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Original Research

High local type-2 inflammation is linked to response in severe asthma treated with anti-Interleukin-5 receptor

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ABSTRACT

Background: Benralizumab is an anti-IL-5 receptor (IL-5R) therapy linked to a huge improvement of the condition of patients with severe eosinophilic asthma. The goal of this study was to identify baseline airway markers of remission and response after anti-IL5R therapy.
Methods: This observational study included 45 patients initiated with an anti-IL-5R. Remission was defined as: no oral corticosteroids intake, no exacerbation, a good asthma control (ACQ <1.5 and/or ACT >19) and a good lung function ($FEV_1 \geq 80$ % predicted and/or an improvement ≥10 %). Components of remission were also assessed individually to evaluate the response of patients. Sputum levels of mediators implicated in inflammation and remodeling were measured before treatment.
Results: Among the 45 patients, 12 were classified in remission. These patients were younger at baseline, had a lower smoking exposure, better asthma control and quality of life and a higher FeNO compared to the others. Moreover, baseline blood eosinophil counts were similar but sputum IL-6 and IL-8 levels were significantly higher in the non-remission group. Finally, patients who only improved their ACT or ACQ score had higher baseline FeNO values or sputum eosinophil percentage respectively. Those who increased their $FEV_1 \geq 10$ % presented a higher baseline sputum eosinophil percentage, sputum eotaxin-3 level and a trend for a higher sputum IL-5 level.
Conclusion: High baseline airway T2 markers appeared to be associated with response to anti-IL-5R therapy. Lower sputum IL-6 and IL-8 levels were linked to remission. These results need to be validated in a bigger cohort.

1. Introduction

Asthma is a highly prevalent chronic respiratory disease which affects people of all ages. It affects 262 million people worldwide [1] and is associated with 455, 000 deaths (WHO website). Severe asthma is a heterogeneous and difficult to treat disease defined in the past by an expert committee [2]. More than half of the patients with severe asthma exhibit an eosinophilic airway inflammation [3] despite high doses of inhaled corticosteroids.

Patients with severe eosinophilic asthma can now benefit from new available biotherapies that target the reduction of the eosinophilic inflammation through different molecular processes. One of them, benralizumab, binds to the IL-5 receptor (IL-5R) alpha and is responsible for the eosinophil apoptosis and depletion.

However, patients do not response equally to this therapy. Two randomized controlled trials based on large patient cohorts (CALIMA and SIROCCO) showed that the response to benralizumab, in term of reduction of exacerbations and improvement in lung function, was higher in patients with a blood eosinophil count (BEC) ≥ 300/µl who were characterized at baseline by oral corticosteroid (OCS) use, nasal polyposis, pre-bronchodilation forced vital capacity <65 % predicted,

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Abbreviations list

ACQ	Asthma control questionnaire
ACT	Asthma control test
AQLQ	Asthma quality of life questionnaire
ATS	American Thoracic Society
BEC	Blood eosinophil count
BMI	Body mass index
CRP	C reactive protein
DTT	Dithiothreitol
ERS	European Respiratory Society
FeNO	Fraction of exhaled nitric oxide
FEV_1	Forced expiration volume in 1s
FVC	Forced vital capacity
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IgE	Immunoglobulin E
IGFBP1	Insulin-like growth factor-binding protein 1
IGFBP3	Insulin-like growth factor-binding protein 3
IL-5	Interleukin-5
IL-5R	Interleukin-5 receptor
LLOD	Lower limits of detection
OCS	Oral corticosteroids
MMP-7	Matrix Metalloproteinase 7
ROC:	Receiver-operating characteristic
TNF-alpha	Tumor necrosis factor-alpha
TSLP	Thymic stromal lymphopoietin
YKL-40	Chitinase 3-like 1

≥3 exacerbations in the previous year and an age at diagnosis ≥18 years.

Asthma remission is a relatively new concept which has not been strictly defined, but in the literature, most authors agree on a definition that includes: no chronic treatment with OCS, no exacerbation, a good asthma control and a stable or improved lung function after at least one year follow-up [4–6].

However, even though BEC has been shown to be predictive of response after therapy targeting IL-5 or its receptor, our group has shown that baseline local markers such as sputum eosinophil count and sputum Type 2 (T2) mediators may be more predictive of remission one year after anti-IL-5 treatment [7,8]. Indeed, airway markers reflect what is ongoing at local level, which can be discordant from what is observed at systemic level.

The goal of this study was to highlight local predictors of remission after anti-IL5R treatment in a cohort of patients suffering from severe eosinophilic asthma. We focused our analyses on mediators implicated in T2 immune responses such as interleukin (IL)-4, IL-5, IL-13, IL-25, IL-33, granulocyte-macrophage colony-stimulating factor (GM-CSF), thymic stromal lymphopoietin (TSLP), eotaxin-3 (CCL-26), and Immunoglobulin E (IgE), in those involved in type 1 response such as the IL-1 family member IL-18, pro-inflammatory cytokines as IL-6, IL-8 and Tumor necrosis factor (TNF-) alpha and anti-inflammatory cytokine as IL-10 and finally those with a role in airway remodeling such as Matrix metalloproteinase-7 (MMP-7), Chitinase 3-like 1 (YKL-40), and Insulin-like growth factor-binding protein 1 and 3 (IGFBP1 and IGFBP3). In addition to its role in airway remodeling, MMP-7 was shown to induce T2 inflammation via the cleavage of IL-25 [9].

2. Study design and methods

2.1. Patients

This observational study included forty-five patients with severe asthma initiated with an anti-IL-5R and recruited from our asthma clinic between 2018 and 2024. Inclusion criteria included a diagnosis of severe asthma defined according to European Respiratory Society (ERS)/ American Thoracic Society (ATS) criteria [2]. The presence of nasal polyps was diagnosed by an Ear-Nose-Throat specialist. For all patients, the treatment was stable at the time of the sampling collection and the average time from baseline to the next evaluation was 18 months. Benralizumab was administrated at the dose of 30 mg each month for the first 3 months and then every 2 months thereafter. We did not include patients who just switched from another biotherapy. Most of the patients were naïve and for the minority who were treated with another biologic in the past, the interval between the last injection of the previous biotherapy and the start of benralizumab was greater than 6 months.

Remission after treatment was defined as: no oral corticosteroids intake, no exacerbation within the last 12 months, a good asthma control: asthma control questionnaire (ACQ) < 1.5 and/or asthma control test (ACT) > 19 and a good or improved lung function: Forced expiration volume in 1s (FEV_1) ≥ 80 % predicted and/or an improvement ≥10 %.

An exacerbation was defined as an increase in symptoms requiring a treatment with oral corticosteroids (OCS) for at least 3 days or an admission to an emergency room.

