

Basophil activation and serum IL-5 levels as possible monitor biomarkers in severe eosinophilic asthma patients treated with anti-IL-5 drugs

To the Editor,

Interleukin-5 (IL-5) is one of the main cytokine involved in the pathogenesis of eosinophilic asthma (EA) and exerts pleiotropic activities on various target cells expressing its receptor, including eosinophils and basophils. Drugs targeting circulating IL-5 or its receptor, such as mepolizumab and benralizumab, allowed the expansion of the treatment options for severe EA.¹ Nevertheless, the response rates reported in phase III regulatory trials^{2,3} make it highly desirable to identify biomarkers able to predict response to these biological treatments in EA patients. Although targeted therapies have been increasingly integrated into treatment strategies for severe asthma, only a few inflammatory biomarkers have been validated for clinical application.⁴ A recent real-life study also suggested that the peripheral absolute eosinophil count (AEC) may not reliably predict the response to anti-IL-5 drugs.⁵

Although the physiologic role of basophils is not completely known yet, recent studies in humans and murine systems have shown that basophils perform non-redundant effector functions and significantly contribute to the development and progression of T_H2 cytokine-mediated inflammation, contributing to the pathogenesis of different diseases such asthma.⁶

Here, we investigated the correlation between clinical response to mepolizumab or benralizumab and potential predictive biomarkers such as the serum IL-5 levels and the phenotype of circulating basophils in EA patients. We enrolled 19 patients older than 18 years, who came to the Allergy Unit of the Columbus Hospital in Rome between January 2018 and June 2019 because of severe EA. For each patient clinical and laboratory evaluations were performed at baseline (T0) and after 3 (T3) and 6 months of treatment (T6). Asthma control test (ACT) scores, FEV_1 , fractional exhaled nitric oxide (FeNO) values, number of exacerbations, and oral corticosteroid (OCS) consumption were used as parameters of clinical response, while peripheral AEC was used to define a laboratory response. Written informed consent to participate to the study was obtained from each patient. The protocol was approved by the institutional review board. Serum IL-5 levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA) (Bio-Techne). The phenotype of basophils activated with anti-Fc ϵ RI antibody (Allergenicity kit,

BeckmanCoulter) was evaluated by flow cytometry (Navios Flow Cytometer, BeckmanCoulter); basophils were identified as CD3-negative/prostaglandin receptor (CRTH2)-positive cells and activation was evaluated by the expression of CD203c, CD63, and IL-5R α (CD125) (Figure S1).

Statistical analysis was performed using SPSS V. 20.0 (SPSS, Chicago) and Prism software (GraphPad) (further details are available in the online Appendix S1).

Patients were treated with mepolizumab or benralizumab according to data sheet (GINA and ERS/ATS guidelines). Table 1 shows the demographic and clinical characteristics of EA patients enrolled in the study.

A complete response (see Appendix S1 for details) was observed in 13 patients (68.4%) and a partial response in 6 (31.6%), regardless of the treatment used (ie, mepolizumab or benralizumab). All patients experienced a remarkable improvement of lung function with an increase of FEV_1 from a mean value of 72%-83.8% of the theoretical value (+12%). FeNO values decreased from a median value of 65 ppb at T0 to 10 ppb at T6 ($P = .01$). Moreover, all patients showed a better disease control at T6 and an ACT score improvement from 14 (10.0-18.0) at T0 to 22 (20-23) at T6 ($P < .0001$).

During the 6 months of follow-up, neither exacerbations nor hospitalization was observed. OCS was progressively reduced and finally stopped in 14 (73.7%) out of 19 patients at T6; moreover, a substantial reduction was achieved in the others.

Peripheral AEC progressively decreased during treatment in all patients, reaching the lowest values, as expected, after 6 months and in the benralizumab group (Figure S3).

During the follow-up, serum IL-5 levels showed a significant increase, almost exclusively in the mepolizumab group (Figure 1A), as previously observed.^{7,8} Serum IL-5 levels did not differ at T0, T3, and T6 among patients with different treatment responses (not shown). As regards to the detection of activated basophils, no difference was observed in the distribution of CD3^{neg}CRTH2^{pos}CD203c^{pos} and CD3^{neg}CRTH2^{pos}CD203c^{pos}CD63^{pos} at T0 in patients with partial or complete responses at T6 (not shown). Interestingly, EA patients who achieved a complete response to anti-IL5 treatment showed a rate of activated basophils CD3^{neg}CRTH2^{pos}CD203c^{pos}CD125^{pos}

