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Letter to the Editor

The impact of mepolizumab on the sputum level of eosinophil-derived protein in three cases of severe asthma

Dear Editor,

Mepolizumab, an interleukin 5 (IL-5) antagonist monoclonal antibody, has been shown to decrease asthma exacerbations and blood eosinophilia in patients with severe eosinophilic asthma.¹ Mepolizumab consistently reduces blood eosinophilia, but its effect on the reduction of sputum eosinophils is controversial. The reduction of sputum eosinophils may be related to the dose of mepolizumab,² however, at the licensed dose, eosinophil concentration may often remain unchanged.³ In this report, we monitored three patients who had initiated mepolizumab therapy for the treatment of severe asthma. These patients showed clinical improvement in symptoms and an obvious reduction in exacerbations, despite the insignificant reduction of sputum eosinophils. Analysis of sputum from these cases revealed that the levels of eosinophilic cationic protein (ECP) and Charcot-Leyden crystal (CLC) protein in sputum supernates were clearly decreased.

Informed consent was obtained for all the patients involved in this study. This study was approved by the Ethics Committee of Niigata University (approval no. 2524).

The clinical background and changes in parameters of the three cases are shown in Table 1. The patient in case 1 was a 54-year-old male, a previous smoker who had been diagnosed with asthma in his 40s. He had eosinophil chronic rhinosinusitis (ECRS) as a comorbidity. Prior to the introduction of mepolizumab, this patient required a high-dose of inhaled corticosteroid (ICS) + a long-acting β agonist (LABA) and 10 mg of oral prednisolone. In the year following mepolizumab initiation, this patient's asthma control test (ACT) score improved from 20 to 25 and exacerbations decreased from 7 episodes per year to zero. The blood eosinophil count was also clearly reduced (1398 cells $\rightarrow$ 93 cells/ μ L), but the sputum eosinophils were not significantly impacted (41.4% $\rightarrow$ 33.0%).

The patient in case 2 was a 54-year-old male, a previous smoker who was diagnosed with asthma in his 30s. He had ECRS as a comorbidity. Prior to the introduction of mepolizumab, he required a high-dose of ICS + LABA and 7.5 mg of oral prednisolone. In the year following mepolizumab initiation, the ACT score improved from 8 to 18 and the number of exacerbations decreased from 12 episodes per year to a single episode in the following year. The blood eosinophil count was also clearly reduced (887 cells $\rightarrow$ 173 cells/ μ L), but the sputum eosinophils were not significantly impacted (83.2% $\rightarrow$ 73.3%).

The patient in case 3 was a 70-year-old female, a non-smoker who was diagnosed with asthma in her 50s. She had ECRS as a

comorbidity. Prior to the introduction of mepolizumab, she had required a high-dose of ICS + LABA. In the year following mepolizumab initiation, the ACT score decreased from 16 to 23, and the number of exacerbations decreased from 6 to 0. The blood eosinophil count was also clearly reduced (889 cells $\rightarrow$ 111 cells/ μ L), but the sputum eosinophils were not significantly impacted (4.0% $\rightarrow$ 10.4%).

The sputum supernates before and after one-year of mepolizumab treatment were used to measure the levels of ECP and CLC protein. Commercially available ELISA testing kits were utilized according to the manufacturers' instructions. ELISA kits used to detect human ECP were obtained from Avisa Bioscience, Inc (Santa Clara, CA, USA) while those for human CLC protein were purchased from Cloud-Clone Corp (Katy, TX, USA). The sputum eosinophils in all three cases showed no significant changes, but the levels of ECP and CLC protein in sputum supernates were clearly reduced one-year after mepolizumab initiation. In particular, the decrease in CLC protein levels were remarkable (Fig. 1).

Table 1

Clinical characteristics and changes in parameters before and after mepolizumab treatment.

	Case 1		Case 2		Case 3	
Age (y)	54		54		70	
Sex (M/F)	M		M		F	
Disease Duration (y)	10		19		13	
BMI (kg/m ²)	24.2		22.8		22.4	
Atopic (Y/N)	Y		N		Y	
Smoking (Never/Past/Current)	Past		Past		Never	
ECRS comorbidity (Y/N)	Y		Y		Y	
	Pre	12M	Pre	12M	Pre	12M
ICS (μ g/day) (Fluticasone equivalent)	1000	1000	800	800	500	500
OCS (mg/day) (PSL equivalent)	10	0	7.5	4	0	0
Exacerbation (episodes/year)	7	0	12	1	6	0
ACT	20	25	8	18	16	23
Blood Eos (cells/ μ L)	1398	93	887	173	889	111
Sputum Eos (%)	41.4	33.0	83.2	73.3	4.0	10.4
IgE (U/L)	911	1180	691	942	1280	973
FeNO (ppb)	167	233	115	223	170	151
%FEV ₁	107.4	98.5	29.7	34.1	91.2	103.4
FEV ₁ /FVC (%)	75.2	70.6	32.3	33.6	69	71

Y, Yes; N, No; y, years; BMI, body mass index; ECRS, eosinophilic chronic rhinosinusitis; Pre, Pretreatment; 12 M, 12 months after initiation of mepolizumab treatment; ICS, inhaled corticosteroid; OCS, oral corticosteroid; PSL, Prednisolone; ACT, Asthma Control Test; Eos, eosinophils; IgE, immunoglobulin E; FeNO, fraction of exhaled nitric oxide; %FEV₁, percent forced expiratory volume in one second; FVC, forced vital capacity; ppb, parts per billion.