Sub-analyses were performed for each parameter individually to assess their predictive values in terms of response such as: an improvement in asthma control based on a gain of ACT >3 or a decrease of 0.5 of ACQ score after treatment, or an improvement in FEV_1 of at least 10 % post-treatment. None of the patients were classified as non-responders as most patients decreased their exacerbation rate (98 % of patients) or OCS dose (100 % of patients) by ≥ 50 % and 92 % had a sputum eosinophil percentage that decreased more than 50 % after treatment. We also looked at the patients who were considered as partial responders defined as patients who were unable to stop OCS intake or who still had exacerbations after 18 months of treatment.

Finally, some results were compared with those obtained from a cohort of 13 healthy subjects matched for demographic characteristics to the asthmatic patients (their characteristics are detailed in the online supplement e-Table 1).

This study has obtained ethical approval from our review board and all subjects gave written informed consent to participate.

2.2. Study design

This study aims to investigate the airway expression of sputum mediators as predictors of remission after anti-IL-5R treatment in a cohort of patients with severe eosinophilic asthma. This was our first study based on mediator measurements in induced sputum of a cohort of patients treated with anti-IL5R and was descriptive and observational, therefore statistical power calculations were not performed. We included all the patients treated with anti-IL5R at our center, for whom we had available sputum supernatant before the initiation of the therapy and who were followed for at least 18 months.

2.3. Respiratory function

Spirometry was performed before and after the inhalation of a bronchodilator agent according to the ATS/ERS standard criteria [10]. Fraction of exhaled nitric oxide (FeNO) was measured using NiOX at a flow rate of 50 mL/s (Aerocrine, Solna, Sweden).

2.4. Blood samples

The blood samples were processed and analyzed by the routine laboratory of our center for leukocyte count, c reactive protein (CRP)

Table 1
Baseline demographic and clinical characteristics of patients with remission (n = 12) vs others (n = 33).

	Remission	No remission	P value
Sex (M/F)	6/6	19/14	0.74
Age (years)	51 ± 13	60 ± 13	<0.05
BMI (kg/m^2)	25 ± 5	26 ± 5	0.71
Smoking status (NS/CS/ES)	10/0/2	15/3/15	0.07
Pack-years	0 (0–0)	6 (0–25)	<0.05
Atopy (yes/no)	8/4	20/13	0.71
OCS cure	2 (1–3)	2 (2–4)	0.17
ACT score	13 (9–18)	10 (8–16)	0.29
ACQ score	2.2 ± 1.2	3.1 ± 1.0	<0.05
AQLQ score	4.5 ± 1.1	3.5 ± 1.1	<0.01
FEV$_1$ (predicted%)	74 ± 18	64 ± 17	0.07
FEV$_1$ post BD (predicted%)	80 ± 19	70 ± 18	0.09
FVC (predicted%)	85 ± 12	76 ± 16	0.07
FVC post BD (predicted%)	89 ± 9	80 ± 16	0.10
FEV1/FVC (%)	69 ± 9	66 ± 12	0.40
FEV1/FVC post BD (%)	72 ± 12	68 ± 12	0.38
Blood neutrophils (/μL)	3511 (2758–4978)	5095 (3367–7216)	0.10
Blood eosinophils (/μL)	455 (330–1384)	455 (295–665)	0.44
Blood eosinophils (/μL) no OCS	428 (317–1433)	434 (272–820)	0.58
Total serum IgE (kU/L)	74 (33–335)	204 (52–504)	0.38
CRP (mg/l)	2.1 (1.2–3.4)	2.8 (1.0–7.2)	0.28
Fibrinogen (g/l)	3.3 (2.9–3.9)	3.4 (3.0–4.1)	0.67
ICS (beclomethasone equivalent)	1500 (1000–2750)	2000 (1600–2000)	0.17
OCS (yes/no)	2/10	8/25	0.71
FeNO (ppb)	71 (47–117)	32 (20–52)	<0.01
Nasal polyposis (yes/no)	5/7	8/25	0.25
Atopic dermatitis/urticaria (yes/no)	0/12	1/32	0.54

Results are presented as median (interquartile range) or mean ± SD; BMI: body mass index; NS: non-Smoker; CS: current smoker; ES: ex-smoker; OCS: oral corticosteroids; FEV$_1$: forced expiration volume in 1s; BD: bronchodilation; coefficient; FVC: forced vital capacity; CRP: C reactive protein; ICS: inhaled corticosteroids; FeNO: fraction of exhaled nitric oxide.

and fibrinogen levels.

2.5. Sputum induction and processing

The sputum was induced and processed as previously described [11, 12].

2.6. Sputum inflammatory mediator measurement

The first panel included interleukin (IL)-4, IL-5, IL-6, IL-10, IL-13, IL-18, IL-25, IL-33, GM-CSF, TSLP and eotaxin-3 which were measured in the sputum supernatant by multiplex electrochemiluminescent assays (Meso Scale Discovery). Spiking recoveries were optimal for all except for IL-33 which was then not use for further analysis. The lower limits of detection (LLOD) are displayed in the e-Table 2 in the online data supplement. The second panel was assessed by multiplex ELISA (Luminex, Biotechne) to measure IL-8, TNF-alpha, MMP-7, YKL-40, IGFBP1 and IGFBP3 and was performed in a sub-group of patients (n = 35) as well as the measurement of IgE levels using the Human IgE ELISA kit from Abcam (Amsterdam, The Netherlands). The detection limits were 1, 1, 35, 90, 54, 247 pg/ml and 0.026 ng/ml respectively.

2.7. Statistical analysis

Categorical variables are presented as numbers or percentages and continuous variables as mean ± standard deviation or as median (25 %–75 %) when appropriate.

The normality of continuous variables was assessed by Shapiro-Wilk

Table 2
Inflammatory characteristics of patients with remission (n = 12) vs others (n = 33).