TABLE 1 Demographic and clinical characteristics of EA patients at baseline

	EA patients (N = 19)
Females, n (%)	15 (79)
Age (years)	59 (34-77)
Disease duration (years)	10 (4-40)
Treatment	
Mepolizumab, n (%)	10 ^a (53)
Benralizumab, n (%)	9 (47)
Atopy history, n (%)	8 (42)
Smoke (former smokers), n (%)	2 (10)
Comorbidities	
CRS, n (%)	13 (68)
CRSwNP, n (%)	8 (42)
OCS: Pulses, n (%)	11 (58)
Chronic use, n (%)	8 (42)
AEC, cells/mm ³	700 (170-3350)
ACT (median-IQ range)	14(6-25)
FEV1 (%)	72 (41-104)
FeNO(ppb)	65(15-90)
BMI (kg/m ²)	25 (18.9-39.5)

Note: Data are expressed as number (percent) of patients or median (range) of values.

Abbreviations: ACT, asthma control test; AEC, peripheral absolute eosinophilic count; BMI, body mass index; CRS, Chronic rhinosinusitis; CRSwNP, chronic rhinosinusitis with nasal polyps; EA, eosinophilic asthma; FeNO, Fractional exhaled nitric oxide; FEV1, Forced expiratory volume in the 1st second; OCS, oral corticosteroids.

^a9 at 100 mg/4 weeks and 1 (EGPA) at 300 mg/4 weeks.

($4.78 \pm 2.26\%$) at T0 significantly lower than that of patients not achieving the complete response ($34.57 \pm 14.01\%$, $P = .05$) (Figure 1B). Moreover, at baseline the rate of CD3^{neg}CRTH2^{pos}CD203c^{pos}CD125^{pos} correlated directly with IL-5 serum levels ($R = 0.635$, $P = .07$).

In our cohort of EA patients, IL-5 levels, at baseline and during treatment, did not correlate with the treatment response, while lower levels were significantly observed at baseline, according to OCS use (OCS pulses at exacerbations vs continuous use), suggesting a correlation between different levels of expression of this cytokine and disease severity (Figure S4).

Previous studies showed that the rate of activated basophils can be used for clinical applications as well as to explore the immune mechanisms of effector cell response. In particular, Siegmund R et al demonstrated that human basophils from subjects allergic to wasp venom undergoing VIT were regulated by cytokines, as shown by the alteration of the expression of cytokine receptors and that the expression of IL-5R (CD125) decreased during immunotherapy.⁹

Interestingly, here, the lower was the rate of the endotype CD3^{neg}CRTH2^{pos}CD203c^{pos}CD125^{pos} of activated basophil at baseline, the better was the clinical response at T6 in patients treated with an anti-IL5 biological therapy.

This pilot study in our cohort of EA patients shows that the rate of activated basophils, in particular of those that express CD125, is inversely related to the effectiveness of anti-IL5/IL5R α drugs, proving to be a biomarker that allows identification of the patient's phenotype that better respond to therapy and, at the same time, the patient's phenotype that requires the concomitant use of OCS. Taking into account study limitations (ie, the small number of subjects enrolled and the limited follow-up period), further studies in large samples are needed to confirm these preliminary data indicating basophils expressing CD125 as a useful biomarker in severe asthmatic patients.

