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Low Counts of Eosinophils After the 4th Dose of Mepolizumab May be an Independent Risk Factor for Sars-CoV-2 Transmission in Patients with Severe Asthma

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ABSTRACT

Objective: Patients with severe asthma require particular attention during the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic, not only due to the underlying airway disease but also in regard to the biological treatments received. Mepolizumab has a negative effect on the number and development of eosinophils, and the assertion that eosinopenia is a poor prognostic factor for Coronavirus disease 2019 (COVID-19) leads to confusion regarding the impact of mepolizumab on the course of COVID-19. Therefore, we aimed to demonstrate the potential risk factors for SARS-CoV-2 infection transmission in patients who were using mepolizumab for the treatment of severe asthma and had SARS-CoV-2 infection during their follow-up in this study.

Materials and Methods: The medical records of 27 adult patients who were being followed-up with a diagnosis of severe asthma and using mepolizumab [Female (F): 17, Male (M): 10] were reviewed to determine whether they had experienced SARS-CoV-2 infection within 1 year after March 2020 (between March 2020 and March 2021).

Results: After 1-year of follow-up, the rate of SARS-CoV-2 PCR positivity among patients receiving mepolizumab was 23.2% (six patients). As a result of univariate analysis, the eosinophil count after the 4th dose of mepolizumab treatment was found to be an independent predictor of SARS-CoV-2 PCR positivity (p:0.026, Odd ratio: 0.957, 95% of confidence interval: 0.920-0.995).

Conclusion: A reduced eosinophil percentage after mepolizumab treatment was found to be a risk factor for SARS-CoV-2 transmission. However, it is obvious that more extensive studies on the effect of low counts of eosinophils on SARS-CoV-2 transmission risk are needed, and clinicians caring for this patient group need to follow-up patients with low eosinophil counts closely for SARS-CoV-2 transmission.

Keywords: Eosinophils, mepolizumab, SARS-CoV-2

INTRODUCTION

Since the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China, it has aggressively spread worldwide and affected the significant majority of the world population, causing one of the greatest pandemics of human history (1, 2). Although some vaccines were introduced by 2021, there is still no consensus on the treatment of Coronavirus disease 2019 (COVID-19). It is therefore important to determine prognostic risk factors and organize the treatment of the patients by taking into consideration these factors, especially in susceptible patient groups with chronic diseases. Patients with severe asthma require particular attention during the SARS-CoV-2 pandemic, not only due to the underlying airway disease but also in regard to the biological treatments received. The virus that infects the respiratory mucosa is a risk factor for asthma exacerbations and many guidelines recommend continuation of treatment with biological agents for the control of asthma exacerbations during the pandemic (3, 4).

Mepolizumab is a monoclonal antibody against interleukin (IL)-5 and has been shown to reduce the frequency

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of asthma exacerbations in patients with severe asthma having a peripheral blood eosinophil count of at least 150 cells/ml (5). There are studies demonstrating that mepolizumab regulates the cellular response triggered by respiratory viruses, has an antiviral effect, and prevents entry of the SARS-CoV-2 virus into respiratory cells (6, 7). On the other hand, its negative effect on the number and development of eosinophils, and the assertion that eosinopenia is a poor prognostic factor for COVID-19, as well as the hindering of various stages of type 2 inflammation by mepolizumab, like all biological agents used for the treatment of asthma, lead to confusion in the impact of mepolizumab on the course of COVID-19 in patients with severe asthma. Therefore, we aimed in this study to compare the clinical and laboratory features of the patients with severe asthma who had been treated with mepolizumab and experienced SARS-CoV-2 infection during their follow-up, and to demonstrate potential risk factors for SARS-CoV-2 infection in this patient group.

MATERIALS and METHODS

The medical records of 27 adult patients who were being followed-up with a diagnosis of severe asthma and using mepolizumab [Female (F): 17, Male (M): 10] were reviewed and whether they had experienced SARS-CoV-2 infection within one year after March 2020, when the SARS-CoV-2 epidemic was declared as a pandemic by WHO (World Health Organization) (between March 2020 and March 2021), was investigated. The patients who were being followed-up with a diagnosis of severe asthma at our clinic by March 2020 and receiving monoclonal antibody treatment were investigated during control visits to determine whether they had experienced the SARS-CoV-2 infection, whether they had been hospitalized, and the treatments they had received. Apart from this information, the patients' work-up results for the diagnosis of severe asthma [complete blood count, eosinophil count, immunoglobulin (Ig) E, allergen-specific IgE values, skin prick test results, etc.] were evaluated retrospectively from the records. When needed, the patients were contacted via phone calls and asked whether they had experienced the SARS-CoV-2 infection.

