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

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ABSTRACT

Introduction. Mepolizumab (MEP) was commercialized in Italy since at least 2 years. Its efficacy was well confirmed in the real-life setting, but a predictive biomarker for efficacy still lacks. It was hypothesized that the magnitude of the response could be positively related to the baseline eosinophil count. Thus, we tested this hypothesis on our available population.

Methods. The analysis was performed on 138 patients, with severe asthma and who had received MEP for at least 1 year. The baseline eosinophil count was correlated to the change (12 months vs baseline) in several parameters: oral corticosteroids intake, FEV_1, asthma control test score, number of exacerbations, fractional exhaled nitric oxide .

Results. We analyzed 138 patients (78 females, 60 males, mean age 58±10 years). By using the Spearman and Pearson tests, no significant correlation could be found between the baseline eosinophil count and the percentage change in the considered parameters (r between -0.091 and 0.093; p= NS).

Conclusion. There is no detectable correlation between the baseline eosinophil count and the magnitude of the effects of MEP, although the efficacy remains confirmed. Under the present conditions, the eosinophil count alone seems not to be a reliable predictive marker of the efficacy of MEP.

Key Words: severe asthma; real life; mepolizumab; eosinophil count; outcome; correlation

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Authorship. All the listed Authors contributed to the clinical work, data collection and analysis. DB and GP designed the study. AM was the biostatistician. All Authors contributed to drafting the manuscript, read the final version and approved its submission.

ABBREVIATIONS:

ACT: Asthma control test

EOS: eosinophils

FEV1: forced expiratory volume in 1 second

FeNO: fractional exhaled nitric oxide

MAbs: monoclonal antibodies

OCS: Oral corticosteroids

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, non-eosinophilic 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, 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/μL) was suggested as a reliable predictive biomarker. The hypothesis that as high is the pre-treatment 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, to assess if 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 randomisation method (permutation or exact test; P_{exact}) or by a Monte Carlo ($P_{Monte\ Carlo}$).The type I error (α) has been set at the 0.01 level. The means were reported with their standard error (±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 non-parametric Correlation Coefficient when appropriate . All statistical analyses were performed using IBM® SPSS 25 (Chicago, IL, US).

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 ($p > 0.05$) were detected between the EOS count at baseline and the change at 12 months versus baseline of the examined parameters (**Supplementary TABLE 1**). Even when log-transformed to achieve normality, no correlation analysis could detect any significant linear association.

The raw data are depicted in **Figure 1 (A-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 bio-marker 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 (e.g. ACT, OCS intake, number of exacerbations, FEV1). The results of a sub-study [4] 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 one 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 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, 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 if 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.

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Legend to Figure 1. The figure shows patient by patient the % change of parameters versus 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 months

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

Characteristics	Baseline	12 months	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)** **Median (SEM)**	 822 (150-3060) 800 (41.78)	 117 (0-600) 100 (8.19)	 -705 (86%)	 <.0001
Exacerbations/12m **Mean (SD)** **Median (IQ RANGE)**	 3.8 (2.5) 3.0 (2.8)	 0.7 (1.1) 0 (1.0)	 -3.1 (81%)	 <.0001
OCS mg/day, mean (SD)	10.1 (9.4)	2.0 (4.2)	-8.1 (80%)	<.0001
FeNO ppb **Mean (SD)** **Median (SEM)**	 75 (53) 64 (5.7)	 42 (37) 30 (4.1)	 -33 (44%)	 < .0001
FEV1 (L±SD) **FEV1 (%)**	2.03 (0.78) 69	2.36 (0.88) 75	0.34 (17%) 5.6 (8%)	.001 < .0001
FVC (L±SD)	3.16 (1.05)	3.32 (1.04)	0.16 (5%)	n.s.

