[Asthma Original Research]

CHEST

Sputum Type 2 Markers Could Predict Remission in Severe Asthma Treated With Anti-IL-5

Catherine Moermans, PhD; C. Brion; G. Bock; S. Graff, PhD; S. Gerday; H. Nekoee, PhD; C. Poulet, PhD; N. Bricmont; M. Henket; V. Paulus; F. Guissard; R. Louis; and F. Schleich

BACKGROUND: Biotherapies targeting IL-5 allow a tangible improvement of asthma. However, all patients do not respond the same way to these treatments. Even if high blood eosinophil counts seem to be associated with a reduction in exacerbations with treatment targeting IL-5, we lack biomarkers for the prediction of remission after these very expensive treatments.

RESEARCH QUESTION: Does the sputum of patients with severe eosinophilic asthma show biomarkers of remission after therapy targeting IL-5?

STUDY DESIGN AND METHODS: This observational study included 52 patients with severe asthma initiated with anti-IL-5 therapy and recruited from the asthma clinic of the CHU of Liege, Belgium. Remission was defined as patients who combined the following at 1 year after therapy: no chronic treatment with oral corticosteroids; no exacerbation; asthma control questionnaire score < 1.5, asthma control test score > 19, or both; FEV_1 of $\geq 80\%$ predicted, improvement of FEV_1 of $\geq 10\%$, or both; and a blood eosinophil count < 300 cells/μL. Eosinophil peroxidase (EPX), IgE, IL-3, IL-4, IL-5, IL-13, IL-25, IL-33, granulocyte-macrophage colony-stimulating factor, thymic stromal lymphopoietin (TSLP), and eotaxin-1 levels were measured in the sputum of these patients before anti-IL-5 treatment.

RESULTS: Among the 52 patients, 11 were classified as being in remission. These patients were characterized by higher sputum eosinophil, macrophage, and lymphocyte counts, whereas the sputum neutrophil percentage was lower than in the nonremission group. In addition, the sputum eotaxin-1, TSLP, IL-5, EPX, and IgE protein levels were higher at baseline in the remission group compared with the nonremission group. Univariate regression analysis revealed that male vs female sex, sputum neutrophil percentage, eotaxin-1, IL-5, and EPX were potential predictors of remission.

INTERPRETATION: Sputum type 2 markers seemed to be potentially predictive of remission after anti-IL-5 therapy in a cohort of patients with severe eosinophilic asthma. These results need validation on a larger cohort.

CHEST 2023; ■(■):■-■

KEY WORDS: asthma; biotherapy; remission; sputum

ABBREVIATIONS: ACQ = asthma control questionnaire; ACT = asthma control test; EPX = eosinophil peroxidase; FENO = fraction of exhaled nitric oxide; IQR = interquartile range; OCS = oral corticosteroids; ROC = receiver operating characteristic; TSLP = thymic stromal lymphopoietin

AFFILIATIONS: From the Giga I3 (C. M., S. Gerday, N. B., R. L., and F. S.), Pneumology Research Group, Liege University, the Department of Pneumology-Allergology (C. M., S. Graff, M. H., V. P., F. G., R. L., and F. S.), CHU of Liege, the Haute École de la Province de Liège (C. B.), the Haute École Charlemagne (G. B.), the Department of Public Health (H. N.), and the Department of Rheumatology (C. P.), CHU and University of Liege, Liege, Belgium.

CORRESPONDENCE TO: Catherine Moermans, PhD; email: c.moermans@chuliege.be

DOI: https://doi.org/10.1016/j.chest.2023.01.037

FLA 5.6.0 DTD ■ CHEST5508_proof ■ 21 February 2023 ■ 2:07 am ■ EO: CHEST-D-22-01152

Take-home Points

Study Question: Does the sputum of patients with severe eosinophilic asthma show biomarkers of remission after therapy targeting IL-5?

Results: This study highlights that baseline type 2 airway inflammation markers can predict remission in severe eosinophilic asthma treated with anti-IL-5 agents.

Interpretation: Sputum markers can be used as surrogate markers of remission 1 year after therapy, but these results need to be validated in a larger cohort.

Refractory asthma still represents between 3% and 10% of patients with asthma.[1,2] These patients represent a high burden in terms of health-care costs. Biotherapies have been developed in recent years to reduce the use of oral corticosteroids (OCS), which are responsible for long-term and costly side effects. Monoclonal antibodies directed against IL-5 or its receptor, IL-5R, are approved to treat severe eosinophilic asthma with tangible improvements in patient conditions.[3,4] However, it seems that the patients do not respond the same way to biotherapies. In a recent article, Mukherjee et al[5] analyzed the predictors of suboptimal response to anti-IL-5 therapies, which was defined as a failure to decrease OCS by 50%, asthma control questionnaire (ACQ) score of $\leq$ 1.5, or exacerbations by 50% with persistent sputum of $>$ 3% and blood eosinophil levels of $\geq$ 400/μL. They found that OCS intake, sinus disease, late-onset asthma, and sputum eosinophil peroxidase IgG were the most predictive of suboptimal response. In another interesting study, Eger et al[6] analyzed a cohort of 114 patients with severe eosinophilia 2 years after anti-IL-5 and IL-5R biologic therapy. They observed that 14% were super responders, defined as patients with no OCS, no exacerbations within 3 months, ACQ score of $<$ 1.5, FEV_1 of $\geq$ 80% predicted, fraction of exhaled nitric oxide (FENO) of $<$ 50 parts per billion, and control of comorbidities. The super response was predicted by shorter asthma duration and higher FEV_1. Harvey et al[7] defined super responders to mepolizumab as the upper 25% of ACQ-5 responders. They represented 24% of the 309 patients followed up. Those patients mostly were female, had a lower BMI, a shorter asthma duration, higher blood eosinophil levels and FENO values, as well as a higher ACQ-5 score. Additionally, those patients were more likely to have a diagnosis of nasal polyps and had fewer comorbidities and less maintenance OCS at baseline. Another retrospective study analyzed 99 patients in the United Kingdom and observed that baseline characteristics associated with response ($\geq$ 50% exacerbation reduction or $\geq$ 50% OCS dose reduction) or superresponse (no exacerbation and no OCS use at 1 year) were the presence of nasal polyps, lower ACQ-6 score, lower BMI, and lower dose of OCS.[8] Finally, mepolizumab also was shown to be more beneficial in patients with severe asthmatic who had nasal polyps (patients with more severe disease and more intense systemic eosinophilic inflammation) than in the patients without nasal polyps in terms of reduction of exacerbations.[9]