The diagnosis of asthma was established by using pulmonary function tests (PFT) in patients with a suggestive history and physical examination findings. Spirometric measurements were obtained using a common protocol with the nSpire ZAN 100 spirometer. FEV1 (Forced expiratory volume in the first second), the FEV1/FVC (forced vital capacity) ratio, and FVC for similar age, sex, race, and height values were recorded.

Each case was subject to an allergy skin test with standardized inhalant allergens (ALK, Madrid, Spain). The skin prick test was performed by using general inhalant allergens, house dust mite (Dermatophagoides farinae, Dermatophagoides pteronyssinus), cat (Felis domesticus), dog (Canis familaris), cockroach (Blatella germanica), fungi (Alternaria, Cladosporium, Aspergillus), and pollen mixtures (tree, weed, grass). The patients with allergen sensitivity were considered to have allergic asthma, whereas those without allergen sensitivity were considered to have non-allergic asthma. House dust mites, mold, cockroach, fungi, and animal dander sensitivity were considered indoor allergen sensitivity.

Complete blood count was performed with the Sheath reagent using the Abbott Cell Dyn 3700 series (Chicago, USA). Quantitative determination of serum IgE was made by means of particle-enhanced immunonephelometry using the Siemens BN II/BN ProSpec system (Erlingen, Germany).

Statistical evaluation was performed by using the SPSS (Statistical Package for the Social Sciences)-25 program. Quantitative data were given as mean ± standard deviation (SD), whereas qualitative data were given as percentages. Nominal (unclassified) variables between the SARS-CoV-2 PCR (+) and SARS-CoV-2 PCR (-) patients were analyzed with the $\chi 2$ independence test. The normality of the numerical data was firstly evaluated with the Shapiro-Wilk test. For nonparametric data, the Kruskal-Wallis independent samples test was used for multiple comparisons and the Mann-Whitney U independent samples test was used for paired comparisons. For parametric data, the Levene test was used for multiple comparisons and Student's t-test was used for paired comparisons. As a result of these statistical comparisons, parameters with a p value <0.2 between SARS-CoV-2 (+) patients and SARS-CoV-2 (-) patients were subjected to regression analysis. Binomial logistic regression analysis was performed to determine independent predictors for SARS-CoV-2 infection. A p value <0.05 was considered statistically significant.

The study protocol was approved by the Ethics Committee (Meeting date: 15.12.2020, Decision number: 2020/019). Informed consent was obtained from all the patients participating in the study.

RESULTS

A total of 27 patients, consisting of 17 women (63%) and 10 men (37%) were included in the study. The mean age of the patients was 45.85 ± 13.22 years. Twelve patients (44.4%) were being followed-up with the diagnosis of allergic asthma, whereas 15 patients (55.6%) were being followed-up with non-allergic asthma. The patients' mean duration of the diagnosis of asthma was 10 years and the mean duration of the asthma treatment was 7.50 (6-12) years. Twenty-five patients (92.6%) had accompanying chronic rhinosinusitis and 21 patients (77.8%) had an accompanying nasal polyp. The rate of SARS-CoV-2 PCR positivity among patients receiving mepolizumab was 23.2% (6 patients). During the 1-year follow-up, there was a significant difference between the SARS-CoV-2 PCR (+) and SARS-CoV-2 PCR (-) patients in terms of eosinophil count (p=0.010). Demographic, laboratory, and clinical features of the patients and comparison of SARS-CoV-2 PCR (+) and SARS-CoV-2 PCR (-) patients are summarized in Table I. Comparison of the eosinophil count of the patients after the 4th dose of mepolizumab is shown in Figure 1.

As a result of the univariate analysis, the eosinophil count after the 4th dose of mepolizumab treatment was found to be an independent predictor of SARS-CoV-2 PCR positivity (p:0.026, Odd ratio (OR): 0.957, 95% of confidence interval: 0.920-0.995) (Table II).

