate of FEV_1 decline in asthma. Both the inflammatory and the remodeling processes are associated with high expression of chemokines, growth factors and other cytokines [8]. There is, however, limited longitudinal data on the potential role of these immunologic biomarkers for identification of airflow declining patients.

The purpose of this study was to highlight potential clinical, biological, and functional risk factors and immunologic biomarkers (EGF, VEGF, FGF, IL5, IL8, TGF-β, and IgE) for accelerated lung function decline in a secondary care asthma population with at least 5-year-follow up.

✉ S. Graff
sgraff@chuliege.be

[1] Department of Respiratory Medicine, CHU Sart-Tilman, GIGA I3 Research Group, University of Liege, GIGA +4; CHU - B34, Avenue de l'Hôpital, 11, 4000 Liège, Belgium

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Methods

Study Design, Setting and Participants

A prospective longitudinal study was conducted on adult (> 18-year-old) asthmatics recruited from the University Asthma Clinic of Liege, Belgium between 2010 and 2014 [9]. Patients came back for a second evaluation 5–10 years later, provided they did not use an oral corticosteroid for exacerbation within the last month (Fig. 1).

Diagnosis of asthma was done by a Pulmonologist [10].

All variables used for the analysis were collected during patient routine visits with approval from the ethics committee of CHU Liège (2005/181) in accordance to the Helsinki Declaration.

Quality of Life was assessed using self-administered Asthma Quality of Life Questionnaire (AQLQ) [11] and Asthma control by the Juniper Asthma Control Questionnaire (ACQ) [12] and an Asthma Control Test (ACT) [13].

Subjects were characterized as atopic if they had one positive specific Immunoglobulin E (IgE) test (0.35 kU.L-1; Phadia, Groot-Bijgaarden, Belgium) for at least one common aeroallergen. Patients underwent Fractional Exhaled Nitric Oxide (FeNO) measurements at flow rate of 50 mL/s according to the ERS/ATS recommendations [14] (NIOX, Aerocrine, Sweden) followed by spirometry with bronchodilation, and sputum induction on the same day.

Sputum induction and processing were performed as previously described [15, 16] using the whole expectorate. Routine laboratory of the University Hospital of Liege performed blood cell count.

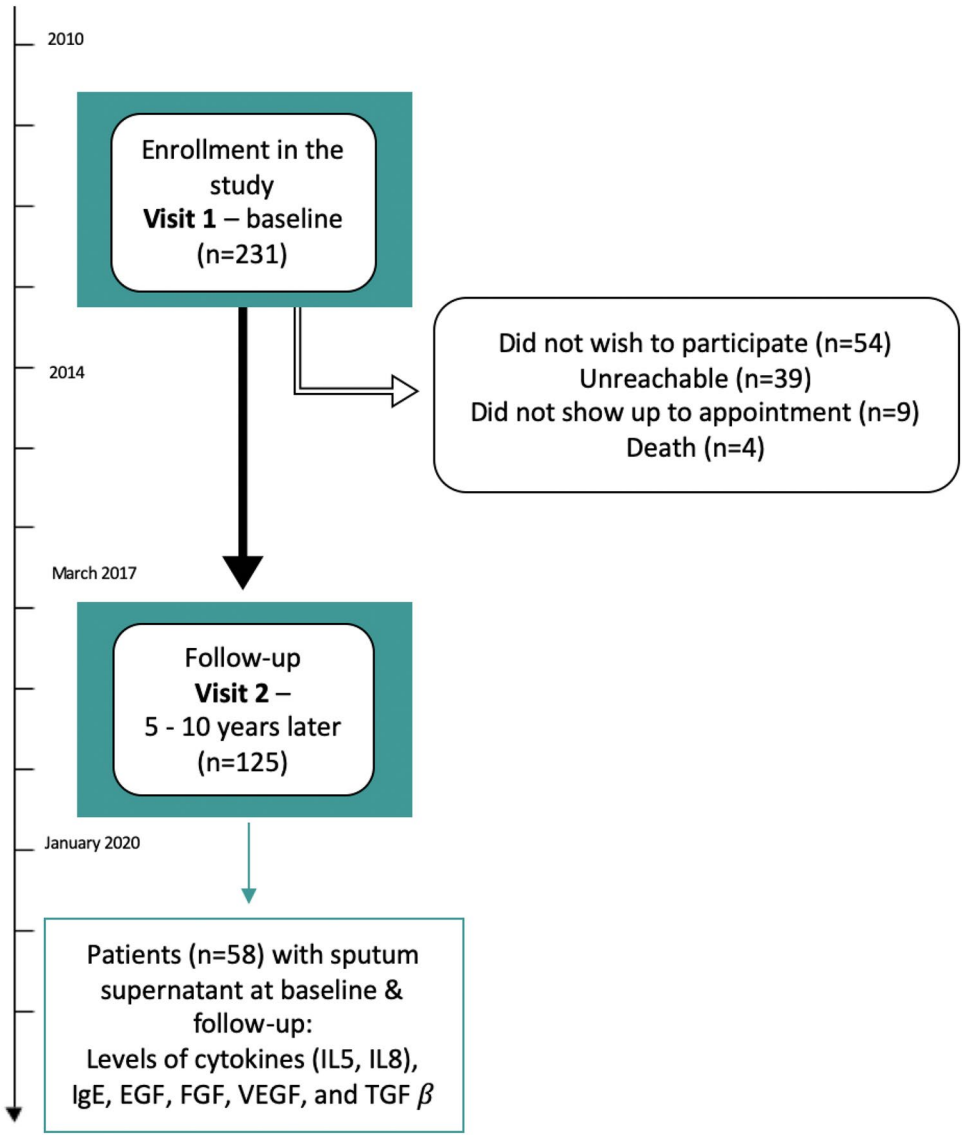

Fig. 1 Study design

None of the patients included in the cohort were treated with biologics.

FEV_1 Decline Definition

Annual post-BD FEV_1 changes obtained for each patient were expressed in % pred.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, divided by the number of months separating the two measurements, and multiplied by 12. Post-BD FEV_1, expressed as a percentage of predicted (% pred.) values for age, sex and height using reference equations from Quanjer GLI 2012 [17], 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.

In order to detect fast decliners, we selected the percentile 75 ($\geq$0.85% pred.y^{-1}) for decline.