In addition to the superresponse, a new concept of asthma remission recently emerged as a therapeutic target to achieve after 12 months of treatment and was defined according to results of randomized control trials as obtaining asthma control, no exacerbation, and no treatment with OCS, although no consensus exists on the importance of improvement in lung function and reduction in type 2 inflammation in the current definition of remission.[10-12]

Post hoc analysis of a randomized control trial looked at the predictive factors of response to benralizumab or mepolizumab in vast cohorts of patients and observed that a high blood eosinophil level was linked to a better improvement in terms of reduction of exacerbation rate.[13,14] Even if blood eosinophil level helps to predict a general response to anti-IL-5 and anti-IL-5R, biomarkers reflecting local inflammation, such as those measured in induced sputum, have a better potential to predict the intensity of response to biologics because they reflect what really happens in the bronchi. So, do biomarkers of remission after therapy targeting IL-5 exist in the sputum of patients with severe eosinophilic asthma? However, other than the study cited previously focusing on suboptimal responders, to our knowledge no studies have analyzed inflammatory mediators as predictors of response after 1 year of anti-IL-5 treatment directly in the sputum itself in patients with severe eosinophilic asthma. The goal of this study was to analyze the role of mediators of the type 2 cascade in the sputum as predictors of remission.

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Study Design and Methods

Patients

Fifty-two patients with severe asthma initiated with an anti-IL-5 agent (51 patients with mepolizumab and one patient with reslizumab) were recruited from our asthma clinic. This observational study was performed at the CHU of Liege, Belgium, between 2014 and 2021. Inclusion criteria included a diagnosis of asthma defined by the Global Initiative for Asthma (http://ginasthma.org/), and severe asthma was defined according to European Respiratory Society and American Thoracic Society criteria.[1] The treatment was stable for all patients at the time of the sampling, and the time between baseline and the next evaluation in average was 1 year. Remission was defined as patients who, in addition to achieving the instauration of the biotherapy, received no OCS therapy; showed no exacerbations; showed an ACQ score of < 1.5, asthma control test (ACT) of > 19, or both; FEV_1 of $\geq 80\%$ predicted, an improvement in FEV_1 of $\geq 10\%$, or both; and a blood eosinophil count of < 300 cells/μL.

Subanalyses also were performed to assess the predictive values of sputum mediators in terms of response for some specific parameters alone, such as: improvement in ACT score of > 19 after treatment, decrease in ACQ score of < 1.5 after treatment, a decrease in sputum eosinophil level of $< 3\%$ or by 50%, stopping OCS after treatment, no more exacerbations after treatment, and improvement in FEV_1 of at least 10% after treatment. A diagnosis of nasal polyps by an ear, nose, and throat specialist and a diagnosis of atopic dermatitis or urticaria made by a dermatologist were reported.

This study was approved by the ethics committee of CHU Liege (Identifier: 2005/181) and all participants gave written informed consent for participation. The study is registered at ClinicalTrials.gov (Identifier: NCT04520165).

Study Design

This study aimed to investigate the airway expression of mediators of the type 2 cascade as predictors of remission after anti-IL-5 treatment in a cohort of patients with severe eosinophilic asthma. To detect a difference between groups with a power of 70% to 80% and a significance level of 5%, the sample size was estimated as 10 to 13 patients per group (https://www.sealedenvelope.com/). These calculations were based on a significant difference of sputum IL-5 obtained as preliminary results. Usually, in the different studies, even if no consensus exists on the remission definition in the asthma context, mention is made of a percentage of approximately 20% of patients exhibiting a good response to anti-IL-5 treatment. Consequently, with a cohort of 52 patients, we expected a sample size of at least 10 patients in the remission group.

Respiratory Function

FENO was measured using NiOX (Aerocrine) at a flow rate of 50 mL/s. Spirometry was performed before and after bronchodilation according to the American Thoracic Society and European Respiratory Society standard criteria.[15]

Blood Samples

Blood samples of patients were analyzed by the routine laboratory of the University Hospital of Liege for leukocyte count, C-reactive protein, and fibrinogen levels.

Sputum Induction and Processing

The sputum was induced and processed as described previously, and the sputum supernatant was collected before the addition of dithiothreitol, which could impact the measurements.[16,17] Cell viability was determined by trypan blue exclusion, and the differential cell count was performed by counting 500 nonsquamous cells on cytospins stained with May-Grünwald-Giemsa.

Sputum Inflammatory Mediators Measurement

Eosinophil peroxidase (EPX) was measured by classic enzyme-linked immunosorbent assay (Mybiosource). Detection limit was 5.4 ng/mL. IgE levels were measured with the Human IgE ELISA kit from Abcam. The detection limit was 30.5 pg/mL. Finally, IL-3, IL-4, IL-5, IL-13, IL-25, IL-33, granulocyte-macrophage colony-stimulating factor, thymic stromal lymphopoietin (TSLP), and eotaxin-1 were measured by multiplex electrochemiluminescent assays (Meso Scale Discovery). This technique already has been used with success in sputum supernatant,[18] and spiking recovery was optimal. The lower limits of detection are displayed in e-Table 1.

