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ABOUT

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Thoracic Research and Practice covers a wide range of topics related to adult and pediatric pulmonary diseases, as well as thoracic imaging, environmental and occupational disorders, intensive care, sleep disorders and thoracic surgery, including diagnostic methods, treatment techniques, and prevention strategies. The journal is interested in publishing original research that addresses important clinical questions and advances the understanding and treatment of these conditions. This may include studies on the effectiveness of different treatments, new diagnostic tools or techniques, and novel approaches to preventing or managing pulmonary diseases.

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Original Article

Comparison of Alectinib/Crizotinib Data in First-Line Therapy in Patients with Anaplastic Lymphomakinase-Positive Nonsmall Cell Lung Carcinoma with Poor Prognostic Features for Alectinib

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Abstract

OBJECTIVE: Alectinib has a much better central nervous system transmission than crizotinib in patients diagnosed with anaplastic lymphoma kinase mutation-positive nonsmall cell lung carcinoma. We aimed to investigate alectinib's efficacy in the treatment and its place in the first-line treatment and report our real-life data.

MATERIAL AND METHODS: The data of 38 patients who were diagnosed with anaplastic lymphoma kinase-positive nonsmall cell lung carcinoma in our clinic between 2016 and 2021, who did not receive any treatment before were retrospectively analyzed.

RESULTS: Of the 19 patients who received alectinib, 14 had multiple, and 6 had pretreatment brain metastases. No newly emerging brain metastases were detected during the treatment period. The progression-free survival of patients was 23.5 ± 4.2 months, and overall survival was 24.6 ± 4.1 months. Progression was observed in 10 (52.6%) patients. Of the 19 patients who received crizotinib, 7 had multiple metastases, and brain metastases were detected in 1 patient before treatment and 6 patients during the treatment period. Progression-free survival of crizotinib patients was 17.1 ± 4.8 months and their overall survival was 26.5 ± 6.1 months. Progression was observed in 17 (89.5%) patients. The second line of alectinib could be given to 8 of these patients. Overall survival after second-line treatment of alectinib was 18.2 ± 7.0 months. Overall survival of the patients who could not receive second-line treatment of alectinib was 4.0 ± 2.0 months.

CONCLUSION: The progression rate was lower in alectinib than the crizotinib patients, although there were more patients with multiple metastases and brain metastases in the alectinib arm.

KEYWORDS: Nonsmall cell lung carcinoma, anaplastic lymphomakinase mutation, alectinib, crizotinib, brain metastasis
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INTRODUCTION

The survival of patients with metastatic nonsmall cell lung carcinoma (NSCLC) is very low. However, it has been significantly prolonged with the development of targeted therapies in anaplastic lymphoma kinase (ALK) mutation-positive patients with chromosomal rearrangements in the ALK gene.[1] This condition which is observed in 3%-7% of patients with NSCLC and is related to the second chromosome, increases tumor cell proliferation and survival by increasing the tyrosine kinase activity of the ALK receptor.[2] Anaplastic lymphoma kinase positivity, first described by Soda et al[3] in 2007, is found more frequently in young patients with adenocarcinoma who have never smoked or rarely smoked.[4]

Brain metastasis is observed in approximately 20%-30% of patients with ALK-positive NSCLC at the time of diagnosis and in 50% of the patients during the course of the disease.[5] During the progression of the disease, brain metastases are most common.[6] The presence of brain metastases is also closely associated with high morbidity and mortality.[7] With the targeted therapies, the overall survival (OS) has increased in this patient group with frequent brain metastases.[8]

Crizotinib, the first ALK receptor inhibitor, has been used successfully for a long time due to its superiority over standard chemotherapy.[9] However, brain metastases were commonly seen during the treatment course due to poor central nervous system (CNS) transmission.[10] As a result of the development of drug resistance and brain metastases under treatment, usually within the first year, new searches have started for treatment of these groups of patients.[2,11,12]

Alectinib, a second-generation tyrosine kinase inhibitor (TKI), has a very good CNS transmission due to its lipophilicity. Therefore, it prevents the development of brain metastases and the progression of existing metastases, provides

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a longer progression-free survival (PFS) than crizotinib and has lower toxicity.[13] Due to the absence of a substrate for the *p*-glycoprotein that maintains the flow in the blood–brain barrier, it can penetrate the CNS effectively.[14] Despite its very strong efficacy, resistance is definitely developing against alectinib, too.[15]

The aim of our study is to evaluate the efficacy of alectinib and crizotinib on survival and brain metastasis in first-line therapy in real life.

MATERIAL AND METHODS

The data of 38 patients diagnosed with NSCLC patients with ALK-positive in our clinic between June 2016 and April 2021 were treated with ALK TKI as a first-line treatment. They did not receive any chemotherapy regimen retrospectively analyzed. Patients who were not followed up and treated in our oncology clinic were not included in the study.

Demographic characteristics of the patients, stages, metastasis status, treatments, PFS, and OS times of the patients were recorded. Patients were categorized according to crizotinib or alectinib administration in first-line therapy and whether they received second-line therapy in the event of progression. Progression-free survival and OS were calculated for patients who did not progress and survived until April 30, 2021, and all data were recorded.

The study is approved by the ethics committee of Dr. Suat Seren Chest Diseases and Surgery Training and Research Hospital at the 10th meeting dated May 26, 2021, with the first decision number. The study was conducted in accordance with the Helsinki Declaration. An informed consent form is not required due to the fact that it is a retrospective study.

Statistical Analyses

Analyses were performed with Statistical Package for Social Sciences (SPSS) software version 22 (IBM Corp.; Armonk, NY, USA). The normality of the data was evaluated with Shapiro–Wilk and Kolmogorov–Smirnov tests. After descriptive statistics, the chi-square test was used to compare categorical variables between groups. Mann–Whitney *U*-test and Student's *t*-test were used for comparing continuous variables. Results were given as median (min-max), mean ± SD, number, and percentage (%). Kaplan–Meier analysis was used to evaluate the effect of parameters on survival. Results were presented with both mean and median values, with 95% confidence intervals. *P*-value < .05 was considered statistically significant in all analyses.

MAIN POINTS

- In lymphoma kinase mutation-positive nonsmall cell lung carcinoma, alectinib is superior to crizotinib in the first-line treatment.
- Although the number of metastases is higher, alectinib provides more benefits than crizotinib.
- Alectinib is also effective in existing brain metastases.
- Alectinib reduces the risk of developing brain metastases.

RESULTS

Thirty-eight ALK-positive NSCLC patients who were treated with ALK TKI as a first-line treatment were included in the study. The mean age of the patients was 56.0 ± 11.5, 24 (63.2%) of them were male, and 8 (21.1%) of them had no history of smoking. All of the patients had adenocarcinoma type and were all in stage 4. The most common sites of metastasis were pleura in 15 patients (39.5%), bone in 11 (28.9%), and contralateral lung in 10 patients (26.3%), respectively. Brain metastases were detected in 7 (18.4%) patients at admission and 6 (15.8%) during the treatment period. Nineteen patients were each given crizotinib or alectinib as first-line therapy. For 8 patients who progressed after crizotinib therapy, alectinib had administered as second-line therapy. More detailed data of the patients are shown in Table 1.

A comparison of demographic and clinical findings between alectinib and crizotinib is shown in Table 2. There were 14 multiple metastatic patients in the alectinib therapy, while this number was 7 in the crizotinib therapy ($P = .022$). There were 6 patients with brain metastases before treatment with alectinib; no new brain metastases were observed during the treatment period. There was only 1 patient with brain metastases before treatment with crizotinib; 6 patients had developed brain metastases during the treatment period ($P = .006$).

Progression was observed in 10 (52.6%) patients in the alectinib group and in 17 (89.5%) patients in the crizotinib group ($P = .012$).

Although PFS was 23.5 ± 4.2 months, OS was 24.6 ± 4.1 months in the alectinib group, PFS was 17.1 ± 4.8 months and OS 26.5 ± 6.1 months in the crizotinib group, it was not statistically significant in terms of both PFS and OS ($P = .187$, $P = .383$, respectively) (Table 3).

As seen in Table 4, PFS was 34.9 ± 6.8 months in female patients and was 13.5 ± 3.2 months in males, and OS was 43.4 ± 7.2 months in females and 22.2 ± 5.1 months in males for all patients who received alectinib or crizotinib ($P = .021$ and $P = .035$). However, when analyzed by drug arms, female patients had longer PFS and OS in both drug arms, while PFS was significantly longer in female patients using only crizotinib ($P = .042$).

The mean OS was 18.2 ± 7.0 in 8 patients treated with alectinib treatment as second-line therapy after progression, while the mean OS of 12 patients who could not be treated with alectinib was 4.0 ± 2.0 months, which was significantly shorter ($P = .004$) (Table 5).

DISCUSSION

Many studies showed that TKIs used in ALK-positive patients with NSCLC were superior to chemotherapy, and alectinib was more effective on both PFS and OS than crizotinib.[16] In our patient group, we tried to answer whether alectinib, which we have used so far in first-line therapy, is superior to crizotinib and whether alectinib is effective against brain metastases or, more importantly, does it prevent their occurrence.

Table 1. Clinical Features of the Patients

Age, mean ± SD (minimum–maximum)	**56.0 ± 11.5 (32.0-75.0)**
Sex, n (%)	
Female	14 (36.8)
Male	24 (63.2)
Smoking status, n (%)	
Current	17 (44.7)
Ex-smoker	8 (21.1)
Never	13 (34.2)
Disease stage at baseline, n (%)	
Stage IV	38 (100)
Metastases, n (%)	
Solitary	17 (44.7)
Multiple organs	21 (55.3)
CNS metastasis, n (%)	7 (18.4)
Bone metastasis, n (%)	11 (28.9)
Liver metastasis, n (%)	7 (18.4)
Surrenal metastasis, n (%)	9 (23.7)
Contralateral lung metastasis, n (%)	10 (26.3)
Pleura metastasis, n (%)	15 (39.5)
LAP metastasis, n (%)	6 (15.8)
The other organ metastasis, n (%)	9 (23.7)
CNS metastasis, n (%)	
No	25 (65.8)
At admission	7 (18.4)
After treatment	6 (15.8)
First-line treatment, n (%)	
Alectinib	19 (50.0)
Crizotinib	19 (50.0)
Second-line treatment, n (%)	
No	30 (78.9)
Alectinib	8 (21.1)
Crizotinib	0 (0.0)
Progression, n (%)	27 (71.1)
Mortality, n (%)	22 (57.9)

CNS, central nervous system; LAP, lymphadenopathy.

Table 2. Comparison of Demographic and Clinical Features Between Drug Groups

	Alectinib (n = 19)	Crizotinib (n = 19)	*P*
Age (mean ± SD)	58.1 ± 10.4	53.9 ± 12.4	.276
Sex, n (%)			
Female	7 (36.8)	7 (36.8)	1.000
Male	12 (63.2)	12 (63.2)	
Smoking status, n (%)			
Current	6 (31.6)	11 (57.9)	
Ex-smoker	6 (31.6)	2 (10.5)	.167
Never	7 (36.8)	6 (31.6)	
Disease stage, n (%)			1.000
Stage IIIB	0 (0.0)	0 (0.0)	
Stage IV	19 (100.0)	19 (100.0)	
Metastasis, n (%)			
Solitary	5 (26.3)	12 (63.2)	.022
Multiple organ	14 (73.7)	7 (36.8)	
CNS metastasis, n (%)	6 (31.6)	1 (5.3)	.042
Bone metastasis, n (%)	7 (36.8)	4 (21.1)	.283
Liver metastasis, n (%)	5 (26.3)	2 (10.5)	.405
Surrenal metastasis, n (%)	7 (36.8)	2 (10.5)	.124
Contralateral lung metastasis, n (%)	5 (26.3)	5 (26.3)	1.000
Pleura metastasis, n (%)	8 (42.1)	7 (36.8)	.740
LAP metastasis, n (%)	3 (15.8)	3 (15.8)	1.000
Other organ metastasis, n (%)	4 (21.1)	5 (26.3)	1.000
CNS metastasis, n (%)			
No	13 (68.4)	12 (63.2)	
At admission	6 (31.6)	1 (5.3)	.006
After treatment	0 (0.0)	6 (31.6)	
Progression, n (%)	10 (52.6)	17 (89.5)	.012
Mortality, n (%)	9 (47.4)	13 (68.4)	.189

CNS, central nervous system; LAP, lymphadenopathy.

We retrospectively analyzed the data of 38 ALK-positive NSCLC patients who were diagnosed, treated, and followed up in our oncology clinic. The presence of 19 patients in both the alectinib and the crizotinib group was a good coincidence, in our opinion, in terms of ensuring homogeneous distribution. Patients in the alectinib group had a greater mean age, higher metastasis rates ($P = .022$), and more patients who had brain metastases before treatment ($P = .006$). However, progression was observed in 10 (52.6%) patients in the alectinib arm and 17 (89.5%) patients in the crizotinib arm ($P = .012$). This shows that alectinib administered to the older group, which had more brain metastases and multiple metastases, was more successful than crizotinib in disease control. Our results are consistent with Alex's study; the incidence of progression or death was 41% with alectinib and 68% with crizotinib, following the administration of alectinib and crizotinib for similar durations.[6]

There was only 1 patient who had brain metastasis before treatment, and 6 patients had developed brain metastases during the treatment in the crizotinib group, while in alectinib group there were 6 patients with brain metastases before treatment; no new brain metastases were detected during the treatment period $P = .006$). This difference shows alectinib is a lipophilic agent, and it can easily cross the CNS barrier, which prevents the formation of new metastases in the CNS. Our data showing the superiority of alectinib in brain metastasis showed similarity with Alex's study evaluating the CNS efficacy.[14]

Table 3. Comparison of PFS and OS Between Drug Groups

	PFS Mean		PFS Median		
	Estimated	**95% CI**	**Estimated**	**95% CI**	***P***
Alectinib	23.5 ± 4.2	15.4-31.7	39.3	6.4-72.2	.187
Crizotinib	17.1 ± 4.8	7.8-26.5	5.8	1.6-9.9	
	OS Mean		OS Median		
	Estimated	95% CI	Estimated	95% CI	*P*
Alectinib	24.6 ± 4.1	16.5-32.7	-	-	.383
Crizotinib	26.5 ± 6.1	14.6-38.4	9.2	0.0-24.6	

OS, overall survival; PFS, progression-free survival.

Table 4. The Effect of Gender, Smoking, and Metastasis Status on PFS and OS

	PFS Mean		PFS Median		
	Estimated	**95% CI**	**Estimated**	**95% CI**	***P***
Sex					.021
Female	34.9 ± 6.8	21.5-48.2	39.3	6.9-71.7	
Male	13.5 ± 3.2	7.3-19.7	4.1	0.0-8.5	
Smoking status					.062
Current	12.8 ± 3.6	5.7-19.9	4.9	0.7-9.1	
Ex	18.9 ± 6.5	6.2-31.6	4.1	0.0-24.9	
Never	35.7 ± 7.4	21.1-50.3	54.5	6.6-102.3	
Metastasis					.412
Solitary	24.5 ± 6.0	12.7-36.3	15.6	1.7-29.4	
Multiple	20.2 ± 5.2	10.0-30.3	10.0	0.0-21.6	
	OS Mean		OS Median		
	Estimated	95% CI	Estimated	95% CI	*P*
Sex					.035
Female	43.4 ± 7.2	29.3-57.4	-	-	
Male	22.2 ± 5.1	12.2-32.1	9.2	0.0-19.9	
Smoking status					.235
Current	22.9 ± 6.1	10.9-34.8	9.2	2.1-16.2	
Ex	20.4 ± 6.1	8.5-32.3	17.9	0.0-39.1	
Never	41.5 ± 7.7	26.5-56.5	-	-	
Metastasis					.689
Solitary	31.5 ± 6.5	18.8-44.3	22.0	0.0-44.7	
Multiple	29.6 ± 6.3	17.2-42.0	14.8	5.2-24.4	

OS, overall survival; PFS, progression-free survival.

Table 5. OS Between Second-Line Treatment Groups

	OS Mean		OS Median		
	Estimated	**95% CI**	**Estimated**	**95% CI**	***P***
Best supportive treatment (n = 12)	4.0 ± 2.0	0.3-8.0	0.2	0.0-1.1	.004
Alectinib (n = 8)	18.2 ± 7.0	4.4-32.0	4.0	0.0-8.0	
Overall	8.3 ± 3.1	2.3-14.3	1.2	0.4-1.9	

OS, overall survival.

In the alectinib group, PFS was calculated as 23.5 ± 4.2 months, and in crizotinib group, PFS was 17.1 ± 4.8 months. Although alectinib provided a greater PFS like 6 months, this time was not statistically significant. However, we still think that this period should not be underestimated and may be marked by an increase in the number of patients.

In ALEX study[1], the independent investigator evaluated PFS 34.8 versus 10.9 months HR = 0.43, and alectinib has been shown to be superior to crizotinib. The lack of statistically significant difference between the 2 groups in our study is related to the higher number of patients with baseline brain metastases and multiple metastases with poor prognostic features in the alectinib arm and the low number of patients in both arms. Also, there were 9 patients in the alectinib arm, while 2 patients in the crizotinib arm did not progress at the time of data entry. This is also related to the fact that we were able to give alectinib to the last patients despite the fact that crizotinib treatment was given to the first patients because alectinib was reimbursed too late by the government so that the crizotinib data were finalized. However, the alectinib data needed more time to be finalized. Our real-life data for alectinib will be more accurate in the coming years. Khan et al, in a meta-analysis of 12 studies involving 3297 patients showed that alectinib and other new-generation ALK inhibitors were significantly superior to crizotinib in PFS.[17]

In our study, OS was 24.6 ± 4.1 months in the alectinib group and 26.5 ± 6.1 months in the crizotinib group. When Mok et al[1] updated the OS data of ALEX study, the mean OS for alectinib was 48.2 months versus 23.3 months for crizotinib, which made alectinib significant in patients with and without CNS metastases. Moreover, 34.9% of the patients in the alectinib group and 8.6% in the crizotinib group were still on treatment. The difference between this study and ours was that patients who progressed with crizotinib were not received alectinib.[1] Although it was not statistically significant, the reasons for the poor survival in the alectinib group in our study were thought to be due to the high number of patients with poor prognostic features such as brain metastases. Also, approximately half of the patients in the crizotinib arm had received alectinib treatment after progression.

When we evaluated the PFS and OS of both alectinib and crizotinib together by gender, it was statistically significantly better in female patients (P = .021 for PFS and P = .035 for OS). However, when we examined it separately according to the drug groups, we found this positive effect in the female gender only for PFS in crizotinib patients (P = .042). The female gender created the fact that we could not find statistically significant in other groups in both PFS and OS for all the remaining cases of the inadequacy of the number of patients, which was the most critical limiting factor in our study. Similar to our research, Zou et al[18] also defined crizotinib as an influential factor for longer PFS in women (P = .003), but they could not find the same relationship with alectinib.

Of the 38 patients in our study, we found that 10 (52.6%) of them progressed while using alectinib during treatment and 17 (89.5%) while using crizotinib (P = .012). Alectinib could be given to 8 of 17 patients who progressed with crizotinib as second-line therapy. The remaining 19 patients did not receive any treatment. Overall survival of the patients was 18.2 ± 7.0 months who received alectinib after progression, while it was 4.0 ± 2.0 months in the patients who did not receive any treatment (P = .004). In other words, alectinib worked well in patients who developed crizotinib resistance. Then, we may wonder if it would be more logical to save alectinib for the second-line treatment for patients who develop resistance to crizotinib. However, we think that we should not forget the 9 patients who were not given any treatment due to performance impairment. Alex clinical research investigator Peters et al's study found that the rate of 12-month progression-free disease was 68.4% with alectinib and 48.7% with crizotinib, which supported our results.[6] In ALUR study[19], patients who progressed with crizotinib were received alectinib or chemotherapy in the second line of treatment. The alectinib group provided statistically significantly better PFS in both intracranial metastases extracranial metastases compared to the chemotherapy group.

The limitations of our study are that it is retrospective, there are imbalances between the groups in terms of basal characteristics, the number of patients is low, and until recently, only chemotherapy could be used in the progression after alectinib treatment in our country.

In conclusion, in light of all these data, we saw that in patients with ALK-positive NSCLC, alectinib was superior to crizotinib in patients with poor prognostic features in first-line treatment, with or without brain metastasis. In the future, this study will be even more meaningful as the number of patients increases, and the real data of the patients who received alectinib have emerged.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Dr. Suat Seren Chest Diseases and Surgery Training and Research Hospital (Approval No: 2021-15-28).

Informed Consent: An informed consent form is not required due to the fact that it is a retrospective study.

Peer-review: Externally peer-reviewed.

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Original Article

An Analysis of Coronavirus Disease 2019 Infection-Related Characteristics of Chronic Obstructive Pulmonary Disease Patients with and without Inhaler Training: A Case–Control Study

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Abstract

OBJECTIVE: Patients with chronic obstructive pulmonary disease are among the most risky groups for Coronavirus Disease 2019. The study was conducted with a case–control group design in order to determine the coronavirus disease 2019 infection-related characteristics of chronic obstructive pulmonary disease patients with and without inhaler training.

MATERIAL AND METHODS: A total of 106 patients, being case group (n = 53) and control group (n = 53), were included in the study sample through purposive and simple random sampling methods. While the control group did not receive training, the case group received inhaler training in 2017-2018. Inhaler training was conducted face-to-face using the demonstration method. Patients were evaluated according to their coronavirus disease 2019 (approved by a physician positive polymerase chain reaction) status from the beginning of the pandemic to the time they were included in the study (June 2021).

RESULTS: The rate of incorrect inhaler use was determined to be 39.6% of the patients in the control group, which is statistically different from the case group ($P < .001$). It was also determined that 17.9% of the patients in the study sample had contracted coronavirus disease 2019. It was found that the presence of symptoms for at least 1 month following the negative polymerase chain reaction result was significantly higher in the control group ($P = .018$). It was determined that the case group patients were more careful compared to the control group in terms of coronavirus disease 2019 measures ($P < .031$). The patients in the case group reported that inhaler medicines were also effective in protecting against other respiratory system diseases ($P = .006$).

CONCLUSION: Few patients with chronic obstructive pulmonary disease have been infected. It was concluded that the coronavirus disease 2019 symptoms lasted longer in the control group than in the case group.

KEYWORDS: COPD, COVID-19, inhaler, inhaler training
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INTRODUCTION

The new virus, which emerged in 2019, was quite similar to the severe acute respiratory syndrome-coronavirus. It was named SARS-CoV2 because of this similarity.[1] Severe acute respiratory syndrome-coronavirus, which led to a severe epidemic in 2003 and caused thousands of people to lose their lives, appeared again in China in December 2019 and spread all over the world in January 2020. The first case in Turkey was reported on March 11, 2020, and on the same day, the World Health Organization (WHO) declared COVID-19 as a lethal pandemic.[2] Due to COVID-19, more than 490 million people were infected and over 6 million people lost their lives. The number of confirmed cases in Turkey reached 15 million, and the number of those who died was close to 100 000.[3] The high prevalence of the disease is related to its being highly contagious.

