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Serum and urine eosinophil-derived neurotoxin (EDN) levels predict biologic response in severe asthma

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ABSTRACT

Background: Eosinophils are crucial in allergic inflammation, and their correlation with asthma severity has made them a focal point in predicting treatment outcomes. Blood eosinophil count is a commonly utilized marker. However, its limitations have prompted alternative biomarker exploration, such as eosinophil-derived neurotoxin (EDN).

Objective: This research was conducted over 24 weeks on 56 patients with severe asthma treated with mepolizumab, reslizumab, and dupilumab. We aimed to evaluate the clinical significance of blood eosinophil count and their potential, including those of blood EDN levels and urine EDN values as biomarkers for predicting treatment response.

Methods: The analysis encompassed examining correlations between biomarkers and clinical features, including exacerbation rates and lung function, through ELISA assays and subsequent statistical analyses. The study protocol is registered at ClinicalTrials.gov (NCT05164939).

Results: The findings underscore strong correlations between serum EDN levels, blood eosinophil counts, and treatment responses, with EDN demonstrating comparable predictive capabilities to blood eosinophil counts to determine treatment responses. Different biologics exhibited varying efficacy regarding baseline eosinophil counts and EDN levels.

Conclusions: Blood eosinophil counts and EDN levels show potential as predictive markers for treatment responses in patients with severe asthma undergoing biologic therapies. However, further comprehensive studies are warranted to enhance the reliability and applicability of EDN as an effective asthma treatment biomarker.

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Keywords: Eosinophils, Allergic inflammation, Asthma, Eosinophil-derived neurotoxin, Blood eosinophil counts, Biologic therapies, Exacerbation rates, Lung function, Biomarker

INTRODUCTION

Eosinophils are crucial in allergic inflammation, influenced by cytokines in type 2 (T2) inflammation. These cytokines regulate various aspects of eosinophil behavior, including their proliferation, blood migration, interaction with endothelial cells, and tissue migration.[1–5] Consequently, the eosinophil count serves as a valuable marker for T2 inflammation, particularly associated with asthma, owing to its influence on symptoms and their severity.[6–9] Blood eosinophil counts typically rise with asthma severity.[10–12] Recently, a growing interest in biologic agents targeting T2 inflammation has been observed in clinical research and practice. These treatment outcomes are closely linked to eosinophil counts.[13–19]

However, employing the absolute eosinophil count (AEC) as a biomarker has certain limitations. AEC is subject to diurnal variations and offers limited insight into eosinophil activation. Moreover, its applicability extends beyond asthma to other eosinophilic diseases, posing challenges to its effectiveness as a practical biomarker in a real-world scenario.[20–22] Studies suggest eosinophil granule proteins, such as eosinophil cationic protein and eosinophil-derived neurotoxin (EDN), may demonstrate a stronger association with eosinophil airway inflammation. When eosinophils are activated, triggered by cytokines and other pro-inflammatory mediators, they degranulate and release cationic proteins that can attract other immune cells, such as mast cells.[23–25] EDN is one of the cationic proteins which known to be involved in lung epithelial damage, mucus hypersecretion, airway remodeling, and inflammation.[26] Previous studies suggested that measurement of EDN might be a more sensitive way of assessing activated eosinophils that tend to affect target organs and induce pathological changes.[27–30] This could potentially exert a greater influence on bronchoconstriction and airway hyperresponsiveness than blood eosinophil count.[22,31,32] EDN also could be useful not only as a predictor of asthma severity and control status, but also as a tool for monitoring treatment response to biological agents.[23,33] Particularly noteworthy is the stability of EDN, which remains stable without diurnal fluctuations, marking it a potential candidate as a biomarker for eosinophil count and activation status.[20,34]

While peripheral blood AEC has been explored for assessing asthma activity and predicting treatment responses to biologic agents, research on whether AEC or EDN values can reliably predict treatment responses in asthma remains limited.

Therefore, this study aimed to explore the following: 1) The clinical relevance of blood eosinophil count in predicting treatment responses, 2) potential of blood eosinophil count, blood EDN levels, and urine EDN values (collected at baseline, 1 month, and 6 months of follow-up) as biomarkers for predicting treatment responses in patients administered biologic agents: mepolizumab, reslizumab, and dupilumab, and 3) determine whether any of these markers, individually or in combination, can effectively serve as predictive biomarkers for treatment response.

The findings of this study could contribute to understanding the role of eosinophil-related biomarkers in predicting treatment outcomes for patients with asthma undergoing biologic therapy. The findings may underscore the clinical significance and potential of these biomarkers as more reliable and specific indicators of eosinophil activity than absolute eosinophil count.

METHODS

Participants

Overall, 56 patients diagnosed with severe asthma were included in this analysis. They were administered 3 different biologics—mepolizumab, reslizumab, and dupilumab—across various tertiary hospitals in Korea from April 2020 to May 2021.

The patients were monitored for 24 weeks to assess their response to treatment. Demographic data of the patients were extracted from electronic patient records. The study protocol is registered at ClinicalTrials.gov (NCT05164939).