Immunoassays

In a subpopulation of 58 non-smoking or ex-smokers (< 20 PAQY) patients, and not treated with systemic corticosteroids or biotherapy, cytokines and total Transforming Growth Factor beta (TGF-β1) levels were measured in sputum supernatant [18] at both visits (baseline and 5–10 year-follow-up) (Fig. 1). All samples were stored at -80 °C and processed at the same time.

The concentrations of cytokines contained in the sputum supernatant were assessed by Human XL cytokine Luminex Performance assay (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. The tested cytokines included interleukin (IL-) IL-5, IL-8 (/CXCL8), endothelial growth factor (EGF), fibroblast growth factor (FGF)-2, and vascular endothelial growth factor (VEGF). Detection limits were 3.0, 0.71, 13.3, 14.9 and 8.9 pg/ml for IL-5, IL-8, EGF, FGF-2 and VEGF respectively**.** Data were acquired using Luminex MAGPIX analyzer (Luminex corp, USA) to determine mean fluorescence intensity and analyte concentrations (pg/mL). IgE levels were quantified by Human IgE SimpleStep ELISA kit (Abcam, Cambridge, UK). Both active and total transforming growth factor beta (TGF-β) were measured in sputum supernatant as previously described [19].

Statistical Methods

Continuous variables are presented as mean and SD when normally distributed or as median and interquartile range (IQR) when not normally distributed. Categorical variables were presented as frequencies and percentages. Continuous variables were compared by t- test when normally distributed or by Wilcoxon Mann–Whitney test when non-parametric. Fisher exact test was used to compare qualitative variables. ANOVA or Kruskal Wallis analyses were conducted in order to compare more than 2 groups (categories).

A sample size of 100 provides 80% statistical power in detecting a correlation coefficient of 0.28, assuming a two-sided alpha risk of 0.05. We addressed the problem of missing data by using the Multiple Imputation Chained Equation (MICE) technique under the assumption that missing values are missing at random [20]. Stata 15's 'mi impute chained' command generated 20 imputed datasets [21]. Analyses run on each dataset were pooled according to Rubin's (1987) rules. Factors associated with annual FEV_1 decline were assessed by conventional linear regression using independent variables such as asthma onset, BMI, atopy, and disease duration at baseline or a delta (change from baseline to follow-up visit) for smoking status, FeNO, SEC, SNC, BEC, BNC, ACT, exacerbations, ICS dose, and chronic OCS use. Decline in lung function per year (in % pred.y^{-1}) was used as the dependent variable. A multivariable analysis was done including all independent variables. We constructed receiver-operating characteristic (ROC) curves for cytokine levels associated with accelerated decline to determine the cut-off which best identified FEV_1 decline ($\geq$0.85% pred.y^{-1}) in asthma. Optimal cutoff points were determined by the method of the nearest point to (0,1). 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).

Results

Patients' Characteristics at Baseline

A hundred and twenty-five patients, seen between February 2010 and November 2014, came back for a visit 5–10 years later. Baseline demographic and clinical data of these patients are presented in Table 1.

Baseline demographic and clinical data of the 58 patients that had both sputum supernatant samples at baseline and 5–10 years later are presented in Table 1 online supplement.

Lung Function Decline

Mean (SD) post-BD FEV_1 decline was 0.06 ± 2.44% pred.y^{-1} or 22.1 ± 65.3 mL. y^{-1} for the overall population.

Median (IQR) time between visits was 66 (62–86) months. Fifty-seven percent of the population had slow (< 30 mL. y^{-1}), 24% intermediate (30–60 mL.y^{-1}), and 19% fast (> 60 mL.yr^{-1}) post-BD FEV_1 decline.

Among 125 patients who were included in the analyses, 32 (26%) patients presented with accelerated annual post-BD

Table 1 Demographic and clinical data at first visit (baseline) (A) and evolution over time (delta (Δ)) (n = 125) (B)

A	
Characteristics at baseline	
N	125
Female (%)	74 (59)
Age (years)	51 ± 14
BMI (kg/m^2)	27 ± 5
Disease duration (years)	19 ± 18
Age of asthma onset (years) (n = 116)	33 ± 22
Asthma onset categories (n = 116)	
Early (< 12) (%)	27 (23)
Intermediate (12–40) (%)	38 (33)
Late (> 40) (%)	51 (44)
Time between visits (months)	66 (62–86) (Min:52 – Max:160)
Smoking status (n = 123)	
Non-smokers (%)	59 (48)
Current smokers (%)	29 (24)
Ex-smokers (%)	35 (28)
Number of packyears	0 (0–15)
Atopy (%) (n = 122)	71 (58)
Pre-BD FEV_1 (mL)	2.35 ± 0.85
Pre-BD FEV_1 (% pred.)	79 ± 21
Post-BD FEV_1 (mL)	2.58 ± 0.87
Post-BD FEV_1 (% pred.)	86 ± 19
Post-BD FEV_1/FVC (%)	73 ± 12
Reversibility (%)	10.8 ± 11.8
PC20M (mg/mL) (n = 50)	2.42 (0.63–10.23)
FeNO (ppb) (n = 112)	31.3 (16.6–51.8)
SEC (%) (n = 121)	3.4 (0.8–17.3)
SEC (/µL) (n = 117)	44.1 (7.4–342.2)
SNC (%) (n = 121)	46.4 (28.7–71.2)
SNC (/µL) (n = 117)	451.0 (175.0–1663.0)
Sputum inflammatory phenotypes:	
Paucigranulocytic (%)	36 (30)
Eosinophilic (%)	60 (50)
Neutrophilic (%)	20 (16)
Mixed (%)	5 (4)
Serum IgE (kU/L) (n = 119)	156 (66–404)
CRP (mg/L) (n = 96)	1.65 (0.60–5.33)
Fibrinogen (g/L) (n = 91)	3.21 (2.77–3.77)
BEC (/µL) (n = 119)	214 (118–375)
BNC (/µL) (n = 119)	4059 (3162–5541)
ACT score (n = 81)	14.9 ± 5.1
ACQ score (n = 70)	2.08 ± 1.27
AQLQ score (n = 120)	4.51 ± 1.36
ICS dose (µg/day) (n = 124)	1000 (400–2000)
ICS categories (n = 124)	
Steroid naïve (%)	27 (22)
Low dose (%)	20 (16)
Moderate dose (%)	28 (23)
High dose (%)	49 (39)

Table 1 (continued)