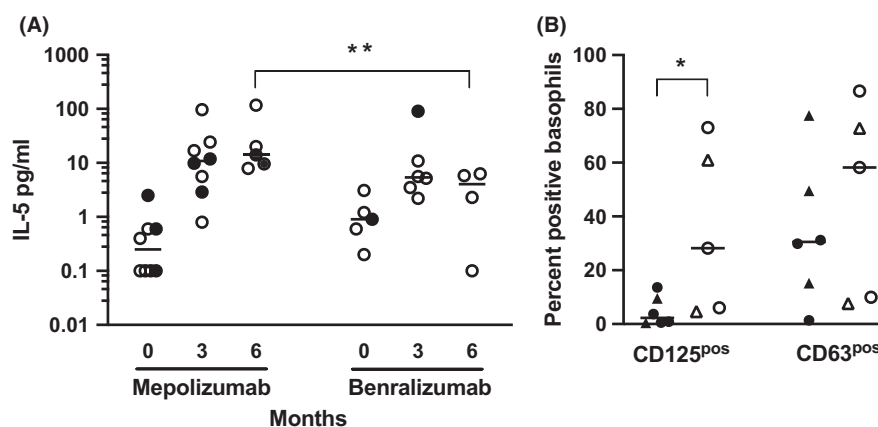


FIGURE 1 Serum IL-5 and phenotype of basophils activated with anti-Fc ϵ R in EA patients treated with anti-IL-5 therapy. (A) Serum IL-5 levels are significantly higher at month 6 of therapy in patients treated with mepolizumab rather than with benralizumab; closed circles denote partial responders to therapy. (B) Partial responders showed significantly higher proportions of CD203c^{pos}CD125^{pos} activated basophils than complete responders at baseline, while the proportions of CD203c^{pos}CD63^{pos} basophils are not significantly different; closed symbols denote complete responders and open symbols partial responders; triangles denote patients treated with mepolizumab. Lines denote medians; * $P < .05$; ** $P < .02$

KEYWORDS

asthma, basophil, eosinophils, interleukins

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Appendix S1

Supporting information

We enrolled subjects older than 18 years attending the Allergy Unit of the Columbus Hospital, Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome between January 2018 and June 2019 because of severe EA. Clinical and laboratory evaluations were performed at baseline and at months 3 and 6 of treatment. Asthma control test (ACT) scores, FEV1, fractional exhaled nitric oxide (FeNO) values (1), number of exacerbations and oral corticosteroid (OCS) consumption (2) were used as parameters of clinical response, while peripheral absolute eosinophil count (AEC) was used to define a laboratory response (3).

All patients had at least two exacerbations during the previous 12 months, that required OCS use (pulses) or up-dosing in those who were in chronic corticosteroid treatment (8/19); 9 (47.4%) EA patients had also experienced at least one episode of hospitalization for asthma. Thirteen patients (68.4%) suffered from chronic rhinosinusitis (CRS) and 8 of them from nasal polyposis. Clinical characteristics of our patients were very similar to those of the SANI (Severe Asthma Network in Italy) population (4). Eight patients (42.1%) had a history of atopy.

We considered as complete responders patients who, at T6, presented both a laboratory and clinical response, i.e. reduction of eosinophilic count, improvement in ACT score and FEV1 value, reduction of FeNO, no exacerbations (no OCS pulses), withdrawal of chronic OCS. Partial responders were patient who didn't achieve all these targets (i.e. sparing of OCS but no withdrawal).

Prior to the study, all subjects received information about the protocol, and written informed consent was obtained from each patient. The study was approved by the institutional review board.

Methods

Serum IL-5 measurement

Blood samples were collected at baseline (T0) and after 3 (T3) and 6 (T6) months of treatment; sera were stored at - 80°C until analysis. Serum levels of IL-5 were measured by Enzyme-Linked Immunosorbent Assay (ELISA) (Bio-Techne, UK). The sensitivity of the test was 1.08 pg/ml.

Basophil phenotype assessment

Flow cytometry studies were performed within two hours from blood collection using the Allergenkit (Beckman Coulter). Briefly, anticoagulated peripheral blood aliquots (100 µl) were stimulated at 37 °C for 15 minutes with 20 µl of antibody directed to the high affinity IgE receptor (FcεRI) (Beckman Coulter) or PBS as negative control, and then stained with 20 µl of mixture of monoclonal antibodies [CRTH2-FITC (clone BM16); CD203c-PE (clone 97A6) and CD3-PC7 (clone (UCHT1))] with 10 µl of CD63-PC5.5 (clone H5C6; Biolegend) and 10 µl of CD125-APC (clone 26815; Beckton Dickinson). After incubation, the reaction was terminated by adding the stop solution and erythrocytes were lysed with lysing solution. After washing and fixation, leucocytes were analysed using a ten-colour flow cytometer (Navios, Beckman Coulter). The gating strategy is illustrated in supplementary Figure 1; basophils were selected as CRTH2^{high}/SSC^{low}/CD3^{neg} cells and evaluated for CD203c, CD63 and CD125 expression.