FVC (%)	88	92	4.4 (5%)	.021
FEV1/FVC (±SD)	0.65 (0.11)	0.69 (0.11)	0.04 (6%)	.002
ACT (±SD)	17 (5)	22 (3)	5 (34%)	<.0001

Figure 1 A

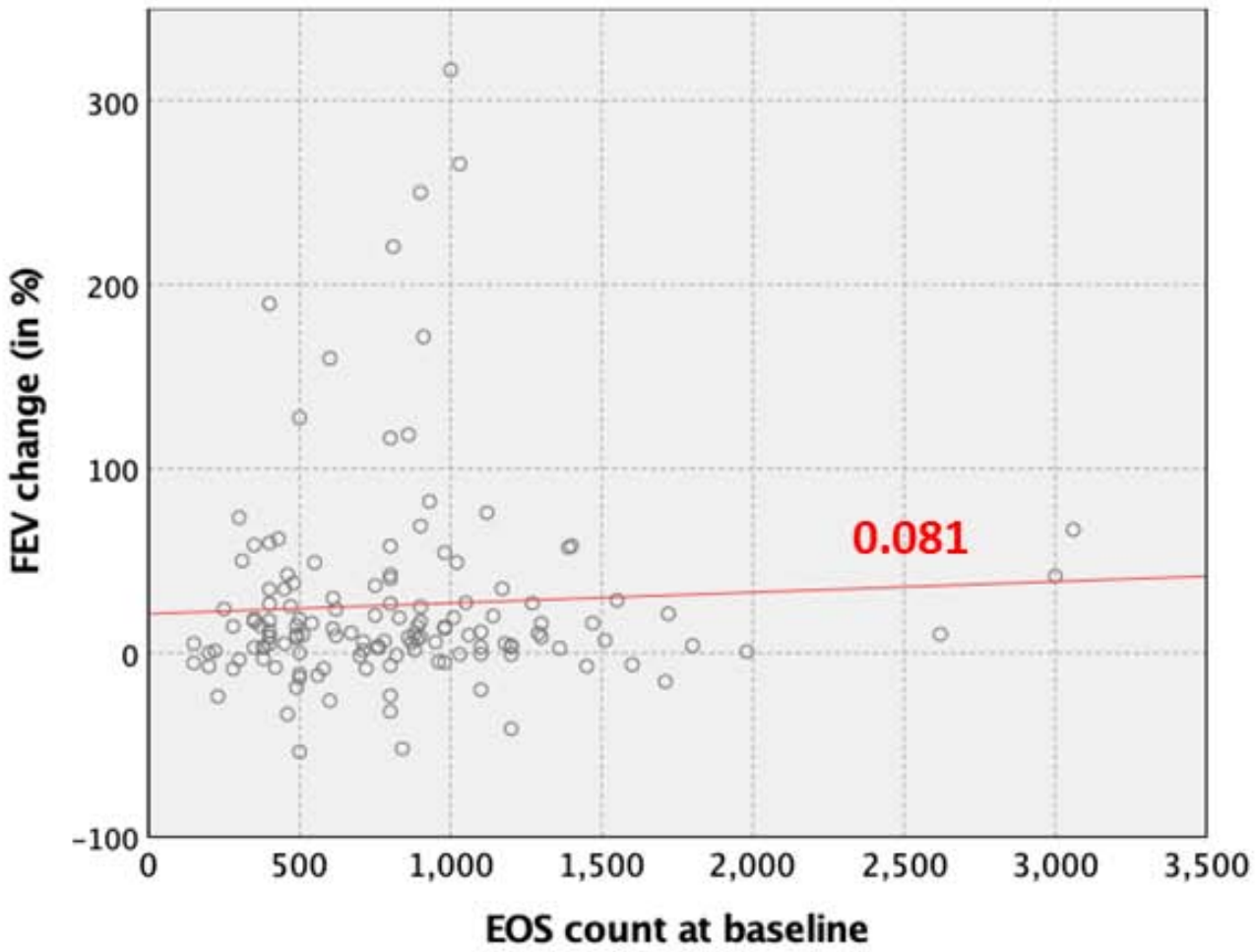

FEV change (in %)
300
200
100
0
-100
0
500
1,000
1,500
2,000
2,500
3,000
3,500
EOS count at baseline
0.081