The COVID-19 infection is contracted through droplets or direct contact. Not taking protective measures such as the use of a face mask and shield in crowded environments or touching the mouth, nose, eyes, and ear cavities without sufficient hand hygiene causes the virus to enter the body easily. The virus is usually involved in the respiratory system, and symptoms such as dyspnea and cough are intensively observed. In addition, fever, fatigue, arthralgias, and loss of taste and smell have also been reported a lot.[4,5] Individuals above the age of 65 and those with a chronic disease are among the risk groups that COVID-19 affects the most. Due to the involvement of the virus in the respiratory system, patients with chronic obstructive pulmonary disease (COPD) are at great risk in terms of COVID-19. World Health Organization reports that COVID-19 constitutes a high risk for respiratory system diseases which increase the need for oxygen.[6] In a meta-analysis study conducted, it was found that the most risky group was the COPD patients among 1558 COVID-19 patients with comorbid diseases.[7]

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In addition to its involvement in many vital organs, COVID-19 exacerbates the existing respiratory system diseases in patients due to the damage it causes.[8] It has been reported that COVID-19 occurring in COPD patients has more bilateral lung involvement, that the duration from the start of the symptoms to presenting to the hospital is short, that dyspnea is severe, that the number of respirations per minute is high, and that their need for intensive care is 1.88 times higher.[9] It has been found that the risk of severe disease in COPD patients infected by COVID-19 is 4 times as much and that their rates of hospitalization in ICU and mortality are higher.[10] Based on the information mentioned above, it is obvious that COPD patients are a high-risk group in the COVID-19 pandemic process. In this process, it is highly important to continue the treatments of patients more carefully and to provide patients with training more frequently.

The most effective treatment for COPD is inhaler medicines. On the condition that patients use their medicines correctly and timely, they experience the symptoms caused by COPD more mildly.[11] However, it is known that most patients use their medicines incorrectly.[12] This situation continues to keep the negative effects of the ongoing COVID-19 epidemic on COPD patients on the agenda. In a systematic compilation study published in 2020, it was emphasized that COPD patients should continue taking their medicines in the same regimen and that if they are infected by COVID-19 in this process, they should not stop using their inhaler medicines.[13] Similarly, Global Initiative for Chronic Obstructive Lung Disease (GOLD) has announced in its report that there is no evidence suggesting that COPD patients should stop using their medicines, and therefore, they should continue their treatment.[14] Moreover, Chinese Thoracic Society has stated in its report that COPD patients should continue using inhaler medicines and that they should do it with a correct technique.[15] However, the studies conducted are new and limited, and information about COPD patients who are infected by COVID-19 is inadequate. Especially, no study was encountered which examined the correct or incorrect use of inhalers by COPD patients in the COVID-19 process and the effects of this situation on COVID-19. Therefore, it is believed that an original contribution will be made to the literature by determining how COPD patients who were provided with training on inhaler use by the researchers were affected by COVID-19. It was aimed in the study to determine COVID-19-related characteristics of COPD patients with and without training on inhaler use.

MAIN POINTS

- One of the chronic diseases most affected by the pandemic is chronic obstructive pulmonary disease patients.
- The importance of regular and correct use of inhaler medications by patients has come to the fore again.
- However, due to the intensity of the pandemic, both hospital admissions and inhaler training of patients with chronic obstructive pulmonary disease were disrupted.
- It was considered important to investigate the effects of correct inhaler use in patients with coronavirus disease 2019 infection.

The aim of this study was to explain the answers to the following questions: (1) does using inhaler medicines positively contribute to COPD patients in terms of thinking protection against COVID-19? And (ii) can COPD patients who received inhaler medicine use training be protected better against COVID-19 in comparison to those who did not receive training?

MATERIAL AND METHODS

Research Type

The study was conducted as a case–control group research.

Participants

The population of the study consisted of COPD patients who presented to Kırşehir Ahi Evran University Training and Research Hospital Thoracic Diseases Outpatient Clinic. The research was conducted between March and June 2021. One hundred six patients were included in the sample, with 53 patients in each of the case group and control group (Figure 1). In determining the case group, the purposive sampling method was used, and the COPD patients who had been given training on inhaler use by Ceyhan and Tekinsoy Kartin[16] in their study conducted between 2017 and 2018 were considered. In the previous study, a total of 67 COPD patients were provided with inhaler use training. The patients whose contact information was available were contacted again. Thus, the case group was formed with 53 patients. The inhaler usage skills were questioned again before starting this study. It was checked how the patients applied according to each type of inhaler they used. The fact that the patients continued the correct application was associated with the application method of the previous study. In the previous study,[16] each patient was shown the use of one-to-one inhaler by demonstration method and then patients were called twice a week for 4 weeks and it was followed whether the applications were performed correctly. Therefore, at the end of the study, all patients reached the ability to use the correct inhaler. Patients continued to apply correctly because they also benefited from the skill they gained in their symptoms. In addition, it was very pleasing to obtain the result that highly accurate inhaler applications were maintained approximately 2 years after the study.

The control group, on the other hand, was chosen by using simple random sampling method. The patients in the control group were determined among COPD patients who were in the registration system of the physician cooperating in the study and visited the hospital for examination in the last 1 year according to random numbers table. The patients whose contact information was available were contacted, and those who agreed to participate in the study formed the control group of the study.

Inclusion Criteria for the Study

Chronic obstructive pulmonary disease patients who had previously received inhaler use training, who were accessed through their contact information, who still used inhaler medicines in correct steps, and who volunteered to participate in the study were included in the case group. Chronic obstructive pulmonary disease patients who were followed up by the same chest diseases physician, who showed similar

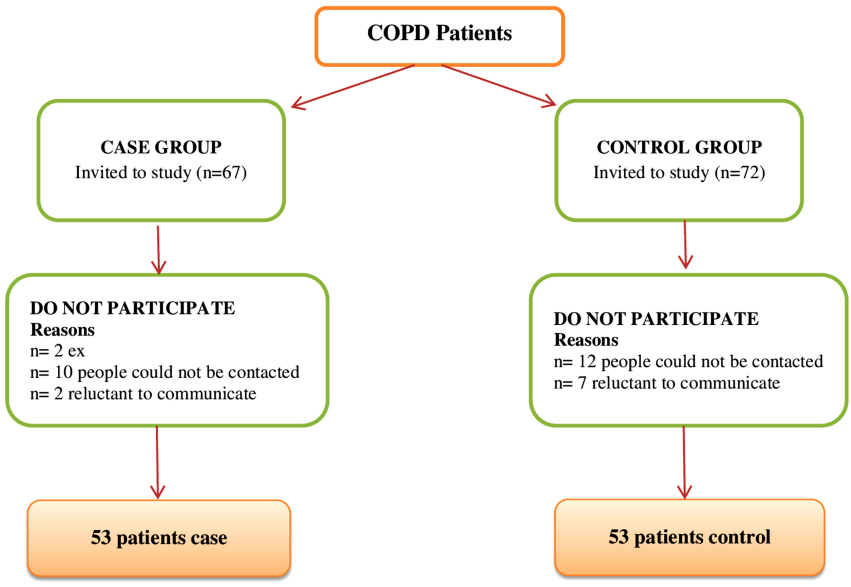


Figure 1. Sample diagram.

characteristics to the case group, who received similar treatment, who had previously not received inhaler use training, who could be accessed, and who agreed to participate in the study were included in the control group.

Data Collection Tools and Their Properties

The study data were collected through a survey form prepared by the researchers by reviewing the literature.[4,5,14,17,18] The form included questions inquiring about the patients' sociodemographic information and their knowledge of COVID-19, their status of contracting COVID-19, the severity of the disease, and the effects of inhaler medicines on COVID-19.

The data were collected through phone interviews. Each interview with the patients lasted approximately 15 minutes. Those who wanted to have the interview when they were available were called later. This method was preferred in order to isolate the patients from the risks of the clinical environment during the pandemic period. The Canadian Thoracic Society has recommended in its report that the training and rehabilitation programs for COPD patients should be continued during the pandemic period and that this should be carried out by using telehealth technologies.[19] Thus, the patients in the study were not exposed to any risk of infection.

Statistical Analysis

The data were analyzed through Statistical Package for the Social Sciences Statistics version 23.0 software (IBM Corp.; Armonk, NY, USA). Compatibility with normal distribution was examined through Kolmogorov–Smirnov test. In the comparison of the data with normal distribution for pairwise groups, independent 2-sample *t*-test was used, and in the comparison of the data without distribution, the Mann–Whitney *U*-test was employed. In the comparison of categorical variables according to groups, chi-square and Fisher's exact test were used, and multiple comparisons of the rates were analyzed with Bonferroni adjusted *Z*-test. Analysis results were presented as mean ± SD and median (minimum–maximum) for quantitative data and as frequency (percentage) for categorical variables.

Ethical Approval

Ethical rules and principles of the Declaration of Helsinki were followed at every stage of the study. Before starting the research, an ethical approval was obtained from the Clinical Research and Publication Ethics Committee of Kırşehir Ahi Evran University (protocol no.: 2021-05/46, date: March 9, 2021). In addition, COPD patients were asked to confirm their participation in the study via telephone.

RESULTS

It was determined that there was no statistically significant difference between the patients in the case and control groups in terms of mean age ($P = .114$) and median duration of COPD diagnosis ($P = .058$). It was found that there was a statistically significant difference between the groups in terms of their distribution according to their employment status ($P = .027$), and that the difference resulted from the retired individuals in the case group. It was determined that 79.2% of the patients in the case group and 50.9% of the patients in the control group were retired. A statistically significant difference was found between the distributions of COPD symptoms according to the groups ($P = .019$), and the difference was found to have originated from the cough and fatigue symptoms in the control group. While cough and fatigue were detected in 30.2% and 17% of the case group, respectively, cough and fatigue were determined in 52.8% and 35.8% of the control group, respectively. It was found that there was a statistically significant difference between the groups in terms of correct use of inhaler ($P < .001$) and that the difference originated from the case group. While 100% of the case group reported correct use of inhaler, this rate was found to be 60.4% in the control group (Table 1).

The condition of the patients caught in COVID 19 was questioned. It was noted that this was diagnosed by the doctor

Table 1. Comparison of the Groups According to Their Sociodemographic and Clinical Characteristics (n = 106)

Characteristics	Case	Control	Total	Stats.	*P*
Age	66.0 ± 7.7	63.0 ± 11.2	64.5 ± 9.7	1.596	.114*
	65.0 (45.0-84.0)	64.0 (33.0- 88.0)	65.0 (33.0-88.0)		
Gender					
Female	4 (7.5)	11 (20.8)	15 (14.2)	3.805	.051*
Male	49 (92.5)	42 (79.2)	91 (85.8)		
Education					
Illiterate	0 (0)	3 (5.7)	3 (2.8)	4.423	.490*
Literate	0 (0)	1 (1.9)	1 (0.9)		
Primary school	36 (67.9)	32 (60.4)	68 (64.2)		
Secondary school	7 (13.2)	6 (11.3)	13 (12.3)		
High school	6 (11.3)	6 (11.3)	12 (11.3)		
Bachelor's degree	4 (7.5)	5 (9.4)	9 (8.5)		
Marital					
Married	52 (98.1)	47 (88.7)	99 (93.4)	—	.113**
Single	1 (1.9)	6 (11.3)	7 (6.6)		
Working					
Housewife	4 (7.5)	9 (17)	13 (12.3)	10.984	**.027***
Worker	0 (0)	3 (5.7)	3 (2.8)		
Officer	1 (1.9)	4 (7.5)	5 (4.7)		
Retired	42 (79.2)[a]	27 (50.9)[b]	69 (65.1)		
Self-employment	6 (11.3)	10 (18.9)	16 (15.1)		
Time of diagnosis	9.2 ± 9.2	7.8 ± 9.9	8.5 ± 9.5	11.060	.058**
	6.0 (2.0 - 50.0)	5.0 (1.0 - 52.0)	5.0 (1.0 - 52.0)		
Symptoms of COPD***					
Dyspnea	50 (94.3)	53 (100)	103 (97.2)	18.384	**.019***
Cough	16 (30.2)[a]	28 (52.8)[b]	44 (41.5)		
Sputum	24 (45.3)	27 (50.9)	51 (48.1)		
Fatigue	9 (17)[a]	19 (35.8)[b]	28 (26.4)		
Wheezing	17 (32.1)	17 (32.1)	34 (32.1)		
Perspiration	2 (3.8)	5 (9.4)	7 (6.6)		
Insomnia	3 (5.7)	6 (11.3)	9 (8.5)		
Hoarseness	2 (3.8)	0 (0)	2 (1.9)		
Regular use of inhaler					
No	3 (5.7)	5 (9.4)	8 (7.5)	—	.716**
Yes	50 (94.3)	48 (90.6)	98 (92.5)		
Terms of use of inhaler					
Incorrect	0 (0)	21 (39.6)	23 (21.7)	20.045	**<.001***
Correct	53 (100)	32 (60.4)	83 (78.3)		
Chronic disease					
None	18 (34)	19 (35.8)	37 (34.9)	0.042	.839*
Have	35 (66)	34 (64.2)	69 (65.1)		
Which chronic disease***					
Hypertension	32 (91.4)	26 (76.5)	58 (84.1)	0.042	.839*
Diabetes mellitus	9 (25.7)	6 (17.6)	15 (21.7)		
Heart disease	16 (45.7)	17 (50)	33 (47.8)		
Goiter	1 (2.9)	0 (0)	1 (1.4)		
Cancer	0 (0)	2 (5.9)	2 (2.9)		
Rheumatoid arthritis	1 (2.9)	1 (2.9)	2 (2.9)		

*Chi-square test.
**Fisher's exact test,
***Multiple response.
[a-b]There is no difference between groups with the same letter in each line.Values with $P < .05$ are significant.
COPD, chronic obstructive pulmonary disease.

according to the positive PCR test. Six individuals in the case group and 13 individuals in the control group contracted COVID-19. About 94.7% of the patients recovered from the COVID-19 infection with various symptoms.

The disease-related characteristics of the patients infected with COVID-19 are presented in Table 2. A statistically significant difference was found between the groups in terms of the distribution of long-term presence of symptoms (*P* =

Table 2. Comparison of COVID-19-Related Variables According to the Groups (n = 19)

COVID-19-Related Variables	Case	Control	Total	*P*
Hospitalization				
No	4 (66.7)	9 (69.2)	13 (68.4)	1.000**
Yes	2 (33.3)	4 (30.8)	6 (31.6)	
Intensive care (n = 6)				
No	2 (100)	1 (25)	3 (50)	—
Yes	0 (0)	3 (75)	3 (50)	
Oxygen use at home				
No	4 (66.7)	7 (53.8)	11 (57.9)	1.000**
Yes	2 (33.3)	6 (46.2)	8 (42.1)	
Pneumonia due to COVID-19				
No	4 (66.7)	9 (69.2)	13 (68.4)	1.000**
Yes	2 (33.3)	4 (30.8)	6 (31.6)	
Long-term presence of symptoms (symptom persisting for the last month despite negative PCR)				
No	6 (100)	5 (38.5)	11 (57.9)	—
Yes	0 (0)	8 (61.5)	8 (42.1)	
Use of inhaler medication in the COVID-19 process				
No	0 (0)	3 (23.1)	3 (15.8)	.517**
Yes	6 (100)	10 (76.9)	16 (84.2)	
Feeling improvement in symptoms with inhalers				
No	0 (0)	3 (23.1)	3 (15.8)	.517**
Yes	6 (100)	10 (76.9)	16 (84.2)	
Thinking that using the right inhaler is beneficial in the COVID-19 process				
No	1 (16.7)	7 (53.8)	8 (42.1)	.177**
Yes	5 (83.3)	6 (46.2)	11 (57.9)	
COVID-19 precautions***				
Mask	47 (100)	40 (100)	87 (100)	**.031***
Distance	47 (100)	40 (100)	87 (100)	
Cleaning	45 (95.7)	39 (97.5)	84 (96.6)	
Reducing exit from home	45 (95.7)	39 (97.5)	84 (96.6)	
Avoiding the crowd	46 (97.9)	38 (95)	84 (96.6)	
Avoiding the family visitation	43 (91.5)	37 (92.5)	80 (92)	
Regular use of inhaler medicines	11 (23.4)[a]	2 (5)[b]	13 (14.9)	
Use of disinfectants	12 (25.5)[a]	1 (2.5)[b]	13 (14.9)	
Routine doctor checkup	3 (6.4)	0 (0)	3 (3.4)	
Thinking that inhaler medicines provided protection against other respiratory system diseases (n = 106)				
No	35 (66)	43 (81.1)	78 (73.6)	**.006***
Yes	18 (34)[a]	10 (18.9)[b]	28 (26.4)	

*Chi-square test.
**Fisher's exact test.
***Multiple response.
[a-b]There is no difference between groups with the same letter in each line.Values with $P < .05$ are significant.
COVID-19, coronavirus disease 2019; PCR: polymerase chain reaction.

.018), and it was determined that the difference originated from the case group. It was determined that long-term symptoms were not present in 100% of the case group and 38.5% of the control group. When the groups' distribution in terms of COVID-19 precautions was examined, it was found that there was a statistically significant difference between the groups (P = .031) and that this difference resulted from the regular use of inhaler medicines and use of disinfectants for cleaning purposes in the case group.

It was determined that 23.4% of the case group and 5% of the control group used their medicines regularly. Disinfectant use rate was 25.5% in the case group and 2.5% in the control group. In terms of thinking that inhaler medicines provided protection against other respiratory system diseases, a statistically significant difference was found between the groups (P = .006), and the difference resulted from the case group patients'

DISCUSSION

In the study, in which the COVID-19-related characteristics of COPD patients were examined according to their receiving inhaler use training, the study sample consisted of mostly male patients above the age of 65. It is known that COPD frequently develops in males at advanced ages. In the GOLD 2022 report, it has been stated that airflow restriction increases more after bronchodilator in males above the age of 40 compared to females and that the highest prevalence is observed in patients over the age of 60.[14] This information is consistent with the study in that 85.8% of the patients were male and at advanced ages.

It has been reported that the COPD-related symptoms that patients complain about the most are dyspnea, cough, phlegm, and wheezing. These complaints observed in the patients are also the most reported symptoms in COPD patients.[14] In the study conducted by Wang et al[20] on COPD patients over the age of 60 who were infected with COVID-19, it was reported that the infection led to serious problems in the respiratory system, progressed very fast, and displayed a poor prognosis. When the symptom distribution of the case group and control group is examined, it is noteworthy that the control group experienced more symptoms and that there was a statistically significant difference between the groups in this regard (Table 1). The reason for this is thought to be related to the correct and regular use of inhaler. Inhaler medicine use is the most effective way in alleviating/eliminating symptoms in COPD patients.[11] Inhaler medicines should be prepared appropriately, be inhaled down to the terminal bronchioles, and be kept here for an adequate period. In order to follow these steps successfully, it is necessary to have inhaler use skills. Regarding the correct inhaler use rates in the patients, while it was 96.2% in the case group, this rate was as low as 60.4% in the control group, which was statistically significant. The fact that the patients in the case group had received inhaler training between 2017 and 2018 and that the great majority of them still had inhaler use skills with correct steps also shows the effectiveness of the training they had received. As a result of the training given on the correct use of inhaler, fewer symptoms were observed in the case group in comparison to the control group. In the literature, there are similar studies conducted which showed that COPD symptoms were mitigated as a result of the patients gaining inhaler medicine use skills. Göriş et al[17] reported in their study that the correct use of inhaler was effective in reducing the frequency of attacks and dyspnea. In the study conducted by Gregoriano et al[18] it was reported that following the inhaler use training, cough, dyspnea, and feeling of obstruction while walking or climbing the stairs were significantly reduced in the patients who used to use inhaler with an incorrect technique. These findings support the findings obtained in the present study.

It is positive that although all COPD patients participating in the study were in the high-risk group, the number of those who were infected with COVID-19 was low. Similar to the present study, it has been reported that in the pandemic period, presentation to hospitals with COPD and inflammation has generally decreased.[14] This situation may have resulted from the patients' being careful about face mask/shield use and measures such as social distancing and hygiene. It has also been reported that the patients' concern about being exposed to the infection during hospital presentation may have caused them to decrease the number of follow-ups.[14] These reports demonstrate the patients' making the correct decisions about their own health and managing the disease appropriately. It is expected that patients who show an effort to manage their disease can be open to training and willing to improve themselves through innovations. Therefore, it is thought that the patients, who wanted to receive training on their disease and grasped the importance of being followed up by healthcare professionals, may have adapted more to the measures against the epidemic. Accordingly, it is an expected situation that the patients in the case group were infected less and had higher levels of adaptation to the measures taken in order to control the epidemic. The fact that the number of patients in the case group who were infected with COVID-19 in the present study was lower compared to the control group patients may have been the result of the previous training that the case group patients had received. In addition, the statistically significant difference being in favor of the case group in terms of regular use of medicines and disinfectants against the COVID-19 epidemic supports our stance (Table 2).

In the case group, there were no symptoms in the patients within the last month following the negative PCR test result after COVID-19 infection, while it was reported that 61.5% of the control group suffered from symptoms (P < .05) (Table 2). This result can be explained by the fact that the patients in the case group continued the correct use of inhaler when they were infected with COVID-19, while the majority of the control group patients did not have this skill. In reports published, it has been emphasized that COPD patients should continue to use inhalers effectively, especially during the pandemic period and when they contracted the COVID-19 infection[13,14], and thus potential inflammations could be prevented.[19] Although the patients in the control group stated that they continued to use inhaler in their COVID-19 infection process (Table 2), it is thought that the medicines that were not used in the correct steps would have little effect

on the symptoms. In a report published on this issue, it was stated that using the inhaler medicines correctly was effective in controlling the symptoms of the respiratory system caused by the COVID-19 infection in COPD patients.[21] All information stated above supports the findings obtained in the present study. In addition, the patients in the case group believed that the inhaler used provided protection against other respiratory system diseases other than COPD (Table 2). This result created a difference compared to the control group (P = .006). Thus, it is seen that there is a correlation between this line of thinking in the case group and correct inhaler use skills. These patients meticulously continued to use inhaler medicines even under the pandemic conditions that affected the whole world while fighting against COPD which they had been suffering from for years, and they benefited from this. Hence, the importance of using inhaler medicines correctly and regularly has been understood once more.

CONCLUSION

As a result of the study, it was determined that the patients who had previously participated in a training program on inhaler use behaved more carefully about their disease and followed treatment options more meticulously. Chronic obstructive pulmonary disease patients with inhaler training contracted COVID-19 less compared to those without training and experienced the infection less severely. Moreover, it was observed that the patients with training protected themselves against the infection better and obeyed the restrictions more. It was also determined that thanks to the correct use of inhaler medicines, the patients in the case group were affected less by other disorders of the respiratory system. According to these results, it can be recommended that COPD patients should be informed about their diseases through appropriate training programs, that they should be provided with information on the importance and correct use of medicines, that the number of rehabilitation programs should be increased, and that the patients should be followed up in the long term.

