Elevated mepolizumab levels in patients with severe asthma responsive to 1 year's mepolizumab treatment

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Background: Asthma involves variable airflow limitation and persistent airway inflammation. Eosinophilic asthma, characterized by cytokine-mediated type 2 inflammation, is generally treated with inhaled corticosteroids. However, patients with severe asthma may require biologics, such as mepolizumab, which targets IL-5 and can manage uncontrolled eosinophilic asthma.

Objective: We investigated the relationship between serum mepolizumab concentrations and treatment response in patients with severe asthma.

Methods: Patients with mepolizumab-treated severe asthma were enrolled onto this prospective cohort study. Baseline assessments were conducted and repeated at 3, 6, and 12 months. Those with response were categorized on the basis of improvements in asthma control test score, lung function, and asthma exacerbations. We quantified the serum concentration of mepolizumab at 3, 6, and 12 months after treatment by liquid chromatography coupled with tandem mass spectrometry.

Results: Twenty-five adult patients aged 20 years and older with severe asthma were included in the analysis. Serum mepolizumab concentrations significantly increased at 6 and 12 months compared with those at 3 months, particularly in those with disease that responded to therapy. Furthermore, the relative change in mepolizumab concentration was significantly higher in those with response than in those with no response. Body size parameters were negatively correlated with mepolizumab concentration. In those with response, there were inverse correlations between mepolizumab concentration and baseline body size parameters.

Conclusions: The study observed a yearlong increase in mepolizumab concentrations, particularly in those with response, indicating a potential mepolizumab surplus. Correlations between mepolizumab concentrations and baseline characteristics suggested differing mepolizumab requirements between those with response and those with no response. Further research is needed to validate these findings and optimize treatment strategies for patients with severe asthma. (J Allergy Clin Immunol Global 2025;4:100410.)

Key words: *Asthma, mepolizumab, concentration*

Asthma is a prevalent chronic disease characterized by variable airflow limitations and bronchial hyperresponsiveness. It is also associated with persistent heterogeneous chronic inflammation of the airways, influenced by various host factors, including genetic and external factors.[1-3] More than 50% of patients with asthma have eosinophilic asthma, characterized by numerous eosinophils in both the peripheral blood and airways, contributing to asthma exacerbations and airflow limitation.[4,5] Although a standard definition of eosinophilic asthma remains elusive, most clinical trials have used peripheral blood eosinophil counts of ≥150 or ≥300 cells/μL to characterize eosinophilic asthma, which can be easily identified in a clinical setting.[6-9] Type 2 inflammation, mediated by type 2 cytokines such as IL-4, IL-5, and IL-13, is the underlying mechanism of eosinophilic asthma.[6,10] Among these cytokines and inflammatory mediators, IL-5 plays a crucial role in various eosinophil functions.[6,11] The primary cellular sources of IL-5 are T helper 2 lymphocytes and group 2 innate lymphoid cells. The biological effects of IL-5 are mediated through its selective interaction with the IL-5 receptor, IL-5R. The binding of IL-5 to IL-5Rα activates the transcription of numerous genes involved in eosinophil proliferation, differentiation, maturation, activation, and degranulation. IL-5 also works synergistically with eotaxins to recruit eosinophils to asthmatic airways. Additionally, IL-5 inhibits eosinophil apoptosis.

Inhaled corticosteroids (ICS) induce eosinophil apoptosis at clinically achievable drug concentrations through their effect on the glucocorticoid receptor, making them effective in treating eosinophilic asthma.[12,13] However, patients with the most severe clinical phenotype of eosinophilic asthma, who also experience steroid-resistant refractory asthma, may have a limited response to ICS. The effectiveness of biologics targeting the IL-5 pathway, such as mepolizumab, in reducing the eosinophil count in the blood and airway tissues, as well as in inhibiting eosinophilic airway inflammation, has been demonstrated when symptom management is challenging and steroid resistance is present.[8,14,15]

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Abbreviations used
ACT: Asthma control test
BMI: Body mass index
BSA: Body surface area
FENO: Fractional exhaled nitric oxide
FEV_1: Forced expiratory volume in 1 second
ICS: Inhaled corticosteroids
MAIT: Mucosal-associated invariant T
NK: Natural killer
nSMOL: Nano-surface and molecular-orientation limited

Mepolizumab, a humanized anti–IL-5 monoclonal IgG_1 antibody, is available in Japan and has shown favorable results in the management of uncontrolled eosinophilic asthma.[7,9,16-19] We reported that 1 year of mepolizumab treatment resulted in improvements in the number of asthma exacerbations, unscheduled visits due to asthma exacerbations, asthma control test (ACT) scores, and airflow limitations in most patients.[19] This study suggests a reduction in the number of peripheral blood eosinophils and basophils, serum periostin and total IgE levels, and number of $CD69^+$ innate immune cells, including ILC1, ILC3, natural killer (NK), and mucosal-associated invariant T (MAIT) cells. Additionally, $CD69^+$ MAIT cells, neutrophils, and serum periostin levels have been identified as potential predictors of mepolizumab treatment effectiveness in patients with severe asthma.

Drugs exhibit variable effects, and even when the same drug is administered, blood concentrations can substantially differ between individuals. The measurement of drug blood concentrations in each patient allows for the precise assessment of drug efficacy and potential adverse effects. Individualized dosage adjustments are crucial to achieve optimal therapeutic blood levels. However, evaluation of drug concentrations, particularly in the context of biologics for patients with severe asthma, remains uncertain. Therefore, we evaluated the relationship between serum mepolizumab concentrations and treatment response in patients with severe asthma.