A	
Characteristics at baseline	
LABA user (%)	94 (75)
LAMA user (%)	13 (10)
OCS user (%)	14 (11)
LTRA user (%)	32 (26)
Exacerbations in last 12 months (n = 122)	0 (0–1)
Exacerbations categories	
0 (%)	80 (66)
1 (%)	33 (27)
≥ 2 (%)	9 (7)
Hospitalizations in last 12 months (n = 73)	0 (0–0)
Decline post-BD FEV_1 ($mL.y^{-1}$)	22.1 ± 65.3
Decline post-BD FEV_1 (% $pred.y^{-1}$)	0.06 ± 2.44
B	
Characteristics—evolution over time	
N	125
Δ Smoking status	
Never (%)	53 (43)
Stable (%)	55 (45)
Never becoming current (%)	4 (3)
Current becoming Ex (%)	11 (9)
Δ FeNO (ppb)	− 4 (− 22–5)
Δ SEC (%) (n = 146)	− 1.1 (− 5.8–2.2)
Δ SNC (%) (n = 146)	12.3 (− 4.3–28.0)
Δ BEC (/µL) (n = 156)	− 12.0 (− 109.0–90.5)
Δ BNC (/µL) (n = 156)	115.5 (− 785.0–1215.0)
Δ ACT score	2.12 ± 5.95
Δ Exacerbations	0 (0–0)
Δ ICS dose (µg/day)	0 (− 500; 250)
Δ ICS dose categories	
Steroid naïve (%)	13 (10)
Stable (%)	53 (42)
Increased (%)	27 (22)
Decreased (%)	32 (26)
Δ LABA	
Naïve (%)	14 (11)
Stable (%)	82 (66)
Added (%)	17 (13)
Discontinued (%)	12 (10)
Δ LAMA	
Naïve (%)	107 (86)
Stable (%)	6 (5)
Added (%)	4 (3)
Discontinued (%)	7 (6)
Δ OCS	
Naïve (%)	103 (82)
Stable (%)	5 (4)
Added (%)	8 (6)

Table 1 (continued)

B	
Characteristics—evolution over time	
Discontinued (%)	9 (7)

Vx, Follow-up visit; V1, baseline visit; BMI, body mass index; BD, bronchodilation; SEC, sputum eosinophil count; SNC, sputum neutrophil count, BEC, blood eosinophil count; BNC, blood neutrophil count; ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; CRP, C reactive protein; ICS, inhaled corticosteroid; LABA, long-acting beta2 agonist; LAMA, long-acting muscarinic antagonist; LTRA, Leucotriene Receptor Antagonist; OCS, oral corticosteroid; PC20M, provocative concentration of metacholine causing a 20% fall in FEV1; ppb, parts per billion; Delta (Δ), Vx value minus V1 value

FEV_1 decline (% pred.$y^{-1} \geq 0.85$). A comparison of the clinical characteristics of accelerated airflow declining group compared to others is presented in Table 2. Patients with accelerated decline were older (56 ± 12 years versus 49 ± 15; p = 0.0063), had a later onset of asthma (43 ± 21 years versus 29 ± 21; p = 0.0039) and tend to have more patients with late-onset (> 40 years). Although baseline post-BD FEV_1 were similar in both groups, post-BD FEV_1/FVC ratio (% pred.) was lower in patients with accelerated decline (67 ± 11 versus 75 ± 11; p = 0.0119). Sputum neutrophil counts (%) were higher at baseline in patients with accelerated decline (57.0 (45.4–77.2) versus 41.9 (24.4 – 66.2); p = 0.0181). Although smoking status were not statistically different at baseline, the group of patients with accelerated decline over time tend to have more patients with history of smoking (65%) compared to non-decliners (48%), and they maintain their status over time, while 11% of patients in the non-decliner group discontinue smoking during the follow-up period (p = 0.038).

Other characteristics at baseline were not different between groups. Moreover, there was no difference between groups in terms of evolution (change) of biomarkers, treatments, or exacerbations over time.

Factors Associated with Lung Function Decline

The univariate linear regression analysis (Table 3) revealed a positive association between FEV_1 decline and Δ smoking (Reg.Coef. (95%CI): 1.34 (0.44 to 2.24), p = 0.004), meaning that current smokers (or ex-smokers) that do not change smoking status over time decline significantly faster than never smokers. Δ BEC was also positively associated with FEV_1 decline (0.002 (0.001 to 0.003), p < 0.0001), meaning that an increase in blood eosinophils during follow-up is associated with faster decline.

The multivariable analysis showed that an increase in blood eosinophils over time (Δ BEC) (0.002 (0.001 to 0.004), p = 0.005) and onset of asthma (0.04 (0.003 to 0.07), p = 0.036) were associated with an accelerated FEV_1 decline independently from other variables such as BMI, disease duration, atopic status, change in exacerbations, change in smoking status, change in ACT score, change in FeNO level, change in SEC, change in SNC, change in BNC, change in ICS dose, or change in chronic OCS use status over time.

Cytokines, Angiogenic Factors and Immunoglobulin E

Levels of cytokines, angiogenic factors, and IgE were measured in sputum in a sub-population of 58 patients whose characteristics are given in Tables 1 and 2 online supplement. Levels of measured IL8 at baseline were higher (499.7 (408.9—603.2) pg/ml, p = 0.0040) in the declining group (FEV_1 decline ≥ 0.85% pred.y^{-1}) compared to baseline IL8 levels in non-declining group (143.9 (88.5—308.8)). Levels of EGF, VEGF, IgE, and TGF beta in the declining group (n = 15) were not statistically different from levels in non-declining group (n = 43) (Table 4). Paired analyses between baseline and follow-up visit did not reveal a difference between groups either (Table 3 online supplement). FGF and IL5 were not detectable in our samples.

Constructing a ROC curve revealed that the IL8 level in sputum supernatant was able to identify accelerated decline (FEV_1 decline ≥ 0.85% pred.y^{-1}) with the best cut-off point of 385.14 (pg/ml) providing an 83% sensitivity and 89% specificity (AUC 0.7810, p = 0.0040, Fig. 2).

Discussion

In this prospective study, we have confirmed that in a secondary care asthma clinic, patients with an increase in blood eosinophil counts over 5–10 year-follow-up duration are more at risk of accelerated annual lung function decline than others. Moreover, we show that later onset and high sputum IL8 levels are associated with accelerated decline.