	Remission	No remission	P value
Sputum weight (g)	3.3 (2.3–6.3)	2.1 (1.2–3.5)	<0.05
Squamous cells (%)	13 (5–35)	10 (3–22)	0.73
Viability (%)	72 (68–92)	86 (75–92)	0.34
Cell number (10^6 cells/g)	0.56 (0.46–1.93)	1.55 (0.49–3.11)	0.22
Macrophages (%)	11.8 (7.6–18.5)	14.0 (5.4–20.4)	0.92
Macrophages (10^3/g)	76 (38–123)	124 (74–403)	0.11
Neutrophils (%)	53 ± 25	58 ± 27	0.57
Neutrophils (10^3/g)	288 (195–544)	828 (214–2001)	0.32
Eosinophils (%)	21 (5–49)	8 (3–36)	0.22
Eosinophils (10^3/g)	180 (38–909)	124 (22–870)	0.87
Epithelial cells (%)	2 (1–2)	3 (1–6)	0.37
Epithelial cells (10^3/g)	11 (4–30)	31 (8–134)	0.09
Lymphocytes (%)	0.0 (0.0–0.0)	0.0 (0.0–0.8)	0.06
Lymphocytes (10^3/g)	0 (0–0)	0 (0–5)	0.09
Eosinophilic phenotype	8/12	19/33	0.64
Neutrophilic phenotype	0/12	4/33	
Mixed phenotype	2/12	7/33	
Paucigranulocytic phenotype	1/12	3/33	
Eotaxin-3 (pg/ml)	16.2 (6.7–23.3)	17.8 (5.3–67.6)	0.76
Eotaxin-3 (detectable/no detectable)	11/2	28/5	0.98
GM-CSF (pg/ml)	0.0 (0.0–0.5)	0.0 (0.0–0.3)	0.47
GM-CSF (detectable/no detectable)	5/7	11/22	0.61
TSLP (pg/ml)	0.8 (0.0–1.6)	1.7 (0.5–2.7)	0.13
TSLP (detectable/no detectable)	8/4	26/7	0.40
IL-4 (pg/ml)	0.0 (0.0–0.1)	0.0 (0.0–0.2)	0.83
IL-4 (detectable/no detectable)	3/9	9/24	0.88
IL-5 (pg/ml)	2.7 (2.0–7.3)	4.0 (1.5–8.5)	0.51
IL-5 (detectable/no detectable)	8/4	27/6	0.28
IL-6 (pg/ml)	10.3 (5.6–17.5)	17.8 (10.6–50.2)	<0.05
IL-6 (detectable/no detectable)	12/0	33/0	
IL-8 (pg/ml)	215 (102–379)	482 (177–1017)	<0.05
IL-8 (detectable/no detectable)	10/0	25/0	
IL-10 (pg/ml)	0 (0–0)	0 (0–0)	0.49
IL-10 (detectable/no detectable)	1/11	7/26	0.32
IL-13 (pg/ml)	0 (0–0)	0 (0–0)	>0.99
IL-13 (detectable/no detectable)	1/11	4/29	0.72
IL-18 (pg/ml)	130 (28–238)	173 (74–397)	0.14
IL-18 (detectable/no detectable)	12/0	33/0	
IL-25 (pg/ml)	0.0 (0.0–1.7)	0.0 (0.0–1.6)	0.79
IL-25 (detectable/no detectable)	3/9	9/24	0.89
IgE (ng/ml)	0.3 (0.2–0.4)	0.3 (0.2–0.4)	0.90
IgE (detectable/no detectable)	9/1	23/2	0.85
IGFBP1 (pg/ml)	965 ± 428	1185 ± 485	0.22
IGFBP1 (detectable/no detectable)	10/0	25/0	
IGFBP3 (pg/ml)	9275 ± 3650	10089 ± 3351	0.53
IGFBP3 (detectable/no detectable)	10/0	25/0	
TNF-alpha (pg/ml)	10 (7–15)	14 (9–19)	0.12
TNF-alpha (detectable/no detectable)	10/0	25/0	
YKL-40 (pg/ml)	3430 (1854–5205)	3681 (1647–9910)	0.82
YKL-40 (detectable/no detectable)	10/0	25/0	
MMP-7 (pg/ml)	5611 (4088–18134)	12577 (5850–18988)	0.21
MMP-7 (detectable/no detectable)	10/0	25/0	

test. Chi-square or Fisher exact test was used for categorical variables comparisons and Student *t*-test or Mann-Whitey *U* test was performed for continuous variables when appropriate. Correlations were performed with Spearman correlation for non-parametric variables.

Univariate logistic regression (presented using odds ratios and corresponding 95 % confidence intervals) was considered to model the association between patient remission status and demographic, clinical and biological parameters.

All tests were performed 2-sided and $p < 0.05$ was considered significant. All statistical analyses were performed with GraphPad Prism 7 (GraphPad Software San Diego, CA, USA).

3. Results

3.1. Remission

Among the 45 patients treated with anti-IL-5R, 12 were classified in remission 18 months post-treatment according to our criteria which represents 27 % of the patients (their baseline characteristics are detailed in Tables 1 and 2). These 12 patients in remission were characterized, by a younger age (51 ± 13 vs 60 ± 13, $p < 0.05$), lower pack-year values ((0-(0-0) vs 6 (0–25), $p < 0.05$)), a better asthma control assessed by ACQ (2.2 ± 1.2 vs 3.1 ± 1.0, $p < 0.05$), a better quality of life assessed by AQLQ score (4.5 ± 1.1 vs 3.5 ± 1.1, $p < 0.01$) and a higher FeNO value in ppb ((71 (47–117) vs 32 (20–52), $p < 0.01$)). When we looked deeper in the differences in ACQ and AQLQ between groups of patients, we found that in both questionnaires, the questions relative to a limitation of activity were impacted and the scores were better in the remission group (ACQ3: 2.5 ± 1.4 vs 3.5 ± 1.3, $p < 0.05$ and AQLQ mean of questions 12-13-14-15: 4.4 ± 1.0 vs 3.3 ± 1.4, $p < 0.05$). The baseline lung function values appeared higher in the remission group compared to the other, but these results were not statistically significant. The BEC were remarkably comparable between groups which was also the case if we removed patients taking OCS, however, even if not significant, the median sputum eosinophil percentage was higher in the remission group compared to the other ((21 (5–49) vs 8 (3–36) %, $p = 0.22$)). Overall, in the entire cohort, we noticed a significant positive correlation between the FeNO values and the sputum eosinophil percentages but not with the BEC ($r = 0.41$, $p < 0.01$ and $r = 0.18$, $p = 0.26$ respectively, data not showed). In addition, the patients in remission had a higher sputum weight than the other group ((3.3 g (2.3–6.3 vs 2.1 g (1.2–3.5), $p < 0.05$)) and showed a lower but not significant sputum macrophage and neutrophil proportions. Moreover, they exhibited a significantly lower level of sputum IL-6 ((10.3 (5.6–17.5) vs 17.8 (10.6–50.2) pg/ml, $p = 0.0283$)) and IL-8 ((215 (102–379) vs 482 (177–1017) pg/ml, $p = 0.0493$)) at baseline compared to the non-remission group. We performed Spearman correlations to check the relationship between sputum IL-6 and IL-8 and all the other parameters in the whole cohort of asthmatic patients (E-Fig. 1, supplementary material). Both cytokines were strongly positively correlated to each other, and they displayed significant negative correlations with FeNO value and lung function parameters while the correlations were positive for age, for the different sputum cell populations expressed in absolute value (stronger for sputum macrophage and neutrophil counts) as well as with the sputum IL-10, IL-18, IgE, IGFBP1, MMP-7, TNF-alpha and YKL-40 levels.