METHODS

Patients

This prospective, noninterventional, and observational cohort study has previously reported some results.[19] Patients aged 20 years or older with severe asthma and newly prescribed mepolizumab were enrolled onto this study. These patients had uncontrolled asthma symptoms and exacerbations of asthma that required oral corticosteroids and that could not be controlled by their existing treatment options despite receiving high-dose ICS plus long-acting β_2 agonists with another controller, and were eligible for mepolizumab treatment through Japanese medical insurance. Recruitment was carried out in the outpatient clinic at Juntendo University Hospital (Tokyo, Japan). The diagnosis of asthma was based on a clinical history of episodic symptoms with airflow limitation and monitored variation in pulmonary function measured using forced expiratory volume in 1 second (FEV_1) or peak expiratory flow, following the guidelines of the Global Initiative for Asthma.[20] Patients who met any of the following criteria were excluded from the study: (1) diagnosed with eosinophilic granulomatosis with polyangiitis, interstitial pneumonia, infectious disease, or cancer; (2) received other antibody preparations; or (3) were considered inappropriate candidates by the investigators. The Juntendo University research ethics committee reviewed and approved the study. Before participating in the study, each patient provided a written informed consent. This study was registered with the UMIN Clinical Trial Registry between May 2016 and March 2019 (www.umin.ac.jp, UMIN000024886).

In Japan, mepolizumab is administered as a 100 mg subcutaneous injection once per month, and the patients in this study maintained this monthly treatment regimen for 1 year. On the date of initial mepolizumab administration, an ACT, pulmonary function test, fractional exhaled nitric oxide (FENO) level measurement, and blood sampling were performed. These assessments were repeated at 3 months, 6 months, and 1 year after administration. FENO levels were measured with an electrochemical handheld nitric oxide analyzer, following the recommendations of the American Thoracic Society, at a constant flow of 0.05 L/s against an expiratory resistance of 20 cm of water (NIOX VERO; Aerocrine, Solna, Sweden).

Criteria for mepolizumab response

According to previous studies, patients were classified as experiencing response to therapy on the basis of their ACT scores, lung function, and asthma exacerbations.[21-27] To be classified as having therapy-responsive disease after 1 year of mepolizumab treatment, patients had to meet at least 2 of the following 3 criteria without experiencing substantial deterioration in any other criterion: (1) an improvement in the ACT score of at least 3 points (including patients with an ACT score of 25 points) because an increase of 3 points was previously identified as the minimum clinically important difference; (2) a reduction in the number of asthma exacerbations (including patients who had no exacerbations before and after treatment); and (3) an improvement in FEV_1 of at least 100 mL.[27-30] Significant deterioration of a criterion was determined by (a) a decrease in the ACT score of at least 3 points, (b) an increase in the number of exacerbations, and (c) a decrease in FEV_1 of at least 100 mL.

Quantification of frequency of circulating lymphocytes in patients with asthma

Flow cytometry was conducted as previously described.[19,31] Complete details of the flow cytometry methods are included in the Methods section in the Online Repository available at www.jaci-global.org.

Quantification of serum cytokine and chemokine levels

Serum periostin levels were measured with an ELISA (Shino Test, Kanagawa, Japan), as previously described.[32] Additional details are provided in the Methods section in the Online Repository.

Proteolysis and liquid chromatography coupled with tandem mass spectrometry

We used nano-surface and molecular-orientation limited (nSMOL) proteolysis coupled with liquid chromatography electrospray ionization–mass spectrometry with a triple quadrupole analysis (LCMS-8050; Shimadzu, Kyoto, Japan) to quantify the concentration of monoclonal antibodies in the serum samples.