Measurement of serum and urine EDN levels

Serum EDN levels were assessed using induced sputum samples collected through inhalation of sterile nebulized saline solution, followed by coughing and expectoration of airway secretions. Serum and urine EDN levels were quantified using a K EDN enzyme-linked immunosorbent assay (ELISA) kit obtained from SKIMS-BIO Co., Seoul, Korea, according to the manufacturer's instructions. This ELISA utilized in this study enables the detection of human EDN within a detection range of 6.0–400 ng/mL.[35] Urine EDN levels were determined by analyzing spot urine samples and adjusting for the serum creatinine level.

Definition of responders

After 24 weeks, treatment response was evaluated based on criteria, including the maintenance oral corticosteroid dose and annualized exacerbation rate. Responders were categorized into 2 groups: responders and super responders. However, patients who did not fall into these categories were classified as nonresponders. Table 4 provides details of these classifications. First, following 48 weeks of treatment, patients were classified as responders or nonresponders based on specific criteria. The response was defined as a $\geq$50% reduction in the annualized exacerbation rate or, for patients requiring mOCS therapy, a reduction of $\geq$50% in the daily mOCS dose. Patients requiring a mOCS dose of $\leq$5 mg owing to adrenal insufficiency were categorized as not requiring mOCS therapy for asthma.[36] Additionally, a subgroup analysis was conducted on super responders, consisting of responders who were completely free from exacerbation and no longer required mOCS therapy for asthma.[36,37] Finally, the following criteria were included in our analyses to identify patients demonstrating a good response to each biologic agent: $\geq$50% reduction in the annualized exacerbation rate; a pre-bronchodilator (BD) FEV1 increase of $\geq$100 mL; and an improvement of $\geq$3 points in the ACT score.[38]

Serum EDN, adjusted urine EDN, and blood eosinophil counts as parameters

Various parameters using baseline and 1-month measurement of serum EDN, adjusted urine EDN, and blood eosinophil counts were defined to predict treatment response at 6 months (Supplemental Table 1). This included baseline levels, 1-month levels, and alterations in these levels.

Statistical analyses

Continuous variables were expressed as mean $\pm$ standard deviation (SD), while categorical variables were presented as frequency and percentage (%). The Kruskal–Wallis test was employed to assess non-normally distributed variables. The correlations between Serum and Urine EDN levels and clinical features of asthma at baseline were evaluated using the Wilcoxon rank-sum test and Spearman correlation coefficient. Log transformations were employed to standardize skewed main exposures for comparison. Treatment responses were defined by alterations in exacerbation numbers, daily average maintenance OCS dose, ACT scores, and pre-bronchodilator FEV1 between baseline and 6 months. T-tests were used to compare the main exposure values across different treatment response groups. Multivariate logistic regression models were utilized to calculate the sensitivity, specificity and area under the curve (AUC) of receiver operating characteristic (ROC) curve for main exposures and identify the most effective predictor for treatment outcome. Statistical analyses were conducted using SAS (SAS Institute v.9.4, Cary, NC). A p-value $\leq$0.05 was considered statistically significant.

RESULTS

Baseline characteristics and serum/urine EDN

Of the total 56 patients with severe asthma, 29 (51.8%) were male, with an average age of 53.48 $\pm$ 10.83. The average duration of asthma at enrollment was 11.68 $\pm$ 8.41 years. Additionally, 35 patients (62.5%) had experienced acute exacerbations in the previous 12 months, with an average number of 4.41 $\pm$ 6.36 acute exacerbations. Nineteen (33.9%) patients used maintenance OCS. Furthermore, 42 (75%) and 30 (53.6%)

patients exhibited allergic rhinitis and chronic rhinosinusitis, respectively. Table 1 presents other baseline characteristics. The frequencies of patients treated with biologic agents were 13 (23.2%), 23 (41.1%), and 20 (35.7%) for Mepolizumab, Reslizumab, and Dupilumab, respectively.

When looking at the blood eosinophil count, serum EDN level, and urine EDN level of each biologic agent at baseline, the first month, and the sixth month of treatment, the blood eosinophil count, serum, and urine EDN levels for mepolizumab and reslizumab all tended to decrease as treatment progressed. However, in the case of dupilumab, blood eosinophil count and serum EDN level tended to increase and decrease after treatment. Urine EDN level did not decrease but continued to increase (Fig. 2, Supplemental Table 2).

Association between baseline serum EDN, urine EDN, and asthma clinical features

We assessed the association between baseline serum EDN, urine EDN, and asthma clinical features (Table 2). A notable finding revealed significantly lower urine EDN levels in patients >60 years old than in their younger counterparts (60.44 ± 46.33 vs. 179.37 ± 392, P = 0.008). However, serum EDN levels did not show significant variance. Patients with a body mass index (BMI) < 25 exhibited markedly higher serum and urine EDN levels than those with a BMI ≥25 (serum EDN: 166.86 ± 84.45 vs. 101.73 ± 72.11, P = 0.002; urine EDN: 212.01 ± 460.81 vs. 81.81 ± 81.58, P = 0.011). Additionally, patients diagnosed with allergic rhinitis demonstrated significantly elevated levels of serum and urine EDN than those without this comorbidity (serum EDN: 145.31 ± 77.56 vs. 115.18 ± 104.26, P = 0.042; urine EDN: 184.84 ± 400.68 vs. 61.02 ± 40.73, P = 0.01).