Figure 1 B

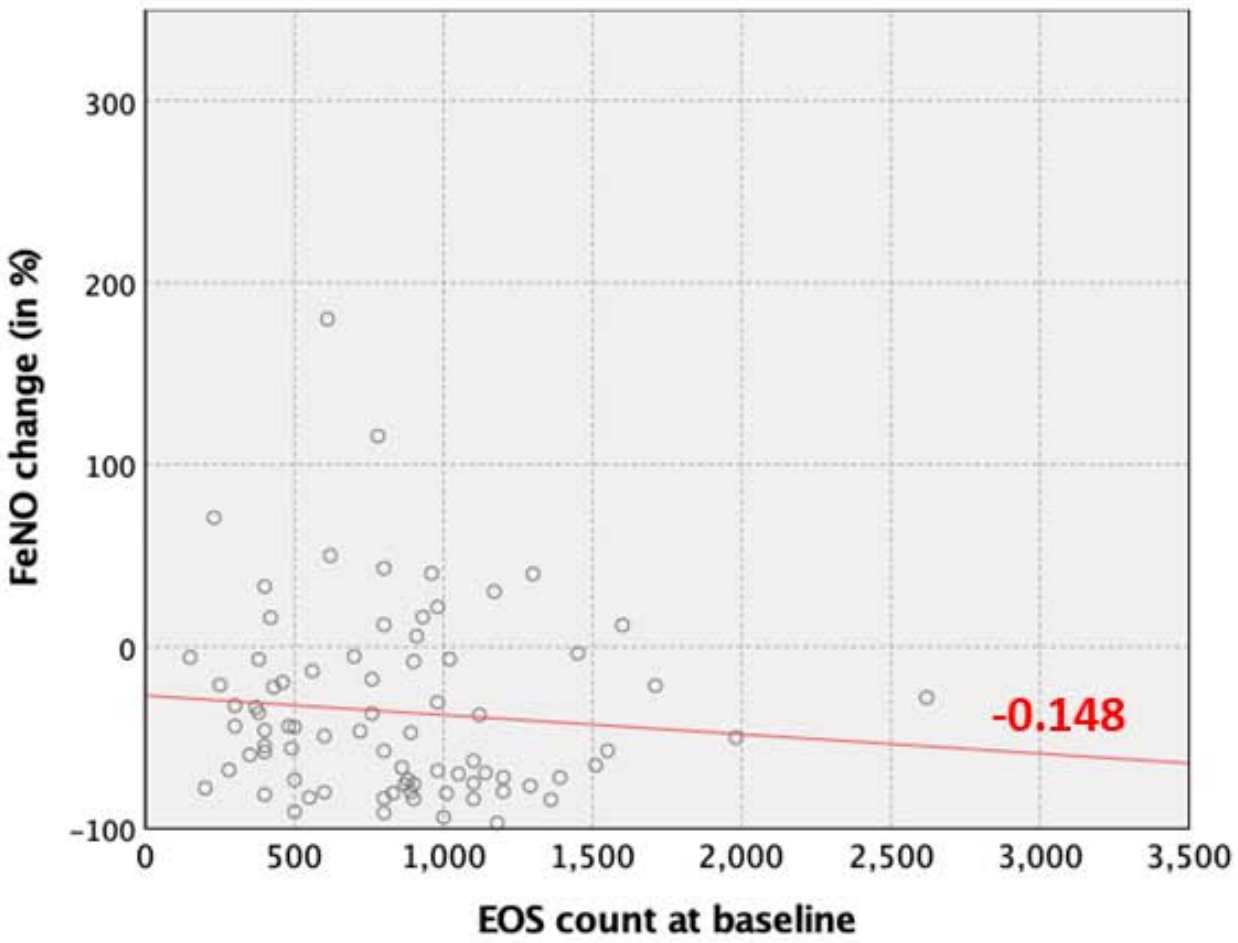

FeNO change (in %)
300
200
100
0
-100
0
500
1,000
1,500
2,000
2,500
3,000
3,500
EOS count at baseline
-0.148