TABLE I. Baseline characteristics of 25 study participants

Characteristic	No. (%) or median (interquartile range)
Sex (M/F)	5 (20%)/20 (80%)
Age (years)	57 (46.5-66.5)
Age at asthma onset (years)	48 (22.5-51)
Duration of asthma (years)	13 (7.5-24.5)
Height (cm)	156.1 (149.2-161.9)
BW (kg)	59.7 (50.4-66.8)
BMI (kg/m^2)	23.8 (21.35-25.15)
BSA (m^2)	1.6 (1.5-1.7)
Smoking history (never/former)	20 (80%)/5 (20%)
NERD	3 (12.0%)
Atopic dermatitis	10 (40.0%)
Allergic rhinitis	19 (76.0%)
Chronic sinusitis	8 (32.0%)
Daily dose of ICS (FP equivalent dose, μg)	1000.0 (900.0-1000.0)
Oral corticosteroid treatment	3 (12.0%)
Previous omalizumab treatment	14 (56.0%)
Asthma exacerbations (per year)	2.0 (0.0-3.0)
Unscheduled visits (per year)	1.0 (0.0-2.0)
Hospitalizations (per year)	0
ACT score points	19 (16.0-22.5)
FENO (ppb)	25.0 (15.0-78.5)
FVC (L)	2.9 (2.33-3.50)
%FVC (predicted, %)	98.2 (92.8-110.7)
FEV_1 (L)	2.1 (1.48-2.66)
%FEV_1 (predicted, %)	92.5 (74.7-103.0)
FEV_1% (%)	75.7 (60.4-84.2)
Peripheral neutrophils (%)	61.5 (56.4-65.3)
Peripheral neutrophils (×10^2 cells/μL)	41.7 (31.6-49.2)
Peripheral eosinophils (%)	4.1 (3.1-7.4)
Peripheral eosinophils (cells/μL)	269.0 (161.0-528.5)
Peripheral basophils (%)	0.6 (0.4-0.75)
Peripheral basophils (cells/μL)	39.0 (22.0-49.6)
Peripheral lymphocytes (%)	27.9 (23.6-31.6)
Peripheral lymphocytes (×10^2 cells/μL)	18.4 (13.9-22.2)
Total IgE	241.0 (108.5-499.5)
Periostin (ng/ mL)	95.0 (74.0-149.0)
Tenascin-C (ng/mL)	34.1 (23.2-51.1)
IL-4 (pg/mL), n = 24	1.1 (0.8-1.5)
IL-8 (pg/mL), n = 20	7.4 (6.1-8.5)
Eotaxin-1 (pg/mL)	73.1 (50.7-82.7)
IP-10 (pg/mL)	550 (451.5-747.0)
MCP-1 (pg/mL), n = 24	20.8 (14.5-23.4)
MIP-1α (pg/mL)	1.4 (1.2-2.1)
MIP-1β (pg/mL)	74.5 (67.8-81.0)
RANTES (ng/mL)	5.0 (4.6-5.6)
T_H1 cells (% of T_H cells, %), n = 24	18.6 (15.4-27.1)
T_H2 cells (% of T_H cells, %), n = 24	5.0 (3.2-6.3)
T_H17 cells (% of T_H cells, %), n = 24	5.0 (3.2-6.3)
Treg cells (% of T_H cells, %), n = 24	5.4 (4.3-6.6)
ILC1 (% of ILC cells, %)	66.3 (54.2-73.9)
CD69$^+$ ILC1 (% of ILC1, %)	9.2 (6.7-14.0)
ILC2 (% of ILC cells, %)	23.5 (14.6-33.5)
CD69$^+$ ILC2 (% of ILC2, %)	10.1 (7.6-12.9)
ILC3 (% of ILC cells, %)	10.1 (6.5-13.4)
CD69$^+$ ILC3 (% of ILC3, %)	11.7 (9.6-16.5)
NK cells (% of lymphoid cells, %)	13.7 (7.0-19.2)
CD69$^+$ NK cells (% of NK, %)	9.5 (7.5-14.3)
γδ T cells (% of CD3$^+$ cells, %)	2.5 (1.5-5.0)
MAIT cells (% of CD3$^+$ cells, %)	1.8 (0.8-2.6)
CD69$^+$ MAIT cells (% of MAIT cells, %)	30.3 (23.4-40.0)

FEV$_1$%, FEV_1/FVC; *FP,* fluticasone propionate; *FVC,* forced vital capacity; *IP-10,* IFN-γ–inducible protein 10; *MCP,* monocyte chemotactic protein; *MIP,* macrophage inflammatory protein; *NERD,* nonsteroidal anti-inflammatory drug–exacerbated respiratory disease; *RANTES,* regulated on activation normal T-cell expressed and secreted.

The nSMOL method, which we only summarize here, has been previously described in more detail elsewhere.[33] Before analysis, the nSMOL method was validated using trastuzumab-, bevacizumab-, or nivolumab-spiked plasma.[34,35] A detailed description is provided in the Methods section in the Online Repository.

Statistical analysis

The D'Agostino-Pearson test was used to assess sample normality. Various statistical tests, including Welch *t* test, paired *t* test, Mann-Whitney *U* test, Wilcoxon signed-rank test, and Fisher exact test, were used to determine the significance of differences in parameters between populations, as appropriate. For comparisons among multiple groups, 1-way repeated ANOVA measurements with Tukey multiple comparison test and Friedman test with Dunn multiple comparison test were used. When applicable, Pearson and Spearman rank correlation coefficients were used to examine the correlations between variables. Statistical significance was set as $P \leq .05$. All statistical analyses were performed by GraphPad Prism v6 software (GraphPad Software, La Jolla, Calif).

RESULTS

Baseline characteristics

The 27 included patients received mepolizumab treatment for severe asthma that remained uncontrolled even with existing treatments. However, 2 of the initial 27 patients were excluded from the analysis because they discontinued mepolizumab treatment after 5 months because of worsening asthma symptoms, and their mepolizumab concentration could not be measured. The baseline characteristics of the included patients are shown in Table I. The median (interquartile range) patient age was 57 (46.5-66.5) years. The median peripheral blood eosinophil count was 269 cells/μL. Twenty-three patients (92%) had a blood eosinophil count of ≥150 cells/μL at the beginning of the study or ≥300 cells/μL in the previous year. The remaining 2 patients received treatment with omalizumab or oral corticosteroids before initiating mepolizumab treatment.