When the baseline results of the patients who will achieve remission were compared with the cohort of healthy subjects, their IL-6 and IL-8 sputum levels were not significantly different ((10.3 (6.0–17.5) vs 16.6 (6.4–21.8) pg/ml, $p = 0.4696$ and 215 (102–379) vs 170 (128–370) pg/ml, $p = 0.9758$)) respectively. Only the sputum IL-8 level in the cohort of healthy subjects was different from the group of patients who will not achieve remission ((170 (128–370) pg/ml vs 482 (177–1017) pg/ml, $p = 0.0082$, Fig. 1)).

None of the other sputum mediators differed between groups.

A receiver-operating characteristic (ROC) curve was plotted to assess the ability of FeNO, BEC, FeNO + BEC and sputum IL-6 and IL-8 to predict remission and showed that FeNO, sputum IL-6 and IL-8 performed well to distinguish between patients with remission versus not (AUC ≥0.7) in contrast to BEC which was close to the diagonal and not significant (Fig. 2 and Table 3). Moreover, the addition of BEC to FeNO did not bring any improvement in term of AUC compared to FeNO alone.

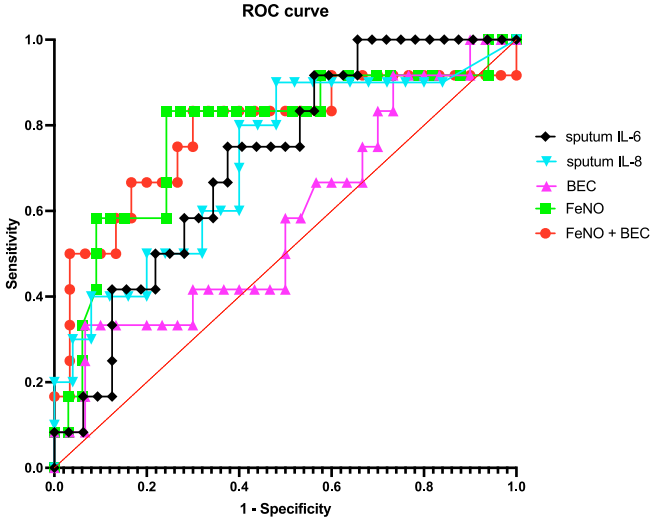

Fig. 2. ROC curves of sputum IL-6, IL-8 protein level, FeNO, BEC and FeNO + BEC.

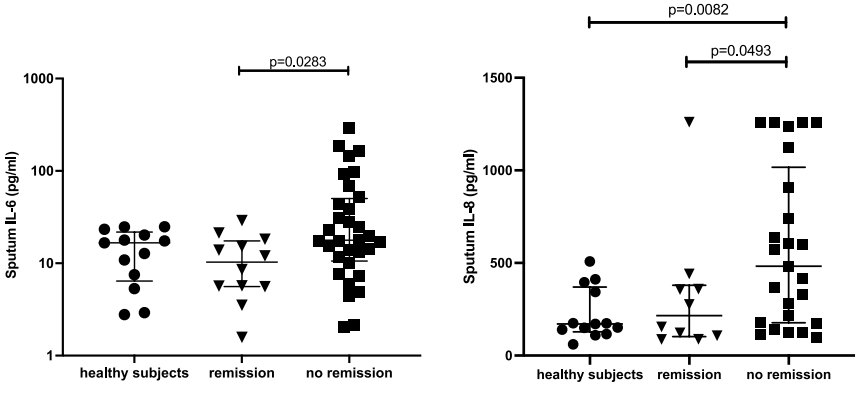

Fig. 1. Baseline sputum IL-6 and IL-8 concentrations in patients with remission versus others. The bars represent the median of the data and the interquartile range.

Table 3
ROC curves details.

Mediator	AUC (95 %CI)	Threshold	Sensitivity (95 %CI)	Specificity (95 %CI)	Likelihood ratio	P value
BEC	0.58 (0.38–0.78)	≥1310	33.3 (5.5–57.2)	93.3 (77.9–99.2)	3.75	0.23
FeNO	0.78 (0.61–0.95)	≥47	83.3 (51.6–97.9)	75.8 (57.7–88.9)	3.44	0.01
BEC + FeNO	0.78 (0.60–0.96)	ND	83.3 (51.6–97.9)	73.4 (54.1–87.7)	3.13	0.03
Sputum IL-8	0.72 (0.51–0.92)	≤441	90.0 (55.5–99.8)	52.0 (31.3–72.2)	1.87	0.07
Sputum IL-6	0.72 (0.56–0.88)	≤15.2	75.0 (42.8–94.5)	62.5 (43.7–78.9)	2.00	0.03

BEC = blood eosinophil count in cells/μL. ND: not determined. The Youden cut-off values were used. The p values were calculated based on the logarithm values.

To screen for potential predictors of one-year asthma remission, univariate logistic regression was performed on baseline demographic, clinical and biological biomarkers. We observed that only a good asthma control or quality of life based on ACQ or AQLQ scores and a high FeNO value were significantly associated with remission.

3.2. Components of response

When the improvement after treatment was analyzed for clinical parameters individually, we observed for patients who had a response in terms of improvement in ACT score (gain of at least 3 points) a significantly lower level of sputum IL-6 at baseline compared to those who did not improve ((13.3 (5.7–21.3) vs 25.6 (17.3–76.3), p = 0.0035)) as well as a significant difference in FeNO values at baseline ((47 (29–71) vs 29 (18–41), n = 28 vs 14, p = 0.03, Fig. 3 and E-Table 3)). Patients who improved their ACQ score by 0.5 (n = 29 vs 14) had a higher sputum eosinophil percentage at baseline compared to those who did not improve their ACQ score ((21 (5–41) vs 3 (0–22), p = 0.02)) and a lower sputum IL-6 level compared to the others ((12.6 (5.6–22.2) vs 32.6 (16.2–113.9), p = 0.0030, Fig. 4 and E-Table 4)). For those who ameliorated their lung function by 10 % post treatment (n = 17 vs 28), we observed a significantly higher sputum eosinophil percentage at baseline vs the other group ((31 (10–48) vs 6 (2–24) %, p < 0.05)). Furthermore, the sputum level of eotaxin-3 was higher ((18.5 (15.0–117.6) vs 11.6 (4.0–30.2) pg/ml, p < 0.05)) and a there was a trend towards a higher sputum IL-5 ((6.5 (1.9–16.8) vs 2.7 (1.3–4.8) pg/ml, p = 0.08, Fig. 5 and E-Table 5)).

Finally, the patients with a partial response (n = 13 vs 32) in terms of OCS use and exacerbation rate exhibited a significantly higher level of IGFBP1 at baseline ((1266 (1078–1921) vs 947 (643–1439) pg/ml, p < 0.05)) and a trend towards a higher TSLP sputum level ((2.3 (0.7–3.7) vs 0.8 (0.0–2.0) pg/ml, p = 0.05, Fig. 6 and E-Table 6)) was observed compared to the other patients.