The main limitation of the study is that it was conducted on a small group. Especially, the insufficient number of patients to be included in the case group in the center where the study was conducted and thus including fewer patients in the study than the maximum number is the main limitation of the study. Therefore, it is necessary to provide COPD patients with inhaler use training and to conduct studies in different centers on wider populations. Another limitation of the study is that the patients were contacted over the phone. Although this was to the benefit of the patients under pandemic conditions, it prevented the researcher from observing inhaler use skills. Also, it should be noted that the results of the study are limited to the group on which it was conducted and therefore cannot be generalized.

Ethics Committee Approval: This study was approved by Clinical Research and Publication Ethics Committee of Kırşehir Ahi Evran University (Approval No.: 2021-05/46, Date: March 9, 2021).

Informed Consent: Verbal informed consent was obtained from the patients who agreed to take part in the study.

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Original Article

Validity and Reliability of the Turkish Version of the Nijmegen Questionnaire in Asthma

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Abstract

OBJECTIVE: The Nijmegen Questionnaire (NQ) enables the assessment and identification of symptoms related to respiratory dysfunction and hyperventilation syndrome. The aim was to investigate the validity of the Turkish version of the NQ in asthmatics.

MATERIAL AND METHODS: Fifty-four individuals with asthma were included. Spirometry was performed. Dyspnea was assessed using the modified Borg and modified Medical Research Council scales. Breath-holding time was recorded. End-tidal carbon dioxide was measured using a portable capnograph. Oxygen saturation and heart rate were recorded. Asthma Control Test was used to evaluate the asthma control level. Quality of life was assessed using the Asthma Quality of Life Questionnaire and Nottingham Health Profile. Beck Depression Inventory was used to determine depression.

RESULTS: Bartlett's test of sphericity (360.749, *df* 105, $P < .001$) and Kaiser–Meyer–Olkin criterion (0.752) for 15-item NQ supported a single-factor model with 36.38% of explained variability through principal component analysis and explanatory factor analysis. For 15-item NQ with this single-factor model, Cronbach's alpha was 0.872, and the test–retest reliability was 0.628. There was a significant negative correlation between NQ and Asthma Control Test ($r = -0.448$), and Asthma Quality of Life Questionnaire ($r = -0.743$) and a significant positive association with Beck Depression Inventory ($r = 0.477$), Nottingham Health Profile—energy ($r = 0.370$), Nottingham Health Profile—pain ($r = 0.313$), Nottingham Health Profile—sleep ($r = 0.294$), and Nottingham Health Profile—physical activity scores ($r = 0.406$) ($P < .05$).

CONCLUSIONS: The 15-item Turkish version of the NQ is valid and reliable in asthmatics. Individuals with uncontrolled asthma have higher NQ scores than those with well-controlled asthma. NQ is associated with asthma control level, asthma-related quality of life, health profile, and depression.

KEYWORDS: Asthma, Nijmegen Questionnaire, respiratory dysfunction
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INTRODUCTION

Asthma is a heterogeneous condition characterized by variable respiratory symptoms and airflow limitation. These features may arise through several underlying mechanisms typically associated with airway inflammation and remodeling.[1] Asthma symptoms are non-specific and include wheezing, shortness of breath, chest tightness, and cough.[1]

Hyperventilation syndrome (HVS) is a complex disease defined as breathing beyond metabolic requirements.[2] Hyperventilation syndrome is the most widely recognized dysfunctional respiratory form in which chronic changes in respiratory pattern lead to dyspnea with non-respiratory symptoms.[3] These changes can occur with or in the absence of respiratory, cardiovascular, and other disorders.[3]

The HVS pathophysiology is controversial. It has been stated that its symptoms emerge from hypocapnia associated with excessive breathing and that low carbon dioxide in arterial blood is considered a need for diagnosis.[4]

Hyperventilation syndrome causes decreased carbon dioxide pressure in the arterial blood, respiratory alkalosis, and increased symptoms.[5] Complex symptoms such as shortness of breath, chest tightness, paresthesia, anxiety, and dizziness may occur.[5]

Nijmegen Questionnaire (NQ) allows the assessment and identification of respiratory dysfunction and unexplained respiratory symptoms and signs of HVS.[6,7] The NQ has an essential place in determining and monitoring respiratory dysfunction. The NQ has been validated in non-asthmatics with a diagnosis of HVS.[8] Many physiological, inflammatory, environmental, psychological, and behavioral factors play a role in controlling asthma. The NQ subjectively evaluates breathing and respiratory response to stress and provides a valuable perspective in interpreting the result. The NQ was used to determine HVS prevalence in asthmatics.[9]

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The questions are related to different systems, such as cardiovascular, neurological, respiratory, gastrointestinal, and psychological factors.[8,9] There are 16 items, and each item is answered with a 5-item scale ranging from 0 to 4. The total score ranges between 0 and 64. Higher scores indicate respiratory dysfunction and increasing probability of HVS.[8] The NQ reveals the factors that cause HVS or respiratory pattern disorder. Identifying these factors helps prevent respiratory pattern disorder and correct musculoskeletal and motor pattern changes. In asthma, symptoms may be accompanied by anxiety.

The Turkish version of the NQ may be useful for assessing respiratory dysfunction, unexplained respiratory symptoms, and HVS signs. Turkish version of the NQ for asthmatics may be helpful to guide researchers for further studies to identify appropriate treatment approaches for asthma and develop different management strategies. Therefore, we aimed to investigate the validity and reliability of the Turkish version of the NQ to evaluate respiratory dysfunction in asthma.

MATERIAL AND METHODS

The present study was carried out at Hacettepe University, Faculty of Physical Therapy and Rehabilitation, Cardiopulmonary Rehabilitation Unit, between March 2020 and January 2021.

Hacettepe University Non-Interventional Clinical Research Ethical Board approved the study (approval date: 17.03.2020 and approval number: GO 20/199). All participants were informed about the study protocol and signed an informed consent form.

Inclusion criteria were having been diagnosed with mild–moderate asthma in the Hacettepe University, Department of Chest Diseases Division of Allergy and Clinical Immunology and being referred to Hacettepe University, Faculty of Physical Therapy and Rehabilitation, Cardiopulmonary Rehabilitation Unit for physiotherapy and rehabilitation, being aged ≥18 years, clinically stable, and able to cooperate. Inability to read and write, presence of severe orthopedic, neurological, and cardiovascular disorders, and cognitive problems were determined as exclusion criteria.

Based on the sample size calculation conducted by Bonett DG, with a two-way hypothesis setup accepting a power of 90% (1 - β) and a type 1 error (α) of 0.05, the analysis from the pilot study revealed a Cronbach's alpha coefficient of 0.814 for the H1 hypothesis. Considering the acceptance of the H0 hypothesis as 0.500, the study determined that the participation of 54 individuals was required.[10]

MAIN POINTS

- The 15-item Turkish version of the Nijmegen Questionnaire (NQ) is valid and reliable in asthmatics.
- Individuals with uncontrolled asthma have higher NQ scores than those with well-controlled asthma.
- The NQ provides a useful tool for healthcare professionals in research and clinical settings regarding its association with health status, asthma control, asthma-related quality of life, and depression.

The Brislin method was used in the translation phase of the questionnaire.[11] This method includes the first translation into the target language, the evaluation of the first translation, the translation back to the source language, re-evaluation of the translation, and receipt of expert opinions.[11] In this context, the questionnaire was first translated from English to Turkish by 2 experts whose native language is Turkish and who had good English command. The first translation was evaluated by 2 experts in the questionnaire items' intelligibility field. The Turkish translation of the questionnaire, which was created as a result of the first evaluation of the experts, was translated into English by a linguist whose native language is English. The questionnaire was obtained due to back-translation to the source language and was compared with the original questionnaire. Two experts who have a good command of English re-evaluated the translation in the field. Pre-final NQ was administered to 5 individuals with asthma for intelligibility and perceptibility.[12] In line with expert opinions and all evaluations, the final version was created, used, and re-administered 1 week apart.

The age, height, and weight of the participants were recorded. Smoking history and asthma symptoms were questioned. A pulmonary function test was performed using a spirometer (Spirodoc, Medical International Research, Rome, Italy). The parameters were expressed as percentages of predicted values.[13]

Dyspnea perception at rest was evaluated using a modified Borg scale (mBorg) and a modified Medical Research Council dyspnea scale (mMRC). The mBorg is a category scale evaluating shortness of breath between 0 and 10.[14] Participants were asked to choose the expression that best describes their dyspnea level using mMRC. Scoring in mMRC varies between 0 and 4 points; "0" means that the individual does not have dyspnea, while "4" indicates that dyspnea perception is present during basic daily life activities such as dressing.[15]

Participants were asked to breathe up to tidal volume at the end of light exhalation and hold their breath until the first involuntary movement of respiratory muscles in a sitting position. Three repetitions were performed to measure the breath-holding time (BHT), and an average value was recorded.[16]

A portable capnograph (MD-660P, Comdek Industrial Corp., Taipei, Taiwan) with a nasal cannula was used to measure end-tidal carbon dioxide ($ETCO_2$) and resting respiratory rate (RR) over a 10-minute period. Participants were asked to breathe through their noses and not to speak during measurement.[17] The $ETCO_2$, oxygen saturation (SpO_2), and heart rate were recorded using this device.

Asthma control test (ACT) was used to assess asthma control. The ACT score ranges from 5 (weak control) to 25 (fully controlled).[18] A total score of <20 indicates uncontrolled asthma. Turkish version of the ACT was used.[19]

Global Initiative for Asthma (GINA) assessment of asthma symptom control was determined as well-controlled, partly controlled, and uncontrolled.[20]

Disease-specific quality of life (QoL) using the Asthma QoL Questionnaire (AQLQ) and health-related QoL using Nottingham Health Profile (NHP) were determined.[21,22] The AQLQ is a 32-item disease-specific questionnaire with 4 domains: activity limitation, symptoms, emotional function, and environmental stimuli. The AQLQ has Turkish validity and reliability.[23] The NHP is related to general QoL and consists of 38 items and has 6 sub-sections: energy, pain, emotional reactions, sleep, social isolation, and physical mobility. Scores for each subsection range from 0 to 100. Higher scores indicate more restrictions in QoL. Turkish version of NHP was used.[24]

Beck Depression Inventory (BDI) measures the physical, emotional, cognitive, and motivational symptoms of depression. The BDI is a scale varying between 0 and 63.[25] Turkish validity and reliability of the BDI were conducted. Higher scores indicate a higher level of depression.[26] The permissions to use all the questionnaires were obtained.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM, Armonk, NY, USA) was used to analyze the data. Descriptive statistics were calculated. Explanatory factor analysis was used to determine whether the questionnaire had single or multiple dimensions. The suitability of data for factor analysis was evaluated using the Bartlett sphericity test and Kaiser–Meyer–Olkin (KMO) coefficient.[27] Invariance and internal consistency over time were evaluated to determine the reliability. Test–retest reliability was calculated for invariance against time, and the Cronbach's alpha reliability coefficient was calculated for internal consistency. Structural validity, test–retest reliability, and internal consistency analysis were used to test the validity and reliability of the NQ. Factor analysis was performed to identify the relevant factors. The criteria for eigenvalues above 1.00, scree plot, percentage of variation explained, and content of extracted factors were used to determine the number of extracted factors.[28] The criterion of having a factor load of >0.30 was used to determine the relevant items.[29] A variable's factor load of 0.30 indicates that the variance explained by the factor is 9%. A factor load of ≥0.60 can be defined as high, while a factor load between 0.30 and 0.59 can be defined as moderate.[30] Internal consistency of the NQ was tested using the Cronbach's alpha coefficient. Test–retest reliability was tested using the intraclass correlation coefficient. For cross-sectional validity, the relationships between NQ total score, ACT, $ETCO_2$, BHT, predicted percentage of forced expiratory volume in 1 second (FEV_1%), RR, mBorg dyspnea score, AQLQ, Beck Depression Inventory, and NHP were analyzed using the Spearman correlation test. The Mann–Whitney *U* -test and Kruskal–Wallis *H*-test were used to compare 2 or more independent variables. Wilcoxon signed-rank test was used to compare 2 related samples. The probability of error was taken as $P < .05$.[27]

RESULTS

Fifty-four individuals with asthma aged between 19 and 65 years were included. The participants' characteristics, spirometric parameters, dyspnea perception, BHT, heart rate, RR, SpO_2, $ETCO_2$, ACT, AQLQ, NHP, and BDI values are given in Table 1.

Table 1. The Characteristics of Participants

	Asthma (n = 54)	
Parameter	**Mean ± SD**	**(Minimum–Maximum)**
Age (years)	42.59 ± 13.71	19-65
Sex (female/male), n (%)	43/11	79.6/20.4
Height (m)	1.63 ± 0.09	1.49-1.94
Weight (kg)	72.07 ± 17.89	48-138
Body mass index (kg/m^2)	27.15 ± 6.38	16.14-47.75
Smoking history (package year)	4.33 ± 10.75	0-45
Duration of asthma (years)	9.80 ± 7.95	1-31
Level of asthma control		
Well-controlled, n (%)	26	48.1
Not well-controlled, n (%)	28	51.9
FVC (%)	97.35 ± 16.51	65-141
FEV_1 (%)	89.88 ± 17.21	56-126
FEV_1/FVC	80.39 ± 10.49	63-127
PEF (%)	81.11 ± 20.52	26-133
$FEF_{25-75\%}$ (%)	68.46 ± 23.58	20-126
mMRC (0-4)	1.25 ± 0.78	0-3
Modified Borg dyspnea score (0-10)	2.73 ± 1.84	0-7
Breath-holding time (s)	23.55 ± 14.34	4.11-77.33
Heart rate (bpm)	78.72 ± 12.98	54-104
Respiratory rate (breath/min)	17.37 ± 5.09	7-32
SpO_2 (%)	96.87 ± 1.15	94-99
$ETCO_2$ (mmHg)	20.27 ± 3.73	10-29
Asthma Control Test (5-25)	18.66 ± 4.09	8-25
Asthma Quality of Life Questionnaire (1-7)	5.21 ± 1.08	2.42-7
Symptom	5.62 ± 1.05	3.25-7
Activity	5.01 ± 1.22	1.9-7
Emotional	5.41 ± 1.26	1.8-7
Environment	4.69 ± 1.59	1-7
Nottingham Health Profile (0-100)		
Energy	41.95 ± 35.75	0-100
Pain	20.89 ± 29.61	0-100
Emotional reactions	27.54 ± 26.15	0-100
Social isolation	17.70 ± 24.50	0-100
Sleep	18.57 ± 20.10	0-72.74
Physical activity	21.47 ± 17.14	0-54.47
Beck Depression Inventory (0-63)	12.53 ± 10.50	0-45

$ETCO_2$, end-tidal carbon dioxide; $FEF_{25-75\%}$, forced expiratory flow at 25%-75% of the vital capacity; FEV_1, forced expiratory volume in 1 second (FEV_1); FVC, forced vital capacity; mMRC: modified Medical Research Council dyspnea scale; PEF, peak expiratory flow; SpO_2, saturation of oxygen.

Table 2. Comparison of Nijmegen Questionnaire Scores According to Sex, Asthma Duration, Asthma Control Test, GINA Asthma Control Level, Smoking History, Breath-Holding Time, and End-Tidal Carbon Dioxide Values

			Nijmegen Questionnaire Score	
Parameter		**n**	**Mean ± SD**	***P***
Sex	Female	43	16.77 ± 9.58	.569
	Male	11	15.36 ± 11.48	
Asthma duration	<8 years	29	16.93 ± 9.85	.683
	≥8 years	25	15.96 ± 10.13	
Asthma Control Test	Not well-controlled (≤19)	28	20.39 ± 10.70	**.005***
	Well-controlled (≥20)	26	12.27 ± 6.96	
Asthma control level (GINA)	Well controlled	17	12.59 ± 6.94	.074[k]
	Partly controlled	16	15.25 ± 8.35	
	Uncontrolled	21	20.57 ± 11.71	
Asthma control level (GINA)	Well-controlled and partly controlled	33	12.59 ± 6.94	**.036***
	Uncontrolled	21	20.57 ± 11.71	
Asthma control level (GINA)	Well controlled	17	12.59 ± 6.94	**.029***
	Uncontrolled	21	20.57 ± 11.71	
Smoking	Yes	18	17.11 ± 11.83	.985
	No	36	16.17 ± 8.95	
BHT	<30 s	43	16.14 ± 9.67	.675
	≥30 s	11	17.82 ± 11.15	
$ETCO_2$ (mmHg)	≤35 mmHg	54	16.48 ± 9.89	—
	>35 mmHg	0	0	

Mann–Whitney *U*-test. [k]Kruskal–Wallis *H*-test.
*$P < .05$.
BHT, breath-holding time; $ETCO_2$, end-tidal carbon dioxide; GINA, Global Initiative for Asthma.

The comparison of the NQ scores according to sex, asthma duration, asthma control, asthma severity, smoking history, BHT, and $ETCO_2$ values are shown in Table 2. The NQ scores were similar according to sex, asthma duration, asthma severity, smoking history, BHT, and $ETCO_2$ ($P > .05$), except for asthma control ($P < .05$) (Table 2). There was a significant difference in NQ scores of well-controlled and not-well-controlled asthmatics according to ACT ($P = .005$, Table 2). According to GINA classification, there was a significant difference in NQ scores of well-controlled and uncontrolled asthmatics ($P = .029$, Table 2).

Bartlett's test of sphericity (373.202, df 120, $P < .001$) and the KMO criterion (0.735) provided support for the single-factor model, which accounted for 34.26% of the explained variability.[27] Fifteen items (excluding item 14, cold hands and feet) showed favorable high factor load (>0.30) and commonality. A scree plot supported the decision to keep a single-factor model. A single factor was determined for 15 items and performed for explanatory factor analysis. A single-factor model with 36.38% of the explained variability was supported by Bartlett's test of sphericity (360.749, *df* 105, $P < .001$) and KMO criterion (0.752). For 15 items with a single-factor model, Cronbach's alpha was 0.872, and test–retest reliability was 0.628. No significant difference was found between test and retest NQ items ($P > .05$, Table 3). Loadings and commonalities of 15 items according to the single-factor model were presented in Table 4.

The NQ was re-applied to 51 of the participants 1 week later. The comparison of test and retest values was performed using Wilcoxon signed-rank test, and there was no significant difference between the NQ test and retest values ($P > .05$, Table 5).

The Cronbach's alpha coefficient was found to be 0.872. Intraclass correlation coefficient was determined as 0.628 (Table 5).

Correlations were evaluated between NQ and age, FEV_1%, smoking history, mMRC, $ETCO_2$, BHT, RR, mBorg dyspnea score, BDI, NHP, ACT, and AQLQ scores (Table 6). There was a positive correlation between NQ and BDI ($r = 0.477$, $P < .001$) (Table 6). The NQ was positively correlated with NHP—energy ($r = 0.370$, $P = .006$), NHP—pain ($r = 0.313$, $P = .021$), NHP—sleep ($r = 0.294$, $P = .031$), and NHP—physical activity ($r = 0.406$ $P = .002$) (Table 6). The NQ showed a negative relationship with ACT and AQLQ ($P < .05$, Table 6).

DISCUSSION

The 15-item Turkish version of the NQ is valid and reliable in asthmatics. The individuals with uncontrolled asthma have higher NQ scores compared to the ones with well-controlled asthma. The NQ was related to health status, asthma control, asthma-related QoL, and depression.

The Turkish version of the NQ was determined to fit the single-factor model, and an item (item 14-cold hands and

Table 3. Descriptive Statistics and Comparison of Test and Retest Items of the Nijmegen Questionnaire

	Nijmegen Questionnaire -1 (n = 54)		Nijmegen Questionnaire -2 (n = 51)		
Items	**Mean ± SD**	**Minimum–Maximum**	**Mean ± SD**	**Minimum–maximum**	***P***
Item 1	1.00 ± 1.04	0-4	0.86 ± 1.09	0-4	.494
Item 2	1.50 ± 1.12	0-4	1.31 ± 1.28	0-4	.601
Item 3	0.75 ± 1.02	0-3	0.37 ± 0.79	0-3	.083
Item 4	1.01 ± 1.12	0-4	0.94 ± 0.96	0-4	.758
Item 5	0.98 ± 1.18	0-4	1.00 ± 1.05	0-4	.808
Item 6	1.57 ± 1.07	0-4	1.68 ± 1.24	0-4	.398
Item 7	1.64 ± 1.15	0-4	1.56 ± 1.23	0-4	.868
Item 8	1.03 ± 1.08	0-3	0.84 ± 1.10	0-4	.352
Item 9	1.27 ± 1.25	0-4	1.39 ± 1.29	0-4	.482
Item 10	1.05 ± 1.07	0-3	0.94 ± 1.19	0-4	.872
Item 11	1.18 ± 1.15	0-3	0.96 ± 1.09	0-4	.231
Item 12	0.57 ± 0.96	0-4	0.45 ± 0.85	0-3	.495
Item 13	0.35 ± 0.75	0-3	0.31 ± 0.76	0-3	.537
Item 15	1.16 ± 1.14	0-4	0.96 ± 1.19	0-4	.409
Item 16	1.25 ± 1.23	0-4	1.27 ± 1.28	0-4	.903

Wilcoxon signed-rank test.

feet) should be removed. Asthma-like respiratory disorder symptoms were reported to have a higher score of coldness in hands or feet (3.0 vs. 1.6) than asthmatics.[31] Li Ogilvie et al[32] defined item 14 as an incompatible item to evaluate HVS between males and females. The same study reported that physiotherapists should use the revised 15-item NQ for clinical and research purposes and 15-item NQ represented participants' symptom severity more accurately than original scoring.[32] Similar to the aforementioned study, item 14 was removed and Turkish version of 15-item NQ was created in our study.

Table 4. Fifteen-Item Nijmegen Questionnaire Factor Loadings and Communalities

Items	Factor Loading	Factor Communality
Item 9	0.735	0.540
Item 15	0.679	0.461
Item 13	0.665	0.443
Item 8	0.633	0.401
Item 7	0.629	0.396
Item 16	0.625	0.390
Item 1	0.620	0.385
Item 5	0.610	0.372
Item 2	0.601	0.362
Item 11	0.589	0.347
Item 10	0.587	0.345
Item 3	0.545	0.298
Item 6	0.538	0.290
Item 4	0.482	0.232
Item 12	0.443	0.197

An increase in alveolar ventilation causes hyperventilation compared to metabolic carbon dioxide production.[33] As a result, alveolar carbon dioxide pressure tends to drop below normal levels. Hyperventilation includes rapid and deep breathing, or it may be in the form of a combination of both, resulting in a 1-minute ventilation increase above the metabolic requirement of the organism.[5,33] Hyperventilation differs from hyperpnea, which increases minute ventilation without a change in carbon dioxide partial pressure. End-tidal carbon dioxide levels below 35 mmHg typically indicate that breathing is in the hypocapnic range.[33] In our study, the mean $ETCO_2$ of asthmatics was 20.27 ± 3.73 mmHg. Reduced carbon dioxide partial pressure and symptoms suggestive of hyperventilation have been frequently observed in asthmatics.[33] Hypocapnia has adverse effects on asthma and

Table 5. Comparison of Test–Retest Values and Internal Consistency and Intraclass Correlation of the Nijmegen Questionnaire

	Asthma (n = 54)		
Parameter	**First Test, Mean ± SD**	**Second Test, Mean ± SD**	***P***
NQ score	16.48 ± 9.89	14.94 ± 10.25	.446[φ]

Internal consistency and intraclass correlation of the Nijmegen Questionnaire

	Cronbach's α	ICC	95% CI
NQ score	0.872	0.628	0.349-0.788

[φ]Wilcoxon signed-rank test.
ICC, intraclass correlation coefficient; NQ, Nijmegen Questionnaire.