Correlation between baseline serum EDN, urine EDN, blood eosinophil count, and asthma clinical features

We assessed the correlation between baseline serum EDN, urine EDN, and asthma clinical features (Table 3). Serum and urine EDN demonstrated a negative correlation with BMI (−0.472, P=<0.0001 and −0.353, P = 0.008). Additionally, urine EDN was negatively correlated with baseline ACT scores (−0.304, P = 0.024). Both serum and urine EDN levels positively correlated with blood eosinophil count (/μL) and sputum eosinophil (%). A moderate correlation was observed between blood eosinophil count (/μL) and serum EDN (0.729, P=<0.0001). Conversely, a weaker correlation was found between blood eosinophil count (/μL) and urine.

EDN (0.453, P = 0.001) (Fig. 1). Moreover, a weak correlation was observed between sputum eosinophil (%) and serum and urine EDN (Serum EDN: 0.321, P = 0.043; Urine EDN: 0.392, P = 0.012). Nonetheless, no discernible correlation was observed between serum EDN, urine EDN, fractional exhaled nitric oxide (FeNO) (ppb), and total IgE (kU/L).

Prediction of treatment response using serum and urine EDN

We focused on evaluating the potential of serum EDN as a predictor for treatment response, assessed through logistic multivariate regression and the ROC curve. Additionally, we analyzed the AUC of various parameters derived from serum and urine EDN, alongside blood eosinophil count, and compared them against the AUC of blood eosinophil counts, as displayed in Supplemental Table 1.

We explored the potential of serum EDN in predicting treatment response, comparing the AUC of new parameters with that of baseline blood eosinophil count (Table 5). For the entire patient cohort, parameters such as serum EDN/blood eosinophil at 1 month and baseline FeNO exhibited higher AUCs than AUC of baseline blood eosinophil counts for predicting responders (0.875 and 0.872 vs. 0.848). The decreased ratio of serum EDN from baseline to 1 month exhibited a higher AUC than AUC of baseline blood eosinophil counts for predicting super responders and pre-bronchodilator FEV1 responders at 6 months (0.805 vs. 0.780, 0.804 vs. 0.752). Furthermore, the alteration in serum EDN over blood eosinophil count from baseline to 1 month showed a slightly higher AUC than that of baseline blood eosinophil count for predicting ACT scores and pre-bronchodilator FEV1 responders (0.867 vs. 0.841, 0.785 vs. 0.752).

	Total (N = 56)
Age	53.48 ± 10.83
Symptom onset (n = 53)	40.83 ± 12.21
Diagnosis with asthma (n = 53)	41.58 ± 12.20
Duration of asthma (years) (n = 53)	11.68 ± 8.41
Male	29 (51.8)
BMI (kg/m^2)	25.04 ± 3.51
Ever experienced AE (prev 12 mo)	35 (62.5)
Number of AE (prev 12 mo)	4.41 ± 6.36
OCS maintenance	19 (33.9)
Smoking history	
never-smoker	25 (44.6)
ex-smoker (<10 years)	10 (17.9)
ex-smoker (≥10 years)	21 (37.5)
History of asthma exacerbation during the last 1 year	
Unscheduled visit to an outpatient clinic	20 (35.7)
Unscheduled visit to an emergency department	11 (19.6)
Hospitalization due to asthma exacerbation	10 (17.9)
ICU admission due to asthma exacerbation	0 (0)
Disease	
Allergic rhinitis	42 (75)
Chronic rhinosinusitis	30 (53.6)
Atopy	27 (48.2)
Blood tests	
WBC (*10^3/μL)	8.26 ± 2.06
Eosinophils (cells/uL)	601.60 ± 503.66
Eosinophils (%)	7.58 ± 5.67
Neutrophil (%)	57.53 ± 11.27
Total IgE (kU/L) (n = 48)	586.48 ± 1315.95
FeNO (ppb) (n = 53)	70.85 ± 54.77
Induced sputum	
Neutrophil (%) (n = 39)	54 ± 37.72
Eosinophil (%) (n = 40)	25.68 ± 34.21
Lung functions	
pre-BD FEV1 (%) (n = 55)	59.22 ± 17.68
pre-BD FEV1/FVC ratio (n = 55)	0.64 ± 0.15
post-BD FEV1 (%) (n = 51)	63.94 ± 17.83
post-BD FEV1/FVC ratio (n = 51)	0.65 ± 0.14
ACT scores (n = 55)	16.02 ± 5.49