Statistical Analysis

Categorical variables are presented as numbers and percentages, and continuous variables are presented as mean ± SD or as median (interquartile range) when appropriate. The normality of continuous variables was assessed by the Shapiro-Wilk test (normal distribution was found for age, BMI, ACQ score, asthma quality of life questionnaire score, pulmonary function parameters, fibrinogen level, and sputum neutrophil percentage). To investigate baseline characteristics of patients in the remission group, the χ^2 or Fisher exact test was used for categorical variables and the Student t test or Mann-Whitey U test was performed for continuous variables when appropriate. Univariate logistic regression was considered to model the association between patient remission status and demographic, clinical, and biological variables. Results of logistic regression were presented using ORs and corresponding 95% CIs. All tests performed were two-sided, and a P value of $< .05$ was considered significant. All statistical analyses were performed using SAS version 9.4 software (SAS Institute), except for receiver operating characteristic (ROC) curve calculation, for which GraphPad Prism 7 software (GraphPad Software) was used. The P values associated with the ROC curves tested the null hypothesis that the area under the ROC curve equals 0.50.

Results

Among the 52 patients treated with anti-IL-5 agent, 11 patients were classified as being in remission at 1 year according to our criteria (baseline characteristics are detailed in Tables 1 and 2). These 11 patients presented comparable baseline demographic characteristics as the others, except for the proportions linked to gender: more men were in the group of patients in remission compared with the group not in remission ($P = .03$). Both groups of patients exhibited an equivalent control of asthma when we looked at the ACQ and ACT scores as well as similar asthma quality of life questionnaire values. The respiratory function parameters were equal and the blood parameters, including differential leukocyte counts, C-reactive protein, IgE, and fibrinogen levels, were in the same ranges. A trend toward a smaller number of patients

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TABLE 1] Baseline Demographic and Clinical Characteristics of Patients in Remission (n = 11) vs Those Not in Remission (n = 41)

Variable	Remission	Not in Remission	*P* Value
Sex[a]			.03
Male	8	15	
Female	3	26	
Age, y	48 ± 18	53 ± 11	.32
BMI, kg/m^2	27 ± 4	28 ± 6	.60
Smoking status			.51
Nonsmoker	7	26	
Current smoker	0	4	
Former smoker	4	11	
Smoking history, pack-y	0 (0-20)	0 (0-8)	.69
Atopy			.12
Yes	6	32	
No	5	9	
Age at diagnosis, y	38 (9-50)	38 (11-51)	.93
Asthma duration, y	8 (2-23)	15 (6-32)	.16
OCS cure	3 (2-3)	2 (2-3)	.19
ACT score	12.5 (9.2-16.0)	10.0 (8.0-14.7)	.34
ACQ score	2.6 ± 1.2	3.0 ± 1.3	.34
AQLQ score	3.9 ± 1.1	3.4 ± 1.2	.22
FEV_1, % predicted	74 ± 15	67 ± 17	.21
FEV_1 after BD administration, % predicted	81 ± 17	74 ± 19	.28
FVC, % predicted	84 ± 15	80 ± 16	.44
FVC after BD administration, % predicted	87 ± 17	84 ± 15	.58
FEV_1 to FVC ratio, %	72 ± 10	70 ± 11	.54
FEV_1 to FVC ratio after BD administration, %	75 ± 10	72 ± 13	.52
Blood neutrophil, cells/μL	4,072 (3,567-4,866)	4,901 (3,746-6,422)	.13
Blood eosinophil, /μL	519 (398-1201)	494 (333-705)	.34
Total serum IgE, kU/L	263 (30-442)	182 (91-739)	.42
CRP, mg/L	1.2 (0.9-10.9)	2.9 (1.1-5.9)	.85
Fibrinogen, g/L	3.3 ± 0.6	3.5 ± 0.7	.63
ICS, beclomethasone equivalent	2,000 (2,000-3,450)	2,000 (2,000-3,200)	.77
OCS treatment			.07
Yes	0	10	
No	11	31	
F_{ENO}, ppb	62 (24-118)	33 (19-65)	.14
Nasal polyposis			.74
Yes	5	16	
No	6	25	
Atopic dermatitis or urticaria			.28
Yes	5	11	
No	6	30	

Results are presented as median (interquartile range) or mean ± SD. ACQ = asthma control questionnaire; ACT = asthma control test; AQLQ = asthma quality of life questionnaire; BD = bronchodilation; CRP = C-reactive protein; F_{ENO} = fraction of exhaled nitric oxide; ICS = inhaled corticosteroids; OCS = oral corticosteroids.

[a]■■■

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TABLE 2] Inflammatory Characteristics of Patients in Remission (n = 11) vs Those Not in Remission (n = 41)

Variable	Remission	Not in Remission	*P* Value
Sputum weight, g	1.8 (1.3-2.6)	2.1 (1.4-3.2)	.42
Squamous cells, %	7 (1-13)	8 (4-25)	.28
Viability, %	61 (39-73)	67 (45-82)	.48
Cell No., 10^6 cells/g	3.7 (1.4-5.3)	1.8 (0.9-4.0)	.28
Macrophages			
%[a]	18 (8-26)	11 (8-16)	.34
10^3/g[a]	526 (296-988)	193 (92-504)	.02
Neutrophils			
%	39 ± 24	61 ± 22	.007
10^3/g	798 (437-3689)	943 (544-2604)	.83
Eosinophils			
%	29 (6-47)	8 (2-30)	.07
10^3/g[a]	494 (235-1663)	156 (16-362)	.006
Epithelial cells			
%	4 (3-12)	3 (1-6)	.21
10^3/g	133 (31-470)	54 (15-192)	.10
Lymphocytes			
%	0.6 (0.2-4.3)	0.4 (0.0-1.4)	.17
10^3/g[a]	32 (7-144)	4 (0-31)	.04
Eotaxin-1, pg/mL[a]			
Median (IQR)	71 (56-204)	54 (0-87)	.046
Detectable	11	29	.09
Not detectable	0	10	. . .
GM-CSF, pg/mL			
Median (IQR)	0.0 (0.0-0.6)	0.0 (0.0-0.5)	.79
Detectable	3	14	.73
Not detectable	8	25	. . .
TSLP, pg/mL			
Median (IQR)[a]	3.2 (2.4-6.5)	2.2 (1.0-3.6)	.04
Detectable	11	37	> .99
Not detectable	0	2	. . .
IL-3, pg/mL			. . .
Median (IQR)	13.1 (11.6-14.8)	11.8 (0.0-17.0)	.45
Detectable	10	23	.08
Not detectable	1	15	. . .
IL-4, pg/mL			. . .
Median (IQR)	0.2 (0.0-0.3)	0.2 (0.0-0.2)	.57
Detectable	8	24	.72
Not detectable	3	15	. . .
IL-5, pg/mL			. . .
Median (IQR)[a]	11.5 (3.5-22.2)	2.7 (1.3-4.8)	.002
Detectable	11	37	> .99
Not detectable	0	1	. . .