Table 6. The Relationship Between Nijmegen Questionnaire and Evaluated Parameters

	Nijmegen Questionnaire (n = 54)	
Parameter	***r***	***P***
Age	−0.064	.644
FEV_1%	−0.064	.645
Smoking history	−0.003	.986
mMRC	0.163	.240
$ETCO_2$	0.062	.654
Breath-holding time	−0.010	.941
Respiratory rate	−0.100	.470
Modified Borg dyspnea score	0.254	.063
Beck Depression Inventory	0.477	**<.001***
NHP—Energy	0.370	**.006***
NHP—Pain	0.313	**.021***
NHP—Emotional reactions	0.240	.080
NHP—Social isolation	0.249	.069
NHP—Sleep	0.294	**.031***
NHP—Physical activity	0.406	**.002***
Asthma Control Test	−0.448	**<.001***
AQLQ—Total	−0.743	**<.001***
AQLQ—Symptom	−0.804	**<.001***
AQLQ—Activity	−0.629	**<.001***
AQLQ—Emotional	−0.504	**<.001***
AQLQ—Environment	−0.638	**<.001***

Spearman correlation analysis.
*P< .05.
AQLQ, Asthma Quality of Life Questionnaire; $ETCO_2$, end-tidal carbon dioxide; FEV_1%, predicted percentage of forced expiratory volume in 1 second; mMRC, modified Medical Research Council dyspnea scale; NHP, Nottingham Health Profile.

can lead to symptom exacerbations, but there is no clarity on the origin of hypocapnia in asthma.[33]

For internal consistency, the difference between the answers given to items measuring the same concept is calculated using the Cronbach's alpha coefficient and correlation between answers. Cronbach's alpha coefficient close to 1 indicates high internal consistency.[34] We found that 15-item NQ has good internal consistency with a high Cronbach's alpha (0.872), meaning that the NQ can be used reliably in this population. We found that the Cronbach's alpha coefficient was slightly lower than that achieved in the Greek version of the NQ (0.920).[9] In Korean version of the NQ, the Cronbach's alpha coefficient was determined as 0.878.[35] On the other hand, we found a higher Cronbach's alpha coefficient than that of the Iranian version of the NQ (0.702).[36]

The relationship with other variables includes a correlation of assessment tool results with other outcomes that may be similar. If there is a previously accepted gold standard measurement, the outcomes are associated with the results obtained with the gold standard. If there is no gold standard, a comparison is performed with other assessments, such as similar questionnaires that seem reasonable.[34] There is no gold standard for the evaluation of HVS.[9] Our study evaluated correlations between NQ and FEV_1%, $ETCO_2$, RR, mBorg dyspnea score, BHT, BDI, NHP, ACT, and AQLQ. Similar to our results, Courtney et al[37] showed no relationship between NQ with RR and BHT. The NQ had a negative correlation with ACT and AQLQ total scores. In the Greek version study, there was a negative correlation between ACT and NQ following our results.[9]

Although there was a significant relationship between $ETCO_2$ and NQ score (r = −0.680, P < .01) in Greek and Iranian versions of NQ (r = −0.783),[9,36] no relationship was found between $ETCO_2$ and NQ in our study, this may be due to our study's smaller sample size than these studies and the absence of any participant with an $ETCO_2$ value >35 mmHg. There was no significant relationship between $ETCO_2$ and NQ scores in another study (r = −0.12, P = .27), similar to ours.[37] The lack of a significant relationship between $ETCO_2$ and NQ score was reported not to indicate the absence of a relationship between respiratory dysfunction symptoms and $ETCO_2$, and it may be due to a complex relationship between these parameters, and this relationship may be affected by other factors such as anxiety.[37] In addition, the variability of relationship between NQ score and hypocapnia and specifying NQ as a measure of hyperventilation is generally weak.[9,37,38] The correlation between NQ and carbon dioxide pressure is reported to be highly variable.[38] Therefore, defining the NQ score to measure functional respiratory complaints was recommended.[38]

Most asthmatics have high NQ scores.[9,39] High NQ scores have been associated with poor asthma control.[9,40] We found that individuals with uncontrolled asthma had higher NQ scores than individuals with well-controlled asthma. The negative relationship between NQ and asthma control level confirmed this.

Women with asthma have higher NQ scores.[9] It is explained by the decrease in carbon dioxide partial pressure in the second half of the menstrual cycle with the effect of progesterone.[5,9] In our study, most participants were female (79.6%), and there was no difference between NQ scores according to sex. The higher number of females compared to males in our study may explain the lack of significant difference between $ETCO_2$ values of participants according to sex.

Respiratory functional disorder term is used instead of hyperventilation.[38,39] It has led to the concept that breathing can be impaired functionally, beyond or without hypocapnia.[38] Respiration has many functions, such as gas exchange, diffusion, expansion, biomechanical function for posture and movement, and subjective breathing experience, each of which may be impaired.[38] Impairment in any of these functions may cause dyspnea.[38] Considering the multidimensionality of respiration, the necessity of multi-component evaluation is emphasized in evaluating respiratory dysfunction.[38]

The NQ is used as a diagnostic method in most studies evaluating epidemiology to diagnose respiratory dysfunction.[3] The HVS prevalence in the general population is around 6-10.[3]

The HVS prevalence shows a variable range in studies. It may have arisen due to cultural differences, the selection of participants included in the studies from different populations, measurement differences, and different cutoff scores.[9,39] In a study with asthmatics, HVS prevalence was reported as 34%.[9] In the Greek version study, the cutoff score was determined as 17.[9] Thomas et al[41] reported HVS frequency as 29% in participants without an objective asthma diagnosis, using a cutoff score of 23. Van Dixhoorn et al[38] stated the best value as 19 points, without distinguishing individuals with HVS. Thus, they emphasized that complaints are a part of common minor ailments and not a part of normal life.[8,38] In our study, when ≥19 was used as the cut-off point, 20 individuals (37%) had NQ scores of ≥19.

Considering the limitations, the absence of participants with an $ETCO_2$ >35 mmHg limits the generalizability of the findings to this population. In addition, the relationship was determined using similar questionnaires due to the lack of a standard method for diagnosing HVS. Further studies with greater sample sizes, including individuals with severe asthma, are needed to draw a firm conclusion regarding associations.

CONCLUSION

The 15-item Turkish version of the NQ is valid and reliable in asthmatics. Individuals with uncontrolled asthma have higher NQ scores than those with well-controlled asthma. The NQ is associated with asthma control level, asthma-related QoL, health profile, and depression.

Ethics Committee Approval: Hacettepe University, Non-Interventional Clinical Research Ethical Board approved the study (Approval date: 17.03.2020 and approval number: GO 20/199).

Informed Consent: All participants were informed about the study protocol and signed an informed consent form.

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Original Article

Association of IL-17F Gene Polymorphism and Its Serum Level with SARS-CoV-2 Infection

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Abstract

OBJECTIVE: Although multiple studies have addressed the clinical outcomes of coronavirus disease, little data exist regarding the definition of immune and inflammatory profiles associated with this infection. Its clinical manifestations often worsen in association with hypercytokinemia (elevated interleukin 8 and interleukin 17). We conducted this research to elucidate the effect of interleukin 17 levels and interleukin 17F gene polymorphism on the severity and outcomes of coronavirus disease.

MATERIAL AND METHODS: Ninety patients with confirmed coronavirus disease and 30 healthy controls were enrolled. Coronavirus disease cases were classified into nonsevere, severe, and critical according to the World Health Organization definition. Approximately 10 mL peripheral blood sample was collected from all patients and controls by venipuncture in-plane and ethylenediaminetetraacetic acid tube. Enzyme-linked immunosorbent assay kits were used for calculating serum interleukin 17 levels, whereas real-time polymerase chain reaction was used for genotyping using the 5'-nuclease allelic discrimination assay for single nucleotide polymorphisms genotyping.

RESULTS: As regards interleukin 17 levels, there was a significant elevation of interleukin 17 in coronavirus disease cases compared to control healthy persons ($P < .001$). Moreover, serum interleukin 17 levels tended to be significantly higher with increased disease severity ($P = .004$). Patients with critical diseases expressed a significant rise of interleukin 17 compared to severe ($P = .03$) and nonsevere cases ($P = .02$). We noted no significant difference between the critical, severe, and nonsevere cases regarding different interleukin 17F genotypes.

CONCLUSION: Coronavirus disease is associated with elevated levels of interleukin 17, which tended to be considerably higher with disease severity. However, different interleukin 17F genotypes do not affect either the predisposition or the severity of coronavirus disease.

KEYWORDS: Interleukin 17, COVID-19, IL-17F, genotyping, gene polymorphism
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INTRODUCTION

In 2019, coronavirus disease (COVID-19) emerged as a worldwide pandemic that is caused by "severe acute respiratory syndrome coronavirus 2" or SARS-CoV-2. The mainstay management of this infection is based on symptomatic management (antipyretics for fever and oxygen therapy for desaturation), while mechanical ventilation is only indicated for patients with respiratory failure.[1]

The current literature is rich with studies, reports, and clinical trials describing the microbiological, epidemiological, clinical, and outcome characteristics of this infection. Nonetheless, this clinical entity is associated with immunological and inflammatory changes that significantly impact patient outcomes. These changes need to be well-defined for a better understanding of disease pathophysiology.[2]

The proinflammatory T-helper 17 (Th17) cells, which are a subtype of CD4+ T-cells), play a crucial role in the human immune system, especially against extracellular pathogens. In fact, these subtypes of immune cells secreted interleukin (IL) 17A and IL-17F. In addition, it organizes inflammatory cell recruitment in the infected area, including macrophages and neutrophils.[3] Therefore, abnormal Th17 regulation must have a role in the pathogenesis of numerous autoimmune and other inflammatory diseases.[4]

COVID-19 patients with worsened clinical presentation usually express a significant rise of proinflammatory cytokines in their serum (cytokine storm or cytokine release syndrome). These cytokines include IL6, 8, 17, and 1B. It is believed that the upregulation of Th17 cytokines (including IL-17 and IL-17F) is the main mediator of immunopathology and subsequent respiratory distress in such patients.[3]

Genetic polymorphism has been found to play an essential role in understanding the basis of many diseases, including pathophysiology, spread, and prevention. Single nucleotide polymorphism (SNP) is a common type of the previous

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phenomena, and it has a crucial impact on disease pathogenesis. It has a clear impact on organism attachment to the host cell, host susceptibility, resistance to diseases, and disease severity.[5]

The current investigation was done to evaluate the effect of IL-17 levels and IL-17F gene polymorphism on the severity and outcomes of COVID-19.

MATERIAL AND METHODS

A total of 90 patients with confirmed COVID-19 admitted at our quarantine hospital were enrolled in this case–control study, in addition to 30 healthy controls. Patients below 18 years old were excluded. This was done after gaining approval from the Mansoura University Scientific and Ethical Committee (code number: R.21.03.1275). Informed written consent was taken from all enrolled patients.

Nasopharyngeal swabs were used to confirm COVID-19 by identifying the specific RNA of the SARS-CoV-2 virus. First, the extraction kits (Qiagen, Foster City, USA) were used to isolate viral RNA. Then, it was identified via the real-time SARS-CoV-2 kit according to the manufacturer's instructions.

According to the World Health Organization definition, COVID-19 cases were classified into nonsevere, severe, and critical.[6] Critical infection was established if the patient developed acute respiratory distress syndrome, sepsis, septic shock, or the need for vasopressors. Severe infection was defined when the patient had less than 90% oxygen saturation on room air, respiratory rate >30 breaths per minute, or signs of severe respiratory distress. In addition, outcome assessment was recorded, including improved clinical data and mortality.

The control group included health service personnel and blood donors who had no systemic comorbidities and were free from any respiratory illness for the past year. Laboratory investigations were ordered to ensure infection clearance, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). The subject was enrolled as a control when CRP was negative, and ESR was lower than 20 mm/h.

Measurement of IL-17 Levels and Genotyping

Approximately 10 mL peripheral blood sample was collected from every patient (before the beginning of any therapy) and from the healthy controls, by venipuncture, in both plane and ethylenediaminetetraacetic acid (EDTA) tubes.

Enzyme-linked immunosorbent assay kits (Bioassay Technology Laboratory) were used to quantify serum IL-17 levels (ng/L).

A crucial quality control step in genetic experiments is to undertake Hardy–Weinberg equilibrium (HWE) estimates. Variations from HWE in the group may reflect significant challenges involving selection bias, population stratification, and genotyping errors.[7]

Real-time polymerase chain reaction (PCR) was used for genotyping using the 5'-nuclease allelic discrimination (TaqMan) SNP Genotyping Assays (Applied Biosystems; Thermo Fisher Scientific, Foster City, USA). To identify IL-17F polymorphism, we utilized TaqMan Universal PCR Master Mix at rs763780 loci using StepOne Real-Time (Applied Biosystems) Figure 1.

The SNP ID is C__2234166_10 for IL-17F rs763780, and the chromosomal location is Chr.6:52101739. The context sequence [VIC/FAM] was GTGGATATGCACCTCTTACT GCACA[C/T] GGTGGATGACAGGGGTGACGCAGGT.

Two TaqMan® probes: One probe labeled with VIC® dye detects the Allele 1 (C) sequence AND the other probe labeled with FAM™ dye detects the Allele 2 (T) sequence.

The protocol used was managed blindly with a final volume of 20 µL containing 5 µL of DNA template (200 ng/µL), 1 µL of TaqMan SNP Genotyping Assay Mix, and 10 µL of TaqMan Universal PCR Master Mix. Thermal cycling conditions were performed as follows: initially, denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and at 60°C for 30 seconds, and final extension was set at 60°C for 2 minutes. In each run, the proper negative controls were used.[8]

Allelic discrimination was carried out by measuring fluorescence intensity at the endpoint. The results of the measurement were analyzed using SDS software version 1.7 (Applied Biosystems, Foster, Calif, USA), and the genotype was determined.

Each sample is interpreted according to the 2 alleles and genotypes (homozygous or heterogygous), an increase in either FAM or VIC dye fluorescence indicates homozygosity for FAM- or VIC-specific alleles (C:C or T:T), and an increase in the fluorescence of both dyes indicates heterozygosity (C:T).

Statistical Analysis

All data were tabulated and analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corp, Armonk, NY, USA). As the alleles and genotype frequencies are considered categorical data, they were presented as numbers and percentages. Comparing 2 or more groups with categorical data was performed by the chi-square test (Fisher's exact test/Monte-Carlo test as a correction). Heredity equilibrium was assessed by Hardy–Weinberg test. Pearson's chi-square goodness-of-fit test was used to determine if SNP genotype frequencies in controls were in Hardy–Weinberg equilibrium (HWE). The SHEsis software was used to calculate

MAIN POINTS

- The mainstay management of COVID-19 is based on symptomatic control.
- The proinflammatory T-helper 17 (Th17) cells, a subtype of CD4+ (T-cells), play a crucial role in the human immune system.
- COVID-19 patients with worsened clinical presentation usually express a significant rise of proinflammatory cytokines including IL-17.
- The current investigation was done to evaluate the effect of IL-17 levels and IL-17F gene polymorphism on the severity and outcomes of COVID-19 infection.

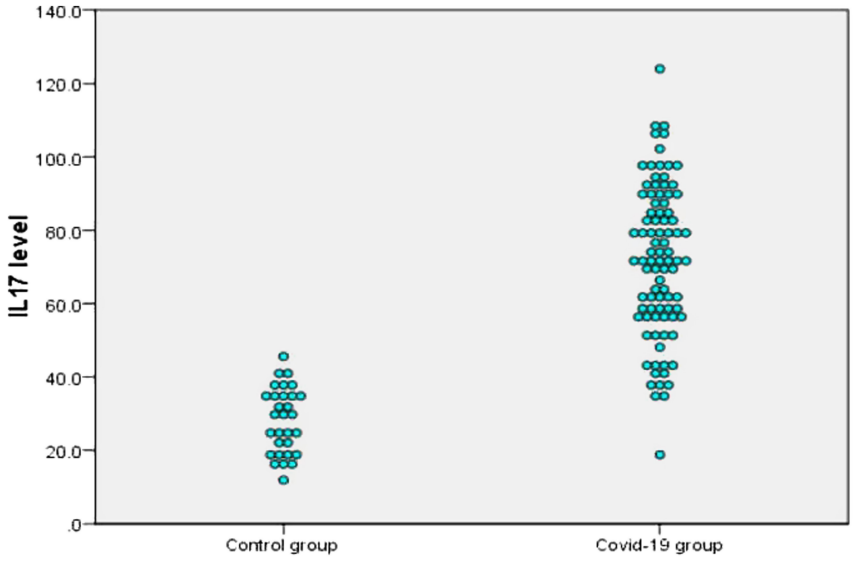


Figure 1. Real-time fluorescence curves of real-time PCR assay targeting analysis for IL-17F rs6505162.

haplotype frequencies and measure linkage disequilibrium (LD) between IL37 gene SNPs. This is the LD coefficient (D′). D′ ranges from 0 (no LD) to 1.0 (complete LD). The odds ratio (with the 95% CI) was performed by logistic regression. In the case of multiple comparisons, the *P*-value was corrected using the Bonferroni test. To specify optimal cutoff values of IL-17 level, receiver-operating characteristic (ROC) curve analysis was applied. All tests were performed using two-tailed probabilities with considering the $P \leq .05$ as the cutoff point for significance.

RESULTS

Ninety patients with confirmed COVID-19 (median age 61 (19-79) years) were enrolled. 57.7% of them were males. In addition, 4.5% were classified as nonsevere COVID-19, 53.3% had severe disease, and 42.2% had critical COVID-19. In-hospital mortality was reported in 56.7% of the studied patients (Table 1).

As regards IL-17 level, there was a significant elevation of IL-17 in COVID-19 cases compared to control healthy persons (mean values 71.8 ± 20.5 vs. 28.1 ± 8.9, respectively, $P < .001$) (Figure 2). Moreover, a positive correlation was noted between IL-17 levels and disease severity ($P = .004$). Cases with the critical disease significantly elevated IL-17 compared to cases with severe and nonsevere disease ($P = .03$ and .02, respectively). Patients with severe disease had comparable IL-17 values to nonsevere cases ($P = .337$) (Table 2).

Table 1. Clinical Severity of COVID-19 and Its Outcome among Studied Patients

Parameter		n, (%)
COVID-19 clinical manifestation	Nonsevere	5 (5.6%)
	Severe	48 (53.3%)
	Critical	37 (42.1%)
Outcome	Improved	39 (43.3%)
	Dead	51 (56.7%)

ROC analysis was conducted to identify the optimal IL-17 level for the prediction of COVID-19. IL-17 best cutoff value for the prediction of COVID-19 was 37.2 ng/L. Therefore, the area under the curve (AUC) was 0.979 ($P \leq .001$) (Table 3 and Figure 3).

The genetic characteristics of the studied SNPs followed the data of the National Center for Biotechnology Information

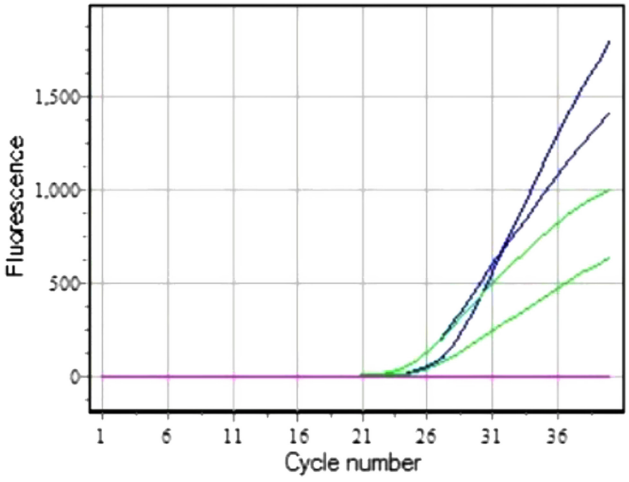


Figure 2. IL-17 levels (ng/L) in COVID-19 patients and control.

Table 2. IL-17 Levels and Clinical Severity of COVID-19 among Studied Patients

	Nonsevere Group (n = 5)	Severe Group (n = 48)	Critical Group (n = 37)	*P*
IL-17 (ng/L), mean ± SD	53.3 ± 17.04	68.04 ± 19.5	79.2 ± 19.7	$P^1 = .337$
				$P^2 = .020$
				$P^3 = .031$
				$P = .004$

P^1, comparison between nonsevere and severe; P^2, comparison between nonsevere and critical; P^3, comparison between severe and critical; *P*, comparison between 3 groups; SD, standard deviation.

Table 3. IL-17 Level for Prediction of COVID-19

	AUC	SE	*P*	95% CI	Cutoff (ng/L)	Sensitivity (%)	Specificity (%)
IL-17	0.979	0.011	<.001	0.958-1.00	37.2	96.7	83.3

AUC, the area under the curve.

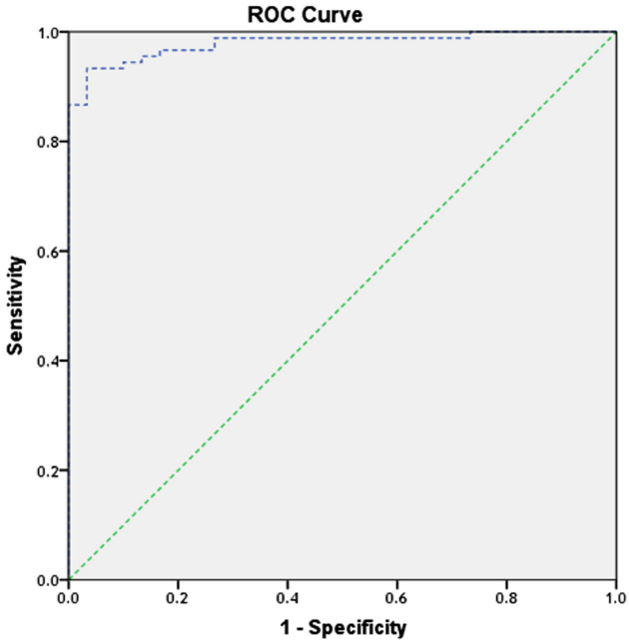


Figure 3. ROC analysis to identify the optimal IL-17 level for the prediction of COVID-19.