Figure 1 C

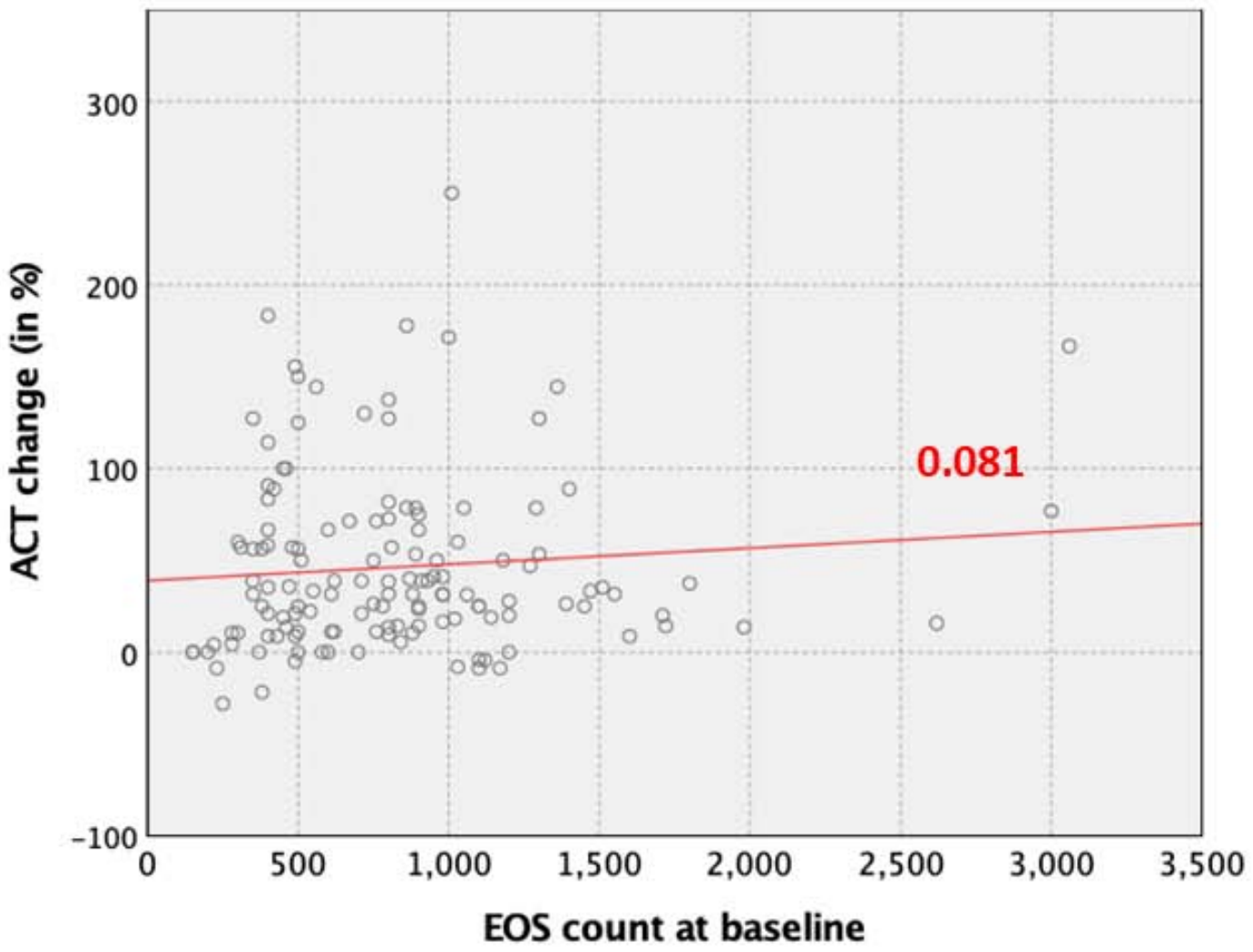

ACT change (in %)
300
200
100
0
-100
0
500
1,000
1,500
2,000
2,500
3,000
3,500
EOS count at baseline
0.081