General features of the patients who were found to be SARS-CoV-2 PCR (+) during follow-ups are summarized in Table III.

DISCUSSION

The COVID-19 pandemic continues to cause mortality and morbidity worldwide and it had caused about 2,800,000 deaths by the 1st anniversary of the declaration of the pandemic (March 2020) (8). Although there is still no consensus, several associations and guidelines recommend continuation of treatment with biological agents in patients with severe asthma in order to control the disease and prevent exacerbations (3, 4). Mepolizumab, which is an IL-5 monoclonal antibody used for the treatment of severe asthma, reduces blood and sputum eosinophilia and thus reduces the eosinophil count below normal (9). There are studies demonstrating that mepolizumab has effects on the macrophage, B-cell, and neutrophil responses triggered by the rhinovirus and prevents entry of the SARS-CoV-2 virus into respiratory cells (7, 10). On the other hand, the use of mepolizumab in this patient group has been brought into doubt due to the studies from the beginning of the pandemic showing that eosinopenia is a risk factor for mortality from COVID-19 (11, 12). In the current study, 23% of the patients with severe asthma receiving mepolizumab were determined to be SARS-CoV-2 PCR (+) by the end of the 1-year follow-up. The eosinophil count after the 4th dose of mepolizumab was determined to be lower in SARS-CoV-2 PCR (+) patients compared to SARS-CoV-2 PCR (-) patients. Furthermore, the eosinophil count after the 4th dose of mepolizumab was found to be an independent risk factor for SARS-CoV-2 infection.

Eger et al. reported a diagnosis of COVID-19 in nine (1.42%) of 634 patients with severe asthma who were be-

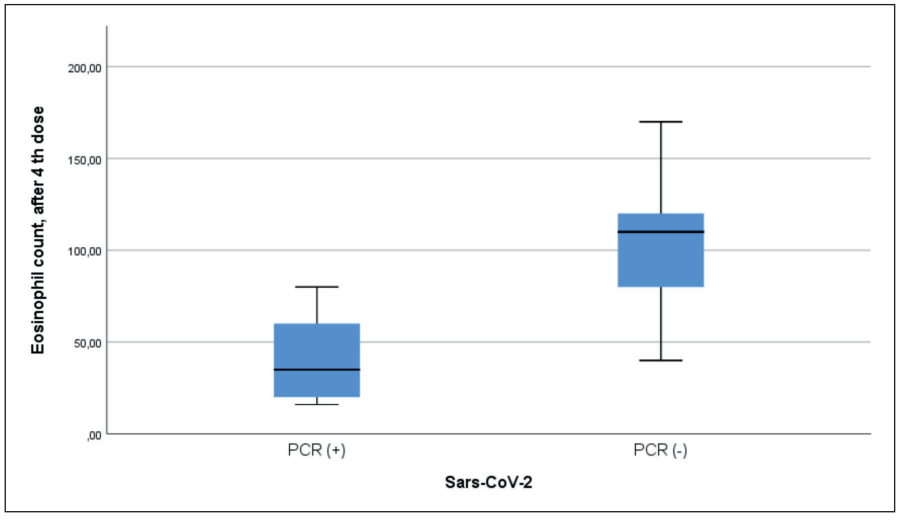

Figure 1. Comparison of eosinophil count of patients after the 4th dose of mepolizumab.

Table I: Demographic, clinical, respiratory and laboratory parameters of the patients.