Table 4. Genetic Features of Studied SNPs According to the National Center for Biotechnology Information (NCBI)

SNP ID	rs763780
Alleles	T/C
Ancestral Allele	T
Cytogenetic location	6p12.2
Gene	Interleukin-17F
Nucleotide change	T to C substitution at the third exon of the IL-17 F gene in the coding region (+7488)
Amino acid change	His-to-Arg (H161R) substitution in position 161

(NCBI), as shown in Table 4. When the Hardy–Weinberg equation was applied at random from the population, it revealed that rs763780 genotypes in COVID-19 patients and control healthy persons were in Hardy–Weinberg equilibrium (HWE) (*P* = .9 and .6) (Table 5).

Table 6 shows the distribution of rs763780 genotype variants alleles in cases compared to controls. We did not detect any significant differences between them regarding genotypes and alleles.

Regarding different genotypes in the cases group, no significant differences were seen between either nonsevere, severe, or critical COVID-19 patients (*P* = .7). Also, no significant differences were noted between improved and dead patients (*P* = .4) (Table 7).

DISCUSSION

In this study, we assessed the effect of IL-17 levels and IL-17F gene polymorphism on the prediction, severity, and outcomes of COVID-19. We noticed a significant elevation of IL-17 in coronavirus disease cases compared to control healthy persons. Moreover, serum IL 17 levels tended to be significantly higher with increased disease severity—also, no significant difference between the critical, severe, and nonsevere cases regarding different IL-17F genotypes.

In the same context, Huang et al[9] noted a significant increase in IL-17 levels in COVID-19 patients who needed intensive care unit (ICU) admission, compared to others who did not, as well as healthy controls. Additionally, Liu et al[10] detected a positive correlation between serum IL-17 levels and disease severity, including COVID-19, middle east respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In this study, IL-17's best cutoff value for the prediction of COVID-19 was 37.2 ng/L. However, a cutoff value of >25 ng/mL was determined by Fathy et al[11] to differentiate between the patients' groups and the control group with 93.94%

Table 5. Assessment of Hardy–Weinberg Equilibrium for Studied IL-17F Genes

		Control (n = 30)		COVID-19 Patients (n = 90)	
Frequency		**Observed**	**Expected**	**Observed**	**Expected**
rs763780	TT	29	29.0	82	82.2
	CT	1	1.0	8	7.6
	CC	0	0.0	0	0.2
	P (HW)	0.962		0.659	

HW, Hardy–Weinberg.

Table 6. Distribution of rs763780 Genotype Variants and Alleles in all COVID-19 Patients Versus Control

				Relative Risk of COVID-19			
		Control (n = 30)	COVID-19 Patients (n = 90)	OR	95% CI		*P*
TT	Count (%)	29 (96.7%)	82 (91.1%)	1	–	–	*R*
CT	Count (%)	1 (3.3%)	8 (8.9%)	2.82	0.339	23.608	.336
CC	Count (%)	0 (0.0%)	0 (0.0%)	0.357	0.006	18.43	.609
CT+CC	Count (%)	1 (3.3%)	8 (8.9%)	2.82	0.339	23.608	.336
T	Count (%)	59 (98.3%)	`172 (95.5%)	2.74	0.336	22.405	.346
C	Count (%)	1 (1.6%)	8 (4.5%)				

OR, odds ratio.

Table 7. Comparison of Clinical Severity of COVID-19 and Outcome Regarding Different Genotypes in COVID-19 Patients

Parameter		TT Group (n = 82)	CT Group (n = 8)	*P*
COVID-19 clinical severity	Nonsevere	5 (6.1%)	0 (0.0%)	.714
	Severe	44 (53.7%)	4 (50.0%)	
	Critical	33 (40.2%)	4 (50.0%)	
Outcome	Improved	37 (45.1%)	2 (25.0%)	.458
	Dead	45 (54.9%)	6 (75.0%)	

sensitivity and 65% specificity. Fatemeh et al[12] determine cutoff value of 169.2 pg/mL for o differentiating mild from severe COVID-19.

COVID-19 has different inflammatory responses between individuals. A possible genetic background could explain this phenomenon. Multiple polymorphisms have been reported in the rs2275913 locus IL-17A gene based on the tested population. At the same previous locus, the AG genotype is present in the populations of Norway, Poland, Finland, Czechia, Japan, India, Iran, and China, whereas the GG genotype is present in the population of Egypt, Tunisia, Germany, Turkey, Netherlands, Spain, Brazil, and Mexico. On the other hand, other studies handling the rs763780 locus noted that all populations had the TT genotype.[13] Therefore, we proposed to investigate rs763780 loci polymorphism and its association with the COVID-19 severity and outcome.

Though, there is uncertainty regarding the fact that IL-17 upregulation is a direct consequence of COVID-19 infection. Recent studies discovered that COVID-19 open reading frame 8 (ORF8) causes activation of the IL-17 signaling pathway via binding to the IL-17 receptor. In previous experimental research, mice infected with SARS-CoV2 ORF8 pseudo-virus decreased pulmonary and hepatic inflammation when treated with IL-17RA blocker (antibody).[14]

In this study, the tested genotypes and alleles showed no significant difference between cases and controls. Also, in comparison between gene polymorphism and COVID-19 severity and outcome among studied groups, no statistically significant difference could be detected.

However, in the study conducted by Karcioglu Batur and Hekim,[13] rs763780 locus TT and TC genotypes showed a significant correlation with COVID-19 prevalence, whereas rs2275913 locus AG genotype showed a significant correlation with COVID-19-related mortality. This was noticed in the tested population from many countries, including Egypt.

Xie et al[15] noted that patients with gene polymorphisms causing decreased IL-17 production had better 30-day survival rates. Contrarily, patients with polymorphisms causing increased IL-17 production were associated with increased disease severity and related mortality.[15]

The main limiting element of this study is the number of patients involved. Further investigations must be done to identify the relationship between IL gene polymorphisms and COVID-19 outcomes. These future investigations must be analyzed to predict potential drugs blocking IL-17RA and reducing IL-17-mediated systemic inflammatory response associated with COVID-19 infection. This would help us to improve disease outcomes.

Ethics Committee Approval: This study was approved by the Ethics Committee of Mansoura University (code number: R.21.03.1275.).

Informed Consent: Written and verbal informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – H.S., S.Y.; Design – A.H., S.S.; Supervision – E.A., H.M.; Resources – G.S., E.A.; Materials – H.S, S.Y.; Data Collection and/or Processing – A.H., S.S.; Analysis and/or Interpretation – E.A. Literature Search – A.H., S.S.; Writing – A.H., H.S.; Critical Review – A.H.

Declaration of Interests: The authors have no conflict of interest to declare.

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Original Article

Serum Vitamin D Is Inversely Associated with Blood Eosinophil Count Among Adults with Allergic Asthma

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Abstract

OBJECTIVE: To evaluate the role that serum vitamin D concentration plays in the number of eosinophils in peripheral blood in adults with allergic asthma.

MATERIAL AND METHODS: A total of 142 patients were categorized based on different cutoff points for eosinophils: ≥200, ≥300, ≥400, and ≥500 cells/mL. The vitamin D concentration was stratified into <20 and ≥20 ng/mL. The association between vitamin D (independent variable) and eosinophils (dependent variable) was explored using multivariate analysis.

RESULTS: The average number of eosinophils in the included patients was 418 cells/mL, and 33.8% of the included patients had vitamin D concentrations ≥20 ng/mL. Asthmatic patients with vitamin D< 20 ng/mL had a higher mean concentration of eosinophils than did asthmatic patients with vitamin D ≥20 ng/mL (464 ± 377.7 eosinophils/mL vs. 327.8 ± 247.2 eosinophils/mL, *P* = .025). We also observed that vitamin D was inversely correlated with eosinophil count (rho = 0.244, *P* = .003). In the multivariate analysis, vitamin D <20 ng/mL showed a significant inverse association with each cutoff value for eosinophilia (odds ratio >1).

CONCLUSION: Vitamin D concentrations <20 ng/mL are associated with a significant increase in the number of eosinophils in blood. Studies that analyze the use of vitamin D supplements as complementary therapy for the treatment of asthma are needed.

KEYWORDS: Allergy, asthma, eosinophils, vitamin D
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INTRODUCTION

Among the most frequent hypovitaminosis worldwide is vitamin D (VD) deficiency. In the adult population, the prevalence of VD deficiency (<20 ng/mL) is approximately 40%.[1-3] Vitamin D is mostly recognized for its influence on calcium and phosphorus metabolism as well as bone metabolism; however, an increasing number of extraosseous effects are increasingly drawing attention. 25-hydroxyvitamin D (25[OH]D) (calcitriol), the active form of VD, participates in the regulation of innate and adaptive immune responses. It favors the production of defensins and cathelicidins and improves chemotaxis and the function of physical barriers, among other things. In the adaptive immune response, calcitriol inhibits the production of Th1 cytokines (IL-12, IFN-γ, IL-6, IL-8, TNF-α, and IL-9) and increases the production of anti-inflammatory cytokines Th2 (IL-4, IL-5, and IL-10); in addition, it inhibits the proliferation of Th1 cells and induces the production of Treg cells.[4] At the level of eosinophils, VD reduces necrosis and, consequently, the secondary release of cytotoxic granules from eosinophils in tissues.[5] Eosinophils cultured in a VD-free medium exhibit spontaneous activation; in contrast, the levels of activation products are reduced in the presence of VD.[6]

In Latin America, there is little information on the relationship between VD and blood eosinophils in adults with asthma. Our primary objectives were, first, to determine the prevalence of alterations in serum VD concentration and, second, to evaluate the role of serum VD concentration on the number of eosinophils in peripheral blood.

MATERIAL AND METHODS

Study Site and Patients

Our teaching hospital is located in the metropolitan area of Guadalajara in western Mexico. In this metropolitan area, the average elevation is 1540 m above sea level; the climate is temperate most of the year, with an average temperature of 18°C; and the months with the greatest solar radiation are from March to August, with a pronounced peak during May.[7]

In this cross-sectional study, only adults aged 18 years or older were included, and all were residents of the metropolitan area. Patients with a history of respiratory disease exacerbated by aspirin or near-fatal asthma, those who were using

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VD supplements, systemic steroids, anticonvulsants, rifampicin, or antiretrovirals during the previous 4 weeks, and those who had an asthma attack 1 month prior to the study were not included. The patient inclusion period was from October 2017 to April 2019.

Serum Vitamin D, IgE, and Eosinophils

Vitamin D, total serum IgE, and eosinophils in peripheral blood were quantified in the central laboratory of the hospital on the same day that the blood sample was obtained. The magnetic particle-based chemiluminescence technique was used to quantify VD (AccessTotal®, Beckman Coulter) and total IgE (AccessTotal IgE®, Beckman Coulter) concentrations; the former is expressed in ng/mL, and the latter is expressed in IU/mL. Flow cytometry (CELL-DYN Ruby™; Abbott Diagnostics Division, Abbott Laboratories, IL, USA) was used to quantify the total eosinophil concentration, expressed as cells per microliter (µL).

Skin Testing and Forced Spirometry

Allergic sensitization was determined by the presence of at least 1 positive skin test to 1 or more of the tested aeroallergens (house dust mites, dander, cockroach mix, pollen from grasses, trees and weeds, and fungal spores). Histamine was used as a positive control, and glycerin was used as a negative control. The aeroallergens were placed on both volar surfaces of the forearms, and a Hollister Stier® lancet was used for puncture; skin test results were interpreted in accordance with international guidelines.[8]

Respiratory function (forced expiratory volume [FEV_1] and forced vital capacity [FVC]) was assessed via spirometry using a Master Screen-Body PFT spirometer (Jaeger®, CareFusion, Baesweiler, Germany). Each participant performed a maximum of 8 maneuvers or until 3 tests met the criteria of acceptability and reproducibility. In addition, a challenge test with a short-acting bronchodilator inhaler (salbutamol 0.4 mg) was performed. This test served to assess the reversibility of airway obstruction.

Definitions

The following cutoff points were used to classify eosinophilia in blood: ≥200, ≥300, ≥400, and ≥500 cells/µL.

Serum VD concentrations ≥20 ng/mL were considered sufficient to maintain calcium homeostasis.[9]

MAIN POINTS

- The frequency of vitamin D concentration ≥20 ng/mL in patients with asthma was 33.8%.
- The prevalence of eosinophilia in blood based on the different cutoff values were: eosinophils ≥200 cells/µL, 75.4%; eosinophils ≥300 cells/µL, 54.9%; eosinophils ≥400 cells/µL, 40.8%; and, eosinophils ≥500 cells/µL, 30.3%.
- Asthmatic patients with vitamin D concentration <20 ng/mL had higher blood eosinophil concentrations than patients with asthma and vitamin D ≥20 ng/mL.
- There was an inverse association between vitamin D concentration and blood eosinophils.

Serum levels of total IgE ≥100 IU/mL were considered outside the normal range.

Allergic asthma was considered the presence of symptoms compatible with the disease, in addition to evidence of reversible airway obstruction and at least 1 positive skin test to one of the tested aeroallergens.

Ethics

Our study was approved by the ethics committee of the New Civil Hospital of Guadalajara "Dr. Juan I. Menchaca"; each of the patients had to sign a written informed consent form to participate in the study, another to undergo spirometry, and another to undergo skin prick tests.

Statistical Analyses

Quantitative variables with a normal distribution are presented as the mean and SD. Categorical variables are presented as frequencies and proportions. The Spearman rho correlation test was performed to detect an association between the variables. Finally, to identify variables associated with eosinophilia, different multivariate logistic regression analysis models were performed, 1 for each eosinophilia cutoff value. A value of $P < .05$ was considered statistically significant. Data analysis was performed with the Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corp.; Armonk, NY, USA).

RESULTS

In this study, a total of 142 patients were included (75% were women), and the mean age was 35 years (Table 1). The frequencies of allergic rhinitis and atopic dermatitis in patients with asthma were 82% and 10%, respectively; 37% had a personal history of hospitalization for asthma during the previous year. Based on the results of asthma control tests (ACT), 28% of the subjects perceived their asthma as controlled. Mites and cockroaches were the allergens to which patients were most sensitized, with atmospheric fungi being the allergen to which patients were least sensitized. Regarding respiratory function, the mean baseline FVC and FEV_1 were 88% and 71% of the predicted value. Notably, asthmatics with VD concentrations <20 ng/mL had significantly lower FVC and FEV_1 than patients with VD greater than ≥20 ng/mL. The mean total serum IgE concentration was 418 IU/mL; 67% of the patients had concentration equal to or greater than 100 IU/mL. The mean VD concentration was 18.4 ± 8.1 ng/mL, and in 33.8% of the patients, the concentration was ≥20 ng/mL. The mean and median absolute blood eosinophil counts were 418 cells/µL and 340 cells/µL, respectively. When comparing the median and mean concentration of eosinophils between patients with asthma and VD <20 ng/mL and patients with VD ≥20 ng/mL, there was a statistically significant difference, i.e., 359 eosinophils/mL vs. 240 eosinophils/µL ($P = .023$) and 464 ± 378 eosinophils/µL vs. 327.8 ± 247 eosinophils/µL ($P = .025$), respectively.

The following provides the prevalence of eosinophilia in blood based on the different cutoff values: eosinophils ≥ 200 cells/µL: n = 107 (75.4%, 95% CI 67.6% to 81.7%); eosinophils ≥300 cells/µL: n = 78 (54.9%, 95% CI 46.7% to 62.9%); eosinophils ≥400 cells/µL: n = 58 (40.8%, 95% CI 33.1% to

Table 1. Characteristics of the Population with Asthma

	Total (n = 142)	Vitamin D <20 ng/mL (n = 94)	Vitamin D ≥20 ng/mL (n = 48)	*P*
Age (years), mean ± SD	34.6 ± 10.4	36.0 ± 10.2	31.8 ± 10.3	.024
Sex, female, n (%)	106 (74.6)	69 (73.4)	37 (77.1)	.634
Current smoking, n (%)	8 (5.6)	6 (6.4)	2 (4.2)	.717
Alcohol consumption, n (%)	33 (23.2)	18 (19.1)	15 (31.3)	.106
Atopic comorbidity, n (%)				
Allergic rhinitis	117 (82.4)	74 (78.7)	43 (89.6)	.108
Food allergy	27 (19.0)	18 (19.1)	9 (18.8)	.954
Atopic dermatitis	13 (9.2)	8 (8.5)	5 (10.4)	.709
Urticaria	7 (4.9)	2 (2.1)	5 (10.4)	.044
BMI (kg/m^2), mean ± SD	29.1 ± 6.2	29.8 ± 6.7	27.9 ± 4.9	.078
Hospitalization in the previous year, n (%)	52 (36.6)	32 (34.0)	20 (41.7)	.372
ACT, points				
Mean ± SD	15.2 ± 5.4	14.6 ± 5.4	16.2 ± 5.3	.103
≥20, n (%)	40 (28.2)	24 (25.5)	16 (33.3)	.328
Allergic sensitization, n (%)				
Mites	94 (66.2)	60 (63.8)	34 (70.8)	.404
Cockroaches	70 (49.3)	45 (47.9)	25 (52.1)	.635
Trees	67 (47.2)	43 (45.7)	24 (50.0)	.631
Epithelia	57 (40.1)	37 (39.4)	20 (41.7)	.791
Weeds	60 (42.3)	36 (38.3)	24 (50.0)	.182
Grass	43 (30.3)	29 (30.9)	14 (29.2)	.836
Fungi	18 (12.7)	12 (12.8)	6 (12.5)	.964
Respiratory function, mean ± SD				
% predicted baseline				
FVC	87.9 ± 18.3	85.1 ± 18.9	93.4 ± 16.0	.011
FEV_1	71.2 ± 19.2	68.8 ± 20.55	75.9 ± 15.4	.039
FEV_1/FVC	67.1 ± 10.3	66.7 ± 11.2	67.9 ± 8.1	.493
Total serum IgE, IU/mL				
Median (P_{25}-P_{75})	211 (74-502)	204 (67-524)	224 (82-501)	.626
IgE ≥100, n (%)	95 (66.9)	61 (64.9)	34 (70.8)	.477
Vitamin D, ng/mL				
Mean ± SD	18.4 ± 8.1	14.2 ± 3.7	26.5 ± 8.2	<.0001
≥20, n (%)	48 (33.8)	N/A	N/A	N/A
Eosinophils, cells/μL				
Mean ± SD	418.1 ± 344.5	464 ± 378	328 ± 247	.025
Median (P_{25}-P_{75})	340 (205-580)	359 (227-602)	240 (145-477)	.023

ACT, asthma control test; BMI, body mass index; FEV_1, forced expiratory volume; FVC, forced vital capacity; IgE, immunoglobulin E; N/A, not applicable; P_{25}-P_{75}, percentile 25-percentile 75.

49.1%); and, eosinophils ≥500 cells/μL: n = 43 (30.3%, 95% CI 23.3% to 38.3%).

As seen in the correlation matrix shown in Table 2, VD concentration was inversely correlated with blood eosinophil count (*P* = .003, statistical power of 84%) and age (*P* = .037). Blood eosinophil count was inversely correlated with age (*P* = .0398) and ACT (*P* = .006) and positively correlated with IgE (*P* = .002). Additionally, there was a significant correlation between age with body mass index (BMI) (positive correlation), FEV_1 (negative correlation), and IgE (negative correlation) (*P* < .0001). Asthma control test was significantly correlated with FEV_1. There was no significant correlation between VD and BMI, FEV_1, ACT, and IgE. There was also

Table 2. Magnitude of Correlation Between Variables

	Eosinophils	Vitamin D	Age	BMI	FEV_1	ACT	IgE
Eosinophils	1						
Vitamin D	-0.244 *P* = .003	1					
Age	-0.071 *P* = .398	-0.176 *P* = .037	1				
BMI	-0.104 *P* = .217	-0.048 *P* = .570	0.317 *P* < .0001	1			
FEV_1	-0.067 *P* = .428	0.096 *P* = .258	-0.298 *P* < .0001	-0.053 *P* = .513	1		
ACT	-0.230 *P* = .006	0.028 *P* = .743	-0.017 *P* = .842	-0.087 *P* = .305	0.427 *P* < .0001	1	
IgE	0.255 *P* = .002	0.070 *P* = .411	-0.317 *P* < .0001	-0.145 *P* = .086	0.088 *P* = .300	-0.160 *P* = .057	1

Correlation estimated through Spearman's rho.
ACT, asthma control test; BMI, body mass index; FEV_1, forced expiratory volume in the first second, predicted baseline; IgE, immunoglobulin E.

no significant correlation between eosinophils and BMI and FEV_1. There was no significant correlation between the other variables.

Table 3 presents several multivariate models of factors associated with blood eosinophilia based on the following cutoff points: ≥200, ≥300, ≥400, and ≥500 cells/μL. In all adjusted models, VD <20 ng/mL exhibited a statistically significant inverse association with eosinophilia. In contrast, IgE ≥100 IU/mL was identified as a risk factor for eosinophilia for all cutoff points, except when the eosinophil count was ≥500 cells/μL. Other risk factors for eosinophilia were sex (female) with an eosinophil count ≥400 cells/μL and fungal sensitization with an eosinophil count ≥500 cells/μL. Last, age younger than 35 years was inversely associated with an eosinophil count ≥500 cells/μL.

DISCUSSION

In this study, many patients with asthma showed alterations in VD concentration. When we compared the number of eosinophils between asthmatic patients with VD <20 ng/mL and those with VD ≥20 ng/mL, the former had a noticeably higher concentration of eosinophils in peripheral blood.

Based on the results of this study, VD deficiency in patients with allergic asthma is not infrequent, as up to 70% had VD concentrations lower than 20 ng/mL. This result contrasts markedly with those from population studies. In the United States, the National Health and Nutrition Examination Survey 2005-2006 showed that slightly more than 0% of the analyzed population had VD levels <20 ng/dL[3]; similar findings were observed in Mexico, 43.6% of adults also had VD levels <20 ng/dL.[1] There are different reasons to explain why VD may be insufficient in this population, including low exposure to sunlight, skin color, age, use of sunscreen, and low physical activity.[10] In our study, most patients had moderate airway obstruction, which may have contributed to less outdoor physical activity; consequently, they would be less exposed to ultraviolet radiation, thus favoring less VD production. The use of systemic or topical steroids at high doses can also contribute to this same problem.