Data are expressed as the number (percentage) or the median (minimum–maximum).

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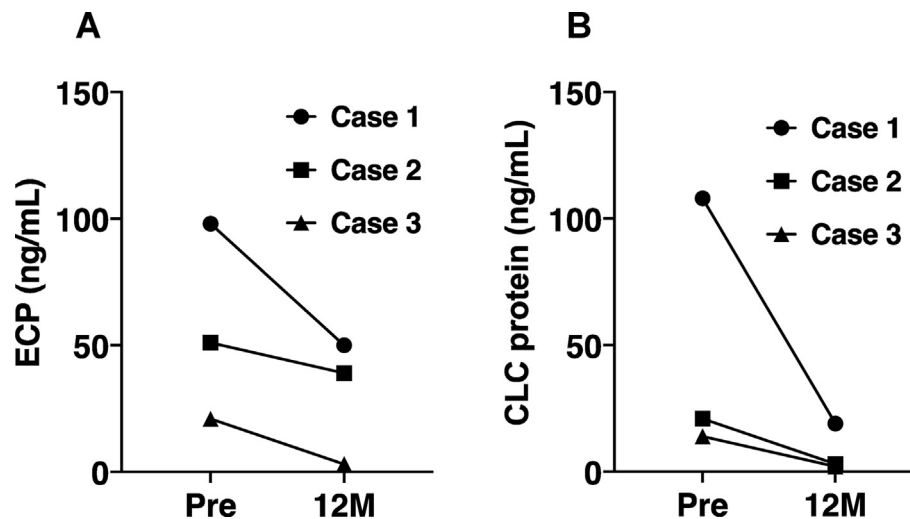


Fig. 1. Changes in the eosinophil-related protein levels in sputum supernates for each case before and 12 months after mepolizumab treatment. Graph (A) represents ECP levels and graph (B) represents CLC protein levels. Pre, before treatment; 12 M, 12 months after treatment; ECP, eosinophilic cationic protein; CLC, Charcot-Leyden crystal.

In our study, there were significant decreases in ECP and CLC protein levels in sputum supernates despite no significant changes of sputum eosinophil levels. In all three cases, blood eosinophils significantly reduced, and their symptoms improved and exacerbations were clearly reduced, indicating that mepolizumab was recognized as clinically effective. From these data, mepolizumab is inadequate in reducing sputum eosinophils entirely, but it seems sufficient in reducing activated sputum eosinophils. However, the levels of FeNO, one of the markers of eosinophilic airway inflammation, were not reduced. It has been reported that the eosinophils have little effect on the expression of induced NO synthase in the airway epithelium. Therefore, the levels of FeNO after mepolizumab use do not change.⁴ In this report, all cases have ECRS, which showed no obvious response to mepolizumab, and NO contamination from the upper respiratory tract cannot, therefore, be ruled out.

In a previous report, administration of a single dose of mepolizumab did not alter the expression of surface markers on eosinophils in bronchial alveolar lavage fluid, or the degranulation of eosinophils in the airways.⁵ However, this study evaluated the effects of mepolizumab after one year of use, and has determined that the long-term use of mepolizumab may induce an alteration in the function of eosinophils.

The CLC protein has been recently reported to be associated with the occurrence of eosinophil extracellular trap cell death (EETosis) and to enhance eosinophilic airway inflammation.^{6,7} Although IL-5 has not been reported to upregulate eosinophil degranulation or EETosis directly, it is known that IL-5 enhances these functions in affiliation with PAF and TNF- α which act as enhancers of eosinophil degranulation and EETosis.^{8,9}

In conclusion, we investigated three patients with severe asthma who displayed clear clinical improvement despite the insignificant reduction of sputum eosinophils following the introduction of mepolizumab. Analysis of sputum from these cases revealed that the levels of ECP and CLC protein in the sputum supernates decreased significantly. From these data, mepolizumab may have a potential in ameliorating eosinophilic airway inflammation by reducing activated eosinophils. In future, EETosis should be directly observed using induced sputum specimens.¹⁰ A larger study group will also be needed to clarify the potential role that mepolizumab plays in the regulation of eosinophils in severe asthma.

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Conflict of interest

The authors have no conflict of interest to declare.

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