Table 1. Patient baseline characteristics

In patients who were administered mepolizumab and reslizumab, the change in serum EDN over blood eosinophil count from baseline to 1 month exhibited higher AUCs for all treatment responders than those of baseline blood eosinophil count. Most parameters showed higher AUCs than that of baseline blood eosinophil count when predicting responders and pre-bronchodilator

		n (%)	Serum EDN	p-value	Urine EDN	p-value
Age (y)	≥60	12 (21.4)	153.33 ± 84.61	0.353	60.44 ± 46.33	0.008
	<60	44 (78.6)	133.54 ± 85.59		179.37 ± 392	
Sex	Female	27 (48.2)	160.89 ± 89.76	0.051	220.31 ± 493.38	0.057
	Male	29 (51.8)	116.26 ± 75.63		92.04 ± 85.71	
BMI	≥25	25 (44.6)	101.73 ± 72.11	0.002	81.81 ± 81.58	0.011
	<25	31 (55.4)	166.86 ± 84.45		212.01 ± 460.81	
Smoking status	Never smoker	25 (44.6)	164 ± 90.19	0.054[a]	232.55 ± 511.37	0.078[a]
	Ex-smoker (<10 years)	10 (17.9)	141.01 ± 79.94		122.37 ± 124.71	
	Ex-smoker (≥10 years)	21 (37.5)	105.03 ± 72.44		75.24 ± 52.05	
Age of symptom onset	≥40	28 (50)	129.08 ± 83.45	0.486	173.83 ± 484.27	0.427
	<40	28 (50)	146.49 ± 87.18		133.94 ± 123.28	
OCS maintenance (%)	Yes	19 (33.9)	110.46 ± 80.29	0.055	97.55 ± 63.93	0.986
	No	37 (66.1)	151.81 ± 84.98		182.81 ± 428.17	
Atopy (%)	Yes	27 (48.2)	127.83 ± 69.48	0.731	129.05 ± 124.01	0.646
	No	29 (51.8)	147.04 ± 97.61		177 ± 475.57	
Allergic rhinitis	Yes	42 (75)	145.31 ± 77.56	0.042	184.84 ± 400.68	0.01
	No	14 (25)	115.18 ± 104.26		61.02 ± 40.73	
Chronic rhinosinusitis	Yes	30 (53.6)	132.61 ± 74.62	0.902	118.80 ± 107.66	0.761
	No	26 (46.4)	143.75 ± 96.80		194.37 ± 503.94	
Nasal polyp	Yes	12 (21.4)	132.12 ± 79.59	0.913	155.35 ± 145.55	0.323
	No	44 (78.6)	139.32 ± 87.24		153.49 ± 389.74	
Control status of asthma	Controlled	6 (10.7)	107.98 ± 95.95	0.315[a]	81.22 ± 43.86	0.125[a]
	Partly controlled	23 (41.1)	126.64 ± 69.40		99.91 ± 113.98	
	Poorly controlled	27 (48.2)	153.89 ± 94.11		216.02 ± 490.93	

Pre-BD FEV1 pred (%)	≥60	29 (51.8)	117.28 ± 69.51	0.079	96.04 ± 79.30	0.431
	<60	27 (48.2)	159.80 ± 95.46		216.02 ± 495.65	
Post-BD FEV1 pred (%)	≥60	31 (55.4)	132.10 ± 82.89	0.531	97.77 ± 76.79	0.668
	<60	25 (44.6)	144.83 ± 88.77		223.47 ± 515.15	
Post-BD FEV1/FVC ratio	≥0.7	17 (30.4)	144.98 ± 104.38	0.859	105.70 ± 94.52	0.887
	<0.7	39 (69.6)	134.64 ± 76.41		174.89 ± 415.65	

Table 2. Association between baseline EDN and asthma clinical features. [a]*p-values were calculated using the Kruskal–Wallis test. (EDN is not normal distribution)*

FEV1, with serum EDN/blood eosinophil at 1 month exhibiting the highest AUC (0.845) for responders and the change in serum EDN over blood eosinophil count from baseline to 1 month demonstrating the highest AUC (0.716).

In patients who were administered dupilumab, the change in serum EDN over blood eosinophil count from baseline to 1 month exhibited the highest AUC, except for predicting responders. For predicting responders at 6 months, the serum EDN ratio from baseline to 1 month demonstrated the highest AUC (0.954) among all parameters, including baseline FeNO.

Furthermore, we explored the potential of urine EDN in predicting treatment response, comparing the AUC of new parameters with that of baseline blood eosinophil count (Table 6). The urine EDN over blood eosinophil count at baseline displayed slightly higher AUCs than those of baseline blood eosinophil count when predicting responders, super responders, and exacerbation responders at 6 months for all patients. Additionally, when analyzing the AUCs of parameters in patients who were administered mepolizumab and reslizumab, all parameters exhibited higher AUCs than those at baseline blood eosinophil count for predicting pre-bronchodilator FEV1 response at 6 months. Similarly, in patients treated with dupilumab, all parameters displayed higher AUCs than the reference for predicting responders and super responders.