Notably, in adult patients with allergic asthma and VD deficiency, there was an inverse association with eosinophil count in peripheral blood. This finding is consistent with previous studies conducted in children and adults with allergic and nonallergic asthma.[11,12] The use of VD supplements in asthmatic patients with VD deficiency has been shown to significantly reduce allergic inflammation.[2] Similar to asthmatic patients, patients with allergic rhinitis and VD deficiency also have increased eosinophil concentrations in blood compared with that in controls.[13] Last, the results herein indicate that a cutoff point of VD ≥20 ng/mL serves as a good predictor of eosinophilia in blood when the eosinophil count is 200 cells/μL or more. The modulating role of VD in the immune response seems to be responsible for its effect on eosinophils by favoring an increase in the production of Th2 cytokines in addition to inhibiting the proliferation of Th1 cells.[14] According to The Global Initiative for Asthma, VD supplementation may reduce the number of asthma exacerbations or improve asthma control, especially when VD levels are <20 ng/mL.[13]

Among other relevant findings in this study is the positive association between IgE concentration and eosinophils in blood. Notably, the relationship was stronger when the amount of eosinophils was ≥200 or ≥300 cells/μL, slightly diminished at ≥400 cells/μL, and finally disappeared at ≥500 cells/μL. A wide network of cytokines, including IL-4, IL-5, IL9, IL-13 and IL-31, is involved in the regulation of IgE expression and eosinophils; however, IL-4 and IL-13 are the main cytokines involved in T2 inflammation.[15] In our study population, all asthmatic patients were sensitized to allergens; thus, different mechanisms that favor eosinophil expression or fungal sensitization, as we present below, are probably involved in the lack of an association between IgE and eosinophil quantities ≥500 cells/μL. Although IgE is a biomarker of T2 inflammation, it has been suggested that IgE concentration does not help identify patients with asthma who will respond better to treatment with type 2 monoclonal antibodies[15]; therefore,

Table 3. Different Multivariate Models to Identify Factors Associated with Eosinophilia in Patients with Asthma

Dependent variable: eosinophils ≥200 cells/μL			
	aOR	**95% CI**	***P***
Vitamin D <20 ng/mL	2.84	1.29-6.26	.020
Female	—	—	.361
Age ≥35 years	—	—	.441
Allergic rhinitis	—	—	.981
ACT ≥20 points	—	—	.578
IgE ≥100 IU/mL	3.18	1.40-7.21	.006
Sensitization to fungi	—	—	.144
Dependent variable: eosinophils ≥ 300 cells/μL			
	aOR	95% CI	*P*
Vitamin D <20 ng/mL	2.62	1.53-6.56	.002
Female	—	—	.227
Age ≥35 years	—	—	.448
Allergic rhinitis	—	—	.806
ACT ≥20 points	—	—	.067
IgE ≥100 IU/mL	3.55	1.67-7.57	.001
Sensitization to fungi	—	—	.896
Dependent variable: eosinophils ≥400 cells/μL			
	aOR	95% CI	*P*
Vitamin D <20 ng/mL	2.20	1.01-4.79	.046
Female	3.22	1.32-7.82	.010
Age ≥35 years	—	—	.133
Allergic rhinitis	—	—	.268
ACT ≥20 points	—	—	.431
IgE ≥100 IU/mL	2.92	1.32-6.49	.008
Sensitization to fungi	—	—	.318
Dependent variable: eosinophils ≥500 cells/μL			
	aOR	95% CI	*P*
Vitamin D <20 ng/mL	3.16	1.27-7.85	.013
Female	—	—	.096
Age ≥35 years	0.40	0.18-0.89	.025
Allergic rhinitis	5.33	1.36-20.93	.016
ACT ≥20 points	—	—	147
IgE ≥100 IU/mL	—	—	.377
Sensitization to fungi	3.65	1.19-11.15	.023

OR obtained by binary logistic regression. All variables were entered dichotomously. The OR was not calculated for those variables that were not included in the model because they did not present a statistically significant association.
ACT, asthma control test; aOR, adjusted odds ratio; IgE, immunoglobulin E.

eosinophils in blood become a better therapeutic target for the control of asthma.

In this study, an eosinophil count ≥500 cells/μL was 60% less frequent in patients with asthma and was only observed in those over 35 years old. The emergence of biological products for the control of T2 asthma redefined the role of eosinophils as a therapeutic target, marking the need to define the best cutoff points to achieve the greatest benefit of their use. Thus, there are some biologics that are indicated when the eosinophil count is ≥150 cells/μL and others that are indicated when the count is ≥400 cells/μL.[16] In our study population, 75% of patients had eosinophil counts ≥200 cells/μL, but only 30% had counts ≥500 cells/μL. It seems that with increasing age, the number of eosinophils decreases, as do their effector functions.[17]

We also observed that allergic rhinitis in asthmatic patients was significantly associated with blood eosinophilia; however, this occurred only when eosinophil counts were ≥500 cells/μL. The mechanisms that could explain this phenomenon are not clear, but it has been shown that the concentration of eosinophils in blood is higher when allergic rhinitis is not controlled than when it is controlled.[18]

Finally, in our study, slightly more than 10% of the patients were sensitized to fungi; this finding is consistent with previously published data.[19,20] Notably, this event was a factor associated with blood eosinophilia, especially when the eosinophil count was ≥500 cells/μL. Colonization by fungi in patients with asthma is recognized for its ability to induce vigorous immune responses, which justifies the intentional search for allergic bronchopulmonary mycosis in patients with allergic sensitization to fungi; for this, new diagnostic criteria have been recently published.[21]

Due to the design characteristics of the study, we were not able to establish if the association between VD and eosinophilia is constant; it has been shown that the levels of eosinophils in blood can vary throughout the day and that, when treating allergic patients, they can be modified by exposure to sensitizing allergens. However, having used different cutoff points to define eosinophilia, it is unlikely that the association remained stable. Another limitation is the use of topical or systemic steroids by some participants at the time of inclusion in the study and the potential effect on blood eosinophils. Notably, we were careful not to include those patients with a history of systemic steroid use 1 month before participants were selected for this study. Regarding the use of inhaled corticosteroids, the patients were not using them regularly; but once the diagnosis of asthma was established, they were instructed to use them regularly and at adequate doses. Finally, we did not investigate the presence of parasitosis concomitant with asthma, diet characteristics, or the use of solar radiation blockers, all factors likely to modify the association between the variables.

CONCLUSION

In summary, several patients with asthma have VD concentrations below 20 ng/mL (more than 70%). In this study, VD levels <20 ng/mL were associated with a greater number of total eosinophils in peripheral blood in adults with allergic asthma. These findings fully justify the analysis of the repercussions of VD deficiency at the eosinophil level, i.e., eosinophil count, and the functional level. Similarly, more research that analyzes the use of VD supplements as complementary therapy for the treatment of asthma is needed.

Ethics Committee Approval: This study was approved by Ethics committee of New Civil Hospital of Guadalajara "Dr. Juan I. Menchaca" (Approval No: CGI/JDI/042/2017).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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Original Article

Lipoprotein(a): A New Intensive Care Unit Admission Predictor in Coronavirus Disease 2019 Patients

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Abstract

OBJECTIVE: Endothelium-related events in patients with coronavirus disease 2019 are linked to a poor prognosis. Lipoprotein(a) plays a role in vascular endothelial cell dysfunction. This research aims to investigate whether baseline serum lipoprotein(a) levels could be a predictor for intensive care unit admission and related clinical parameters in coronavirus disease 2019 patients.

MATERIAL AND METHODS: The research covers 126 patients who were hospitalized in intensive care unit or the non-intensive care unit in our hospital. This prospective cohort study was conducted from January 2021 to June 2021. The patients who were positive for severe acute respiratory syndrome coronavirus 2 according to real-time polymerase chain reaction test results were included in the study. Two groups were created according to the status of intensive care unit admission. Lipoprotein(a) was studied from blood samples taken at the time of hospital admission.

RESULTS: According to the results of the first clinical evaluation, 46 patients were admitted to the intensive care unit and 80 patients were admitted to non-intensive care unit in the hospital. Patients with intensive care unit admission had significantly higher serum lipoprotein(a) levels than patients without intensive care unit admission (40.9 ng/mL and 17.4 ng/mL, $P < .001$, respectively). The regression analysis revealed that serum lipoprotein(a) levels were independently related to intensive care unit admission (odds ratio 1.242, 95% CI 1.109-1.391, $P < .001$). In receiver operating characteristic curve analysis, lipoprotein(a) level ≥31.42 ng/mL had 82.6% sensitivity and 72.5% specificity in predicting intensive care unit admission. The risk of intensive care unit admission was seen to be 12.522-fold higher in cases with lipoprotein(a) level ≥31.42.

CONCLUSION: Lipoprotein(a) could be used as a useful biomarker for the triage of coronavirus disease 2019 patients. Baseline serum lipoprotein(a) levels may serve as a useful prognostic biomarker in patients hospitalized for coronavirus disease 2019.

KEYWORDS: COVID-19, mortality, clinical outcome, lipoprotein(a)
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INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first named and identified by WHO on February 11, 2020.[1] Various clinical outcomes can be seen in the course of the COVID-19 disease, ranging from asymptomatic to death.[2] Clinical symptoms such as coughing, high fever, and dyspnea are frequently observed in patients, while embolic and vascular pathologies are also seen in some patients.[3,4]

During severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral infectious disease, lipid and lipoprotein metabolisms can change, and these changes can be easily identified by biochemical tests. A virus exploits the host organism to satisfy its need to reproduce and can alter lipid and lipoprotein levels.[5,6]

Lipoprotein(a), which is a prothrombotic and anti-fibrinolytic lipoprotein, consists of low-density lipoprotein particles attached to apolipoprotein(a).[7,8] Lipoprotein(a) levels are mainly determined by genetics; however, they may temporarily increase during acute inflammatory episodes.[9] Lipoprotein(a) is an important acute phase reactant in endothelium-related events such as cardiac and non-cardiac clinical situations.[10,11] Our knowledge of how Lp(a) levels change in COVID-19 patients and the clinical use of this change are limited. We know from previous publications that endothelial-related events trigger worse clinical scenarios in COVID-19 patients.[12]

We hypothesized that Lp(a) may be associated with intensive care unit (ICU) admission in COVID-19 disease. The aim of our study was to investigate the relationship between Lp(a) levels at the time of hospitalization and the status of ICU admission in COVID-19 patients.

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MATERIAL AND METHODS

Study Population

Our prospective cohort study was conducted in a single center and included patients who applied to our tertiary health center between January 2021 and June 2021. The Ministry of Health of the Republic of Turkey and the ethics committee of Çanakkale Onsekiz Mart University approved the study (09.12.2020, 2011-KAEK-27/2020-E.2000175652). Our research was carried out in line with the decisions of the Declaration of Helsinki. Both verbal and written informed consent were obtained from the participants.

Our study consisted of 126 patients who were hospitalized and monitored in the clinics of cardiology, infectious diseases, and clinical microbiology and who were positive for COVID-19 according to clinical, laboratory, radiological, and real-time polymerase chain reaction (RT-PCR) tests.

Combined throat/nose swab samples were taken, and SARS-CoV-2 RT-PCR tests were performed in accordance with the guidelines of the Ministry of Health of the Republic of Turkey and the World Health Organization (WHO). Non-ICU and ICU admissions were made in line with the recommendations of the scientific committee in our country.[13]

Patients were hospitalized if any of the following symptoms were present:

1. Moderate/severe pneumonia (patients with bilateral widespread pneumonia findings on their chest x-ray or tomography).
2. Hypotension (<90/60 mmHg) and tachypnea (≥30/min) or an arterial oxygen saturation value (<93%) without supplemental oxygen.
3. Severe laboratory parameters [C-reactive protein (CRP) >40 mg/dL (high sensitivity), lymphopenia, or >1000 ng/mL ferritin] despite unilateral infiltration of the lung.

The patients were admitted to the ICU if any of the following symptoms were present:

1. A respiratory rate of ≥30/min.
2. Oxygen saturation of <90% and partial oxygen of 70 mmHg despite nasal oxygen supplement of >5 L/min.
3. Heart rate >100 beats/min and systolic blood pressure (bp) <90 mmHg or mean arterial bp <65 mmHg.
4. Arterial oxygen partial pressure (PaO_2)/fractional inspired oxygen (FiO_2) ≤300.
5. Lactate levels >2 mmol/L.

MAIN POINTS

- Laboratory parameter abnormalities on admission are common in coronavirus disease 2019 patients.
- Lipoprotein(a) could be used as a useful biomarker for the triage of coronavirus disease 2019 patients.
- Baseline serum lipoprotein(a) levels may serve as a useful prognostic biomarker in patients hospitalized for coronavirus disease 2019.

Patients with renal disease [estimated glomerular filtration rate (eGFR) <30 (mL/min/1.73 m^2)] and heart failure with reduced left ventricular function (left ventricular ejection fraction of 40% or less), thromboprophylaxis contraindications, malignant disease, abnormal liver dysfunction (ALT and AST >3x the upper limit of normal), cerebrovascular disease, chronic atrial fibrillation, a history of heart attack, a history of coronary artery bypass surgery, use of cholesterol-lowering drugs, systemic lupus erythematosus, rheumatoid arthritis, current use of oral contraceptives, steroids or monoclonal antibody drugs, and those with endocrine diseases such as familial hypercholesterolemia that may affect Lp(a) levels were excluded from the study.

Biochemical and hemogram parameters were acquired from peripheral blood samples taken at the time of hospitalization using standard laboratory techniques. Body temperatures were determined as ≥38°C. The recommendations of the WHO were applied in categorizing the patients according to the severity of the disease.[14]

Serum Lipoprotein(a) Measurements

Human Lp(a) test samples and 10 mL peripheral venous blood samples were taken from patients at the time of hospital admission (at the time of initial diagnosis and before starting treatments such as steroids). Blood samples were centrifuged at approximately 1000×g for 20 minutes, and the obtained serum samples were stored at −80°C before further analysis. ELK1564 enzyme linked-immunosorbent assay kits [ELK (Wuhan) Biotechnology CO., Ltd., Hubei, P.R.C] were used for measuring Lp(a) levels. The kit has a sensitivity of 1.48 ng/mL and a detection range of 3.13-200 ng/mL. The inter- and intra-assay variation coefficients are both less than 10% and 8%, respectively, for this test.

Statistical Analysis

The Kolmogorov-Smirnov test was used for the distribution analysis of variables. The data are presented as median and percentiles (25th and 75th percentiles) for continuous variables. Categorical data are expressed in percentages and numbers. The probability values of categorical data were compared using the chi-square test. The Mann–Whitney *U*-test was used to compare non-normally distributed data variables between the groups. The Spearman rank correlation coefficient was used for detecting the correlations between Lp(a) and the variables. The impact of Lp(a) and other factors on ICU admission were investigated using logistic regression analysis. To determine whether Lp(a) could be used as a predictor of ICU admission in COVID-19 patients, a receiver operating characteristic curve was used, and the area under the curve (AUC) was calculated. Data were considered statistically significant in the presence of *P*-values less than .05.

RESULTS

Characteristics of Study Subjects

Our study consisted of a total of 126 patients hospitalized with the diagnosis of COVID-19; 46 of the patients (19 men, 27 women) were admitted to the intensive care unit. The median age of the ICU admission group was 60 (53-73),

and the median age of the non-ICU admission group was 58 (44-67). The basic characteristics of the patients are given in Table 1. Oxygen saturation was lower [88 (86.7-92) vs. 92.5 (91-94), $P < .001$, respectively], and Lp(a) values were higher [40.9 (34.48-48.11) vs. 17.4 (9.18-30.91), $P < .001$, respectively] in patients with ICU admission compared to non-ICU admission group (Table 1).

Antibiotic treatment was added to the standard treatment in 16 patients (34.8%) in the ICU admission group and in only 8 patients (10%) in the non-ICU admission group. Twenty (43.5) patients in the ICU admission group required high-flow oxygen nasal cannula treatment, while only 14 (17.5%) patients in the non-ICU admission group needed it. In the ICU admission group, 23 (50%) patients required invasive mechanical ventilation. There was no need for invasive mechanical ventilation in the non-ICU admission group. There was a significant difference in Lp(a) levels between patients who received and did not receive high-flow oxygen nasal cannula treatment [35.28 (17.34-42.96) vs. 26.11 (12.92-35.81), $P = .016$]. Among the participants in our study, no one had a pulmonary embolism diagnosis.

It was discovered that Lp(a) and C-reactive protein are correlated ($r = 0.479$, $P < .001$) (Table 2).

The duration of treatment and hospital stay was observed to be longer in the ICU admission group compared to the non-ICU admission group [14 (9-15) vs. 8 (7-12) days, respectively, $P < .001$; Table 3]. In the study, 46 of the patients were admitted to the ICU following the initial evaluations and a total of 18 patients died (Table 3).

According to regression analysis, serum Lp(a) levels were independently related to the ICU admission [odds ratio (OR) 1.242, 95% CI 1.109-1.391, $P < .001$] (Table 4).

Table 1. Demographic and Laboratory Findings of the Patients

	ICU Admission		
	Without (n = 80)	**With (n = 46)**	***P***
Age	58 (44-67)	60 (53-73)	.127
Sex (male/female)	42/38	19/27	.226
SaO_2 (%)	92.5 (91-94)	88 (86.7-92)	<.001
SBP (mm Hg)	131 (128-135)	132 (128-136)	.667
DBP (mmHg)	67 (65-77)	67 (65-71.7)	.253
Heart rate (bpm)	90 (89-101)	90 (78-127)	.959
Smokers, n (%)	18 (22.5)	16 (34.8)	.135
Hypertension, n (%)	23 (28.8)	11 (23.9)	.556
Diabetes mellitus, n (%)	9 (11.3)	4 (8.7)	.767
COPD, n (%)	12 (15)	5 (10.9)	.514
Glucose (mg/dL)	116 (100.2-137.7)	109.5 (90-135.2)	.104
Creatinine (mg/dL)	0.77 (0.65-0.83)	0.72 (0.64-0.81)	.355
ALT (U/L)	26 (19-39)	30 (22-43)	.239
AST (U/L)	27.5 (22.2-38)	27 (19-41)	.980
TSH (uIU/mL)	0.63 (0.31-1.27)	0.86 (0.53-1.65)	.134
LDL-C (mg/dL)	96.5 (83-116)	87 (80-103)	.155
Total protein (g/dL)	6.84 (6.68-7.11)	6.8 (6.43-7.13)	.417
Albumin (g/dL)	4 (3.8-4.28)	4 (3-4.2)	.083
WBC ($\times 10^3$ /uL)	6.76 (4.72-8.48)	6.66 (4.77-11.96)	.182
Hemoglobin (g/dL)	12.95 (11.9-14.1)	12.5 (11.3-14)	.190
Neutrophil count ($\times 10^3$/uL)	4.84 (2.66-6.32)	4.95 (2.61-9.84)	.202
Lymphocyte count ($\times 10^3$/uL)	1.19 (0.88-1.76)	1.45 (0.93-1.71)	.602
CRP (mg/dL)	5.67 (2-13.75)	12 (4.84-15)	.012
Fibrinogen (mg/dL)	575 (385-764)	547 (385-792)	.953
D-Dimer (ugFEU/mL)	190 (130-299)	210 (110-521)	.794
Hs-TnT, ng/L	7.69 (6.1-10.3)	6.88 (4.9-9.36)	.672
Lp(a) (ng/mL)	17.40 (9.18-30.91)	40.90 (34.48-48.11)	<.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; DBP, diastolic blood pressure; FEU, fibrinogen equivalent units; Hs-TnT, high-sensitivity troponin T; ICU, intensive care unit; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); SaO_2, arterial saturation; SBP, systolic blood pressure; TSH, thyroid-stimulating hormone; WBC, white blood cells.

Table 2. Correlations Between Lipoprotein(a) and Laboratory Findings

		Age	Hs-TnT	D-Dimer	Fibrinogen	CRP	Albumin	LDL-C
Lipoprotein(a)	Correlation coefficient (*r*)	−0.72	−0.009	0.030	0.013	0.479	−0.126	−0.043
	P	.421	.923	.743	.884	<.001	.159	.634

CRP, C-reactive protein; Hs-TnT, high-sensitivity troponin T; LDL-C, low-density lipoprotein cholesterol.

Receiver operating characteristic curve analysis was performed to evaluate Lp(a) usability in predicting ICU admission. The cutoff value of Lp(a) was 31.42 (AUC: 0.815, 95% CI 0.738-0.891, $P < .001$, with 82.6% sensitivity and 72.5% specificity) (Figure 1). The risk of ICU admission was seen to be 12.522-fold higher in cases with Lp(a) level ≥31.42.

DISCUSSION

In our study, when investigating whether the Lp(a) levels are associated with ICU admission and related clinical parameters in COVID-19 patients, a significant relationship was found between baseline Lp(a) levels and ICU admission. We found that Lp(a) levels were independent predictors of ICU admission in COVID-19 patients. Lp(a) level ≥31.42 ng/mL had 82.6% sensitivity and 72.5% specificity in predicting ICU admission.

Severe acute respiratory syndrome coronavirus 2 causes vascular endothelial damage by stimulating the renin-angiotensin-aldosterone pathway. Endothelial injury decreases the antithrombotic function of cells, making them more prone to thrombus formation.[15,16] Increased fibrinogen levels, platelet activation, and hypoxia cause increased coagulation and complement activation in COVID-19 patients. As a result of all these factors, thrombosis can be triggered, and the dissemination of thrombosis can be accelerated by inflammation.[17] Patients who died from COVID-19 had widespread microthrombi in the peripheral capillaries and arterioles of several organs including the lungs and heart according to autopsy studies,[18] nevertheless, an autopsy is not possible for all of them. Early diagnosis of patients who may have a clinically serious and insidious course is important in reducing undesirable clinical outcomes.

Endothelial damage can be directly caused by SARS-CoV-2 or indirectly by the host's inflammatory response in many organs and by the interleukin-6 (IL-6) secreted by endothelial cells to defend against virus attack.[19] Increases in IL-6 levels can upregulate hepatic apo(a) synthesis, resulting in improved Lp(a) particle assembly and release into the circulation.[20] In addition, due to the increase in IL-6 levels in

Table 3. Clinical Outcomes of Patients

	ICU Admission			
Clinical outcomes	**All (n = 126)**	**With (n = 46)**	**Without (n = 80)**	***P***
ICU admission, n (%)	46 (36.5)	46 (100)	0	<.001
In-hospital mortality, n (%)	18 (14.3)	18 (39.1)	0	<.001
Hospital stay duration (days)	9 (7-14)	14 (9-15)	8 (7-12)	<.001

ICU, Intensive care unit.

Table 4. The Effect of Variables on ICU Admission

Variables	***P***	**Exp(B) Odds Ratio**	**95% CI Lower-Upper**
Age	.069	1.071	0.995-1.154
Hypertension	.055	1.284	0.558-2.952
Diabetes mellitus	.810	1.167	0.331-4.109
COPD	.515	1.447	0.475-4.404
SaO_2	.206	0.850	0.661-1.093
CRP	.706	0.970	0.827-1.137
Fibrinogen	.502	1.001	0.998-1.005
D-Dimer	.520	0.999	0.998-1.001
Hs-TnT	.541	1.016	0.965-1.070
Albumin	.728	0.719	0.112-4.621
LDL-C	.885	0.998	0.964-1.032
Lp(a)	< .001	1.242	1.109-1.391

COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; Hs-TnT, high-sensitivity troponin T; ICU, intensive care unit; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); SaO_2, arterial saturation.