Rates of post-BD FEV_1 decline (in % pred.) previously reported in studies with at least 5 year-follow-up have varied from 2.22% (SE: 0.15%) over a 5-year period in asthmatics [22] to 0.07 units in FEV_1%pred. in a population with an average of 18 year-follow-up [23] which is comparable to our results with 0.06% pred.y^{-1}.

Our data indicate that half of the population does not actually decline which is an important observation and makes

Table 2 Comparison: Airflow decline (> 0.85%) group (n = 59) to no decline (≤ 0.85%) group (n = 66) based on FEV_1 decline % pred. (V_1-V_x). Demographic and clinical data at first visit (baseline) (A) and evolution over time (Δ) (B)

A			
	Non-decliners	Airflow decliners	P value
N (%)	93 (74)	32 (26)	
Gender (F) (%)	54 (58)	20 (63)	0.683
Age (years)	49 ± 15	56 ± 12	0.0063
BMI (kg/m^2)	26 ± 5	27 ± 5	0.4331
Disease duration (years)	21 ± 19	15 ± 18	0.1348
Asthma onset			0.060
< 12 (%)	24 (28)	3 (10)	
12 to 40 (%)	29 (34)	9 (30)	
> 40 (%)	33 (38)	18 (60)	
Age asthma onset (years)	29 ± 21	43 ± 21	0.0039
Time between first and last visit (months)	75 ± 19	78 ± 26	0.5439
Smoking status			0.064
Non-smokers (%)	48 (52)	11 (35)	
Current smokers (%)	23 (25)	6 (19)	
Ex-smokers (%)	21 (23)	14 (45)	
Pack-Year	8.3 ± 13.8	14.3 ± 23.2	0.1850
Atopy (Y) (%)	53 (59)	18 (56)	0.837
Pre-BD FEV_1 (L)	2.44 ± 0.86	2.11 ± 0.79	0.0513
Pre-BD FEV_1 (% pred.)	79 ± 20	77 ± 24	0.5575
Post-BD FEV_1 (L)	2.65 ± 0.87	2.39 ± 0.87	0.1673
Post-BD FEV_1 (% pred.)	86 ± 18	86 ± 23	0.9941
Post-BD FEV_1/FVC (%)	75 ± 11	67 ± 11	0.0119
Reversibility (%)	9.7 ± 11.2	14.0 ± 13.1	0.1089
PC20M (mg/ml)	2.68 (0.57–10.34)	1.65 (0.88–6.09)	0.8648
FeNO (ppb)	33.1 (16.2–55.2)	25.7 (18.0–48.0)	0.5346
SEC (%)	4.8 (0.8–17.3)	2.0 (0.6–19.2)	0.4033
SNC (%)	41.9 (24.4–66.2)	57.0 (45.4–77.2)	0.0181
Sputum inflammatory phenotypes			0.131
Paucigranulocytic (%)	27 (30)	9 (29)	
Eosinophilic (%)	47 (52)	13 (42)	
Neutrophilic (%)	11 (12)	9 (29)	
Mixed (%)	5 (6)	0 (0)	
Serum IgE (kU/L)	196 (67–420)	86 (59–256)	0.1741
BEC (/μL)	215 (123–376)	209 (99–297)	0.5369
BNC (/μL)	4023 (3024–5065)	4752 (3551–6150)	0.1256
ACT score	14.7 ± 5.1	15.8 ± 5.2	0.3795
ACQ score	2.13 ± 1.29	1.97 ± 1.24	0.6641
AQLQ score	4.48 ± 1.37	4.59 ± 1.36	0.7114
ICS dose	1000 (400–2000)	650 (100–2000)	0.3778
ICS category: (%)			0.342
Steroid naïve (%)	19 (21)	8 (25)	
Low dose (%)	12 (13)	8 (25)	
Moderate dose(%)	22 (24)	6 (19)	
High dose (%)	39 (42)	10 (31)	
LABA (Y) (%)	71 (76)	23 (72)	0.639
LAMA (Y) (%)	7 (8)	6 (19)	0.094
OCS (Y) (%)	10 (11)	4 (13)	0.753

Table 2 (continued)

A			
	Non-decliners	Airflow decliners	P value
LTRA (Y) (%)	24 (26)	8 (25)	1.000
Exacerbations in last 12 months	0 (0–1)	0 (0–1)	0.5355
Exacerbations			0.926
0	28 (52)	11 (58)	
1	20 (37)	7 (37)	
≥ 2	6 (11)	1 (5)	
Hospitalizations in last 12 months	0 (0–0)	0 (0–1)	0.4189
B			
	Non − decliners	Airflow decliners	P value
N (%)	93 (74)	32 (26)	
Δ Smoking status			0.038
Never (%)	44 (48)	9 (29)	
Stable (current or ex − smokers) (%)	34 (37)	21 (68)	
Never becoming current (%)	3 (3)	1 (3)	
Current becoming Ex (%)	10 (11)	0 (0)	
Δ FeNO (ppb)	− 4 (− 26; − 6)	− 3 (− 16; − 4)	0.8130
Δ SEC (%)	− 1.4 (− 7.0; − 2.4)	0.2 (− 2.8; − 2.0)	0.3149
Δ SNC (%)	5.3 (− 7.6; − 24.7)	9.2 (1.9; − 19.0)	0.6828
Δ BEC (/μL)	− 16 (− 123; − 76)	0 (− 60; − 169)	0.1732
Δ BNC (/μL)	37 (− 796; − 1427)	193 (− 458; − 750)	0.6658
Δ ACT	3 (− 1; − 6)	0 (− 3; − 3)	0.0828
Δ Exacerbations	0 (− 1; 0)	0 (0; 1)	0.0738
Δ ICS dose	0 (− 500; 0)	0 (0; 775)	0.0677
Δ ICS (category) dose			0.283
Steroid naïve (%)	9 (10)	4 (13)	
Stable (%)	40 (43)	13 (41)	
Increased (%)	17 (18)	10 (31)	
Decreased (%)	27 (29)	5 (16)	
Δ LABA			0.878
Naïve (%)	10 (11)	4 (13)	
Stable (%)	61 (66)	21 (66)	
Added (%)	12 (13)	5 (16)	
Discontinued (%)	10 (11)	2 (6)	
Δ LAMA			0.081
Naïve (%)	84 (90)	23 (74)	
Stable (%)	3 (3)	3 (10)	
Added (%)	2 (2)	2 (6)	
Discontinued (%)	4 (4)	3 (10)	
Δ OCS			0.337
Naïve (%)	79 (85)	24 (75)	
Stable (%)	4 (4)	1 (3)	
Added (%)	4 (4)	4 (12)	
Discontinued (%)	6 (7)	3 (9)	