Serum concentration of mepolizumab and its correlation with baseline characteristics

Serum mepolizumab concentrations were measured after 3, 6, and 12 months of mepolizumab treatment, with median (interquartile range) values of 11.2 (8.0-15.0) μg, 13.1 (10.2-18.0) μg, and 13.8 (11.0-17.6) μg, respectively—significantly higher at 6 and 12 months than at 3 months (Fig 1, *A* and *D*). Furthermore,

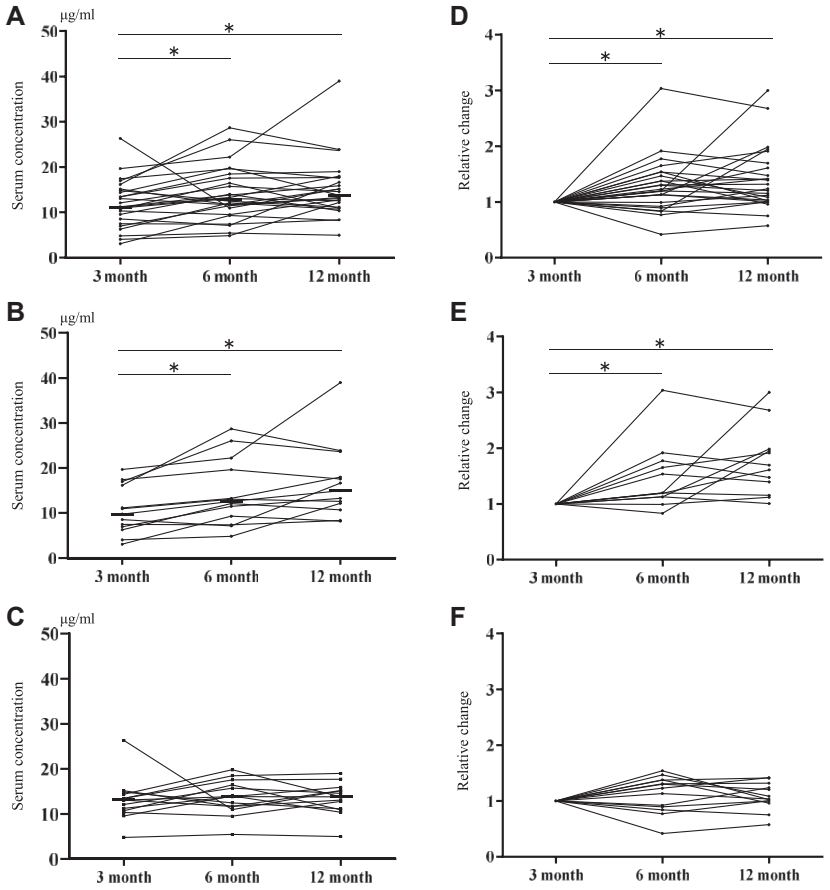

FIG 1. Changes in serum mepolizumab concentration from month 3 to 12 of treatment. Changes in serum concentrations for all patients (**A** and **D**), those with response (**B** and **E**), and those without response (**C** and **F**), including serum concentration value *(A-C)* and relative change with 3-month concentration as one *(D-F)*. Bars represent median values. *$P < .05$.

when serum levels of mepolizumab were examined separately for those with (n = 12) and without (n = 13) response at 12 months, serum levels were significantly higher at 6 and 12 months than at 3 months only in those with disease that responded to therapy (Fig 1, *B* and *E*). However, those with no response showed no significant changes throughout the year, and there were no notable differences in serum mepolizumab concentrations at 3, 6, and 12 months between those with and those without response (Fig 1, *C* and *F,* and data not shown). Therefore, the relative change ratio was calculated by dividing the serum mepolizumab concentration at 12 months by the concentration at 3 months, which revealed that the relative change ratio was significantly higher in those with responsive disease (Fig 2). Table II shows the correlation between each parameter of the baseline characteristics and the concentration of mepolizumab at 1 year. In general, body weight (BW), body surface area (BSA), and total serum IgE levels were negatively correlated with mepolizumab concentration (Table II). In those with response, the frequency of peripheral blood lymphocytes was positively correlated, and BW, body mass index (BMI), BSA, total IgE, and frequency of peripheral blood $CD69^+$ ILC3 were negatively correlated with the concentration of mepolizumab. In those without response, the frequency of ILC1 was positively correlated with the concentration of mepolizumab (Table II and Fig 3). Table II shows the correlation between each characteristic parameter at baseline and the relative

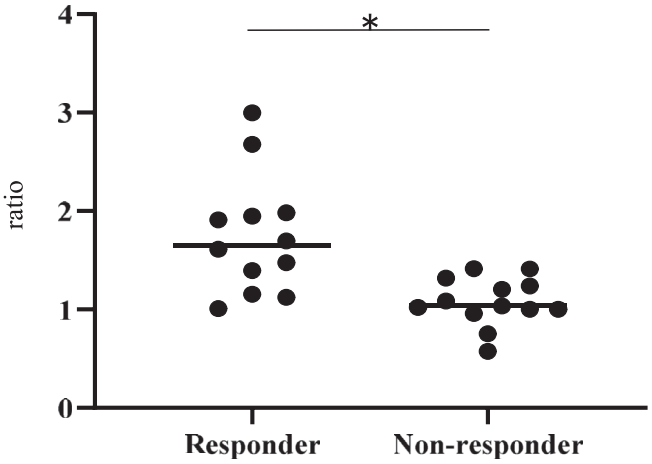

FIG 2. Relative change ratio in serum mepolizumab concentrations in those with response and those with no response. Bars represent median values. *$P < .05$.

changes in the concentration of mepolizumab. Changes in mepolizumab were negatively correlated with peripheral blood neutrophil count and $CD69^+$ MAIT frequency and positively correlated with eosinophil frequency (Table II). These correlations were not evident when analyzing the 2 groups.