In Supplemental Tables 3 and 4, the sensitivity and specificity of blood eosinophil count and parameters consisting of serum EDN, urine EDN, and blood eosinophil count that showed AUC values comparable to blood eosinophil count in predicting various responses were also listed, although not statistically significant. For the entire patient cohort, the AUC of baseline blood eosinophil count in predicting reduction of acute exacerbations and improving pre-bronchodilator FEV1 after treatment was 0.782 and 0.752, respectively. However, sensitivity was confirmed to be 55.8% and 48.3% (specificity 92.3% and 88.5%). Change in serum EDN compared to the change in blood eosinophil at baseline and after one month of treatment had a sensitivity of 77.5% for predicting a decrease in acute exacerbations and a

	Serum EDN		Urine EDN	
	correlation coefficient	p-value	correlation coefficient	p-value
Age (y)	−0.044	0.749	−0.236	0.080
BMI	**−0.472**	<0.0001	**−0.353**	0.008
Age of symptom onset (years)	−0.103	0.462	−0.166	0.235
Asthma duration (years)	0.153	0.274	0.039	0.779
Number of exacerbations (prev. 6 months)	−0.150	0.269	0.025	0.855
ACT score at baseline	0.107	0.437	**−0.304**	0.024
Lung function tests				
Pre-BD FEV1 pred (%)	−0.169	0.218	−0.067	0.627
Pre-BD FEV1/FVC ratio	0.020	0.883	−0.027	0.843
Post-BD FEV1 pred (%)	−0.107	0.454	−0.001	0.997
Post-BD FEV1/FVC ratio	−0.106	0.380	−0.019	0.892
Laboratory tests				
Blood eosinophils (/μL)	**0.729**	<0.001	**0.453**	0.001
Sputum eosinophil (%)	**0.321**	0.043	**0.392**	0.012
FeNO (ppb)	0.097	0.488	0.234	0.092
Total IgE (kU/L)	0.038	0.797	0.064	0.668
Serum EDN (ng/mL)			**0.325**	0.015
Urine EDN (ng/mL)	**0.275**	0.040		

Table 3. Correlation between baseline EDN and asthma clinical features *Correlation coefficient with $p < 0.05$.*

sensitivity of 71.4% for predicting an improvement in pre-bronchodilator FEV1 (specificity 75.0% and 78.3%). In patients who administered mepolizumab and reslizumab, change in serum EDN compared to the change in blood eosinophil at baseline and after one month of treatment showed a higher AUC and sensitivity in predicting responder (0.774 vs. 0.821, 75.8% vs. 93.3%, specificity 99.9% vs. 99.9%). In patients who were administered dupilumab, the sensitivity of baseline blood eosinophil count in predicting responders and reduction of acute exacerbations was 46.7% and 42.9%. In predicting responder, decreased ratio of serum EDN from baseline to 1 month showed sensitivity of 80.0% and specificity of 99.9%.

Treatment response	Definition
Responder	≥50% reduction in the annualized exacerbation or ≥50% reduction in daily mOCS dose[a]
Super responders	No exacerbation and mOCSs during 24 weeks
Exacerbation	≥50% reduction in the annualized exacerbation
ACT scores	≥3 points improvement
Pre-bronchodilator FEV1	≥100 mL increase and 10%

Table 4. Definition of responder and treatment responses. *mOCS: maintenance oral corticosteroids. [a]Excluding patients who were prescribed mOCS for adrenal insufficiency, at a dosage equivalent to prednisone 5 mg*

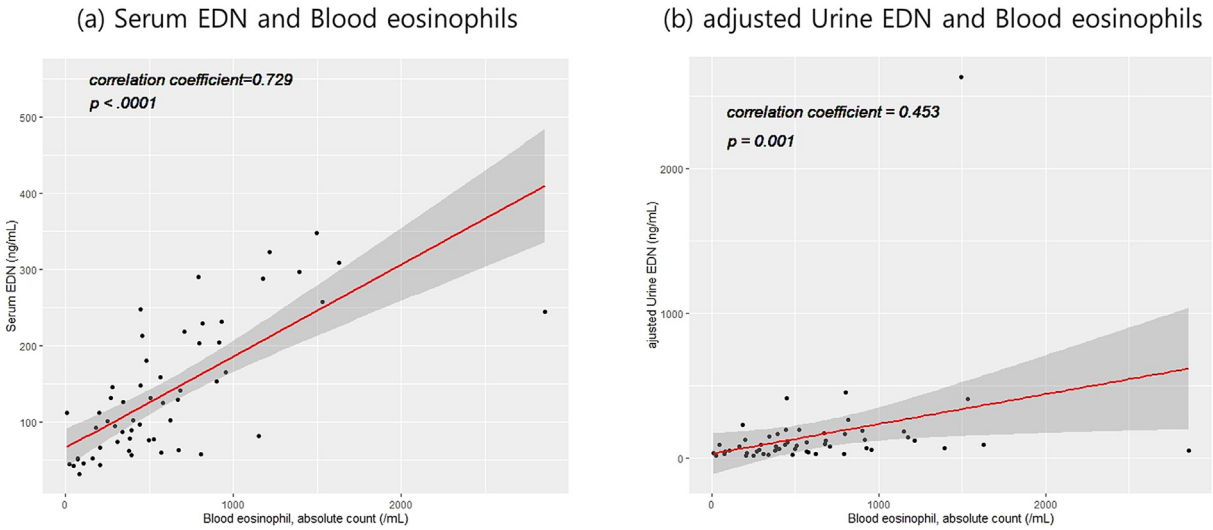


Fig. 1 Correlation between baseline serum and urine EDN and blood eosinophil count. Scatter plot with linear regression line and 95% confidence intervals of (a) baseline serum EDN and blood eosinophil count, (b) baseline urine EDN and blood eosinophil count. Spearman's correlation coefficients are displayed at the top-left of each plot.

