

The importance of being not significant: Blood eosinophils and clinical responses do not correlate in severe asthma patients treated with mepolizumab in real life

To the editor,

The availability of monoclonal antibodies (MAbs) has changed the therapeutic approach to severe asthma, defined by the ERS/ATS document as asthma that remains not controlled despite the maximum inhaled treatment, and that often requires the use of systemic corticosteroids (OCS). So far, we can distinguish a type 2 asthma, characterized by eosinophilic inflammation, high fractional exhaled nitric oxide (FeNO), and frequently IgE sensitization. On the other hand, we have the non-T2 asthma, with poor inflammatory characteristics, noneosinophilic inflammation, and poor response to OCS. The T2-type asthma offered intriguing

strategies, such as anti-IgE (omalizumab) and anti-IL-5(R) MAbs (mepolizumab, reslizumab, and benralizumab).¹ It is necessary to look for one or more biomarkers able to predict the clinical response, in order to prescribe the biological treatment with a reasonable expectancy of success.

In the case of biological agents, some markers have been proposed as possibly predictive, such as peripheral blood eosinophils (EOS), FeNO, periostin, and total IgE. In the specific case of T2 asthma, where the antagonism to IL-5 (either circulating or receptor-directed) is considered of primary relevance, the eosinophil count (cells/ μ L) was suggested as a reliable predictive biomarker. The

TABLE 1 Main characteristics of the patients at baseline and after 12 mo of MEP treatment. The parameters for which the % change from baseline was considered in the statistical analysis are highlighted (from 11)

Characteristics	Baseline	12 mo	Difference	P-value
Number of patients	138	138	n.a.	n.a.
Age (SD, range)	58 (10; 21-81)	59 (10; 22-82)	-	n.s.
Age onset (SD)	34 (17)			
Female (%)	78 (57%)	78 (57%)	n.a.	n.a.
Blood eosinophils				
Mean (range)	822 (150-3060)	117 (0-600)	-705 (86%)	<.0001
Median (SEM)	800 (41.78)	100 (8.19)		
Exacerbations/12 mo				
Mean (SD)	3.8 (2.5)	0.7 (1.1)	-3.1 (81%)	<.0001
Median (IQ RANGE)	3.0 (2.8)	0 (1.0)		
OCS mg/d, mean (SD)	10.1 (9.4)	2.0 (4.2)	-8.1 (80%)	<.0001
FeNO ppb				
Mean (SD)	75 (53)	42 (37)	-33 (44%)	<.0001
Median (SEM)	64 (5.7)	30 (4.1)		
FEV1 (L $\pm$ SD)	2.03 (0.78)	2.36 (0.88)	0.34 (17%)	.001
FEV1 (%)	69	75	5.6 (8%)	<.0001
FVC (L $\pm$ SD)	3.16 (1.05)	3.32 (1.04)	0.16 (5%)	n.s.
FVC (%)	88	92	4.4 (5%)	.021
FEV1/FVC ($\pm$ SD)	0.65 (0.11)	0.69 (0.11)	0.04 (6%)	.002
ACT ($\pm$ SD)	17 (5)	22 (3)	5 (34%)	<.0001

Abbreviations: ACT, asthma control test; EOS, eosinophils; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; MAbs, monoclonal antibodies; OCS, oral corticosteroids.