TABLE II. Correlation of serum concentration of mepolizumab and baseline characteristics

	Serum concentration at 12 months						Relative changes in concentration (12 months/3 months)					
	Total (N = 25)		Response (n = 12)		Nonresponse (n = 13)		Total (N = 25)		Response (n = 12)		Nonresponse (n = 13)	
Characteristic	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Age (years)	0.023	.910	−0.042	.904	0.083	.788	0.032	.880	0.013	.969	0.527	.065
Height (cm)	−0.198	.343	−0.116	.720	−0.335	.263	0.010	.964	−0.265	.405	0.155	.614
BW (kg)	−0.453	.023*	−0.818	.002*	−0.299	.321	0.296	.152	0.305	.335	0.033	.914
BMI (kg/m^2)	−0.328	.110	−0.706	.013*	−0.028	.928	0.305	.138	0.481	.113	−0.091	.768
BSA (m^2)	−0.426	.034*	−0.751	.006*	−0.329	.273	0.249	.230	−0.029	.932	0.099	.747
Asthma exacerbations (per year)	0.212	.310	0.174	.586	0.186	.544	0.126	.550	−0.117	.717	0.080	.796
ACT score points	−0.292	.156	−0.336	.283	−0.163	.594	−0.180	.390	0.009	.977	0.464	.110
FENO (ppb)	−0.191	.359	−0.123	.704	−0.063	.838	0.226	.278	0.137	.672	0.259	.390
FVC (L)	−0.137	.514	−0.070	.835	−0.286	.344	−0.016	.939	−0.412	.183	−0.101	.742
%FVC (predicted %)	0.247	.234	0.322	.309	0.087	.779	0.075	.720	0.004	.990	0.010	.975
FEV_1 (L)	−0.101	.632	−0.077	.817	−0.158	.607	−0.115	.585	−0.309	.328	−0.124	.686
%FEV_1 (predicted %)	0.154	.463	−0.077	.817	0.070	.821	−0.085	.688	0.011	.972	0.021	.946
FEV_1% (%)	0.015	.945	−0.049	.886	−0.048	.877	−0.028	.893	−0.016	.961	−0.127	.681
Peripheral neutrophils (%)	−0.063	.764	−0.431	.163	0.500	.082	−0.182	.384	0.294	.354	0.107	.728
Peripheral neutrophils (cells/μL)	−0.165	.432	−0.455	.140	0.246	.418	−0.411	.041*	0.014	.966	−0.151	.623
Peripheral eosinophils (%)	0.120	.569	0.186	.561	−0.259	.392	0.467	.019*	−0.110	.734	0.239	.431
Peripheral eosinophils (cells/μL)	0.078	.711	0.063	.852	−0.258	.395	0.274	.185	−0.116	.720	0.126	.682
Peripheral basophils (%)	0.070	.740	0.275	.384	−0.201	.507	−0.107	.610	−0.473	.122	−0.266	.376
Peripheral basophils (cells/μL)	0.103	.624	0.280	.379	−0.192	.529	−0.233	.262	−0.406	.190	−0.385	.196
Peripheral lymphocytes (%)	0.205	.327	0.587	.049*	−0.159	.604	0.015	.945	−0.213	.507	−0.203	.505
Peripheral lymphocytes (cells/μL)	−0.101	.632	0.322	.309	−0.352	.238	−0.254	.221	−0.296	.350	−0.300	.320
Total IgE (IU/ mL)	−0.459	.021*	−0.678	.019*	0.035	.910	0.023	.913	0.042	.904	−0.337	.260
Periostin (ng/mL)	−0.149	.479	−0.035	.921	−0.228	.450	0.225	.279	−0.262	.411	0.190	.532
Tenascin-C (ng/mL)	−0.225	.279	−0.217	.499	−0.119	.699	−0.072	.731	−0.302	.341	0.158	.606
IL-4 (pg/mL)	0.010	.962	−0.193	.546	0.273	.365	0.164	.435	−0.033	.920	0.424	.149
IL-8 (pg/mL)	−0.224	.281	−0.434	.162	−0.014	.968	0.164	.435	0.238	.457	0.228	.450
Eotaxin-1 (pg/mL)	−0.162	.438	−0.420	.177	0.039	.899	−0.081	.701	−0.448	.147	0.532	.062
IP-10 (pg/mL)	0.205	.327	0.259	.417	−0.132	.667	0.312	.129	0.043	.886	0.376	.205
MCP-1 (pg/mL)	−0.376	.064	−0.385	.218	−0.460	.114	0.183	.381	0.532	.079	−0.166	.588
MIP-1α (pg/mL)	−0.323	.115	−0.469	.125	−0.210	.487	0.155	.459	0.158	.623	0.185	.542
MIP-1β (pg/mL)	−0.121	.565	−0.021	.956	−0.192	.529	−0.028	.893	−0.175	.588	0.011	.978
RANTES (pg/mL)	−0.028	.893	−0.196	.543	0.013	.967	0.192	.357	0.396	.202	−0.319	.288
T_H1 cells (% of T_H cells, %), n = 24/12/12	0.370	.075	0.441	.154	0.350	.266	−0.174	.416	−0.379	.225	0.014	.974
T_H2 cells (% of T_H cells, %), n = 24/12/12	−0.082	.704	−0.147	.651	0.056	.869	−0.077	.719	0.336	.287	−0.173	.591
T_H17 cells (% of T_H cells, %), n = 24/12/12	−0.134	.533	−0.252	.430	−0.126	.700	−0.096	.657	0.056	.862	0.042	.904
Treg cells (% of T_H cells, %), n = 24/12/12	−0.198	.353	−0.133	.683	−0.266	.404	0.286	.175	0.315	.319	0.310	.327
ILC1 (% of ILC cells, %)	0.293	.156	0.035	.921	0.626	.025*	0.319	.120	−0.129	.690	0.505	.079
ILC2 (% of ILC cells, %)	−0.221	.289	−0.133	.683	−0.473	.106	−0.214	.305	0.232	.469	−0.482	.096
ILC3 (% of ILC cells, %)	0.169	.420	0.126	.700	0.390	.189	−0.128	.543	−0.200	.534	0.506	.081
$CD69^+$ ILC1 (% of ILC1, %)	−0.112	.596	−0.203	.528	0.038	.906	−0.245	.237	−0.331	.293	0.140	.649
$CD69^+$ ILC2 (% of ILC2, %)	0.135	.520	0.126	.700	0.115	.710	0.105	.616	0.270	.396	0.179	.559
$CD69^+$ ILC3 (% of ILC3, %)	−0.260	.209	−0.657	.024*	0.270	.369	0.158	.451	0.418	.176	0.255	.402
NK cells (% of lymphoid cells, %)	−0.050	.812	0.112	.733	−0.264	.384	0.189	.365	0.479	.116	0.248	.414
$CD69^+$ NK cells (% of NK, %)	−0.229	.270	−0.245	.443	−0.104	.737	−0.333	.104	−0.483	.115	−0.159	.604
γδ T cells (% of lymphoid cells, %)	0.112	.596	0.357	.256	−0.143	.643	−0.111	.598	0.097	.764	−0.434	.141
MAIT cells (% of $CD3^+$ cells, %)	−0.079	.708	0.203	.528	−0.528	.067	−0.182	.383	−0.532	.079	−0.451	.122
$CD69^+$ MAIT cells (% of MAIT cells, %)	−0.337	.099	−0.420	.177	−0.080	.796	−0.544	.005*	−0.448	.147	−0.171	.577