DISCUSSION

In this study, we investigated several aspects of patients with severe asthma who were treated with biologic agents. Consequently, we confirmed the following outcomes in patients with severe asthma treated with biologic therapies (mepolizumab, reslizumab, and dupilumab): (1) We observed a robust correlation (correlation coefficient of 0.729) between serum EDN levels and blood eosinophil counts. ROC curve AUC analysis revealed comparable predictive power in predicting treatment responses when employing baseline blood eosinophil counts as the benchmark. (2) Urine EDN levels positively correlated with blood eosinophil counts, although the correlation was weak (correlation coefficient, 0.453). Moreover, ROC curve analysis notably revealed similar predictive capabilities to baseline blood eosinophil counts in determining treatment responses.

Eosinophilic inflammation is a hallmark feature of asthma and a primary target for its treatment.[39] Prior studies underscore the predictive capacity of blood eosinophil counts in determining treatment responses to various biologics. For instance, in the case of mepolizumab targeting IL-5, the baseline blood eosinophil count is associated with the clinical efficacy of mepolizumab, including reduced acute exacerbations, enhanced lung function (FEV1), and improved asthma control.[40-45] Moreover, higher baseline eosinophil counts were associated with reduced risks of acute exacerbation, regardless of whether FeNo levels were high or low FeNO. Similar research findings have been established for reslizumab, another IL-5 targeting agent.[46] Reslizumab did not show marked improvements in lung function and symptom control in patients with baseline blood eosinophil counts <400/μL.[47] Studies exploring benralizumab–an IL-5 receptor antagonist–have demonstrated heightened efficacy in reducing acute exacerbations in patients with higher baseline blood eosinophil counts.[48] These findings are consistent across several studies examining acute exacerbations and improvement in lung function.[49,50] Dupilumab, serving as an IL-4 receptor antagonist, demonstrated a reduction in acute exacerbation and FEV1 enhancement across various baseline eosinophil count levels. However, these effects were particularly pronounced in cases with elevated blood eosinophil counts and FeNO values.[19,51-53]

While the blood eosinophil count correlates with treatment effects in biologic therapy for patients with severe asthma, it does not clearly correspond to eosinophilic inflammation. Recent studies suggest that evaluating eosinophil granule proteins may surpass assessing eosinophilic airway inflammation in efficacy.[54] EDN is considered a valuable marker for evaluating eosinophilic inflammation.[33,34] EDN levels might serve as a valuable biomarker for determining the administration and effectiveness of

	Responders		Super responders		Exacerbation		ACT scores		Prebronchodilator FEV1	
	AUC	*P*	AUC	*P*	AUC	*P*	AUC	*P*	AUC	*P*
All (n = 56)										
Baseline blood eosinophil, absolute count (/mL)	0.848	ref	0.780	ref	0.782	ref	0.841	ref	0.752	ref
Baseline serum EDN (ng/mL)	**0.851**	0.90	0.773	0.70	0.743	0.24	0.778	0.07	0.724	0.16
Serum EDN decreased ratio from baseline to 1 m	**0.887**	0.25	**0.805**	0.49	0.730	0.42	0.794	0.42	**0.804**	0.37
Serum EDN/Blood eosinophil at baseline	**0.854**	0.88	0.769	0.50	0.773	0.82	**0.851**	0.79	0.726	0.25
Serum EDN/Blood eosinophil at 1 m	**0.875**	0.42	0.769	0.76	0.739	0.30	0.819	0.69	**0.771**	0.57
ΔSerum EDN/ΔBlood eosinophil at 1 m	**0.854**	0.75	0.777	0.88	0.768	0.73	**0.867**	0.69	**0.785**	0.50
Baseline fractional exhaled nitric oxide (FeNO)	**0.872**	0.53	0.758	0.28	0.746	0.46	0.774	0.32	0.719	0.20
Mepolizumab & reslizumab (n = 36)										
Baseline blood eosinophil, absolute count (/mL)	0.774	ref	0.783	ref	0.820	ref	0.843	ref	0.644	ref
Baseline serum EDN (ng/mL)	**0.821**	0.52	0.783	1.00	0.800	0.69	0.737	0.13	**0.676**	0.34
Serum EDN decreased ratio from baseline to 1 m	**0.786**	0.69	0.768	0.71	0.767	0.49	0.793	0.57	**0.680**	0.63
Serum EDN/Blood eosinophil at baseline	**0.821**	0.51	0.778	0.91	0.820	1.00	**0.874**	0.67	0.644	1.00
Serum EDN/Blood eosinophil at 1 m	**0.845**	0.48	0.763	0.70	0.773	0.69	0.742	0.32	**0.689**	0.43
ΔSerum EDN/ΔBlood eosinophil at 1 m	**0.821**	0.61	**0.823**	0.44	**0.860**	0.65	**0.960**	0.13	**0.716**	0.07
Baseline fractional exhaled nitric oxide (FeNO)	**0.786**	0.76	**0.838**	0.46	0.773	0.55	0.732	0.33	**0.671**	0.33
Dupilumab (n = 20)										
Baseline blood eosinophil, absolute count (/mL)	0.662	ref	0.727	ref	0.736	ref	0.840	ref	0.896	ref
Baseline serum EDN (ng/mL)	**0.708**	0.65	**0.779**	0.52	0.736	1.00	**0.864**	0.29	0.818	0.48
Serum EDN decreased ratio from baseline to 1 m	**0.954**	0.04	**0.779**	0.68	0.736	1.00	**0.914**	0.44	0.870	0.57
Serum EDN/Blood eosinophil at baseline	0.631	0.63	**0.740**	0.74	0.722	0.81	**0.852**	0.69	0.883	0.63
Serum EDN/Blood eosinophil at 1 m	**0.815**	0.38	**0.818**	0.42	**0.778**	0.63	**0.951**	0.26	**0.935**	0.42
ΔSerum EDN/ΔBlood eosinophil at 1 m	**0.769**	0.44	**0.805**	0.59	0.722	0.86	0.840	1.00	**0.909**	0.89
Baseline fractional exhaled nitric oxide (FeNO)	**0.739**	0.72	**0.792**	0.51	**0.792**	0.58	**1.000**	0.15	0.831	0.36