4. Discussion

In this study, we reported that 27 % of our patients were considered to be in remission 18 months after starting anti-IL-5R treatment according to a strict definition that included no exposure to systemic corticosteroids, normal or improved lung function and symptoms control. These patients were characterized by a younger age, a lower pack-year, a better asthma control and quality of life and a higher FeNO value. These results are in line with previous findings observed in a study [13] where the authors used an exhaustive remission definition close to our criteria (no exacerbation, no OCS, ACT ≥20 and FEV1 ≥ 80 %). In this study, they found through multivariate analysis that better asthma control and quality of life and less tobacco use were all associated with a higher chance of remission. They also observed that younger patients (<65 years) had a greater improvement in FEV_1 and ACT scores one year after benralizumab compared to patients older than 65 years.

Interestingly, the ACQ and AQLQ scores differed for questions related to activity limitation. It is indeed plausible that this difference is due to the fact that patients in the non-remission group suffer from peripheral muscle deconditioning which may explain their underestimation of asthma control and quality of life [14].

High FeNO was already shown to be associated with a good response after benralizumab (more than 50 % reduction in the annualized exacerbation rate or more than 50 % reduction in daily mOCS dose) versus non-responders [15]. Furthermore, Watanabe et al. showed that a high FeNO was associated with a better response after 48 weeks of benralizumab [16], although their definition of response differed from ours (reduction of ≥50 % in exacerbation frequency or OCS use in patients with past exacerbations or OCS use; and for patients without, an improvement of ≥0.5 in ACQ and ≥3 in ACT scores, or of ≥10.38 % of FEV1). However, they also found that BEC predicted a good response which was not the case in our study. Another study from the UK registry based on a cohort of 1111 patients reported the same observations, namely a higher rate of remission in patients with higher T2 markers such as FeNO values and BEC [6]. Their definition of remission was close to ours and included no OCS, no exacerbation, an ACQ<1.5 and a stable lung function.

We previously showed that, with a similar BEC, the remission status may be related to a more local type of inflammation [7] in case of anti-IL-5 treatment and here we confirmed this finding in the context of anti-IL5R therapy as FeNO is the only marker associated with remission in this cohort. Indeed, our patients in remission and not in remission

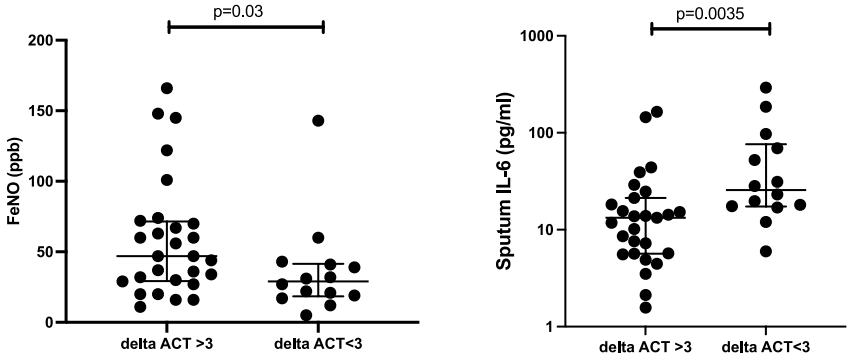

Fig. 3. Baseline FeNO values and sputum IL-6 levels for those who improved the ACT score by 3 points after treatment vs others. The bars represent the median of the data and the interquartile range.

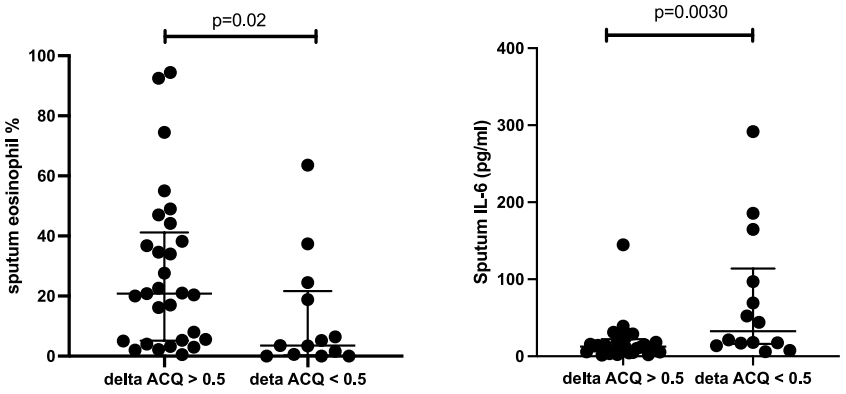

Fig. 4. Baseline sputum eosinophil percentage and sputum IL-6 levels for those who improved their ACQ score by 0.5 vs others. The bars represent the median of the data and the interquartile range.

Table 4
Univariate logistic regression analysis of potential predictors of asthma remission.