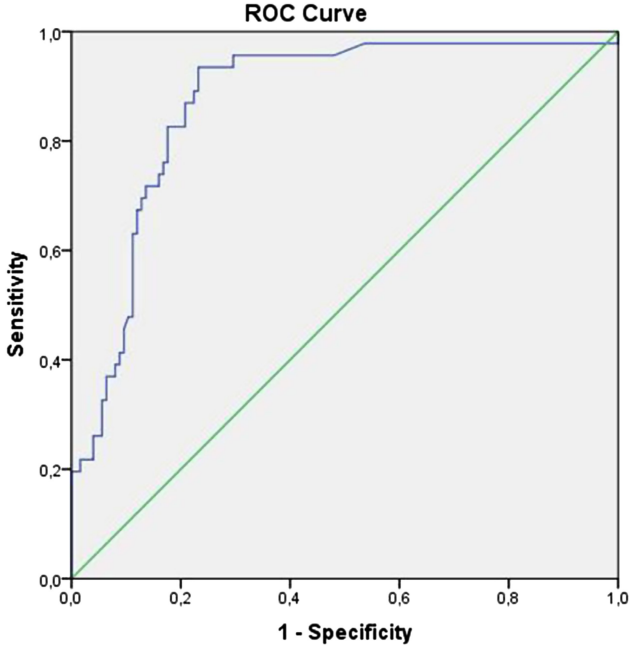


Figure 1. Receiver operating characteristic curve of lipoprotein(a) for ICU admission of Coronavirus Disease 2019 patients.

SARS-CoV-2 infection, an increase in CRP levels, which is an acute phase reactant, is observed secondarily in the liver.[21] Systemic inflammation with cytokine storm may cause vascular damage, causing disruptions in lipid transport.[22] In light of the above information, we think that both increased Lp(a) levels and impaired metabolism of Lp(a) are the main causes of poor clinical outcomes in COVID-19 patients. In our study, a positive correlation was observed between CRP and significantly higher Lp(a) levels in the ICU admission group. In addition, higher Lp(a) levels were detected in those who needed high-flow oxygen nasal cannula therapy.

D-Dimer has been shown to be a predictor of poor clinical outcomes including ICU admission and death in patients with COVID-19.[23] In another study, Huang et al[24] found that D-dimer levels were significantly higher in the ICU admission group than in non-ICU admission patients with COVID-19. However, in a recent study, researchers reported that D-dimer may have a lower accuracy than CRP for prognosis in patients with COVID-19.[25] In another recent study, it was reported that normal or slightly increased D-dimer and fibrinogen levels could be found. Obtaining different results from studies in the literature in terms of the main role of leukocytes in the thrombo-inflammatory process during bacterial infections shows that the main role of platelets in COVID-19 is not clear.[26] It is possible to see this difference in laboratory parameters. As seen in our study, no differences were observed between the groups in D-dimer and fibrinogen levels, which indicates that COVID-19 has a unique coagulopathy. However, Lp(a) levels were found to be significantly different between the groups in our study.

Lipoprotein(a) levels are not a simple acute phase reactant but are also likely to be an indirect indicator of complications that may develop in COVID-19 patients.[27] In light of all this information, it is expected that the risk of developing vascular events is higher in individuals with SARS-CoV-2 infection. The direct or indirect effect of the SARS-CoV-2 virus may contribute to endothelial cell damage, and poor clinical outcomes may be seen in COVID-19 patients as a result of endothelial damage. When our study results are evaluated, we believe that basal serum Lp(a) levels will provide clinicians with important information about the prognosis of patients hospitalized with the diagnosis of COVID-19.

In a recent study, it was shown that, despite a significant cardiac biomarker increase in COVID-19 survivors without known cardiovascular disease, mild deterioration in cardiac function may occur although cardiac biomarker increase has no effect on patient management or short-term prognosis.[28] As seen in our study, the results we obtained with cardiac troponin between both groups support the findings in the literature.

There were some limitations of our research. Considering our study results and current literature studies, it is not known how effective medical treatment that can impact Lp(a) levels may be in ICU admission. Serum Lp(a) samples were obtained from each patient at the first admission, and 1-time measurements were made. Therefore, we do not know whether there are dynamic changes in Lp(a) levels. However, the lack of a relationship between the time from the onset of symptoms to the time of diagnosis and Lp(a) levels is an important advantage of our study. We do not know how long it will take to change Lp(a) levels in recovering patients who have been discharged and how much higher Lp(a) levels may be associated with future COVID-19-related clinical conditions in this patient group compared to the control group. Multicenter studies are needed to support our study results and eliminate deficiencies.

Lipoprotein(a) can assist the clinician in the triage of COVID-19 patients. In line with these results, Lp(a) levels measured at hospital admission will provide clinicians with important information about which COVID-19 patients will require close monitoring.

Ethics Committee Approval: This study was approved by Ethics committee of Çanakkale Onsekiz Mart University University (Approval No: 2011-KAEK-27/2020-E.2000175652, Date: December 9, 2020).

Informed Consent: Both verbal and written informed consents were obtained from the participants.

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Original Article

Peripheral Blood Eosinophilia and Neutrophil Lymphocyte Ratio in the Choice of Antibiotic and/or Steroid in Patients Hospitalized with Acute Exacerbations of Chronic Obstructive Pulmonary Disease

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Abstract

OBJECTIVE: The choice of steroids and antibiotics is optional for the management of acute exacerbation of chronic obstructive pulmonary diseases according to international guidelines. The study hypothesized that the steroid and antibiotic choice can be decided by using the neutrophil–lymphocyte ratio and peripheral blood eosinophilia in patients with acute exacerbation of chronic obstructive pulmonary diseases. This would reduce the rate of re-hospitalization in 28 days.

MATERIAL AND METHODS: Patients were hospitalized due to acute exacerbation of chronic obstructive pulmonary diseases from February 1, 2018, to January 31, 2019. Patients were divided into 2 groups: Sureyyapasa protocol group and conventional group. In the Sureyyapasa protocol group, patients were divided into 4 subgroups according to peripheral blood eosinophilia and neutrophil–lymphocyte ratio values. Treatment success was defined as 5-7 days acute exacerbation of chronic obstructive pulmonary diseases treatment was enough to discharge and no re-hospitalization within 28 days. Treatment failure was defined that the hospital stay was longer than 7 days or transport to intensive care and death or readmission to the hospital due to acute exacerbation of chronic obstructive pulmonary diseases within 28 days after discharge.

RESULTS: The Sureyyapasa protocol group (n = 96) and the conventional group (n = 95) were randomly selected. The conventional group and Sureyyapasa protocol group had similar hospital stay ($P = .22$), and antibiotic and steroid uses were significantly higher in the conventional group than the Sureyyapasa protocol group (antibiotic use 100% vs. 83%, $P < .001$ and steroid use 84% vs. 29%, $P < .001$, respectively). Treatment failure in the conventional Group (n = 23, 24%) is higher than the Sureyyapasa protocol group (n = 17, 18%).

CONCLUSIONS: Initiating treatment by evaluating eosinophilia and neutrophil–lymphocyte ratio in patients with acute exacerbation of chronic obstructive pulmonary diseases in the ward reduces unnecessary antibiotic and steroid use and cost rates in hospitalizations.

KEYWORDS: Respiratory infections, COPD, interstitial lung disease, tuberculosis, respiratory intensive care

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INTRODUCTION

Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) increases morbidity and mortality of patients especially if needed hospitalization.[1,2] The most common treatments for AECOPD are antibiotics and corticosteroids.[1] Two-thirds of AECOPD is caused by lower respiratory infections, and two-thirds of these infections are caused by bacterial infections.[3] Due to Antonisen criteria, a high leukocyte count, C-reactive protein (CRP), and other anti-inflammatory biomarkers are partially useful for antibiotic selection.[4-6] However, CRP elevation is not limited to bacterial infections; viral and non-infectious causes also contribute to its elevation. Clinicians need objective criteria in addition to the subjective Antonisen Criteria and CRP elevation to avoid prescribing unnecessary antibiotics.[6-9] According to promising studies; inflammatory biomarkers such as neutrophil–lymphocyte ratio (NLR) and peripheral blood eosinophilia (PBE) may be useful in AECOPD management.[10-14] Studies have shown that steroid use is beneficial in patients with a PBE ratio above 2%.[14-18]

Also the Global initiative obstructive lung diseases (GOLD) 2019 guidelines emphasized the need for inhaled steroids in the treatment of eosinophilic COPD (PBE count >300) and oral systemic steroids in non-life-threatening AECOPD (GOLD).[19-23] Also PBE, NLR, platelet–lymphocyte ratio, mean platelet volume (MPV), and platelet–MPV ratio have been identified in the management of AECOPD patients.[20-25] The physicians have advised to "consider" the use of corticosteroids, antibiotics, or combination therapy in the treatment of AECOPD; however, the objective criteria in the guidelines have not been adequately explained. In the current study, it is hypothesized that the treatment protocol, which met the criteria of PBE ratio equal or greater than 2% for systemic steroid therapy, and NLR equal or greater than 4 is for antibiotic therapy, would reduce hospital stay and re-hospitalization in the first 28 days in AECOPD patients.

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This study's primary objective is to establish a standard treatment using the PBE ratio and NLR in hospitalized patients with AECOPD to shorten their length of stay. The secondary objective of this study is to reduce unnecessary antibiotic and steroid usage. The significance of this study is its contribution of objective data in terms of adding systemic steroids and antibiotics to the treatment of hospitalized AECOPD patients.

MATERIAL AND METHODS

This prospective cohort study was conducted between February 1, 2018, and January 31, 2019, at the University of Health Sciences Süreyyapaşa Training and Research Hospital for Chest Diseases.

The research was authorized by the University of Health Sciences Süreyyapaşa Chest Diseases and Thorasic Surgery Training and Research Hospital Ethics Committee (Approval No: 116, Date: 2017). The research conformed to the Declaration of Helsinki.

Patients

Eighty percent power and 95% CI were used to analyze sample size, and each group contained at least 80 patients. Patients were included who were admitted to the hospital with AECOPD. Figures 1 and 2 provide a summary of the treatment strategies for the Sureyyapasa protocol group (SPG). Conventional AECOPD treatment was given as per the 2017 GOLD guideline. Inhalation of short-acting beta 2 agonist as salbutamol and/or anticholinergic ipratropium bromide comprised the majority of bronchodilator treatment. Antibiotic therapy was 4 × 1 g amoxicillin clavulonic acid, and steroid therapy was 1-0.5 mg/kg of methylprednisolone per day, orally administered over a period of 5-7 days. Supplemental oxygen was administered if the pulse oxygen saturation was less than 88%; low molecular weight heparin (LMWH) is also administered to every patient unless there is a contraindication (e.g., platelet count of less than 50 000/mL or a history of hypersensitivity to LMWH).

Definitions

Blood neutrophil to lymphocyte count ratio (NLR) was calculated, and groups were defined based on NLR values; steroids and antibiotics were administered based on PBE ratio and NLR values (Figure 2). Patients in the SPG were divided into 4 subgroups based on their PBE and NLR values. The SPG-1 (symptomatic group): NLR <4 and PBE <2%; SPG-2 (steroid group): NLR <4 and PBE ≥2%; SPG-3 (antibiotic group): NLR ≥4 and PBE <2%; and SPG-4 (combined group): NLR ≥4 and PBE ≥2% (Figures 1 and 2). The selection of steroids and antibiotics for the conventional therapy group (CG) was determined by physicians regardless of PBE and/or NLR values. The treatment was considered successful if the patient was discharged 5-7 days after AECOPD treatment and did not require readmission within 28 days. Treatment failure was defined as a hospital stay longer than 7 days, transport to intensive care unit (ICU), death in or out of hospital, or readmission due to AECOPD within 28 days after discharging (Figure 1). The criteria for patient discharge and

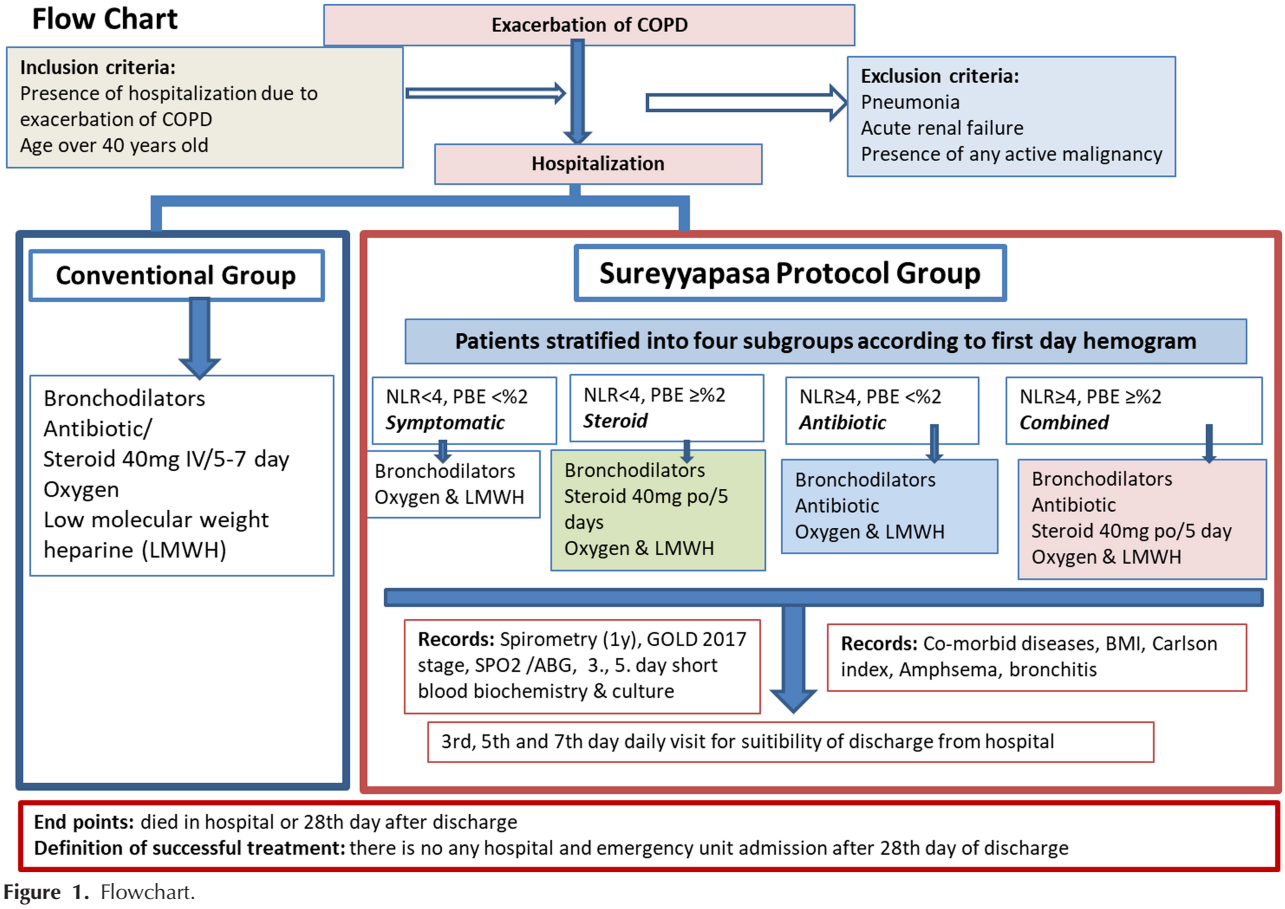


Figure 1. Flowchart.

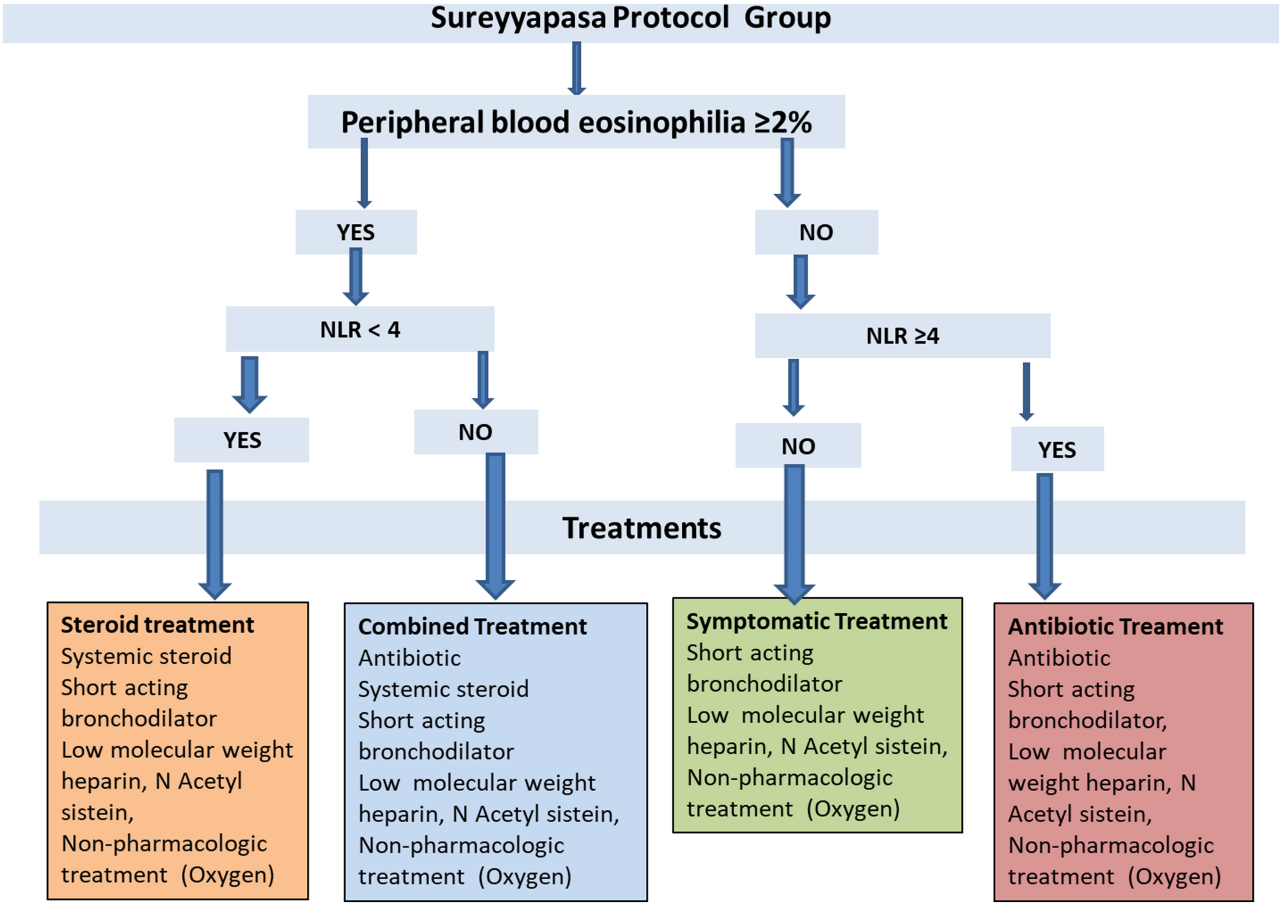


Figure 2. Subgroup classification of the Sureyyapasa protocol group according to peripheral blood eosinophilia and neutrophil–lymphocyte ratios.

recommendations for follow-up were determined in accordance with the 2017 GOLD guidelines.[26] The duration of follow-up after hospital discharge was 28 days.

Recording Data

The patient's file contains the patient's demographic information, co-morbidities, pulmonary function tests (within the past 2 years), hemogram, biochemistry, and arterial blood gas values on the day of hospital admission and discharge. On the first, third, fifth, seventh, and the final day of hospitalization, blood eosinophil, neutrophil, and lymphocyte counts and percentages were recorded.

Statistical Analysis

Statistical analyses were done by Statistical Package for Windows (IBM Corp.; Armonk, NY, USA). The dichotomous variables were analyzed by the Pearson chi-square test. Fisher's exact test was used if number was less than 5. Continuous variables such as age, pulmonary function test results, arterial gas values, and length of hospital stay in days are shown as mean with SD if normally distributed, and the Student's *t*-test was used for 2 group comparisons. Two groups that are not normally distributed were compared by the Mann–Whitney *U*-test. The *P*-value of .05 was considered statistically significant.

RESULTS

Two hundred patients were included in the study who met the inclusion criteria. The exclusion of 9 patients was due to complications that arose after hospitalization. Using random randomization methods, 96 patients with AECOPD were assigned to the SPG and 95 patients with AECOPD were assigned to the CG. There was no statistical difference between these groups in terms of age, gender, body mass index, smoking status, use of respiratory devices, and comorbidities (Table 1). Also, the groups had similar results in spirometric measurements and arterial blood gas values (Table 2). Both groups had moderate to severe volume loss on pulmonary function tests.

Table 3 summarizes the number and ratio of blood eosinophil levels with AECOPD on the day of hospitalization (basal eosinophil) and on the third, fifth, and seventh days and over the seventh day of hospitalization. There were 3 categories of eosinophil level: 2% as ratio and 100 and 300 cells/mL as count.[5] The SPG exhibited a statistically significant increase in blood eosinophil ratio and cell count on the third and fifth day of the hospitalization (Table 3). Table 3 indicates the comparison of NLR and PBE values for each study group. On the first day of hospitalization, the SPG had significantly higher NLR values than the CG (P = .047); on the third day both groups had similar NLR values (P = .11); on the fifth and seventh day of hospitalization, the CG had a significantly higher NLR value than the SPG (P = .001 and P = .005) (Table 3). On the fifth day of hospitalization, platelet lymphocyte ratio (PLR) values in the CG were significantly higher than in the SPG (P = .001). Platelet/MPV ratios were similar in 2 groups on all hospitalization days.

Table 1. Demographic Features of Conventional Therapy and Sureyyapasa Protocol Groups

	Conventional Therapy Group (n = 95)	Sureyyapasa Protocol Group (n = 96)	*P*
Male, n (%)	68 (72)	76 (79)	.22
Age, years, mean (SD)	69 (10)	69 (9)	.90
BMI—kg/m^2 median (25%-75%)	25 (22-29)	25 (22-29)	.65
Biomass exposure n (%)	29 (31)	33 (35)	.57
Smoking history			
Former smoker, n (%)	47 (61)	58 (71)	.16
Current smoker, n (%)	78 (82)	82 (85)	.54
Packet-year, median			
25%-75%	50 (35-60)	50 (30-60)	
LTOT, n (%)	41 (43)	42(44)	.93
NIMV at home, n (%)	20 (21)	21 (22)	.89
mMRC, mean (SD)	3 (1)	3 (1)	.57
Comorbidities, n (%)			
Hypertension	58 (61)	56 (58)	.70
Diabetes mellitus	24 (25)	30 (31)	.36
CHF	19 (20)	25 (26)	.32
CAD	41 (43)	43 (45)	.82
CRF	12 (13)	10 (10)	.63
Liver disease	3 (3)	0 (0)	.07
Hyperlipidemia	7 (7)	6 (6)	.76
Hypo/ Hyperthyroidism	5 (5)	3 (3)	.46
Psychiatric disorders	16 (17)	13 (14)	.53
Cerebrovascular diseases	5 (5)	5 (5)	.97
Tuberculosis history	11 (12)	18 (19)	.17
Osteoporosis	12 (13)	8 (8)	.33
Lung surgery	8 (8)	5 (5)	.38

BMI, body mass index; CAD, coronary artery disease; CHF, congestive heart failure; CRF, chronic renal failure; LTOT, long-term oxygen therapy; mMRC, modified medical research council; NIMV, noninvasive mechanical ventilation.