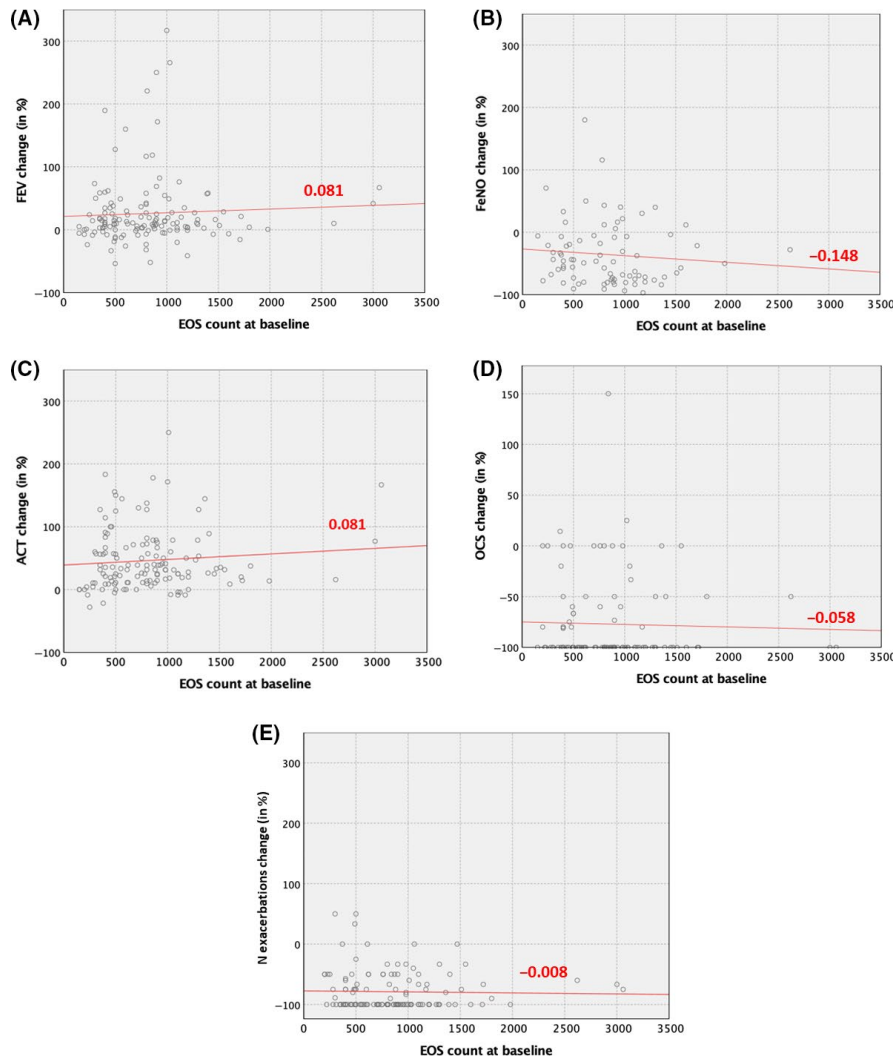


FIGURE 1 The figure shows patient by patient the % change of parameters vs baseline (Y-axis) and the baseline eosinophil count (X-axis). The linear trend is indicated as a red line, and the correlation coefficient is also reported. A: FEV1; B: FeNO; C: ACT score; D: OCS daily dose; E: exacerbations in the past 12 mo

hypothesis that as high is the pretreatment eosinophil count, as better would be the clinical response, was apparently confirmed in the phase III regulatory trials.²⁻⁴ In this context, it must be considered that the patients enrolled in the phase III trials are different from those treated in the real-life context.⁵

In this analysis, we attempted to correlate the baseline EOS count with the clinical, functional, and biological outcome changes, and to assess whether baseline EOS could be a predictive biological marker.

All the patients, diagnosed with severe asthma according to ERS/ATS criteria and which main comorbidities have been sought and treated,⁶ were followed up by several nationwide distributed centers (Genoa, Pietra Ligure, Milan, Cuneo, Turin, Reggio Emilia, Verona, Rome, Arenzano, Brescia, Naples) and received MEP for at least 12 months. The general characteristics and their changes over 12 months of treatment are described elsewhere.⁷ To assess this, the percentage change (12 months–baseline) was calculated for: OCS dose, asthma control test (ACT) score, FEV1, exacerbation rate, and FeNO. The null hypothesis was that the baseline EOS concentration does not influence the mentioned outcomes, as evaluated by the available measurements.