Table 5. Predicting treatment response based on serum EDN and blood eosinophils *Displayed in bold if higher than the AUC value of baseline blood eosinophil count.*

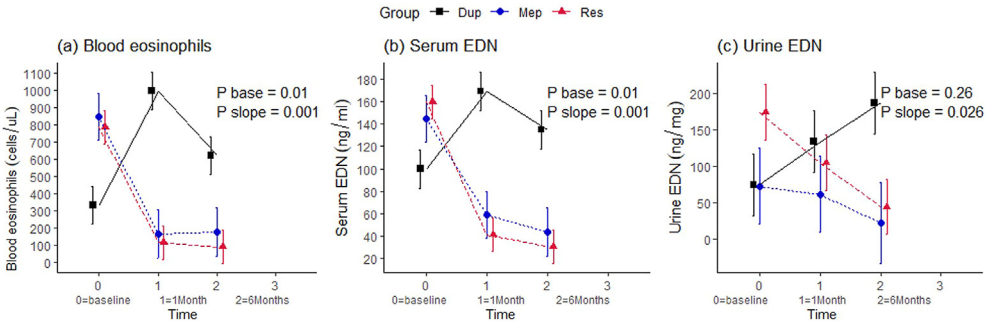


Fig. 2 Change of blood eosinophil count, serum EDN, and urine EDN according to biologics. Time series plots with error bar of (a) blood eosinophil count, (b) serum EDN, and (c) urine EDN. Dup = Dupilumab, Mep = Mepolizumab, Res = Reslizumab.

biologics treatment by assessing the extent of type 2 airway inflammation. Some studies reveal that blood eosinophil counts are a useful biomarker for mepolizumab treatment.[41,55] Another study suggests that EDN levels may be a superior biomarker for evaluating biologic efficacy, as their levels correlate better with control status than blood eosinophil count in ROC analysis.[56] In studies involving omalizumab–which does not target IL-5–a significant correlation was observed between decreased EDN serum levels and the extent of %FEV1 improvement following omalizumab therapy.[29] Elevated EDN levels have been detected in serum, urine, and other body fluids in eosinophil-related diseases.[29,30,33] Compared to blood eosinophil count, EDN exhibits stability with minimal intraday fluctuations and is relatively cost-effective than other eosinophil granule proteins.[23] Furthermore, urine EDN levels can be assessed noninvasively without collecting blood.

A correlation has been observed between EDN levels and asthma symptoms, severity, lung function, and control status.[57-60] However, the effectiveness of monitoring EDN levels for asthma treatment remains incompletely validated. Our study findings confirmed the predictive utility of baseline blood eosinophil counts via ROC curve analysis. In the mepolizumab and reslizumab groups, AUC values $>$ 0.7 were observed for treatment response, exacerbation, and ACT score parameters, indicating reductions in acute exacerbation and OCS usage. The dupilumab group also showed AUC values $>$ 0.7 for parameters other than those predicting responder, but specificity was higher than sensitivity. This low sensitivity and high specificity might be due to the tendency for blood eosinophil count, serum EDN, and urine EDN to rise at the early stage of treatment, as confirmed in this study. Particularly noteworthy was the FEV1 improvement category, where the mepolizumab and reslizumab groups demonstrated an AUC value of 0.644. Conversely, the dupilumab group exhibited a robust predictive power at 0.896. These findings closely align with those from previous studies linking baseline blood eosinophil counts to biologic clinical effectiveness.

This study has some limitations. First, the 24-week duration limits our ability to make long-term predictions for biologic treatments solely based on the data employed in this study. Second, with 56 patients–36 in the mepolizumab/reslizumab groups and 20 in the dupilumab group–the small sample size might compromise predictive accuracy compared to calculated EDN predictive values. Therefore, longer-term and larger-scale studies are imperative in future studies. Finally, EDN levels were assessed solely at baseline, 1 month, and 6 months, and treatment response data were cross-sectional, preventing confirmation of temporal changes or acute exacerbation values. Nevertheless, this study underscores the essential role of eosinophil and EDN biomarkers in predicting treatment responses.