(Continued)

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TABLE 2] *(Continued)*

Variable	Remission	Not in Remission	*P* Value
IL-13, pg/mL			
Median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.91
Detectable	1	6	> .99
Not detectable	10	33	. . .
IL-25, pg/mL			
Median (IQR)	0.0 (0.0-2.1)	0.0 (0.0-2.2)	.72
Detectable	4	16	> .99
Not detectable	7	23	. . .
IL-33, pg/mL			
Median (IQR)	0.4 (0.0-0.6)	0.0 (0.0-0.7)	.31
Detectable	8	17	.17
Not detectable	3	22	. . .
EPX, ng/mL			
Median (IQR)	183 (129-342)	48 (30-99)	.001
Detectable	11	40	> .99
Not detectable	0	0	. . .
IgE, ng/mL			
Median (IQR)	1.2 (0.4-2.4)	0.4 (0.3-0.6)	.006
Detectable	9	31	.32
No detectable	0	8	. . .

Data are presented as No. or median (IQR), unless otherwise indicated. EPX = eosinophil peroxidase; GM-CSF = granulocyte-macrophage colony-stimulating factor; IQR = interquartile range; TSLP = thymic stromal lymphopoietin.

[a] ■■■

treated with OCS maintenance was found in the group of patients in remission compared with the group not in remission ($P = .07$). Regarding the inflammatory markers, the FENO values were similar in both groups. The proportions of patients with nasal polyposis or atopic dermatitis or urticaria were comparable. Also, the absolute numbers of sputum eosinophil count were markedly elevated at baseline in the group of patients in remission ($P = .006$) as well as the sputum macrophage and lymphocyte counts ($P = .02$ and $P = .04$, respectively). Finally, a lower proportion of sputum neutrophils was observed ($P = .007$).

For the mediators at protein level, eotaxin-1, TSLP, IL-5, EPX, and IgE sputum levels were higher in the remission group compared with the other group ($P = .046$, $P = .04$, $P = .002$, $P = .001$, and $P = .006$, respectively), whereas IL-3, IL-4, IL-13, IL-25, IL-33, and granulocyte-macrophage colony-stimulating factor were not significantly different between the groups. A ROC curve was constructed to evaluate the ability of these sputum type 2 markers to predict remission after anti-IL-5 therapy (Fig 2, Table 3). All markers seemed to perform well in distinguishing between patients in remission at 1 year vs those who were not (all AUC $\geq$ 0.7), with EPX and IL-5 showing the best combination of sensitivity and specificity with the best AUC. In contrast, the ROC curve of the blood eosinophil count gave a lower performance (closer to the 45° diagonal) and was not significant.

When the improvement after treatment was analyzed for clinical parameters one at a time, we observed that patients with improved ACT score also showed a significantly higher sputum EPX protein level at baseline (129 ng/mL [interquartile range (IQR), 47-241 ng/mL] vs 48 ng/mL [IQR, 30-148 ng/mL]; n = 15 vs 34; $P = .03$). Also, patients who showed an increase in FEV_1 before bronchodilation by at least 10% demonstrated a higher baseline sputum IL-5 protein level (9 pg/mL [IQR, 2-22 pg/mL] vs 3 pg/mL [IQR, 1-5 pg/mL]; n = 15 vs 34; $P = .04$). These results are summarized in Figure 3. Nothing was noted for the other parameters.

To screen for potential predictor of 1-year asthma remission, univariate logistic regression was