BMI, body mass index; BD, bronchodilation; SEC, sputum eosinophil count; SNC, sputum neutrophil count, BEC, blood eosinophil count; BNC, blood neutrophil count; ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; CRP, C reactive protein; ICS, inhaled corticosteroid; LABA, long-acting beta2 agonist; LAMA, long-acting muscarinic antagonist; LTRA, Leucotriene Receptor Antagonist; OCS, oral corticosteroid; PC20M, provocative concentration of metacholine causing a 20% fall in FEV1; ppb, parts per billion; Delta (Δ), Vx value minus V1 value

Table 3 Factors associated with lung function decline. Results of the linear regression–multivariable analysis. N = 125*

Lung function decline (Post − BD FEV_1% pred.y^{-1})	Total population*					
	Univariate			Multivariable		
	Coeff	95%CI	P value	Coeff	95%CI	P value
BMI	0.03	−0.06 to 0.12	0.567	−0.03	−0.13 to 0.06	0.502
Atopy	0.21	−0.67 to 1.09	0.644	0.81	−0.21 to 1.84	0.119
Disease duration	−0.01	−0.03 to 0.02	0.546	0.02	−0.02 to 0.05	0.370
Onset	0.02	−0.004 to 0.04	0.127	0.04	0.003 to 0.07	0.036
Δ Smoking (Never (%))						
Stable (%)	1.34	0.44 to 2.24	0.004	0.83	−0.12 to 1.78	0.085
Never becoming current (%)	1.94	−0.49 to 4.37	0.118	1.71	−0.86 to 4.28	0.190
Current becoming Ex (%)	0.15	−1.39 to 1.70	0.844	0.15	−1.53 to 1.83	0.858
Δ ACT score	−0.06	−0.15 to 0.03	0.156	−0.05	−0.14 to 0.04	0.234
Δ Exacerbations	0.07	−0.24 to 0.38	0.665	−0.04	−0.39 to 0.32	0.843
Δ FeNO (ppb)	0.01	−0.002 to 0.02	0.136	−0.001	−0.01 to 0.01	0.939
Δ SEC (%)	0.19	−0.006 to 0.04	0.127	0.01	−0.03 to 0.04	0.750
Δ SNC (%)	0.005	−0.01 to 0.02	0.570	0.01	−0.01 to 0.03	0.308
Δ BEC (/µL)	0.002	0.001 to 0.003	< 0.0001	0.002	0.001 to 0.004	0.005
Δ BNC (/µL)	−0.00002	−0.0002 to 0.0002	0.858	−0.0001	−0.0003 to 0.0001	0.475
Δ ICS dose (µg/day)	−0.00007	−0.0006 to 0.0004	0.764	−0.00005	−0.0004 to 0.0005	0.850
Δ OCS (Naïve)						
Stable	−1.38	−3.61 to 0.84	0.221	0.54	−2.04 to 3.12	0.680
Added	0.03	−1.75 to 1.81	0.973	0.85	−0.90 to 2.61	0.337
Discontinued	0.09	−1.32 to 2.06	0.664	0.41	−1.39 to 2.21	0.653

BMI, body mass index; SEC, sputum eosinophil count; SNC, sputum neutrophil count, BEC, blood eosinophil count; BNC, blood neutrophil count; ACT, Asthma Control Test; FeNO, fractional exhaled nitric oxide; ICS, inhaled corticosteroid; LABA, long-acting beta2 agonist; LAMA, long-acting muscarinic antagonist; OCS, oral corticosteroid; ppb, parts per billion; Delta (Δ), V_x value minus V_1 value

Table 4 Comparison of cytokine and growth factors levels (Pg/mL) measured in sputum at baseline in non-decliners and decliners

Baseline	Non-decliners	Decliners	p-value
EGF	65 (42–111) (n = 39)	93 (57–171) (n = 15)	0.1990
IL8	143 (88–308) (n = 35)	499 (408–603) (n = 12)	0.0040
VEGF	189 (141–263) (n = 38)	247 (170–329) (n = 14)	0.2082
TGF total	1536 (873–2480) (n = 43)	1071 (710–2456) (n = 15)	0.4612
TGF active	519 (415–742) (n = 43)	520 (391–776) (n = 15)	0.9222
IgE	0.31 (0.00–0.75) (n = 42)	0.31 (0.00–0.58) (n = 15)	0.9631

worth investigating the factors associated with decline. Hastie et al. also showed that only 20% of the population studied (i.e. patients who combined high sputum eosinophils and neutrophils) had an FEV_1 decline over a 3 years observation period [24].

Based on previous studies [7, 25], it appears that lung function decline should be evaluated in patients with at least 5 year-follow-up. With the current study on asthmatics with at least 5 year-follow-up, we confirm our previous observation linking change in blood eosinophils and change in post-bronchodilation FEV_1 [7]. Our data actually show that a reduction in BEC is associated with prevention of lung function decline and is sometimes related to lung function improvement [26]. Moreover, treatments targeting IL-5 thereby preventing increase in blood eosinophils over time were found to improve lung function in severe eosinophilic asthma [27]. Why change in BEC may be associated with lung function decline while change in SEC is not, remains unclear. It may be that eosinophils coming from the blood play a role in damaging distant part of the airways or parts of the lung itself, two compartments not adequately sampled by sputum analysis but which may critically contribute to FEV_1 decline [28, 29]. Also, recent evidence indicate that eosinophils are not a homogenous cell type and the proportions for different eosinophil types may vary between blood and airway lumen [30, 31].