Variable	OR	LCL-UCL	P value	Variable	OR	LCL-UCL	P value
Sex (male vs female)	0.74	0.19–2.82	0.65	**Lymphocytes (%)**	0.03	7.18^{-006}-0.65	0.18
Age	0.95	0.90–1.00	0.05	**Lymphocytes (10^3/g)**	3.46^{-121}	/-0.10	0.30
BMI	0.97	0.84–1.12	0.71	**Eotaxin-3**	0.10	0.99–1.00	0.45
Smoking status (CS vs NS)	0.87	0.32–2.30	0.77	**Eotaxin-3 detectable (y vs n)**	0.89	0.16–6.92	0.90
Smoking status (ES vs NS)	0.20	0.03–0.92	0.06	**GM-CSF**	2.33	0.15–31.24	0.52
Pack year	0.95	0.88–1.0	0.11	**GM-CSF detectable (y vs n)**	1.43	0.35–5.56	0.61
Atopy (yes vs no)	1.30	0.33–5.69	0.71	**TSLP**	0.66	0.34–1.04	0.14
OCS cure	0.80	0.47–1.17	0.34	**TSLP detectable (y vs n)**	0.46	0.10–2.18 2	0.31
ACT	1.07	0.94–1.23	0.28	**IL-4**	0.42	0.00–67.08	0.75
ACQ	0.45	0.21–0.85	0.02	**IL-4 detectable (y vs n)**	0.89	0.17–3.81	0.88
AQLQ	2.28	1.23–4.82	0.02	**IL-5**	0.96	0.84–1.03	0.35
FEV1 %predicted	1.04	1.00–1.09	0.08	**IL-5 detectable (y vs n)**	1.11	0.21–8.44	0.91
FEV1 post BD%predicted	1.03	1.00–1.08	0.10	**IL-6**	1.11	0.37–3.94	0.11
FVC %predicted	1.05	1.00–1.11	0.08	**IL-6 detectable (y vs n)**	ND	ND	ND
FVC post BD%predicted	1.04	1.00–1.09	0.10	**IL-8**	1.00	1.00–1.00	0.12
FEV1/FVC	1.00	0.98–1.02	0.75	**IL-8 detectable (y vs n)**	ND	ND	ND
FEV1/FVC post BD	1.03	0.97–1.10	0.36	**IL-10**	1.07	0.88–1.31	0.47
Blood neutrophils (/µl)	0.69	0.45–0.97	0.06	**IL-10 detectable (y vs n)**	0.34	0.02–2.23	0.34
Blood eosinophils (/µl)	2.41	0.68–9.45	0.17	**IL-13**	0.99	0.51–1.60	0.96
Blood eosinophils (/µl) no OCS	1.00	1.00–1.00	0.39	**IL-13 detectable (y vs n)**	0.66	0.03–5.10	0.72
Serum IgE	1.00	1.00–1.00	0.46	**IL-18**	1.00	0.99–1.00	0.14
CRP	0.88	0.65–1.01	0.25	**IL-18 detectable (y vs n)**	ND	ND	ND
Fibrinogen	0.65	0.20–1.61	0.40	**IL-25**	1.25	0.64–2.34	0.48
ICS	1.00	1.00–1.00	0.37	**IL-25 detectable (y vs n)**	0.89	0.17–3.81	0.88
OCS (yes vs no)	0.62	0.08–3.06	0.59	**IgE**	0.25	0.00–1.37	0.53
FeNO	1.02	1.01–1.05	0.01	**IgE detectable (y vs n)**	0.78	0.07–18.06	0.85
Nasal polyposis (y vs n)	2.23	0.53–9.15	0.26	**TNF-alpha**	1.01	0.96–1.05	0.70
Sputum weight (g)	1.35	1.01–1.87	0.05	**TNF-alpha detectable (y vs n)**	ND	ND	ND
Squamous cells (%)	1.01	0.97–1.05	0.47	**IGFBP1**	1.00	1.00–1.00	0.22
Viability (%)	0.99	0.96–1.02	0.52	**IGFBP1 detectable (y vs n)**	ND	ND	ND
Cell number (10^6 cells/g)	0.99	0.91–1.03	0.66	**IGFBP3**	1.00	1.00–1.00	0.52
Macrophages (%)	1.00	0.94–1.05	0.88	**IGFBP3 detectable (y vs n)**	ND	ND	ND
Macrophages (10^3/g)	0.02	1.48^{-005}-0.90 0.90	0.19	**MMP-7**	1.00	1.00–1.00	0.27
Neutrophils (%)	0.99	0.97–1.02	0.56	**MMP-7 detectable (y vs n)**	ND	ND	ND
Neutrophils (10^3/g)	0.99	0.91–1.03	0.70	**YKL-40**	1.00	1.00–1.00	0.74
Eosinophils (%)	1.01	0.99–1.04	0.30	**YKL-40 detectable (y vs n)**	ND	ND	ND
Eosinophils (10^3/g)	0.74	0.20–1.94	0.59				
Epithelial cells (%)	0.81	0.56–1.01	0.15				
Epithelial cells (10^3/g)	4.53^{-007}	1.67^{-017}-0.39	0.11				

Y vs n: yes versus no; BMI: body mass index; NS: non-Smoker; CS: current smoker; ES: ex-smoker; OCS: oral corticosteroids; FEV_1: forced expiration volume in 1s; BD: bronchodilation; FVC: forced vital capacity; CRP: C reactive protein; ICS: inhaled corticosteroids; FeNO: fraction of exhaled nitric oxide. ND: not determined, all samples were detectable, no statistical analysis was performed.

exhibited an equal BEC but differed at local level. Type 2 mediators, and particularly IL-5, were not different between groups which may be due to the presence of a certain T2 inflammation also in the airways of patients who did not achieve remission but not only. Indeed, the non-remission group exhibited a higher sputum IL-6 and IL-8 level than the non-remission group. The significant correlations showed that IL-6 was negatively correlated to lung function and positively correlated to sputum neutrophil count, and it has been shown that sputum IL-6 was

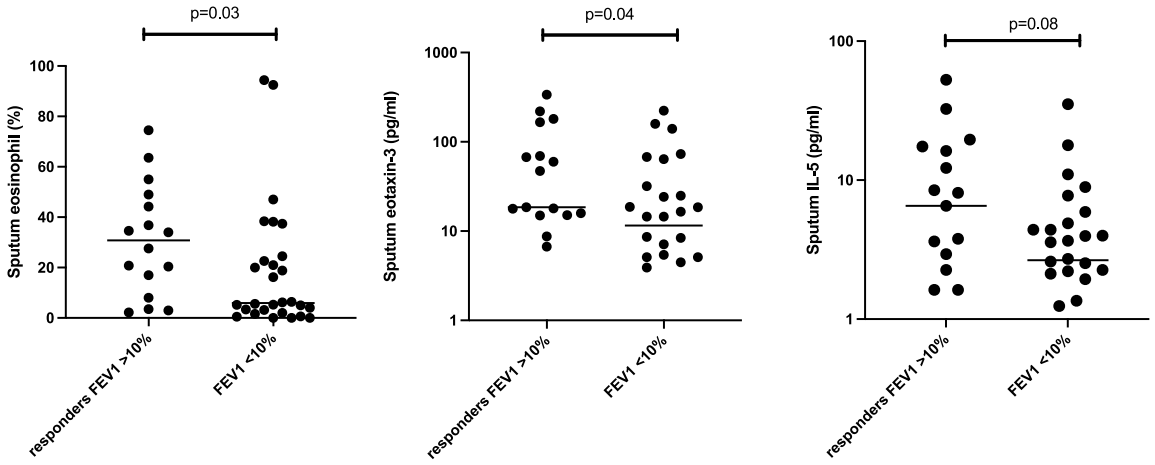

Fig. 5. Baseline sputum eosinophil percentage, sputum eotaxin-3 level and sputum IL-5 level of those who improved FEV1 greater than 10 % post-treatment vs others. The bars represent the median of the data.