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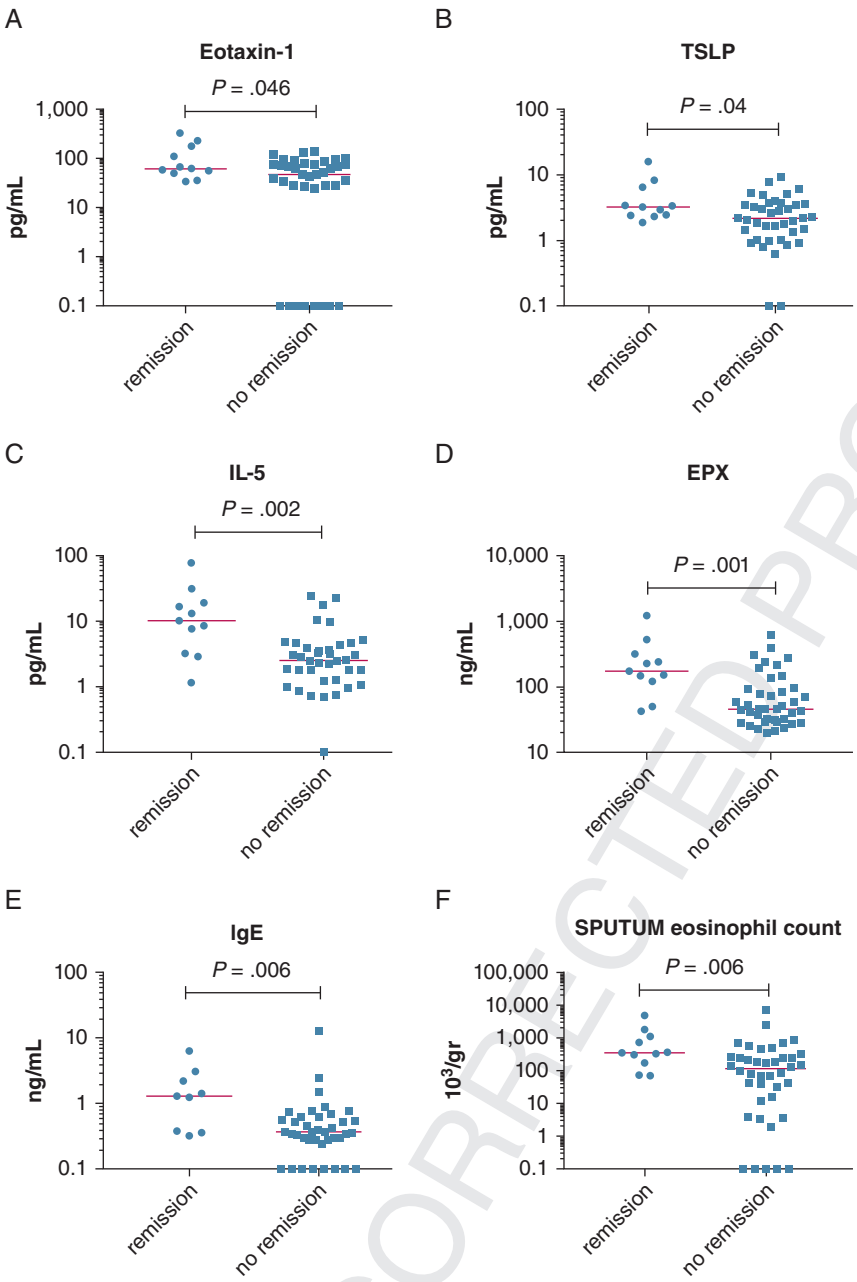

Figure 1 – *Graphs showing baseline sputum type 2 marker concentrations and sputum eosinophil counts in patients in remission vs those not in remission. EPX = eosinophil peroxidase; TSLP = thymic stromal lymphopoietin.*

performed on baseline demographic, clinical, and biological biomarkers. These results are shown in Table 4 with the corresponding ORs and *P* values. We found that male vs female sex (OR, 4.6; 95% CI, 1.1-20.126; *P* = .041), sputum neutrophil percentage (OR, 1.50; 95% CI, 1.08-2.07; *P* = .014; unit, −10), eotaxin-1 level (OR, 1.321; 95% CI, 1.00-1.679; *P* = .023; unit, 20), IL-5 level (OR, 1.727; 95% CI, 1.10-2.67; *P* = .014; unit, 5), and EPX level (OR, 1.264; 95% CI, 1.00-1.576; *P* = .037; unit, 50) were potential predictors of remission.

Discussion

In this study, we reported that 21% of patients were considered to be in remission 1 year after starting anti-IL-5 treatment. These patients more often were men and were characterized by higher sputum eosinophil counts at baseline as well as higher sputum type 2 biomarkers such as eotaxin-1, TSLP, IL-5, EPX, and IgE protein levels.

We observed a higher proportion of men in the group achieving remission. We previously reported that

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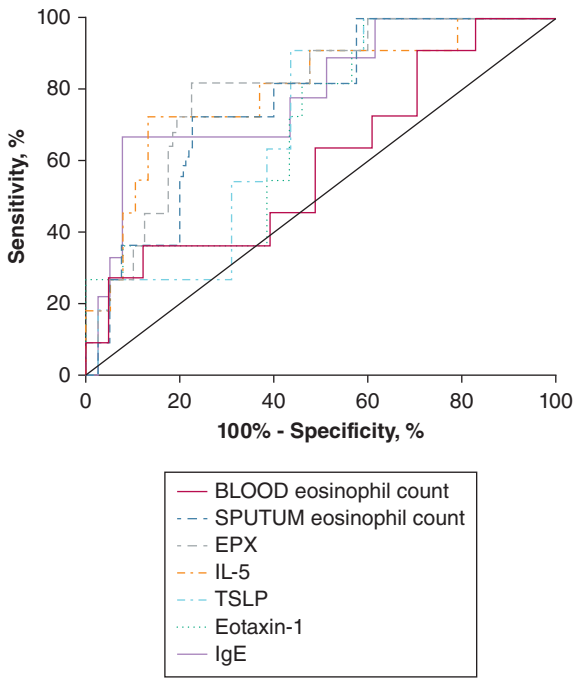

Figure 2 – *Graph showing receiver operating characteristic curve of sputum type 2 marker protein levels. EPX = eosinophil peroxidase; TSLP = thymic stromal lymphopoietin.*

patients combining an increase in both local and systemic eosinophilic inflammation more often were men,[19] and eosinophilic inflammation previously was associated with the response to anti-IL-5 and anti-IL-5R in severe asthma.[13,14] In addition, patients achieving remission were characterized by a trend for a lower proportion of patients treated with OCS maintenance, a finding also observed by another group.[7]

The higher airway macrophage and lymphocyte numbers observed in our study counterbalance a lower neutrophil proportion compared with the group not in remission. Nonresponders to anti-IL-5 therapy indeed are linked to a more intense local neutrophilic inflammation, as shown previously in real life in our clinic.[4]