FEV_1%, FEV_1/FVC; *FP,* fluticasone propionate; *FVC,* forced vital capacity; *IP-10,* IFN-γ–inducible protein 10; *MCP,* monocyte chemotactic protein; *MIP,* macrophage inflammatory protein; *RANTES,* regulated on activation normal T-cell expressed and secreted.
*$P < .05$.

Regarding sex distribution, there was no significant difference in treatment response between the sexes ($P = .645$), with 3 male participants experiencing response and 2 no response; the same values for female participants were, respectively, 9 and 11 (see Fig E1, *A,* in the Online Repository available at www.jaci-global.org). However, after 1 year of treatment, the serum mepolizumab concentrations were significantly higher in women (Fig E1, *B*). Women had significantly lower BSA than men, suggesting that the higher mepolizumab concentrations in women may be attributed to differences in body size (Fig E1, *B*).

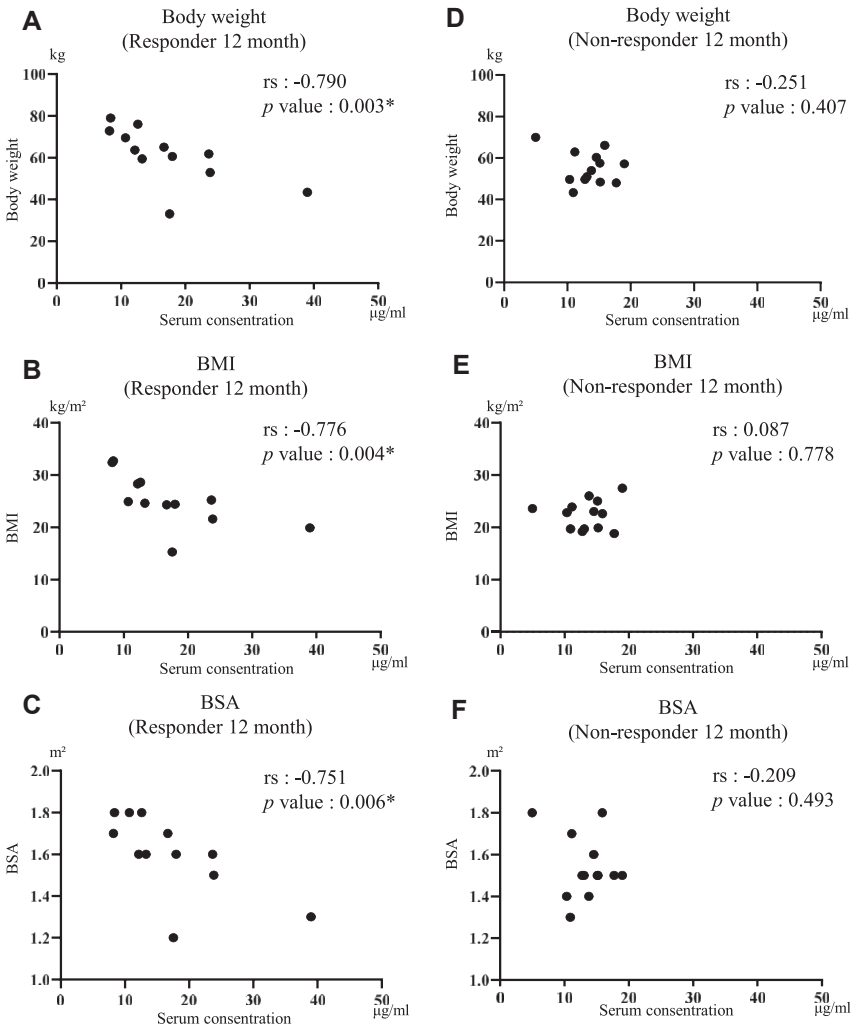

FIG 3. Correlation between serum mepolizumab concentration and body size parameters. In response group **(A-C)**, there was negative correlation between serum mepolizumab concentration and BW *(A)*, BMI *(B)*, and BSA *(C)*. These correlations were not observed in nonresponse group *(D-F)*. *$P < .05$.