In conclusion, eosinophil counts and EDN levels are potential predictive markers for treatment responses in patients with severe asthma undergoing biologic therapies. However, further extensive and prolonged studies are warranted to validate and strengthen the reliability of EDN as an effective asthma treatment biomarker.

	Responders		Super responders		Exacerbation		ACT scores		Prebronchodilator FEV1	
	AUC	*P*	AUC	*P*	AUC	*P*	AUC	*P*	AUC	*P*
All (n = 56)										
Baseline blood eosinophil, absolute count (/mL)	0.854	ref	0.785	ref	0.779	ref	0.840	ref	0.738	ref
Baseline adjusted urine EDN (ng/mL)	**0.860**	0.86	0.779	0.86	0.770	0.85	0.837	0.93	0.712	0.25
adjusted Urine EDN decreased ratio from baseline to 1 m	0.845	0.68	0.756	0.24	0.741	0.35	0.780	0.28	0.722	0.56
adjusted Urine EDN/Blood eosinophil at baseline	**0.881**	0.40	**0.803**	0.56	**0.803**	0.61	0.781	0.26	0.726	0.64
adjusted Urine EDN/Blood eosinophil at 1 m	**0.872**	0.55	0.774	0.68	0.743	0.32	0.778	0.28	**0.748**	0.83
adjusted Urine ΔEDN/ΔBlood eosinophil at 1 m	0.851	0.90	**0.790**	0.84	0.754	0.40	0.826	0.80	0.711	0.48
Baseline fractional exhaled nitric oxide (FeNO)	**0.875**	0.57	0.759	0.19	0.739	0.39	0.778	0.33	0.721	0.49
Mepolizumab & reslizumab (n = 36)										
Baseline blood eosinophil, absolute count (/mL)	0.782	ref	0.802	ref	0.821	ref	0.841	ref	0.604	ref
Baseline adjusted urine EDN (ng/mL)	**0.828**	0.32	0.797	0.93	0.776	0.55	0.826	0.84	**0.663**	0.15
adjusted Urine EDN decreased ratio from baseline to 1 m	**0.793**	0.69	0.763	0.30	0.776	0.58	0.744	0.34	**0.671**	0.13
adjusted Urine EDN/Blood eosinophil at baseline	**0.828**	0.33	**0.841**	0.42	**0.840**	0.68	0.720	0.22	**0.667**	0.16
adjusted Urine EDN/Blood eosinophil at 1 m	**0.793**	0.80	0.754	0.24	0.795	0.69	0.734	0.32	**0.663**	0.16
adjusted Urine ΔEDN/ΔBlood eosinophil at 1 m	**0.885**	0.23	**0.845**	0.52	**0.859**	0.72	0.763	0.28	**0.708**	0.25
Baseline fractional exhaled nitric oxide (FeNO)	**0.805**	0.60	**0.850**	0.52	0.769	0.54	0.739	0.39	**0.617**	0.38
Dupilumab (n = 20)										
Baseline blood eosinophil, absolute count (/mL)	0.662	ref	0.727	ref	0.736	ref	0.840	ref	0.896	ref
Baseline adjusted urine EDN (ng/mL)	**0.785**	0.48	**0.753**	0.74	0.736	1.00	**0.852**	0.48	0.818	0.40
adjusted Urine EDN decreased ratio from baseline to 1 m	**0.692**	0.80	**0.792**	0.44	0.736	1.00	0.840	1.00	0.805	0.37

adjusted Urine EDN/Blood eosinophil at baseline	**0.815**	0.17	**0.753**	0.60	0.722	0.81	**0.852**	0.48	0.844	0.51
adjusted Urine EDN/Blood eosinophil at 1 m	**0.815**	0.37	**0.818**	0.39	**0.778**	0.63	**0.877**	0.62	**0.909**	0.74
adjusted Urine ΔEDN/ΔBlood eosinophil at 1 m	**0.754**	0.62	**0.753**	0.79	0.722	0.86	**0.938**	0.31	0.896	1.00
Baseline fractional exhaled nitric oxide (FeNO)	**0.739**	0.72	**0.792**	0.51	**0.792**	0.58	**1.000**	0.15	0.831	0.36

Table 6. Predicting treatment response based on urine EDN and blood eosinophils *Displayed in bold if higher than the AUC value of baseline blood eosinophil count.*

Abbreviations

T2, type 2; AEC, absolute eosinophil count; EDN, eosinophil-derived neurotoxin; ELISA, enzyme-linked immunosorbent assay; AUC, the area under the curve; BMI, body mass index; FeNO, fractional exhaled nitric oxide.

Author contributions

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Authors' consent for publication

All authors have approved the manuscript and agree with its submission to World Allergy Organization Journal.

Ethics approval

The Institutional Review Board of participating centres approved the study and all patients provided written informed consent (IRB No. 2019–1676). The study protocol is registered at ClinicalTrials.gov (NCT05164939).

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Declaration of competing interest

None. This document is provided for informational purposes only and does not contain any conflicts of interest related to its contents. All authors declare no competing interests.

Availability of data and materials

The data employed in this study was sourced from the Precision Medicine Intervention in Severe Asthma (PRISM) cohorts, comprising patients with asthma.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2024.100990.

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