Previous, but not all, studies[8] reported that blood eosinophil count was correlated with anti-IL-5 general response. However, the systemic eosinophilic inflammation has been shown to be discordant from local eosinophilic inflammation,[19] and both compartments provide additional information on the patient's status. Herein, we demonstrated that with a comparable blood eosinophil level at baseline before biotherapy, the response can be highly variable. In this case, sputum cell proportion and sputum type 2 markers levels could be surrogate markers of response. Indeed, TSLP is an epithelial alarmin and an upstream key actor of type 2 inflammatory cytokines release including IL-5. IL-5 is a central cytokine in the severe eosinophilic asthma phenotype. IL-5 is the cytokine responsible for eosinophil survival and activation, which can explain the concomitant high sputum eosinophil count and EPX sputum level. Also, the higher eotaxin-1 protein level, which is a potent chemoattractant for eosinophils, was higher at baseline in the sputum of patients in remission. If it exists, a concomitant systemic increase of these mediators in the blood compartment was not assessed in this study, but deserves further research because the patients combined both systemic (> 400 cells/μL) and airway (> 3%) eosinophilic inflammation. Although a recent study did not find any difference in blood eosinophil and IL-5 levels in responders (no exacerbation, ≥ 50% OCS dose reduction at week 16, or both) vs nonresponders in a cohort of patients before mepolizumab treatment.[20] Also, the investigation of those mediators in the sputum of patients with only selective

TABLE 3] ROC Curve Details

Mediator	AUC (95% CI)	Threshold	Sensitivity (95% CI)	Specificity (95% CI)	Likelihood Ratio	*P* Value
Blood eosinophil count	0.60 (0.40-0.79)	501.30	0.64 (0.31-0.89)	0.51 (0.35-0.67)	1.3	.33
Sputum eosinophil count	0.76 (0.62-0.91)	398.60	0.73 (0.39-0.94)	0.77 (0.62-0.89)	3.2	.008
Sputum EPX	0.81 (0.67-0.94)	114.30	0.82 (0.48-0.98)	0.77 (0.62-0.89)	3.6	.002
Sputum IL-5	0.80 (0.64-0.95)	7.24	0.73 (0.39-0.94)	0.87 (0.72-0.96)	5.5	.003
Sputum TSLP	0.70 (0.55-0.85)	2.29	0.91 (0.59-1.00)	0.56 (0.40-0.72)	2.1	.043
Sputum eotaxin-1	0.70 (0.53-0.86)	55.26	0.82 (0.48-0.98)	0.54 (0.37-0.70)	1.8	.048
Sputum IgE	0.79 (0.62-0.95)	0.98	0.67 (0.30-0.93)	0.92 (0.79-0.98)	8.7	.007

AUC = area under the receiver operating characteristic curve; EPX = eosinophil peroxidase; ROC = receiver operating characteristic; TSLP = thymic stromal lymphopoietin.

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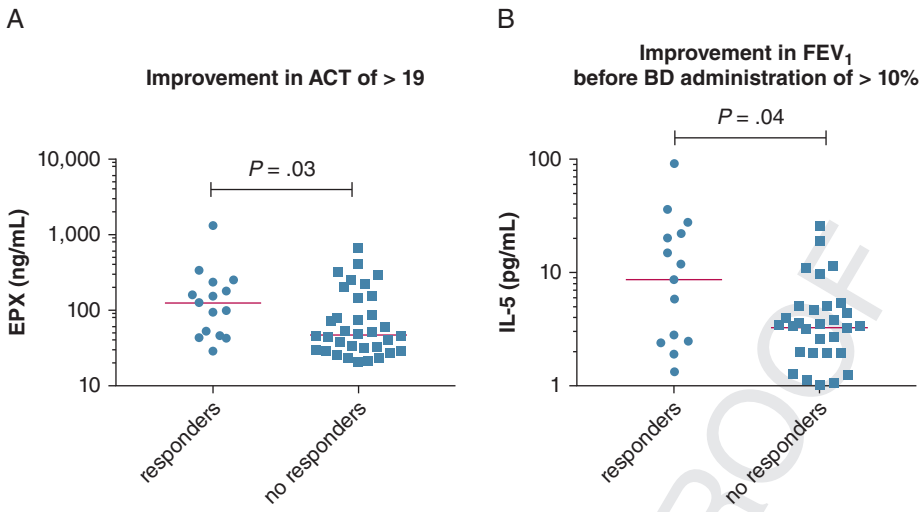

Figure 3 – *A, B, Graphs showing baseline sputum mediator concentrations in patients showing a response vs those not showing a response: improvement in ACT score of > 19 (A) and improvement in FEV_1 before BD administration of > 10% (B). ACT = asthma control test; BD = bronchodilator; EPX = eosinophil peroxidase.*

local eosinophilic inflammation is of interest because those patients represent half of the patients with asthma demonstrating eosinophilic inflammation.[19] An explanation for a higher sputum eosinophil count in the current patients who achieved remission at 1 year would be that in those with an equal mobilizable pool, an extent of eosinophil infiltration exists in the airways because of the release of chemotactic agents such as eotaxin-1.

The higher sputum IgE levels in patients achieving remission are more intriguing. Indeed, the group with remission did not include a higher proportion of patients with atopia compared with the patients not in remission, and blood IgE levels did not differ between them. However, it has been shown that local production of IgE exists even in case of intrinsic asthma and was not linked to atopic status.[21] In addition, our team previously observed that sputum IL-5 levels also were increased in case of high sputum IgE levels and that higher sputum IgE levels were seen in patients exhibiting eosinophilic airway inflammation.[22]

Univariate ROC analyses showed associations between these baseline sputum mediators and the likelihood of achieving remission, and all performed better than blood eosinophil count. These results confirmed the finding that anti-IL-5 therapies are more efficient in patients with a local type 2 high endotype. In addition, the univariate logistic regression analysis mainly identified male sex, sputum neutrophil percentage, eotaxin-1 level, IL-5 level, and EPX level as potential predictors of remission, but not sputum eosinophil count, possibly because of the presence of extreme data. However, these results need to be validated in a larger cohort in a multicenter study. Whether sputum neutrophil percentage is associated with a suboptimal response also needs further investigations.