Correlation between relative changes in serum mepolizumab concentrations and relative changes in parameters

Table III shows the correlations between the relative changes in serum mepolizumab concentrations from 3 to 12 months and from baseline to 12 months for various parameters. The changes in each parameter have been previously reported.[19] Overall, the relative change in forced vital capacity, percentage predicted forced vital capacity, FEV_1, and percentage predicted FEV_1 was positively correlated with the relative change in mepolizumab concentration, and the relative change in the frequency of $CD69^+$ NK cells showed a negative correlation (Table III). In the nonresponse group, the relative change ratio in mepolizumab concentration was negatively correlated with the relative change ratios in ACT scores, serum levels of IL-4 and IP-10 (IFN-γ–inducible protein 10), and the frequency of peripheral blood Treg cells (Table III).

DISCUSSION

In this study, there was an inverse relationship between the mepolizumab concentration and factors, such as BW, BSA, and total serum IgE levels. This suggests that smaller patients have excess serum mepolizumab. Furthermore, in those with response, there is an inverse correlation between mepolizumab concentration and BW, BMI, BSA, and $CD69^+$ ILC3, while no such relationship between body size and blood concentration was observed in those without response. In oncology, most drugs are administered according to body size, not as a fixed dose for all patients. This approach is essential to account for variations in body size, including in children and individuals with obesity.[36] However, it remains unclear whether the same principle applies to biologics used for asthma treatment. The results of this study suggest that those without response may require higher doses of mepolizumab, as they do not exhibit a surplus, while those with response have adequate mepolizumab levels. These observations may also support a similar association between asthma, obesity, and ILC3, as previously reported in a mouse model.[37,38] Furthermore, there is evidence indicating a correlation between $CD69^+$ ILC3 in peripheral blood and BMI in patients with asthma.[31] Those with response in this study exhibited an inverse correlation between mepolizumab concentration and the frequency of $CD69^+$ ILC3 in peripheral blood, similar to the correlation observed with BMI.

TABLE III. Correlation between relative changes in serum mepolizumab concentrations and relative changes in parameters

	Relative change ratios in in serum mepolizumab concentrations (12 months/3 months)					
	Total (N = 25)		Response (n = 12)		Nonresponse (n = 13)	
Relative changes (12 months/0 months)	*r*	*P*	*r*	*P*	*r*	*P*
ACT score points	0.339	.098	−0.105	.744	−0.667	.013*
FENO (ppb)	−0.153	.465	−0.559	.063	−0.063	.837
FVC (L)	0.491	.013*	0.329	.297	0.209	.493
%FVC (predicted %)	0.435	.030*	0.336	.287	0.093	.765
FEV_1 (L)	0.499	.011*	0.204	.526	0.157	.609
$\%FEV_1$ (predicted %)	0.465	.019*	0.224	.484	0.133	.666
$FEV_1\%$ (%)	0.243	.242	0.181	.574	−0.088	.775
Peripheral neutrophils (%)	−0.274	.185	−0.377	.227	−0.077	.806
Peripheral neutrophils (cells/μL)	−0.094	.656	−0.437	.155	−0.011	.971
Peripheral eosinophils (%)	−0.388	.055	−0.416	.179	−0.437	.136
Peripheral eosinophils (cells/μL)	−0.345	.091	−0.420	.175	−0.394	.183
Peripheral basophils (%)	0.320	.118	0.230	.471	−0.072	.815
Peripheral basophils (cells/μL)	0.333	.104	0.427	.169	0.058	.852
Peripheral lymphocytes (%)	0.385	.058	0.287	.366	0.264	.384
Peripheral lymphocytes (cells/μL)	0.241	.246	−0.050	.877	0.271	.372
Total IgE (IU/mL)	0.015	.942	−0.196	.543	−0.517	.074
Periostin (ng/mL)	−0.214	.304	−0.161	.617	0.052	.867
Tenascin-C (ng/mL)	−0.138	.512	0.118	.716	−0.484	.097
IL-4 (pg/mL)	−0.255	.218	0.941	.771	−0.740	.005*
IL-8 (pg/mL)	−0.005	.983	−0.080	.805	−0.319	.289
Eotaxin-1 (pg/mL)	−0.028	.896	0.173	.592	0.033	.921
IP-10 (pg/mL)	−0.369	.070	0.175	.588	−0.676	.014*
MCP-1 (pg/mL), n = 24/11/13	−0.211	.322	−0.071	.836	−0.174	.569
MIP-1α (pg/mL)	0.297	.150	0.378	.228	−0.140	.646
MIP-1β (pg/mL)	0.167	.425	0.055	.866	−0.104	.737
RANTES (pg/mL)	0.163	.436	−0.276	.385	0.264	.384
T_H1 cells (% of T_H cells, %)	−0.170	.438	0.152	.656	0.102	.752
T_H2 cells (% of T_H cells, %)	0.129	.556	−0.109	.755	0.274	.389
T_H17 cells (% of T_H cells, %)	−0.052	.812	−0.073	.839	−0.448	.147
Treg cells (% of T_H cells, %)	−0.165	.452	−0.336	.313	−0.587	.049*
ILC1 (% of ILC cells, %), n = 23/11/12	0.002	.993	0.279	.407	0.105	.747
ILC2 (% of ILC cells, %), n = 23/11/12	−0.219	.315	−0.335	.315	−0.147	.651
ILC3 (% of ILC cells, %), n = 23/11/12	0.066	.764	0.077	.821	−0.427	.169
$CD69^+$ ILC1 (% of ILC1, %), n = 23/11/12	−0.281	.195	0.045	.903	−0.140	.667
$CD69^+$ ILC2 (% of ILC2, %), n = 23/11/12	−0.043	.844	0.034	.920	−0.119	.713
$CD69^+$ ILC3 (% of ILC3, %), n = 23/11/12	−0.400	.059	−0.498	.119	−0.178	.581
NK cells (% of lymphoid cells, %), n = 23/11/12	−0.254	.242	−0.457	.158	−0.375	.230
$CD69^+$ NK cells (% of NK, %), n = 23/11/12	−0.469	.024*	−0.156	.646	−0.525	.084
γδ T cells (% of lymphoid cells, %), n = 24/11/13	0.183	.393	0.082	.818	−0.066	.831
MAIT cells (% of $CD3^+$ cells, %), n = 23/11/12	−0.011	.961	−0.155	.648	0.217	.499
$CD69^+$ MAIT cells (% of MAIT cells, %), n = 23/11/12	−0.133	.544	0.276	.412	−0.450	.142