Changes in the considered parameters were first tested with a *t* test for paired samples or with Wilcoxon test for paired data when appropriate. Furthermore, the probability level for the Wilcoxon test was computed using a complete randomization method (permutation or exact test; P_{exact}) or by a Monte Carlo ($P_{\text{Monte Carlo}}$). The type I error (α) has been set at the 0.01 level. The means were reported with their standard error ($\pm$ SE). The percentage change from baseline of analyzed parameters used to estimate the association with EOS was performed by the Pearson linear correlation analysis or Spearman nonparametric correlation coefficient when appropriate. All statistical analyses were performed using IBM® SPSS 25.

In total, 138 patients (78 female, 60 male, mean age 58 ± 10 years; age range 21–81) were observed. Of them, 27 were allergic (all to mite, and 18 also to pollen allergens). The main demographic and clinical characteristics of those patients, as already described in detail,⁷ are summarized in Table 1.

No significant linear associations ($P > .05$) were detected between the EOS count at baseline and the change at 12 months vs baseline of the examined parameters (Table S1). Even when log-transformed to achieve normality, no correlation analysis could detect any significant linear association.

The raw data are depicted in Figure 1A-E, where it is clear that the slopes of linear trends are minimal. In final, we could not detect any significant association between baseline eosinophils and the changes in the considered parameters.

It was repeatedly hypothesized that the count of peripheral eosinophils could be a predictive biomarker for the response to IL-5 antagonism approach.⁴ In this case, we evaluated the observations made in real-life patients. The basic hypothesis was that, according to literature, as higher were peripheral eosinophils as higher would be the response that can be measured (eg, ACT, OCS intake, number of exacerbations, FEV1). The results of a sub-study⁴ stratified the response on exacerbations rate related to baseline eosinophils, dividing subjects into three groups (2, 3, >4 exacerbations) and showed a significant positive correlation. We performed the same analysis taking into account multiple parameters and using, instead of a group categorization, the continuous linear trend. In the present study, we could detect no clear significant correlation between baseline EOS count and the final outcomes after 1 year of treatment. The major weakness of the study may be the possible confounding action of the OCS treatment used by many patients, as often required in severe asthma. Nonetheless, since we have available several biological agents, the possibility to identify a predictive marker of response for the correct and appropriate prescription would be optimal, and real-life data could be useful for this aim, but currently we have only few observations.^{7,8} Different biomarkers have been proposed (including eosinophils, FeNO, periostin, and total IgE), but so far none of them proved to be applicable. Since eosinophils are considered a good candidate, we attempted to detect associations between the clinical, demographic, and functional data in order to assess whether EOS count could be related to the magnitude of the response (clinical/functional). It is true that this biological treatment could reduce the intake of OCS, the number of exacerbations,⁹ and, therefore, the overall cost of severe asthma; nonetheless, a predictive value of baseline EOS could not be detected in this setting.

Thus, we have to acknowledge that we are still unable to strictly identify a predictive biomarker, that can be easily managed and used in the real-life setting. What do we know for certain is that IL-5 antagonism (MEP in this case) is effective, but that EOS count at baseline, according to the results from our analysis, does not seem to represent a sufficient parameter to predict a more or less satisfactory outcome.

CONFLICT OF INTEREST

None to declare for all authors.

Diego Bagnasco¹
Alessandro Massolo²
Marco Bonavia³
Luisa Brussino⁴
Caterina Bucca⁵
Marco Caminati⁶ 
Giorgio Walter Canonica^{1,7}
Cristiano Caruso⁸

Maria D'Amato⁹
Laura De Ferrari¹
Giuseppe Guida¹⁰
Enrico Heffler⁷
Carlo Lombardi¹¹
Francesco Menzella¹²
Manlio Milanese¹³
Giovanni Paoletti⁷
Anna Maria Riccio¹ 
Giovanni Rolla⁴
Gianenrico Senna⁶
Elisa Testino¹
Giovanni Passalacqua¹ 

¹Allergy and Respiratory Diseases, IRCCS Policlinico San Martino, University of Genoa, Genoa, Italy