The limitations of this study are that the criteria of remission in asthma are not yet universally defined. Also, the limited number of patients may have reduced the power of the subanalyses to detect significant differences between both groups. Furthermore, an analysis based on nonoptimal or suboptimal response predictors would be of interest and should be performed in another multicenter study. In addition, it would be interesting to look at other markers involved in different pathways, but we focused on type 2 markers because a type 2 disease was considered. Finally, the sputum also may not be available in all asthmatic care centers, but expanding its use in clinical practice could be recommended because it allows the collection of valuable supplementary information useful in the management of patients with severe eosinophilic asthma. Indeed, even if preliminary and retrospective, this study is the first to investigate local airway expression of mediators of the type 2 cascade as predictors of remission after anti-IL-5 treatment in a cohort of patients with severe eosinophilic asthma.

Interpretation

Sputum type 2 markers levels could be surrogate markers of response 1 year after anti-IL-5

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TABLE 4] Univariate Logistic Regression Analysis of Potential Predictors of Asthma Remission

Variable	OR	95% CI	*P* Value	Unit
Sex, male vs female[a]	4.6	1.1-20.126	.041	. . .
Age	0.97	0.93-1.03	.314	. . .
BMI	0.97	0.85-1.10	.591	. . .
Smoking status				
Current vs never	0.39	0.01-11.40	.520	. . .
Former vs never	1.38	0.34-5.58	.444	. . .
Pack-y of smoking	1.00	0.97-1.04	.802	. . .
Atopy, yes vs no	0.34	0.08-1.37	.128	. . .
Age at diagnosis	1.00	0.97-1.04	.986	. . .
Asthma duration	0.97	0.93-1.02	.276	. . .
OCS cure	1.05	0.77-1.42	.768	. . .
ACT score	1.05	0.91-1.21	.489	. . .
ACQ score	0.77	0.45-1.32	.337	. . .
AQLQ score	1.40	0.81-2.42	.224	. . .
FEV_1, % predicted	1.03	0.98-1.07	.213	. . .
After BD administration	1.02	0.98-1.06	.275	. . .
FVC, % predicted	1.02	0.97-1.06	.433	. . .
After BD administration	1.01	0.97-1.06	.570	. . .
FEV_1 to FVC ratio	1.02	0.96-1.09	.530	. . .
After BD administration	1.02	0.96-1.08	.508	. . .
Blood neutrophils, /μL	1.00	1.00-1.00	.131	. . .
Blood eosinophils, /μL	1.00	1.00-1.00	.174	. . .
Serum IgE	1.00	1.00-1.00	.306	. . .
CRP	1.02	0.90-1.15	.769	. . .
Fibrinogen	0.68	0.14-3.15	.618	. . .
ICS	1.00	1.00-1.00	.944	. . .
OCS, yes vs no	0.13	0.01-2.68	.184	. . .
F_{ENO}	1.01	1.00-1.03	.095	. . .
Nasal polyposis, yes vs no	1.30	0.34-4.99	.700	. . .
AD or urticaria, yes vs no	2.27	0.58-8.97	.241	. . .
Sputum weight, g	0.77	0.42-1.41	.402	. . .
Squamous cells, %	0.96	0.90-1.02	.172	. . .
Viability, %	1.00	0.97-1.02	.717	. . .
Cell No., 10^6 cells/g	1.02	0.93-1.13	.624	. . .
Macrophages				
%	1.03	0.98-1.09	.212	. . .
10^3/g	1.00	1.00-1.00	.501	. . .
Neutrophils				
%[a]	1.50	1.08-2.07	.014	−10
10^3/g	1.00	1.00-1.00	.875	. . .
Eosinophils				
%	1.02	0.99-1.05	.162	. . .
10^3/g	1.00	1.00-1.00	.269	. . .
Epithelial cells				
%	1.03	0.95-1.12	.465	. . .
10^3/g	1.00	1.00-1.00	.230	. . .

(Continued)

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TABLE 4] *(Continued)*

Variable	OR	95% CI	*P* Value	Unit
Lymphocytes				
%	1.06	0.97-1.15	.200	. . .
10^3/g	1.00	1.00-1.00	.604	. . .
Eotaxin-1[a]	1.321	1.00-1.679	.023	20
Detectable, yes vs no	8.18	0.39-173.41	.177	. . .
GM-CSF	0.85	0.16-4.58	.847	. . .
Detectable, yes vs no	0.67	0.15-2.94	.595	. . .
TSLP	1.29	0.99-1.69	.056	. . .
Detectable, yes vs no	1.53	0.04-67.15	.824	. . .
IL-3	1.02	0.97-1.07	.418	. . .
Detectable, yes vs no	6.52	0.76-56.33	.088	. . .
IL-4	15.90	0.76-332.01	.074	. . .
Detectable, yes vs no	1.67	0.38-7.29	.497	. . .
IL-5[a]	1.727	1.10-2.67	.014	5
Detectable, yes vs no	0.92	0.01-89.18	.971	. . .
IL-13	1.04	0.80-1.34	.779	. . .
Detectable, yes vs no	0.55	0.06-5.13	.600	. . .
IL-25	1.08	0.78-1.50	.634	. . .
Detectable, yes vs no	0.82	0.21-3.28	.781	. . .
IL-33	1.17	0.44-3.10	.755	. . .
Detectable, yes vs no	3.45	0.79-15.01	.099	. . .
EPX[a]	1.264	1.00-1.576	.037	50
Detectable, yes vs no	NA	NA	NA	. . .
IgE	1.26	0.88-1.79	.201	. . .
Detectable, yes vs no	5.13	0.23-115.21	.304	. . .