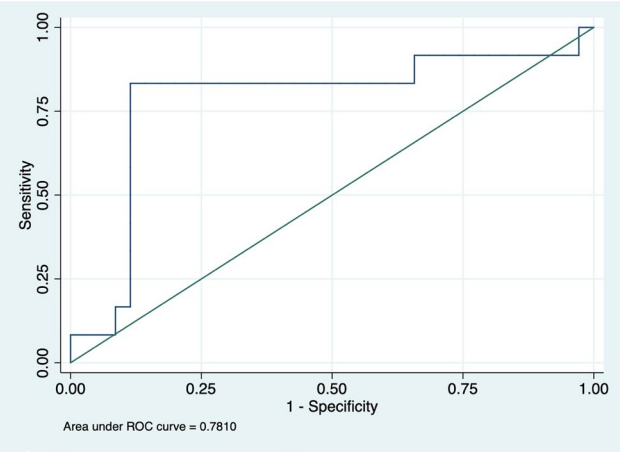

Fig. 2 Receiver operating characteristic (ROC) curves for sputum IL-8 as predictor for FEV_1 decline ($\geq 0.85\%$ pred.y.$^{-1}$) in asthma. Sensitivity 83%, specificity 89%, cut-off: 385 pg/ml, $p = 0.0040$, AUC: 0.7810

We also confirm the observation that later asthma onset is linked to accelerated decline in mild to moderate asthma [32, 33]. In the current study decliners had already clear indication of airway obstruction with significantly altered FEV_1/FVC ratio irrespective of smoking history. This suggests a more aggressive form of the disease likely to be prone to greater airway remodeling, indeed more often associated with comorbidities such as nasal polyposis and more intense local and systemic eosinophilic inflammation [34, 35].

Consistent with prior studies in asthma and chronic obstructive pulmonary disease (COPD) [36, 37] reporting that plasma and sputum levels of IL8 inversely correlate with lung function, we show that higher sputum IL8 levels are associated with accelerated FEV_1 decline over time. Marc-Malovrh et al. [38] had already shown that elevated sputum IL8 at follow-up might be raised in asthma patients with accelerated FEV_1 decline. In our study, we go one step further showing that higher baseline sputum IL8 levels are associated with accelerated lung function decline. Furthermore, we did measure levels of inflammatory cytokines at baseline and at follow-up and found IL8 levels remained high at follow-up. There are several possibilities through which IL-8 could favor lung function decline. IL-8 is a potent chemotactic agent for granulocytes (both neutrophils and eosinophils) recruitment in the airways the role of which have been suggested in persistent airway obstruction [19, 24]. In addition, it has been shown that IL-8 may contribute to the pathogenesis of severe asthma by directly facilitating airway remodeling with an increase bronchial smooth muscle cell migration and proliferation [39, 40]. Although elevated levels of IL-8 are usually associated with neutrophilia [41], we found increased levels of IL8 in decliners at baseline compared to non-decliners despite the lack of difference in SNC numbers. Indeed, even though, decliners in our overall population was older and was more neutrophilic ([42–45]), but in our subpopulation where cytokines were tested, decliners were not older or more neutrophilic. This implies that IL8 can play a role in remodeling independently of neutrophilia. To assess the usefulness of IL8 as a predictor of accelerated decline in asthma, a ROC analysis was performed. We found that, sputum IL8 demonstrated an optimal cutoff point at 385.14 (pg/ml), which is associated with a sensitivity and specificity of 83% and 89% respectively. Treatment with ant-IL8 receptor (CXCR2) inhibitor has however not brought any clinical improvement in moderate to severe asthma over a period of a few months [46]. However, it may be that outcomes such as day to day asthma control, FEV_1 fluctuation and exacerbations are disconnected from loss in post-bronchodilator FEV_1 over long term period. Contrary to our expectation, other cytokines and growth factors measured were not associated with loss of FEV_1 over time.

This study presents some limitations. First, lung function decline calculation was based on 2 measurements only. Second, all disease severities were present in our population which was a heterogenous population of asthmatics representative of a secondary care population, even if the patients were carefully diagnosed by specialists as opposed to self-reported. Third, time between first and last visits was also much variable but decline was annualized to correct for that possible bias. Fourth, treatment was not fixed during the follow-up but left at the discretion of the physicians and adherence to treatment was not recorded. Over all there was however no striking changes in asthma treatment between visit 1 and visit 2.

Conclusion

In this study, we have confirmed in a secondary care asthma clinic that patients with an increase in blood eosinophil counts over 5–10 year-follow-up duration or with later onset of asthma are more at risk of accelerated annual lung function decline than others. Moreover, high sputum IL8 levels are associated with accelerated decline, which makes this cytokine a possible target to counter loss in lung function on the long term.

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Author Contributions Graff S and Schleich F had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Graff S, Schleich F, Moermans C, Gerday S, Henket M, Paulus P, and Guissard F and Louis R contributed

substantially to the study design, data analysis and interpretation, and the writing of the manuscript.

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Data Availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interests Graff S, Moermans C, Gerday S, Henket M, Paulus P, and Guissard F have nothing to disclose. Prof. LOUIS reports grants and personal fees from GSK, grants and personal fees from AZ, grants and personal fees from Novartis, grants from Chiesi, outside the submitted work. Prof. Schleich reports grants and personal fees from GSK, grants from AZ, grants and personal fees from Chiesi, outside the submitted work.

Ethical Approval CHU Liège: 2008/181.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Consent for Publication Not applicable.