$FEV_1\%$, FEV_1/FVC; *FP,* fluticasone propionate; *FVC,* forced vital capacity; *IP-10,* IFN-γ–inducible protein 10; *MCP,* monocyte chemotactic protein; *MIP,* macrophage inflammatory protein; *RANTES,* regulated on activation normal T-cell expressed and secreted.
*$P < .05$.

Furthermore, Table III shows a negative correlation between the relative change ratio in the percentage of $CD69^+$ NK cells and mepolizumab concentration. This suggests that an increased frequency of $CD69^+$ NK cells correlates with lower mepolizumab concentrations. Consequently, patients exhibiting low mepolizumab concentrations and an inadequate response after 1 year may benefit from benralizumab, which leverages NK cell–mediated antibody-dependent cytotoxic activity. However, further investigations are necessary to substantiate this hypothesis. Furthermore, future investigations are needed to determine the negative correlation between the relative change in mepolizumab concentration and the relative change in ACT scores and other parameters, which was observed exclusively in the group with no response.

The correlation between each parameter and the difference in mepolizumab concentration after 12 and 3 months of treatment showed a negative correlation between peripheral blood neutrophil count and the frequency of $CD69^+$ MAIT cells and a positive correlation between eosinophil frequency. The identified negative correlation between neutrophils and $CD69^+$ MAIT cells aligns with our previous findings, in which a low peripheral blood neutrophil count and the frequency of $CD69^+$ MAIT cells were considered potential biomarkers that predicted responses to mepolizumab treatment.[19] The results that serum mepolizumab concentrations were more likely to increase in patients with a high pretreatment peripheral blood eosinophil ratio are consistent with previous reports, which indicate that higher peripheral blood eosinophil counts are

associated with a greater effect of mepolizumab in suppressing asthma exacerbations.[18] However, when patients were divided into response and no-response groups, these correlations, although expected to be more pronounced in those with response, were not observed in either group.

The recombinant human monoclonal antibody for mepolizumab, which is commercially available from Invitrogen (Thermo Fisher Scientific, Waltham, Mass), was tailored for the detection of mepolizumab and can act as a primary antibody for multiple scientific procedures, such as ELISA and SDS-PAGE. Considering the demonstrated promise of serum mepolizumab concentrations as an indicator of mepolizumab response in this study, the use of commercially accessible mepolizumab antibodies for ELISA assessment can prove beneficial in clinical settings. However, further clinical trials are required to confirm these findings.

This study has several limitations. First, this was a single-center, single-arm, open-label observational study with a small sample size. Second, many of the observed changes over the 1-year period remain uncertain and largely phenomenological. Therefore, the evidence or statistical power in this study was insufficient to support the suggestion that those with response may have had a surplus of mepolizumab and those without response might require higher doses. Further research involving larger sample sizes and incorporating multivariate analyses to control for potential confounders is essential to confirm these findings and explore their wider clinical implications. Furthermore, the criteria used to define clinical response in this study were based on expert opinions, but a consensus has not yet been reached. These criteria are likely to evolve over time.

To our knowledge, this is the first observation of a yearlong increase in serum mepolizumab concentration. Notably, this increase was not observed in those without response to mepolizumab treatment, suggesting that those without response may require more mepolizumab. Furthermore, the increase in concentration was more pronounced in those who experienced response who had smaller body sizes, suggesting that these participants might have had a surplus of mepolizumab. The findings of this study suggest that measurement of serum mepolizumab concentrations could be a valuable tool for distinguishing patients whose disease would benefit from continued treatment from patients whose disease might require a change in their treatment plan.

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Key messages

- **Analysis of mepolizumab blood concentrations may be useful for optimizing treatment, but its relevance to asthma remains uncertain.**
- **In those with response to mepolizumab treatment, the administered dose appeared adequate, suggesting that measuring serum mepolizumab concentrations might be beneficial for optimizing treatment.**
- **Determining the importance of measuring serum mepolizumab concentrations in asthma treatment is a valuable topic for future clinical practice.**

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