²Ethology Unit, Department of Biology, University of Pisa, Pisa, Italy

³Pneumology Unit, Ospedale La Colletta, Arenzano, Italy

⁴Allergy and Immunology, AO Mauriziano Hospital, University of Turin, Turin, Italy

⁵Azienda Ospedale-Università Città della Salute e della Scienza, S.C. Pneumologia, University of Turin, Turin, Italy

⁶Asthma Center and Allergy Unit, Verona University and General Hospital, University of Verona, Verona, Italy

⁷Personalized Medicine Clinic Asthma and Allergy, Humanitas Clinical and Research Center, Department of Biomedical Sciences, Humanitas University, Milan, Italy

⁸Allergy Unit, Fondazione Policlinico A. Gemelli, IRCCS, Rome, Italy

⁹Department of Pneumology, Monaldi Hospital, Federico II University, Naples, Italy

¹⁰Department of Medical Sciences, University of Turin, Turin, Italy

¹¹Departmental Unit of Allergology & Respiratory Diseases, Fondazione Poliambulanza, Brescia, Italy

¹²Pneumology Unit, Arcispedale Santa Maria Nuova, Azienda Ospedaliera di Reggio Emilia, Italy

¹³Division of Pneumology, S. Corona Hospital, Pietra Ligure, Italy

Correspondence

Giovanni Passalacqua, Allergy & Respiratory Diseases, IRCCS Policlinico S. Martino, University of Genoa, Genoa, Italy
Email: passalacqua@unige.it

ORCID

Marco Caminati  <https://orcid.org/0000-0001-7383-1487>
Anna Maria Riccio  <https://orcid.org/0000-0001-8392-533X>
Giovanni Passalacqua  <https://orcid.org/0000-0002-5139-3604>

REFERENCES

- Patel SS, Casale TB, Cardet JC. Biological therapies for eosinophilic asthma. *Expert Opin Biol Ther*. 2018;18:747-754.

2. Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med*. 2014;371:1198-1207.
3. Chupp GL, Bradford ES, Albers FC, et al. Efficacy of mepolizumab add-on therapy on health-related quality of life and markers of asthma control in severe eosinophilic asthma (MUSCA): a randomised, double-blind, placebo-controlled, parallel-group, multicentre, phase 3b trial. *Lancet Respir Med*. 2017;5(5):390-400.
4. 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;4:549-556.
5. Bagnasco D, Milanese M, Rolla G, et al. The North-Western Italian experience with anti IL-5 therapy and comparison with regulatory trials. *World Allergy Organ J*. 2018;11(1):34.
6. Brussino L, Solidoro P, Rolla G. Is it severe asthma or asthma with severe comorbidities? *J Asthma Allergy*. 2017;10:303-305.
7. Bagnasco D, Caminati M, Menzella F, et al. One year of mepolizumab. Efficacy and safety in real-life in Italy. *Pulm Pharmacol Ther*. 2019;29(58):101836.
8. Bagnasco D, Menzella F, Caminati M, et al. Efficacy of mepolizumab in patients with previous omalizumab treatment failure: Real-life observation. *Allergy*. 2019; <https://doi.org/10.1111/all.13937>
9. Brussino L, Heffler E, Bucca C, Nicola S, Rolla G. Eosinophils target therapy for severe asthma: critical points. *Biomed Res Int*. 2018;25(2018):7582057.

SUPPORTING INFORMATION

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

1 **Supplementary TABLE 1.** Summary of the linear correlation results between the baseline
2 eosinophils concentration and the changes in the outcome considered.
3

	FEV1 (N=138)	FeNO (N=79)	ACT score (N=135)	OCS dose (N=110)	Exacerbations (N=130)
Pearson correlation	0.052	-0.093	0.091	-0.030	-0.026
Significance (2-tailed)	0.547	0.416	0.296	0.753	0.769
Sum of squares and cross-products	194180.397	-160256.579	289478.813	-70866.039	-54366.313
Spearman correlation coefficient	0.081	-0.148	-0.081	-0.058	-0.008
Significance (2 tailed)	0.347	0.192	0.348	0.548	0.926

4
5
6