References

1. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G (1998) A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 339:1194–1200. https://doi.org/10.1056/NEJM199810223391703
2. Contoli M, Baraldo S, Marku B, Casolari P, Marwick JA, Turato G, Romagnoli M, Caramori G, Saetta M, Fabbri LM, Papi A (2010) Fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease: 5-year follow-up. J Allergy Clin Immunol 125:830–837. https://doi.org/10.1016/j.jaci.2010.01.003
3. Broekema M, Volbeda F, Timens W, Dijkstra A, Lee NA, Lee JJ, Lodewijk ME, Postma DS, Hylkema MN, ten Hacken NHT (2010) Airway eosinophilia in remission and progression of asthma: accumulation with a fast decline of FEV(1). Respir Med 104:1254–1262. https://doi.org/10.1016/j.rmed.2010.03.030
4. Talini D, Novelli F, Bacci E, Bartoli M, Cianchetti S, Costa F, Dente FL, Di Franco A, Latorre M, Malagrino L, Vagaggini B, Celi A, Paggiaro P (2015) Sputum eosinophilia is a determinant of FEV1 decline in occupational asthma: results of an observational study. BMJ Open 5:e005748–e005748. https://doi.org/10.1136/bmjopen-2014-005748
5. Sposato B, Scalese M, Ricci A, Rogliani P, Paggiaro P, The Omalizumab Italian Study Group, Sposato B, Migliorini MG, Di Tomassi M, Olivieri C, Perrella A, Camiciottoli G, Maselli R, Pelaia G, Busceti MT, Sabato E, Cagnazzo MG, Colombo F, Palumbo L, Ravazzi A, Bucca C, Caiaffa MF, Berra A, Calabrese C, Stanziola AA, Schino P, Di Gioacchino M, Rogliani P, Cazzola M, Segreti A, Pastorello EA, Scibilia G, Vianello A, Marchi MR, Paladini L, Baglioni S, Abbritti M, Almerigogna F, Matucci A, Vultaggio A, Maggi E, Maestrelli P, Guarnieri G, Steinhilber G, Bonavia M, Rottoli P, Bargagli E, Senna G, Caminati M, Macchia L, Bellia V, Scichilone N, Paggiaro P, Novelli F, Latorre M, Vergura L, Masieri S, Scalese M, Rosati Y, Milanese M, Folletti I, Pio R, Pio A, Maccari U, Maggiorelli C, Scala R, Vignale L, Pulerà N, Carpagnano GE, Foschino Barbaro MP (2021) Persistence of both reversible airway obstruction and higher blood eosinophils may predict lung function decline in severe asthma. Clin Respir J 15:237–243. https://doi.org/10.1111/crj.13325
6. Newby C, Agbetile J, Hargadon B, Monteiro W, Green R, Pavord I, Brightling C, Siddiqui S (2014) Lung function decline and variable airway inflammatory pattern: longitudinal analysis of severe asthma. J Allergy Clin Immunol 134:287–294. https://doi.org/10.1016/j.jaci.2014.04.005
7. Graff S, Demarche S, Henket M, Paulus V, Louis R, Schleich F (2019) Increase in blood eosinophils during follow-up is associated with lung function decline in adult asthma. Respir Med 152:60–66
8. Cianchetti S, Cardini C, Puxeddu I, Latorre M, Bartoli ML, Bradicich M, Dente F, Bacci E, Celi A, Paggiaro P (2019) Distinct profile of inflammatory and remodelling biomarkers in sputum of severe asthmatic patients with or without persistent airway obstruction. World Allergy Organ J 12:100078. https://doi.org/10.1016/j.waojou.2019.100078
9. Schleich FN, Zanella D, Stefanuto P-H, Bessonov K, Smolinska A, Dallinga JW, Henket M, Paulus V, Guissard F, Graff S, Moermans C, Wouters EFM, Van Steen K, van Schooten F-J, Focant J-F, Louis R (2019) Exhaled volatile organic compounds are able to discriminate between neutrophilic and eosinophilic asthma. Am J Respir Crit Care Med 200:444–453. https://doi.org/10.1164/rccm.201811-2210OC
10. GINA-Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2018. Available from: www.ginasthma.org
11. Juniper EF, Guyatt GH, Epstein RS, Ferrie PJ, Jaeschke R, Hiller TK (1992) Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. Thorax 47(2):76–83
12. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR (1999) Development and validation of a questionnaire to measure asthma control. Eur Respir J 14:902–907
13. Nathan RA, Kosinski M, Pendergraft TB, Jhingran P (2006) Asthma control test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. J Allergy Clin Immunol 117:549–556
14. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR (2011) American thoracic society committee on interpretation of exhaled nitric oxide levels (FENO) for clinical applications an official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med 184(5):602–615. https://doi.org/10.1164/rccm.9120-11ST
15. Guiot J, Demarche S, Henket M, Paulus V, Graff S, Schleich F, Corhay J-L, Louis R, Moermans C (2017) Methodology for