ACQ = asthma control questionnaire; ACT = asthma control test; AD = atopic dermatitis; AQLQ = asthma quality of life questionnaire; BD = bronchodilation; CRP = C-reactive protein; EPX = eosinophil peroxidase; FENO = fraction of exhaled nitric oxide; GM-CSF = granulocyte-macrophage colony-stimulating factor; ICS = inhaled corticosteroids; NA = all patients were detectable so no statistical analysis was performed; OCS = oral corticosteroids; TSLP = thymic stromal lymphopoietin.

treatment. Sputum indeed is now recommended by American Thoracic Society and European Respiratory Society guidelines in the management of severe asthma and should be reimbursed by local authorities because it could predict patients who will achieve remission after administration of these very costly biologics.

Funding/Support

GSK and AstraZeneca provided funding support for this study (ClinicalTrials.gov Identifier: NCT04520165).

Financial/Nonfinancial Disclosures

The authors have reported to *CHEST* the following: R. L. and F. S. received educational and research grants from GSK, AstraZeneca, and Chiesi; received consulting fees from GSK and AstraZeneca (national and international advisory boards); and received lecture fees from GSK, AstraZeneca, and Chiesi. None declared (C. M., C. B., G. B., S. Graff, S. Gerday, H. N., C. P., N. B., M. H., V. P., F. G.).

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Acknowledgments

Author contributions: C. M. takes the responsibility for the content of the manuscript, including the data and analysis. C. M. participated in the study design, performed the research and data analysis, and wrote the manuscript. C. B. and G. B. performed the research and participated in data analysis. H. N. and C. P. performed the statistical analysis. S. Graff, S. Gerday, N. M., M. H., V. P., and F. G. participated in the sputum induction and processing and patient data collection. F. S. and R. L. designed the study and interpreted the data. All authors participated in manuscript review, gave final approval of the manuscript, and ensured that questions related to the accuracy or integrity of any part of the work were investigated and resolved appropriately.

Role of sponsors**:** GSK was provided the opportunity to review this manuscript draft for factual accuracy, but the authors are solely responsible for final content and interpretation.

Additional information: The e-Table is available online under "Supplemental Data."

References

1. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*. 2014;43(2):343-373.
2. Hekking PPW, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. *J Allergy Clin Immunol*. 2015;135(4):896-902.
3. Carstens DD, Katial R, Young J, et al. Real-world effectiveness of benralizumab on asthma exacerbations: results from the ZEPHYR 1 study 2021; 2021;128(6):669-676.
4. Schleich F, Graff S, Nekoee H, et al. Real-word experience with mepolizumab: does it deliver what it has promised? *Clin Exp Allergy*. 2020;50(6):687-695.
5. Mukherjee M, Forero DF, Tran S, et al. Suboptimal treatment response to anti-IL-5 monoclonal antibodies in severe eosinophilic asthmatics with airway autoimmune phenomena. *Eur Respir J*. 2020;56(4):2000117.
6. Eger K, Kroes JA, ten Brinke A, Bel EH. Long-term therapy response to Anti–IL-5 biologics in severe asthma—a real-life evaluation. *J Allergy Clin Immunol Pract*. 2021;9(3):1194-1200.
7. Harvey ES, Langton D, Katelaris C, et al. Mepolizumab effectiveness and identification of super-responders in severe asthma. *Eur Respir J*. 2020;55(5):1902420.
8. Kavanagh JE, d'Ancona G, Elstad M, et al. Real-world effectiveness and the characteristics of a "super-responder" to mepolizumab in severe eosinophilic asthma. *Chest*. 2020;158(2):491-500.
9. Howarth P, Chupp G, Nelsen LM, et al. Severe eosinophilic asthma with nasal polyposis: a phenotype for improved sinonasal and asthma outcomes with mepolizumab therapy. *J Allergy Clin Immunol*. 2020;145(6):1713-1715.
10. Menzies-Gow A, Szefler SJ, Busse WW. The Relationship of asthma biologics to remission for asthma. *J Allergy Clin Immunol Pract*. 2021;9(3):1090-1098.
11. Thomas D, McDonald VM, Pavord ID. Asthma remission—what is it and how can it be achieved? *Eur Respir J*. 20222102583. https://doi.org/10.1183/13993003.02583-2021
12. Menzies-Gow A, Bafadhel M, Busse WW, et al. An expert consensus framework for asthma remission as a treatment goal. *J Allergy Clin Immunol*. 2020;145(3):757-765.
13. Ortega HG, Yancey SW, Mayer B, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. *Lancet Respir Med*. 2016;4(7):549-556.
14. FitzGerald JM, Bleecker ER, Menzies-Gow A, et al. Predictors of enhanced response with benralizumab for patients with severe asthma: pooled analysis of the SIROCCO and CALIMA studies. *Lancet Respir Med*. 2018;6(1):51-64.
15. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-338.
16. Delvaux M, Henket M, Lau L, et al. Nebulised salbutamol administered during sputum induction improves bronchoprotection in patients with asthma. *Thorax*. 2004;59(2):111-115.
17. Guiot J, Demarche S, Henket M, et al. Methodology for sputum induction and laboratory processing. *J Vis Exp*. 2017;130:56612.
18. Couillard S, Shrimanker R, Chaudhuri R, et al. Fractional exhaled nitric oxide nonsuppression identifies corticosteroid-resistant type 2 signaling in severe asthma. *Am J Respir Crit Care Med*. 2021;204(6):731-734.
19. Schleich FN, Chevremont A, Paulus V, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J*. 2014;44(1):97-108.
20. Šokić MK, Rijavec M, Korošec P, et al. Heterogeneous response of airway eosinophilia to anti-IL-5 biologics in severe asthma patients. *J Pers Med*. 2022;12(1):70.
21. Mouthuy J, Detry B, Sohy C, Pirson F, Pilette C. Presence in sputum of functional dust mite-specific IgE antibodies in intrinsic asthma. *Am J Respir Crit Care Med*. 2011;184(2):206-214.
22. Manise M, Holtappels G, Van Crombruggen K, Schleich F, Bachert C, Louis R. Sputum IgE and cytokines in asthma: relationship with sputum cellular profile. *PLoS One*. 2013;8(3):e58388.

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