Parameters	Study population (n=27)	SARS-CoV-2 PCR (+) (n=6)	SARS-CoV-2 PCR (-) (n=21)	p
Demographic Parameters				
Age, year	45.85 ± 13.22	46.83 ± 14.73	45.57 ± 13.14	0.841
Gender, F, n (%)	17 (63)	3 (50)	14 (66.7)	0.456
BMI	27.91 ± 4.30	28.35 ± 3.34	27.27 ± 4.43	0.587
Smoking, n (%)				
Non-smoker	23 (81.5)	5 (83.3)	17 (81)	0.852
Smoker	3 (11.1)	1 (16.7)	2 (9.5)	
Ex-smoker	2 (7.4)	0	2 (9.5)	
Diagnosis, n (%)				0.756
Allergic asthma	12 (44.4)	3 (50)	9 (42.9)	
Non-allergic asthma	15 (55.6)	3 (50)	12 (57.1)	
Skin prick test, n (%)				
Atopy	12 (44.4)	3 (50)	9 (42.9)	0.557
Indoor allergens	9 (33.3)	3 (50)	6 (28.6)	0.326
Non-atopic	15 (55.6)	3 (50)	12 (57.1)	0.557
Duration of asthma, years	10 (2-29)	7.50 (4-20)	10 (2-29)	0.441
Duration of asthma treatment, years	7.50 (6-12)	6.50 (3-8)	6 (3-12)	0.670
Respiratory Properties				
FEV1, %, before*	60.74 ± 18.15	72.40 ± 10.85	59.67 ± 18.60	0.158
FEV1, %, after*	68.28 ± 17.93	75.40 ± 12.94	68.95 ± 19.39	0.490
FVC, %, before*	64.91 ± 18.56	71.60 ± 15.32	64.38 ± 19.02	0.439
FVC, %, after*	74.34 ± 16.0	77.80 ± 11.39	75.29 ± 17.12	0.759
FEV1/FVC, %, before*	81 (60-91)	81 (78-91)	81 (60-90)	0.950
FEV1/FVC, %, after*	81 (64-103)	80 (78-91)	81 (64-103)	0.950
Laboratory parameters				
Eosinophil count, before*	965 (310-2390)	705 (390-1150)	1000 (310-2390)	0.140
Eosinophil count, after*	95 (10-450)	40 (20-80)	110 (10-450)	**0.010**
Lymphocyte count, before*	2621.15 ± 697.28	2545 ± 729.02	2642.91 ± 704.94	0.768
Lymphocyte count, after*	2665.22 ± 654.15	2224 ± 821.21	2694.50 ± 609.03	0.162
Neutrophil count, before*	4900.44 ± 1359.47	5336.67 ± 2709.05	4890 ± 1381.69	0.582
Neutrophil count, after*	4864.76 ± 1363.92	5336.67 ± 2709.05	4890 ± 1381.69	0.582
Platelet count, $x10^3$, before*	314.78 ± 88.77	273.50 ± 35.95	320.90 ± 91.77	0.232
Platelet count, $x10^3$, after*	288.30 ± 55.69	252.60 ±48.08	300.75 ± 52.44	0.075
IgE, before*	144 (17-1560)	154.50 (40-1560)	119 (17-897)	0.408
IgE, after*	132 (18.50-1130)	114 (38.30-723)	134 (18.5-1130)	0.679

SARS-CoV-2 PCR: Severe acute respiratory syndrome coronavirus 2 polymerase chain reaction, **F:** Female, **BMI:** Body Mass Index, **FEV1:** Forced expiratory volume in 1 second, **FVC:** Forced Vital capacity, **ER:** Emergency Room, **IgE:** Immunoglobulin E, **ACT:** Asthma control test, **Before***: before mepolizumab treatment, **After*:** after 4 doses of mepolizumab treatment.

ing treated with biological agents between 17th March 2020 and 30th May 2020 (13). It was reported that seven of these nine patients were hospitalized due to hypoxemia and oxygen need, and five of these seven hospitalized patients were transferred to the intensive care unit due to the need for intubation and mechanical ventilation with one death being reported (13). In our study, the rate of SARS-CoV-2 PCR positivity among patients receiving mepolizumab was

Table II: Univariate binomial regression analyses demonstrating the relationship between baseline characteristics and SARS-CoV-2 PCR positivity.

Variables	Univariants		Variables	Univariants	
	OR (95% CI)	P value		OR (95% CI)	P value
Eosinophil count, before*	0.998 (0.995-1.001)	0.173	Eosinophil count, after*	0.957 (0.920-0.995)	**0.026**
FEV1, before*	0.951 (0.885-1.022)	0.174	Platelet count, after*	1.020 (0.997-1.044)	0.090
Lymphocyte count, after*	1.001 (0.999-1.003)	0.170			

SARS-CoV-2 PCR: Severe acute respiratory syndrome coronavirus 2 polymerase chain reaction, **FEV1:** Forced expiratory volume in 1 second, **Before*:** Before mepolizumab treatment, **After*:** After 4 doses of mepolizumab treatment.