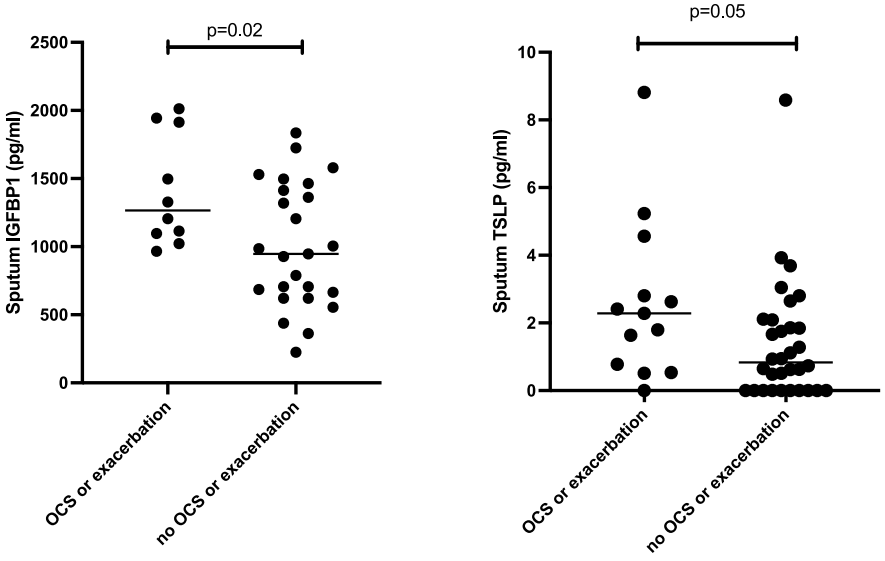

Fig. 6. Baseline sputum IGFBP1 and TSLP levels for those who did not respond in term of OCS use or exacerbation vs others. The bars represent the median of the data.

associated with mixed eosinophilic/neutrophilic airway inflammation and impaired lung function [17,18] which characterized our non-remission group. In addition, sputum IL-6 was found to be correlated to sputum markers of remodeling as YKL-40 and MMP-3 as well as inflammatory markers as IL-8 and IL-1 family proteins which also corroborates our results [19]. Sputum IL-6 was finally shown to be linked to a poorer asthma control in elderly asthmatic patients [20] and with impaired oscillometry parameters in a cohort of smokers [21].

IL-8 strongly correlates with sputum neutrophil count which is consistent with its primary role in attracting neutrophils. Furthermore, in a recent study, our group showed an association with high sputum IL-8 and accelerated lung function decline in asthma [22] which confirm its negative correlation with lung function parameters.

We also observed a higher sputum weight in our patients in remission compared to the others. This finding could be explained by the lower lung function in the non-remission group which induced a lower ability to expectorate due to a reduced airway caliber. In addition, the non-remission patients exhibited a higher neutrophilic type of inflammation which could be responsible for a higher local oxidative stress and DNA release, both linked to a higher sputum viscosity and a decreased mucus clearance [23].

When looking at improvement in term of individual parameter, asthmatic patients who improved in term of ACT or ACQ scores exhibited higher T2 marker values such as FeNO or sputum eosinophil percentage at baseline respectively and patients who improved their lung function by 10 % displayed a higher airway eosinophilic inflammation as well as sputum eotaxin-3 and a trend for IL-5 levels than the others. These data re-affirmed that a high local T2 kind of inflammation is key in identifying patients who will improve after anti-IL-5R therapy.

Finally, patients who exhibited a partial response in terms of OCS use and exacerbation rate were characterized by a higher baseline sputum levels of IGFBP1 and TSLP. IGFBP1 is a steroid inducible gene [24] and it could be increased due to high oral or inhaled corticosteroids doses in these patients facing exacerbations. TSLP is an alarmin produced by the airway epithelium in response to danger signals and is increased due to viral infections, allergens, cigarette smoke exposure or

pro-inflammatory cytokines. Its release is linked to the recruitment of eosinophils and neutrophils, it is then implicated in different asthma phenotypes. In addition, TSLP has been shown to be associated with steroid resistance. At systemic level, its rise was shown to be linked to a higher risk of future exacerbation in a cohort of 132 severe asthmatic patients [25]. We speculated here that this observation might also be valuable at local level.

The limitations of this study include a limited number of patients which may have reduced the power of the univariate analyses, the fact that this is a single-center study, the different treatment strategies used by the different pulmonologists which may have affect the remission evaluation, the small group of patients considered in remission and the impact of steroids. However, all analyses that concerned the BEC were also performed when patients taking OCS were removed and did not show significant results. Finally, the sputum method may not be used in all asthmatic care centers, but this technique adds valuable additional information on the local compartment and was shown to be a feasible trait to optimally manage patients with severe eosinophilic asthma [26].

CRediT authorship contribution statement

Catherine Moermans: Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nicolas Decerf:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Nicolas Javaux:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Adrien Onssels:** Writing – review & editing, Methodology, Data curation. **Noémie Bricmont:** Writing – review & editing, Methodology, Data curation. **Romane Bonhiver:** Writing – review & editing, Methodology, Data curation. **France Regnier:** Writing – review & editing, Methodology, Data curation. **Adeline Rosu:** Writing – review & editing, Methodology, Data curation. **Sophie Graff:** Writing – review & editing, Methodology, Data curation. **Sara Gerday:** Writing – review & editing, Methodology, Data curation. **Makon-Sébastien Njock:** Writing – review & editing, Methodology, Data curation. **Virginie Paulus:** Writing – review & editing, Methodology, Data curation. **Françoise Guissard:** Writing – review & editing, Methodology, Data curation. **Stéphanie Ziant:** Writing – review & editing, Methodology, Data curation. **Carole Sanchez:** Writing – review & editing, Methodology, Data curation. **Renaud Louis:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization. **Florence Schleich:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Florence Schleich reports financial support was provided by Astra Zeneca. Florence Schleich and Renaud Louis reports a relationship with GSK that includes: consulting or advisory, funding grants, and speaking and lecture fees. Florence Schleich and renaud Louis reports a relationship with Astra Zeneca that includes: consulting or advisory, funding grants, and speaking and lecture fees. Florence Schleich and Renaud Louis reports a relationship with Sanofi that includes: funding grants. Florence Schleich and Renaud Louis reports a relationship with Novartis that includes: funding grants and speaking and lecture fees. Florence Schleich and renaud Louis reports a relationship with Chiesi that includes: funding grants and speaking and lecture fees. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2025.108151.