sputum induction and laboratory processing. J Vis Exp JoVE. https://doi.org/10.3791/56612
16. Delvaux M, Henket M, Lau L, Kange P, Bartsch P, Djukanovic R, Louis R (2004) Nebulised salbutamol administered during sputum induction improves bronchoprotection in patients with asthma. Thorax 59:111–115. https://doi.org/10.1136/thorax.2003.011130
17. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, Ip MS, Zheng J, Stocks J (2012) ERS global lung function initiative. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. Eur Respir J 40(6):1324–1343. https://doi.org/10.1183/09031936.00080312
18. Godinas L, Corhay J-L, Henket M, Guiot J, Louis R, Moermans C (2017) Increased production of TGF-β1 from sputum cells of COPD: relationship with airway obstruction. Cytokine 99:1–8. https://doi.org/10.1016/j.cyto.2017.06.018
19. Graff S, Bricmont N, Moermans C, Henket M, Paulus V, Guissard F, Louis R, Schleich F (2020) Clinical and biological factors associated with irreversible airway obstruction in adult asthma. Respir Med 175:106202. https://doi.org/10.1016/j.rmed.2020.106202
20. Schafer JL, Graham JW (2002) Missing data: our view of the state of the art. Psychol Methods 7:147–177
21. Multiple Imputation for Nonresponse in Surveys | Wiley. In: Wiley.com. https://www.wiley.com/en-us/Multiple+Imputation+for+Nonresponse+in+Surveys-p-9780471655749. Accessed 10 Feb 2021
22. Busse WW, Pedersen S, Pauwels RA, Tan WC, Chen Y-Z, Lamm CJ, O'Byrne PM, START Investigators Group (2008) The inhaled steroid treatment as regular therapy in early asthma (START) study 5-year follow-up: effectiveness of early intervention with budesonide in mild persistent asthma. J Allergy Clin Immunol 121:1167–1174. https://doi.org/10.1016/j.jaci.2008.02.029
23. Backman H, Lindberg A, Hedman L, Stridsman C, Jansson S-A, Sandström T, Lundbäck B, Rönmark E (2020) FEV1 decline in relation to blood eosinophils and neutrophils in a population-based asthma cohort. World Allergy Organ J 13:100110. https://doi.org/10.1016/j.waojou.2020.100110
24. Hastie AT, Mauger DT, Denlinger LC, Coverstone A, Castro M, Erzurum S, Jarjour N, Levy BD, Meyers DA, Moore WC, Phillips BR, Wenzel SE, Fahy JV, Israel E, Bleecker ER (2021) Mixed sputum granulocyte longitudinal impact on lung function in the severe asthma research program. Am J Respir Crit Care Med 203:882–892. https://doi.org/10.1164/rccm.202009-3713OC
25. Sposato B (2016) Predicted values for spirometry may underestimate long-standing asthma severity. Open Respir Med J 10:70–78. https://doi.org/10.2174/1874306401610010070
26. Denlinger LC, Phillips BR, Sorkness RL, Bleecker ER, Castro M, DeBoer MD, Fitzpatrick AM, Hastie AT, Gaffin JM, Moore WC, Peters MC, Peters SP, Phipatanakul W, Cardet JC, Erzurum SC, Fahy JV, Fajt ML, Gaston B, Levy BD, Meyers DA, Ross K, Teague WG, Wenzel SE, Woodruff PG, Zein J, Jarjour NN, Mauger DT, Israel E (2021) Responsiveness to parenteral corticosteroids and lung function trajectory in adults with moderate-to-severe asthma. Am J Respir Crit Care Med 203:841–852. https://doi.org/10.1164/rccm.202002-0454OC
27. Schleich F, Graff S, Nekoee H, Moermans C, Henket M, Sanchez C, Paulus V, Guissard F, Donneau A, Louis R (2020) Real-word experience with mepolizumab: does it deliver what it has promised? Clin Exp Allergy 50:687–695. https://doi.org/10.1111/cea.13601
28. Schleich F, Louis R (2019) It needs more than just eosinophils to cause emphysema in COPD. Eur Respir J. https://doi.org/10.1183/13993003.00332-2019
29. Doyle AD, Mukherjee M, LeSuer WE, Bittner TB, Pasha SM, Frere JJ, Neely JL, Kloeber JA, Shim KP, Ochkur SI, Ho T, Svenningsen S, Wright BL, Rank MA, Lee JJ, Nair P, Jacobsen EA (2019) Eosinophil-derived IL-13 promotes emphysema. Eur Respir J 53:1801291. https://doi.org/10.1183/13993003.01291-2018
30. Cabrera López C, Sánchez Santos A, Lemes Castellano A, Cazorla Rivero S, Breña Atienza J, González Dávila E, Celli B, Casanova MC (2023) Eosinophil subtypes in adults with asthma and adults with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. https://doi.org/10.1164/rccm.202301-0149OC
31. Mesnil C, Raulier S, Paulissen G, Xiao X, Birrell MA, Pirottin D, Janss T, Starkl P, Ramery E, Henket M, Schleich FN, Radermecker M, Thielemans K, Gillet L, Thiry M, Belvisi MG, Louis R, Desmet C, Marichal T, Bureau F (2016) Lung-resident eosinophils represent a distinct regulatory eosinophil subset. J Clin Invest 126:3279–3295. https://doi.org/10.1172/JCI85664
32. Ulrik CS, Lange P (1994) Decline of lung function in adults with bronchial asthma. Am J Respir Crit Care Med 150:629–634. https://doi.org/10.1164/ajrccm.150.3.8087330
33. Porsbjerg C, Lange P, Ulrik CS (2015) Lung function impairment increases with age of diagnosis in adult onset asthma. Respir Med 109:821–827. https://doi.org/10.1016/j.rmed.2015.04.012
34. Schleich F, Brusselle G, Louis R, Vandenplas O, Michils A, Pilette C, Peche R, Manise M, Joos G (2014) Heterogeneity of phenotypes in severe asthmatics. the belgian severe asthma registry (BSAR). Respir Med 108:1723–1732. https://doi.org/10.1016/j.rmed.2014.10.007
35. Schleich FN, Chevremont A, Paulus V, Henket M, Manise M, Seidel L, Louis R (2014) Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. Eur Respir J 44:97–108. https://doi.org/10.1183/09031936.00201813
36. Huang A-X, Lu L-W, Liu W-J, Huang M (2016) Plasma inflammatory cytokine IL-4, IL-8, IL-10, and TNF-α levels correlate with pulmonary function in patients with asthma-chronic obstructive pulmonary disease (COPD) overlap syndrome. Med Sci Monit 22:2800–2808. https://doi.org/10.12659/MSM.896458
37. Wilkinson TMA, Patel IS, Wilks M, Donaldson GC, Wedzicha JA (2003) Airway bacterial load and FEV $_1$ decline in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 167:1090–1095. https://doi.org/10.1164/rccm.200210-1179OC
38. Marc-Malovrh M, Camlek L, Škrgat S, Kern I, Fležar M, Dežman M, Korošec P (2020) Elevated eosinophils, IL5 and IL8 in induced sputum in asthma patients with accelerated FEV1 decline. Respir Med 162:105875. https://doi.org/10.1016/j.rmed.2020.105875
39. Kuo P-L, Hsu Y-L, Huang M-S, Chiang S-L, Ko Y-C (2011) Bronchial epithelium-derived IL-8 and RANTES increased bronchial smooth muscle cell migration and proliferation by Krüppel-like factor 5 in areca nut-mediated airway remodeling. Toxicol Sci 121:177–190. https://doi.org/10.1093/toxsci/kfr030
40. Hosoki K, Ying S, Corrigan C, Qi H, Kurosky A, Jennings K, Sun Q, Boldogh I, Sur S (2015) Analysis of a panel of 48 cytokines in BAL fluids specifically identifies IL-8 levels as the only cytokine that distinguishes controlled asthma from uncontrolled asthma, and correlates inversely with FEV1. PLoS ONE. https://doi.org/10.1371/journal.pone.0126035
41. Gibson PG, Simpson JL, Saltos N (2001) Heterogeneity of airway inflammation in persistent asthma : evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 119:1329–1336. https://doi.org/10.1378/chest.119.5.1329
42. Thomas RA, Green RH, Brightling CE, Birring SS, Parker D, Wardlaw AJ, Pavord ID (2004) The influence of age on induced sputum differential cell counts in normal subjects. Chest 126:1811–1814. https://doi.org/10.1378/chest.126.6.1811

43. Ducharme M-E, Prince P, Hassan N, Nair P, Boulet L-P (2011) Expiratory flows and airway inflammation in elderly asthmatic patients. Respir Med 105:1284–1289. https://doi.org/10.1016/j.rmed.2011.04.006
44. Schleich F, Graff S, Guissard F, Henket M, Paulus V, Louis R (2021) Asthma in elderly is characterized by increased sputum neutrophils, lower airway caliber variability and air trapping. Respir Res 22:15. https://doi.org/10.1186/s12931-021-01619-w
45. Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R (2013) Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. BMC Pulm Med 13:11. https://doi.org/10.1186/1471-2466-13-11
46. O'Byrne PM, Metev H, Puu M, Richter K, Keen C, Uddin M, Larsson B, Cullberg M, Nair P (2016) Efficacy and safety of a CXCR2 antagonist, AZD5069, in patients with uncontrolled persistent asthma: a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 4:797–806. https://doi.org/10.1016/S2213-2600(16)30227-2