Table III: Clinical and laboratory properties of Sars-CoV-2 PCR (+) patients.

P	G	A	CRS	NP	E (%), before	E (%), after	E (cell/ml), before	E (cell/ml after	FEV1 (%), before	FEV1 (%), after
1	F	47	+	+	5.70	0.7	390	80	85	91
2	M	63	+	+	8.50	0.5	900	60	79	80
3	F	28	+	-	11.70	0.6	660	40	58	67
4	F	61	+	-	10.20	0.4	1150	30	75	81
5	M	51	+	+	10.10	0.3	750	20	65	58
6	M	31	+	+	3.80	0.1	580	16	63	68

SARS-CoV-2 PCR: Severe acute respiratory syndrome coronavirus 2 polymerase chain reaction, **P:** Patient, **G:** Gender, **A:** Age, **CRS:** Chronic rhinosinusitis, **E:** Eosinophil, **FEV1:** FEV1: Forced expiratory volume in 1 second.

23.2%. Although the study population was small, the inclusion of a 12-month period, the inclusion of only the patient receiving mepolizumab treatment, and potential variants of the virus are thought to have caused this difference.

Eosinophils are tissue-resident leukocytes, with a role in the formation of the type 2 inflammatory environment in the lungs (10). There are hypotheses that type 2 inflammation reduces the level of Angiotensin-converting enzyme 2 (ACE2) receptors in respiratory epithelial surfaces and that type 2 inflammation may thus be protective against SARS-CoV-2 transmission (13). Type 2 inflammation may also contribute to the prevention of the hyperinflammatory process, the major cause of mortality in COVID-19, by reducing interferon responses (13). In addition, eosinophils have been shown to act as antigen-presenting cells in lung tissue, have antiviral activity against influenza virus and respiratory syncytial virus, and reduce viral infectivity in several in vivo and in vitro studies (10, 14, 15). Thus, it can be thought that mepolizumab, which acts by reducing the eosinophil count, can make a negative contribution to the COVID-19 process. When considered from this point of view, it is unsurprising that low eosinophil count after the 4th dose of mepolizumab was a risk factor for SARS-CoV-2 transmission. Interestingly, the course of the disease was relatively mild in SARS-CoV-2 PCR (+) patients with severe asthma receiving mepolizumab. In a study by Azim et al., in which they evaluated four patients who were receiving mepolizumab and were diagnosed with COVID-19, they reported that the eosinophil count was 0 cell/mm^3 in three patients and 100 cells/mm^3 in the other patient at the time of diagnosis of COVID-19 (16). Three patients recovered from COVID-19 with self-isolation, whereas one patient was hospitalized and then discharged without admission to the intensive care unit (16). Aksu et al. also reported one patient with severe asthma receiving mepolizumab who was diagnosed with COVID-19 and recovered from COVID-19 without needing hospitalization (17). In our study, all SARS-CoV-2 PCR (+) patients were treated as outpatients, and none of the patients required hospitalization. Forster-Ruhrmann et al. attributed a relatively mild course of COVID-19 in a patient who was receiving dupilumab for chronic rhinosinusitis and nasal polyp and diagnosed with COVID-19 to the increased eosinophil count after the use of dupilumab (18).

The relatively small study population and presentation of a single-center experience are the major limitations of our study.

In conclusion, the eosinophil count that was reduced after mepolizumab treatment was found to be a risk factor for SARS-CoV-2 transmission. However, more extensive studies on the effect of low counts of eosinophils on SARS-CoV-2 transmission risk are obviously needed, and clinicians caring for this patient group need to follow-up patients with low eosinophil counts closely for SARS-CoV-2 transmission.

Authorship Contributions

Concept: **Emel Atayık, Gökhan Aytekin,** Design: **Emel Atayık, Gökhan Aytekin,** Data collection or processing: **Emel Atayık, Gökhan Aytekin,** Analysis or Interpretation: **Emel Atayık, Gökhan Aytekin,** Literature search: **Emel Atayık, Gökhan Aytekin,** Writing: **Emel Atayık, Gökhan Aytekin,** Approval: **Emel Atayık, Gökhan Aytekin.**

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