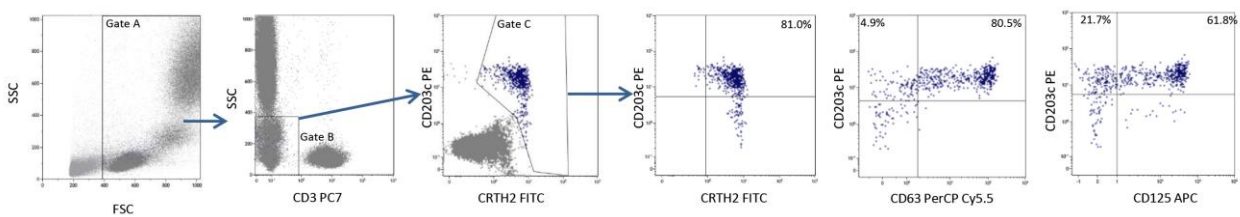
Statistical analysis

Statistical analysis was performed using SPSS V. 20.0 (SPSS, Chicago, Illinois, USA) and Prism software (GraphPad, San Diego, California, USA). Categorical and quantitative variables were described as frequencies; percentage and mean $\pm$ SEM. Demographic, clinical and laboratory data were compared by the Mann-Whitney U test or the Fisher's exact test as appropriate. Spearman's rank correlation test was used for correlations. A Receiver Operating Characteristic (ROC) curve analysis of percentage of basophils activation differentially distributed among patients based on outcome was performed to obtain relevant threshold allowing the prediction of "improvement of clinical and laboratory parameters within 6 months of treatment". ROC plots used all the data making no parametric assumption and provide unbiased estimates of sensitivity and specificity. The optimal cut-off point was determined to yield the maximum corresponding sensitivity and specificity. (supplementary Figure 2). $P < 0.05$ was considered statistically significant and all test were 2-tailed unless otherwise indicated.

References

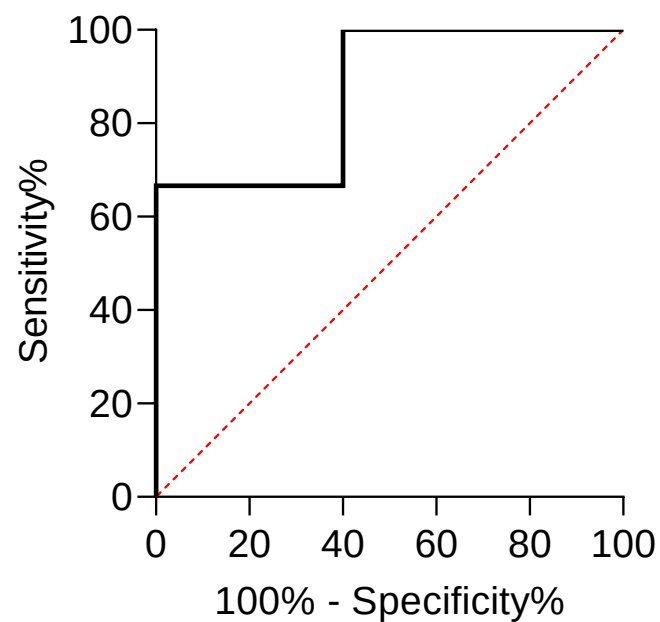
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Supplementary Figure 1



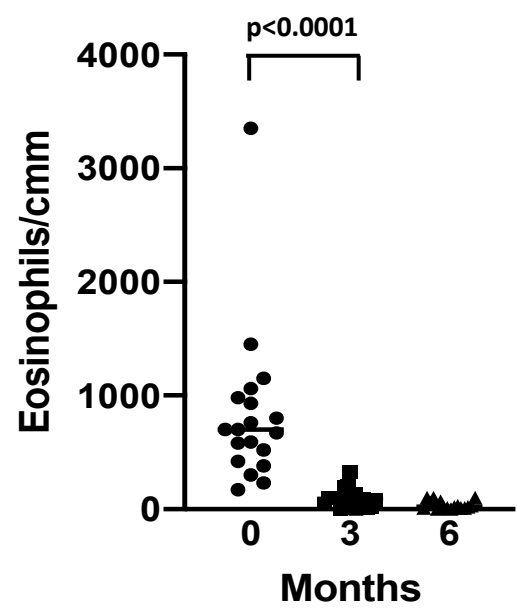
Flow cytometric analysis of basophils phenotype in EA patients, using a ten-colour flow cytometer (Navios, Beckman Coulter). Basophils were selected as CRTH2^{high}/SSC^{low}/CD3^{neg} population (Gates A and B) and CD203c, CD63 and CD125 expression were evaluated. The threshold for positivity (CD203c^{pos}) was set at less than 1% of activated cells in negative control.

Supplementary Figure 2



Receiver-operating characteristic (ROC) curve analysis of basophils CD3neg/CRTH2posCD203cpoCD125pos (%) in patients with partial or complete response to Anti-IL5 treatment. Area under the receiver-operating curve (AUC): 0.186, 95% confidence interval (CI): 0.000-0.439, sensitivity 57.1%, specificity 100%.

Supplementary Figure 3.



Peripheral absolute eosinophil counts in EA patients treated with anti-IL-5 therapy at baseline and after 3 and 6 months of treatment.