Figure 1 D

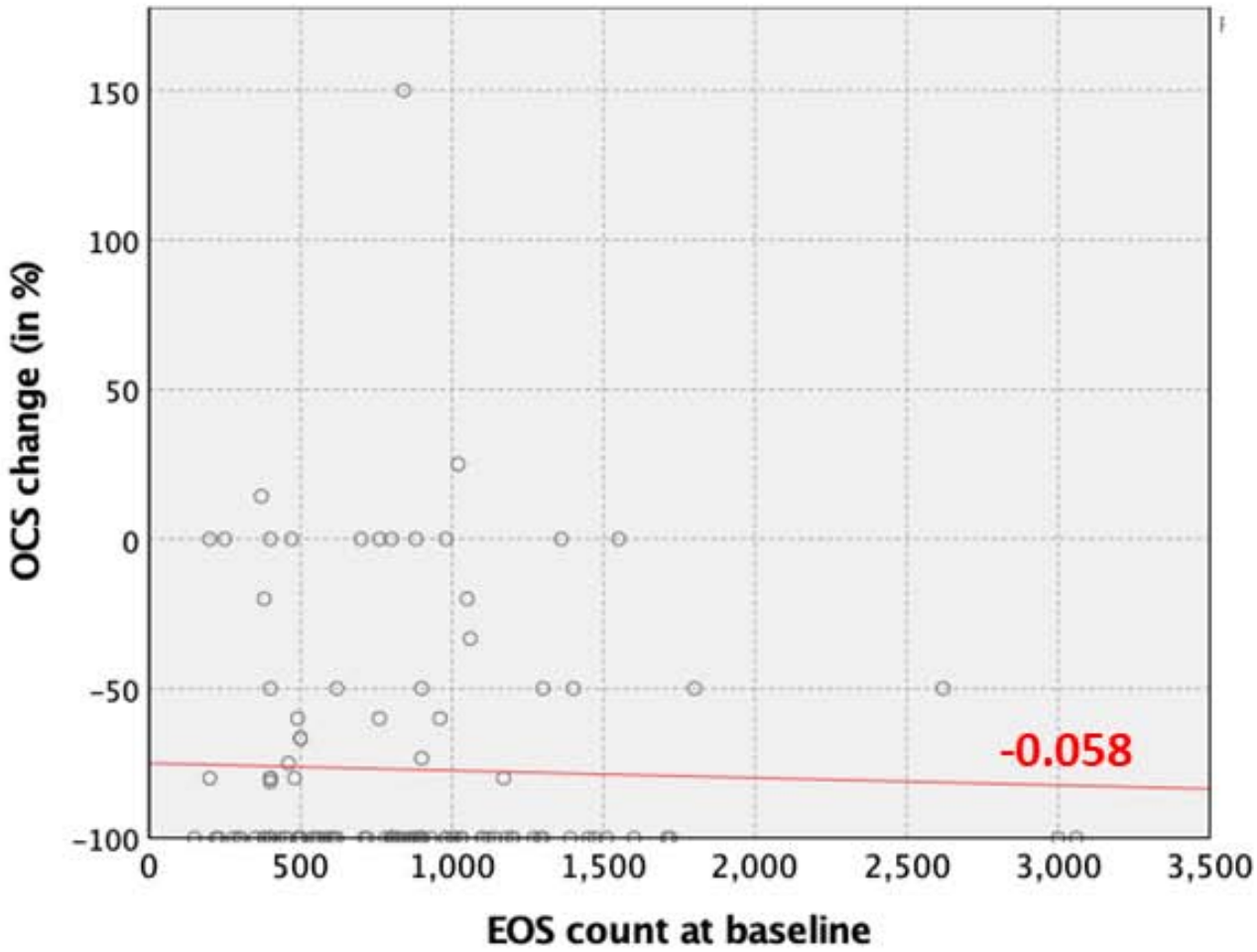
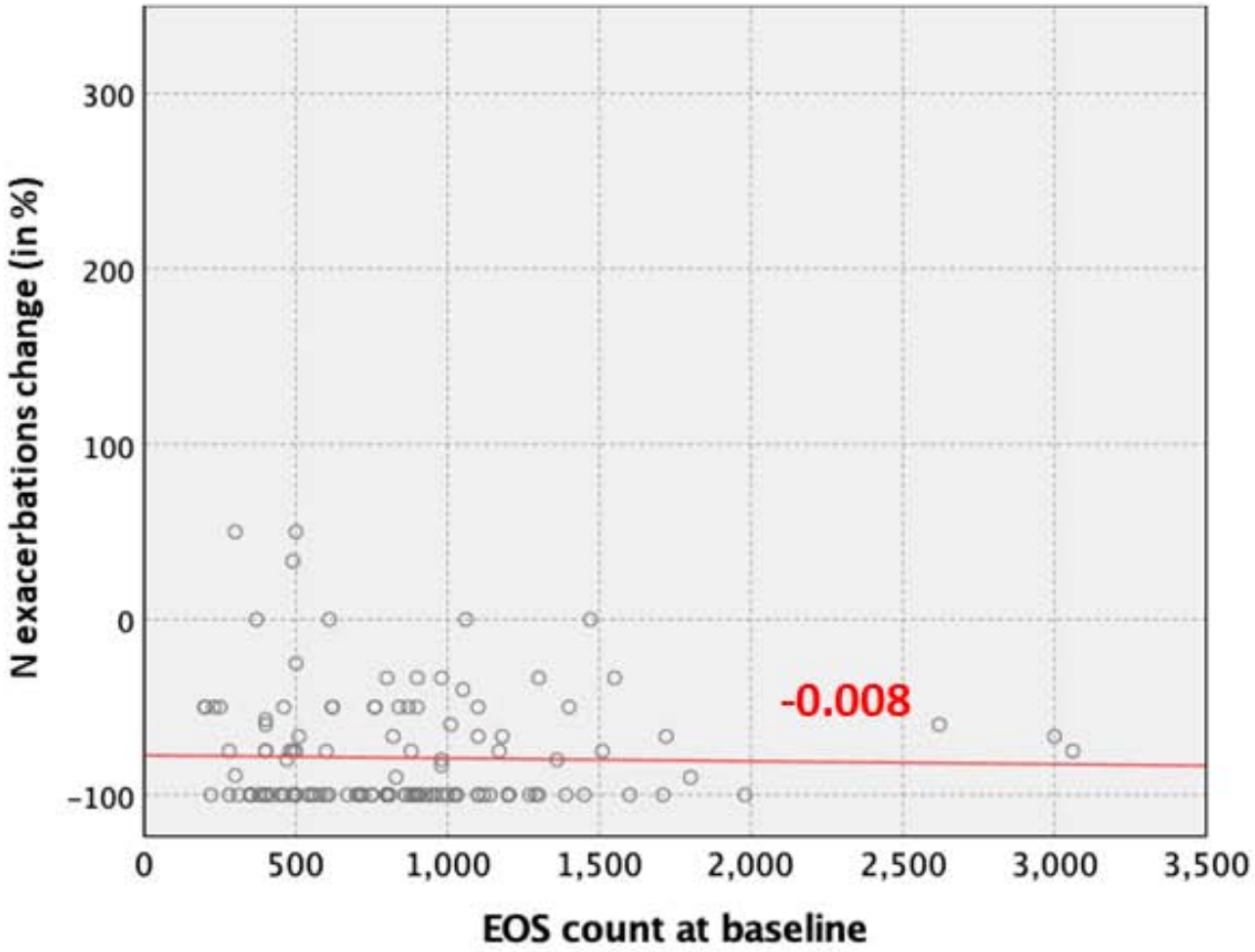

OCS change (in %)
150
100
50
0
-50
-100
0
500
1,000
1,500
2,000
2,500
3,000
3,500
EOS count at baseline
-0.058

Figure 1 E


N exacerbations change (in %)
300
200
100
0
-100
-0.008
0
500
1,000
1,500
2,000
2,500
3,000
3,500
EOS count at baseline