References

[1] Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019, Lancet 396 (10258) (2020) 1204–1222, https://doi.org/10.1016/S0140-6736(20)30925-9.
[2] K.F. Chung, S.E. Wenzel, J.L. Brozek, A. Bush, M. Castro, P.J. Sterk, et al., International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma, Eur. Respir. J. (2014), https://doi.org/10.1183/09031936.00202013.
[3] F. Schleich, G. Brusselle, R. Louis, O. Vandenplas, A. Michils, C. Pilette, et al., Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR), Respiratory Medicine, 2014, https://doi.org/10.1016/j.rmed.2014.10.007.
[4] D.J. Jackson, H. Burhan, H. Rupani, P.E. Pfeffer, I.J. Clifton, S. Faruqi, et al., Overcoming barriers to remission in severe eosinophilic asthma: two-year real-world data with benralizumab, Clin. Exp. Allergy 31 (2024), https://doi.org/10.1111/cea.14544.
[5] I.D. Pavord, Remission in the world of severe asthma, Am. J. Respir. Crit. Care Med. 210 (7) (2024) 855–857, https://doi.org/10.1164/rccm.202405-0894ED.
[6] P.J. McDowell, R. McDowell, J. Busby, M.C. Eastwood, P.H. Patel, D.J. Jackson, et al., Clinical remission in severe asthma with biologic therapy: an analysis from the UK Severe Asthma Registry, Eur. Respir. J. 62 (6) (2023), https://doi.org/10.1183/13993003.00819-2023.
[7] C. Moermans, C. Brion, G. Bock, S. Graff, S. Gerday, H. Nekoee, et al., Sputum type 2 markers could predict remission in severe asthma treated with anti-IL-5. Chest, juin 163 (6) (2023) 1368–1379, https://doi.org/10.1016/j.chest.2023.01.037.
[8] S. Gerday, S. Graff, C. Moermans, F. Guissard, V. Paulus, M. Henket, et al., Super-responders to anti-IL-5/anti-IL-5R are characterised by high sputum eosinophil counts at baseline, Thorax 78 (11) (2023) 1138–1141, https://doi.org/10.1136/thorax-2022-219781.
[9] S. Goswami, P. Angkasekwinai, M. Shan, K.J. Greenlee, W.T. Barranco, S. Polikepahad, et al., Divergent functions for airway epithelial matrix metalloproteinase 7 and retinoic acid in experimental asthma, Nat. Immunol. 10 (5) (2009) 496–503, https://doi.org/10.1038/ni.1719.
[10] M.R. Miller, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, et al., Standardisation of spirometry, Eur. Respir. J. 26 (2005) 319–338, https://doi.org/10.1183/09031936.05.00034805. European Respiratory Society.
[11] M. Delvaux, M. Henket, L. Lau, P. Kange, P. Bartsch, R. Djukanovic, et al., Nebulised salbutamol administered during sputum induction improves bronchoprotection in patients with asthma, Thorax (2004), https://doi.org/10.1136/thorax.2003.011130.
[12] J. Guiot, S. Demarche, M. Henket, V. Paulus, S. Graff, F. Schleich, et al., Methodology for sputum induction and laboratory processing, JoVE J. (2017), https://doi.org/10.3791/56612.
[13] Martinez-Moragon E, Chiner E, Suliana Mogrovejo A, Palop Cervera M, Lluch Tortajada I, Boira Enrique I, et al. Real-world clinical remission of severe asthma with benralizumab in Spanish adults with severe asthma. J. Asthma :1-15. DOI: 10.1080/02770903.2024.2332351.
[14] F. Schleich, S. Ziant, S. Louis, C. Moermans, R. Deroisy, R. Louis, et al., Uncontrolled asthma is associated with increased visceral adipose tissue, decreased bone mineral content, and reduced exercise capacity, J. Asthma Allergy 17 (2024) 1369–1382, https://doi.org/10.2147/JAA.S474667.
[15] J.E. Kavanagh, A.P. Hearn, J. Dhariwal, et al., Real-World effectiveness of benralizumab in severe eosinophilic asthma, Chest 159 (2) (2021) 496–506, https://doi.org/10.1016/j.chest.2020.08.2083 [published correction appears in Chest. 2023 May;163(5):1340. doi: 10.1016/j.chest.2023.03.020.].
[16] H. Watanabe, T. Shirai, K. Hirai, T. Akamatsu, H. Nakayasu, K. Tamura, et al., Blood eosinophil count and FeNO to predict benralizumab effectiveness in real-life severe asthma patients, J Asthma 2 sept 59 (9) (2022) 1796–1804, https://doi.org/10.1080/02770903.2021.1963769.
[17] D.K. Chu, A. Al-Garawi, A. Llop-Guevara, R.A. Pillai, K. Radford, P. Shen, et al., Therapeutic potential of anti-IL-6 therapies for granulocytic airway inflammation in asthma, Allergy Asthma Clin. Immunol. 11 (1) (2015) 14, https://doi.org/10.1186/s13223-015-0081-1.
[18] N. Turan, M.J. Edwards, S. Bates, D. Shaw, K.F. Chung, M.J. Loza, et al., IL-6 pathway upregulation in subgroup of severe asthma is associated with neutrophilia and poor lung function, Clin Exp Allergy. avr 48 (4) (2018) 475–478, https://doi.org/10.1111/cea.13085.

[19] Z. Jevnikar, J. Östling, E. Ax, J. Calvén, K. Thörn, E. Israelsson, et al., Epithelial IL-6 trans-signaling defines a new asthma phenotype with increased airway inflammation, J Allergy Clin Immunol. févr 143 (2) (2019) 577–590, https://doi.org/10.1016/j.jaci.2018.05.026.

[20] P.J. Busse, J.M. Birmingham, A. Calatroni, J. Manzi, A. Goryachokovsky, G. Fontela, et al., Effect of aging on sputum inflammation and asthma control, J Allergy Clin Immunol. juin 139 (6) (2017) 1808–1818.e6, https://doi.org/10.1016/j.jaci.2016.09.015.

[21] O. Freund, M. Rotem-Green, M. Rahat, et al., Nanoparticles in induced sputum - a window to airway inflammation among active smokers, Nanomedicine (Lond). 18 (4) (2023) 303–315, https://doi.org/10.2217/nnm-2022-0269.

[22] S. Graff, C. Moermans, S. Gerday, et al., Increase in blood eosinophil count over time and sputum IL8 are associated with FEV_1 decline in asthma, Lung 203 (1) (2024) 8, https://doi.org/10.1007/s00408-024-00760-9. Published 2024 Nov 30.

[23] R.S. Linssen, G. Chai, J. Ma, A.B. Kummarapurugu, J.B.M. van Woensel, R.A. Bem, et al., Neutrophil extracellular traps increase airway mucus viscoelasticity and slow mucus particle transit, Am J Respir Cell Mol Biol. janv 64 (1) (2021) 69–78, https://doi.org/10.1165/rcmb.2020-0168OC.

[24] H. Bui, Y. Amrani, B. Deeney, R.A. Panettieri, O. Tliba, Airway smooth muscle cells are insensitive to the anti-proliferative effects of corticosteroids: the novel role of insulin growth factor binding Protein-1 in asthma, Immunobiology 224 (4) (2019) 490–496, https://doi.org/10.1016/j.imbio.2019.05.006.

[25] H.K. Ko, S.L. Cheng, C.H. Lin, S.H. Lin, Y.H. Hsiao, K.C. Su, et al., Blood tryptase and thymic stromal lymphopoietin levels predict the risk of exacerbation in severe asthma, Sci Rep. 19 avr 11 (1) (2021) 8425, https://doi.org/10.1038/s41598-021-86179-1.

[26] Y. Hoshino, T. Soma, Y. Uchida, Y. Shiko, K. Nakagome, M. Nagata, Treatment resistance in severe asthma patients with a combination of high fraction of exhaled nitric oxide and low blood eosinophil counts, Front. Pharmacol. 13 (2022) 836635, https://doi.org/10.3389/fphar.2022.836635. PMID: 35517829; PMCID: PMC9065285.