Table 2. Spirometric Measurements and Arterial Blood Gas Values of Conventional Therapy and Sureyyapasa Protocol Groups

Spirometric measurements	Conventional Therapy Group (n = 95)	Sureyyapasa Protocol Group (n = 96)	*P*
FEV1, %, median (25%-75%)	33 (26-47)	33 (25-46)	.63
FEV1, mL, median (25%-75%)	830 (620-1130)	860 (620-1110)	.78
FVC, %, median (25%-75%)	45 (36-62)	45 (36-56)	.74
FVC, mL, median (25%-75%)	1405 (1040-1850)	1510 (1200-1780)	.46
FEV1/FVC, mean (SD)	60 (10)	60 (10)	.78
MEF 25%-75%, median (25%-75%)	20 (13-29)	18 (13-28)	.50
OH SPO_2, mean (SD)	84 (9)	85 (9)	.78
ABG			
PH, mean (SD)	7.40 (0.04)	7.41 (0.04)	.09
PO_2, median (25%-75%)	60 (51-89)	59 (52-76)	.70
PCO_2, mean (SD)	49 (10)	46 (10)	.09
HCO_3, mean (SD)	29.4 (5)	28.4 (5)	.28
BE, median (25%-75%)	4.8 (2.2-7.9)	3.8 (1.3-6.9)	.21
SO_2, mean (SD)	90 (7)	91 (6)	.79
FiO_2, median (25%-75%)	21 (20-40)	21 (20-40)	.41

ABG, arterial blood gases; BE, base excess; FEV1, forced expiratory volume at first second; FiO_2, fractioned oxygen in respiratory air; FVC, forced vital capacity; HCO_3, serum bicarbonate; MEF, mid-expiratory flow; OH SPO_2, room air partial oxygen saturation; PCO_2, partial carbon dioxide pressure; PO_2, partial oxygen pressure; SO_2, oxygen saturation.

The sputum culture obtained cases and their results, antibiotic-free cases, corticosteroid usage rate and length of hospital stay for study groups are summarized in Table 4. One of the 51 patients in the CG with a positive sputum culture for *Pseudomonas aeruginosa*, while the others had positivity for gram-positive bacteria. The SPG group had 60 sputum culture positivity of which 2 cases were positive for *Pseudomonas aeruginosa*, 2 cases were positive for *Klebsiella pneumonia*, and the remaining cases were positive for gram-positive bacteria. There were no significant differences between the groups. According to NLR values, all patients in the CG used antibiotics, whereas 17% of patients in the SPG did not use antibiotics. According to serum eosinophil level, 29% of the SPG and 84% of the CG utilized corticosteroids (*P* = .001). Both groups had similar hospitalization lengths (Table 4). According to NLR criteria, the majority of patients with AECOPD required to be prescribed antibiotics (83%). The majority of patients with AECOPD were discharged from the hospital after 5 days. In the first 28 days after discharge, 38 patients [CG: n = 23 (24.2%); SPG: n = 17 (17.7%)] were admitted to the emergency unit or re-hospitalized. In SPG, the rate of AECOPD patients readmitted within 28 days of discharge was significantly lower than the CG (*P* = .008) (Tables 4 and 5). The readmission rate of the patients who were discharged on the sixth day of the hospitalization was significantly higher in the CG.

DISCUSSION

In this study, considering that prescribing steroids when PBE ratio ≥2% and prescribing antibiotics when NLR ≥4

Table 3. Comparison of NLR and the Mean PBE Levels of the Groups by the Treatment

	Conventional Therapy Group (n = 95)	Sureyyapasa Protocol Group (n = 96)	*P*
Baseline			
Cases of eosinophil ≥2%, n (%)*	24 (25)	30 (31)	.36
Cases of eosinophil ≥100 cc/mL, n (%)†	41 (43)	42 (43)	.93
Cases of eosinophil ≥300 cc/mL, n (%)‡	17 (18)	22 (23)	.39
NLR	4.80 (3-10)	6 (4-11)	.047
Third day			
*Cases of eosinophil ≥2%, n (%)	6 (6)	23 (24)	.001
Eosinophil ≥100 cc/mL, n (%)	14 (15)	36 (38)	<.001
Cases of eosinophil ≥300 cc/mL, n (%)‡	3 (3)	10 (10)	.046
NLR	6.60 (3-11)	4.70 (3-8)	.11
Fifth day eosinophil			
Cases of eosinophil ≥2%, n (%)*	7 (9)	37 (45)	<.001
Cases of eosinophil ≥100 cc/mL, n (%)†	15 (20)	48 (58)	<.001
Cases of eosinophil ≥300 cc/mL, n (%)‡	3 (4)	13 (16)	.015
NLR	6.60 (3-11)	4 (3-6)	<.001
Seventh day			
Cases of eosinophil ≥2%, n (%)*	9 (23)	22 (47)	.018
Cases of eosinophil ≥100 cc/mL, n (%)†	13 (33)	31 (66)	.002
Cases of eosinophil ≥300 cc/mL, n (%)‡	3 (8)	6 (13)	.42
NLR	5.40 (4-14)	3.50 (2-6)	.005
Over 7 days			
Cases of eosinophil ≥ 2%, n (%)*	3 (19)	7 (33)	.32
Cases of eosinophil ≥100 cc/mL, n (%)†	8 (50)	10 (48)	.89
Cases of eosinophil ≥300 cc/mL, n (%)‡	1 (6)	2 (10)	.72
NLR	8.18 (4-19)	5.25 (3-7)	.17

*Ref. 13, 17, and 18.
†Ref. 5.
‡Ref. 5.
NLR, neutrophil–lymphocyte ratio; PBE, peripheral blood eosinophilia.

Table 4. The Sputum Culture Positivity Rates, Antibiotic and Steroid Treatment Ratios, and the Length of Hospitalization Days in the Patients with AECOPD and Hospital Admission after Discharge from Hospital in the First 28 Days

	Conventional Therapy Group	Sureyyapasa Protocol Group	*P*
Sputum culture positivity, n (%)	51 (85)	60 (94)	.069
Antibiotic free, n (%)	0 (0)	13 (17)	<.001*
Corticosteroid usage, n (%)	80 (84)	28 (29)	<.001
Length of hospital stay day, median (25%-75%)	6 (5-8)	7 (5-8)	.22
Length of hospitalization days			
Fifth day and below	37 (39)	33 (34)	.51
Above fifth day	58 (61)	63 (66)	
Length of hospitalization days			
Seventh day and below	71 (75)	64 (67)	.52
Above the seventh day	24 (25)	32 (33)	
First month of admission to hospital, n (%)			
Due to COPD	21 (91)	9 (52)	.008*
Other reasons	2 (9)	8 (48)	
Sixth day discharge and first-month admission	21 (22.1)	9 (9.4)	.016

*Fisher's exact test, if n < 5.
AECOPD, acute exacerbation of chronic obstructive pulmonary disease; COPD, chronic obstructive pulmonary disease.

in patients hospitalized with acute COPD exacerbation, it was shown that it shortened the length of hospitalization and reduced readmission rate in the first 28 days due to chronic obstructive pulmonary (COPD)-related symptoms.

All Anthonisen-eligible CG patients received antibiotics. The SPG reduced unnecessary antibiotic use by 17%. In the CG, 84% of AECOPD patients used steroids based on clinical status and pulmonary oscultation; 29% of AECOPD patients in the SPG group have prescribed steroids whose blood eosinophil ratio was ≥2%.

Bacterial, viral, eosinophilic, and pauciinflammatory AECOPD groups had similar FEV_1 and FEV_1/FVC values.[13] Anthonisen et al[4] studied antibiotic initiation in AECOPD. In GOLD 2017 guidelines, the decision for antibiotic initiation for AECOPD was based on clinical history and "physician opinion" guided by Anthonisen criteria.[26] GOLD 2019 defined eosinophilic COPD endotype and its treatment which led to new treatment approaches in this field.[5] In the current study, COPD endotype distribution was similar between groups (Table 3). If the blood eosinophil ratio is ≥2%, the cell count per liter is between 2 and 1/5. If eosinophil counts were ≥300 cells/L, mortality was found to be 1%-5% (Table 3). As a biomarker for AECOPD risk and treatment response, peripheral blood eosinophil level is being monitored. Recent studies[15–17] show that systemic steroid treatment improves COPD exacerbation symptoms faster. Corticosteroid treatment was more effective when there was peripheral blood

Table 5. First Twenty-Eighth Day Hospital Admission

	Conventional Therapy Group Failure (Readmission to Hospital), n = 23		Sureyyapasa Protocol Group Failure (Readmission to Hospital), n = 17	
	n	%	n	%
Male	17	73.9	13	76.5
Hypertension	17	73.9	11	64.7
Diabetes Mellitus	6	26.1	6	35.3
Hearth failure	8	34.8	5	29.4
Coronary artery disease	10	43.5	8	47.1
Long-term Oxygen user	16	69.6	10	58.8
Home NIV user	10	43.5	6	35.3
Steroid use in AECOPD	19	82.6	6	35.3
Antibiotic use in AECOPD	23	100.0	14	82.4
Sputum pathogen	12	100.0	9	90.0
Sixth day discharge and rehospitalization in the first 28th day	21	91.3	9	52.9
Bradycardia	0	0.0	1	5.9
Home NIV device problem	1	4.3	0	0.0
Ileus	0	0.0	1	5.9
Pneumonia	0	0.0	5	29.4
Discharge EOS 2%	0	0.0	1	33.3
Discharge EOS100	1	33.3	2	66.7
Discharge EOS300	0	0.0	0	0.0

AECOPD, acute exacerbation of chronic obstructive pulmonary disease; EOS, eosinophilia; NIV, noninvasive mechanical ventilation.

eosinophilia (2%). In 4 studies on eosinophilic AECOPD, corticosteroid treatment had a better treatment response, and also intensive care and ward hospitalization rates were lower, noninvasive mechanical ventilation response was better.[15,24,27] In the current study, ICU-admitted AECOPD patients were excluded. Bafadhel et al[28] evaluated peripheral blood eosinophilia in AECOPD. Treatment failures were hospitalization due to AECOPD within 90 days, re-treatment, and death. In that study, patients were categorized by prednisolone treatment and peripheral blood eosinophil ratio (<2% or ≥2%). Non-prednisolone group failed in 66% of patients with a blood eosinophil ratio of ≥2%, while prednisolone group failed in 11% of patients. In that study, patients with a blood eosinophil ratio less than 2% did not differ significantly in treatment failure.[28] In 2018, Aksoy et al[27] reported that 86% of eosinophilic AECOPD patients were followed up through outpatient clinics, 10% of patients were hospitalized in inpatient clinics, and 4% were treated at ICU. The eosinophilic endotype, defined as peripheral blood eosinophil ≥2%, was found in 20% of ward patients, 9.6% of ICU patients, and 5% of outpatients with chronic respiratory failure and home noninvasive mechanical ventilation. A study in 2019 by Müllerová et al[29] recommended multiple inhalers (including inhaler corticosteroid) for exacerbation patients with 150 eosinophil cells/mL and at least 2 severe COPD exacerbations or 1 hospitalization due to COPD exacerbation. In the current study, 28 out of 96 (29%) in the SPG patients received steroids because they had ≥2% peripheral blood eosinophil ratio, whereas 24 out of 95 (26%) in the CG patients had ≥2% peripheral blood eosinophils and 80 patients (84%) were treated with steroids. Also in the 28 patients who were readmitted due to AECOPD, only 8 patients (28.5%) were in the SPG group. The "neutrophilic endotype" increases as COPD exacerbations increase in number and severity.[16,24,27] In Iran, in 2017, patients with NLR ≥4 had a higher mortality rate (9.5% vs. 24.0%, $P = .001$). In the same study, PLR and lymphocyte-to-monocyte ratios were investigated, but no significant difference was found; however, a high NLR was the first positive indicator of in-hospital mortality in AECOPD patients.[30] Recent clinical studies link a high NLR and leukocyte count to cardiovascular disease severity, mortality, and prognosis.[31] Patients with stable COPD and AECOPD had significantly higher NLR values than healthy subjects. In that study, stable COPD patients had NLR values similar to AECOPD patients, and researchers could not find a correlation between NLR values and disease severity. This study revealed a significant correlation between CRP and NLR.[9] In Korea, the study including 148 AECOPD patients, stable COPD, and healthy controls had significantly different NLRs ($P = .001$).[32] In a study of 100 cases of AECOPD, 80 healthy subjects were examined for NLR during the diagnosis, and also NLR was assessed in the stable period for COPD, 3 months after diagnosis. The NLR, CRP, leukocytes, and erythrocyte sedimentation rate correlate during COPD exacerbation.[33] In the same study, AECOPD patients had higher NLR than stable COPD patients and healthy subjects.[33] In the current literature, numerous studies indicated correlation between NLR and CRP. C-reactive protein evaluation is more expensive than hemogram. Cost and feasibility motivate physicians' use of NLR over CRP in AECOPD. Neutrophil–lymphocyte ratio is an easy-to-access, inexpensive biomarker of AECOPD that can help hospitalization decisions.[9,31-33] In our study, AECOPD treatment was based on NLR and blood eosinophil ratio and the SPG's NLR values decreased as expected after treatment. Platelet/MPV and PLR ratios did not differ between groups, except the fifth day PLR. Tanrıverdi et al[34] researched the significance of NLR in AECOPD due to infectious causes and found that NLR was elevated in bacterial infections, with a cutoff value of 11.5, achieving 61% sensitivity and 58% specificity. Procalcitonin is more specific for bacterial infections, but its high cost makes routine use difficult.[34] Neutrophil–lymphocyte ratio can be used more broadly than CRP because CRP is elevated also in noninfectious and viral diseases and requires laboratory equipment and more time.

Our study involves a single center. However, the study center is a specific training and research hospital for chest diseases. Additionally, the SPG for AECOPD with distinct endotypes cannot be applied universally. In the meantime, our research findings can be applied to a larger sample of AECOPD patients with various endotypes.

CONCLUSION

The rate of readmission of COPD patients within 28 days of discharge is reduced if AECOPD is treated with steroids when the blood eosinophil ratio is ≥2% and antibiotics when NLR is ≥4. The SPG reduces the need for steroids and antibiotics. The SPG patients had 90% less re-exacerbation at 28 days. Sureyyapasa protocol group reduced AECOPD steroid use. Neutrophil–lymphocyte ratio was used for antibiotic prescribing and peripheral blood eosinophil ratio was used for steroid selection. This study did not support standardizing treatment using the PBE and NLR to shorten hospital stays for AECOPD patients; however, the study showed that reduced use of antibiotic and steroid in AECOPD patients. This study adds objective data to steroid and antibiotic initiation guidelines for hospitalized AECOPD.

Ethics Committee Approval: This study was approved by Ethics committee of the University of Health Sciences Süreyyapaşa Chest Diseases and Thorasic Surgery Training and Research Hospital (Approval No: 116, Date: 2017).

Informed Consent: Verbal and written informed consent was obtained from the patients who agreed to take part in the study.

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Case Report

Austrian Syndrome: Community-Acquired Pneumonia in an Unusual Triad

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Abstract

Austrian syndrome corresponds to the triad of meningitis, pneumonia, and endocarditis caused by *Streptococcus pneumoniae*, there is no global or local incidence given the infrequency of entity. Scarce cases are published in Latin America, with none of them in Colombia. A case of Austrian syndrome by penicillin-resistant *S. pneumoniae* in an immunocompetent patient is presented. Aortic valve is the most frequent site involved in Austrian syndrome; this patient had an unusual localization of the vegetation on the right coronary artery ostium. The prognosis is poor with a mortality rate of 30% or higher, this patient survived despite systemic complications. Vaccination status impacts in prevention and severity of cases because responsible serotypes are often included in available vaccines. The patient had a serotype covered by available vaccines; however, her vaccination status was unknown. Thus, we present the first case reported in Colombia of Austrian syndrome by a penicillin-resistant *S. pneumoniae*, in a patient with no identified comorbidities or toxicological history, with a successful evolution.

KEYWORDS: Pneumococcal infections, meningitis, pneumococcal. endocarditis, bacterial, pneumonia
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INTRODUCTION

Austrian syndrome was first described in 1862 by the Austrian pathologist Richard Weschl. Later in 1881 Sir William Osler suggested the spread of pneumococcus to the central nervous system as a complication of pneumonia and endocarditis. Some years later Netter emphasized the predisposition due to the aortic valve, and finally the American infectologist Robert Austrian described 8 cases in 1957 revealing that lethality was commonly due to rupture of the aortic valve.[1,2]

Austrian syndrome is composed of the triad of pneumonia, endocarditis, and meningitis, secondary to infection by *Streptococcus pneumoniae*.[3] Considering the introduction of penicillin in 1940 and increasing vaccination against pneumococcus, infections by this pathogen have decreased drastically in years,[4,5] a clear example of this is the change in the incidence of infective endocarditis caused by this pathogen, previously being up to 15% and currently reaching rates less than 3%.[6] Furthermore, this condition is an uncommon event, with less than 100 cases occurring between 1957 and 2017 according to the literature reviewed.[7] Hence, we present the case of a patient with no previous comorbidities who developed Austrian syndrome, with a satisfactory outcome after the interventions performed despite what is described in the literature.

CASE REPORT

The patient gave us her written informed consent to present information about the medical record and diagnostic images. She was a 38-year-old patient with a single personal history of migraine without aura, who was assisted to the emergency department due to four days of moderate-intensity holocranial headache, left otalgia with scant ipsilateral otorrhea, dry cough, general malaise, and fever quantified at 39°C, with two emetic episodes and impaired state of consciousness 24 hours before consultation. Hypotension was documented on admission along with progressive neurological deterioration (Glasgow coma scale of 8/15) with adequate oxygenation indices and ensuring the airway with orotracheal intubation for invasive mechanical ventilation was decided. A simple brain tomography was performed without parenchymal or mastoid findings, and cerebrospinal fluid studies showed polymorphonuclear pleocytosis (32 cells/mm^3) with hyperproteinorrhachia (683 mg/dL) and hypoglycorrhachia (2 mg/dL, with 110 mg/dL of glycemia) being compatible for concurrent bacterial meningoencephalitis with a positive culture for penicillin-resistant *S. pneumoniae*, whence she received management with intravenous ceftriaxone (2 g twice a day) plus vancomycin (1 g twice a day) due to the unavailability of susceptibility test to third generation cephalosporins and quinolones. She also received dexamethasone 10 mg every 6 hours for 4 days scheme as part of the pneumococcal meningitis protocol. The blood laboratory tests were remarkable for mild leukocytosis (14 360 cells/mm^3) with neutrophilia, mild anemia (11.4 g/dL), and elevated C-reactive protein (341 mg/L); liver and renal functions were normal, and the fourth generation HIV antigen test was negative. High-resolution chest tomography

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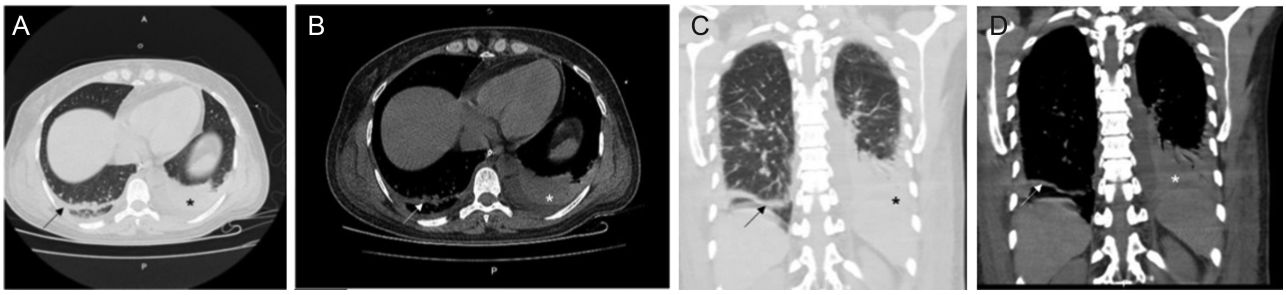


Figure 1. High-resolution computed tomography of the chest. (A-B) Axial section in the lung and mediastinum window shows the presence of atelectasis (arrow) and bibasal pleural effusion, predominantly left (asterisk) with concomitant consolidation. (C-D) Coronal slices using the lung and mediastinum window with the findings referenced in A and B. Source: Authors.

showed left basal consolidation and bilateral pleural effusion compatible with community-acquired pneumonia (Figure 1), while endotracheal tube aspiration cultures were negative. During her stay, she had two peripheral blood cultures positive for penicillin-resistant *S. pneumoniae*, whereby a transthoracic echocardiogram was performed reporting a 4 mm vegetation at the ostium of the right coronary artery, with mild primary aortic insufficiency and left ventricular ejection fraction conserved. In sum, the findings mentioned were compatible with Austrian syndrome. The serotype of *S. pneumoniae* was 19A, which was identified by the Colombian National Institute of Health.

The patient completed 42 days of biconjugate antibiotic management described above, with progressive neurological improvement, ventilatory weaning with the suspension of oxygen therapy, and resolution of bacteremia with negative control peripheral blood cultures. A control chest tomography showed the resolution of left basal pneumonia (Figure 2), and a control transthoracic echocardiogram showed a complete resolution of aortic vegetation. She was discouraged with mild bradypsychia as probably sequalae, without other respiratory or cardiovascular symptoms.

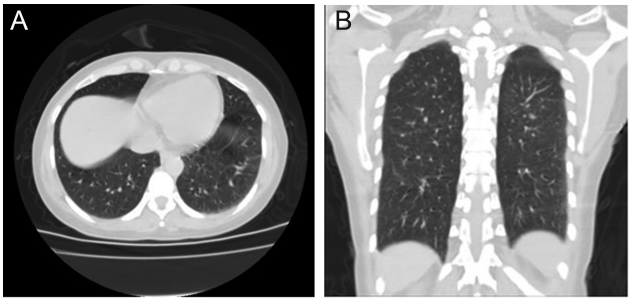


Figure 2. High-resolution computed tomography of the chest after 42 days of antibiotic treatment. (A-B) Axial and coronal section of the lung showing the complete resolution of left basal consolidation, pleural effusion, and atelectasis. Source: Authors.

DISCUSSION

Regarding epidemiological aspects of Austrian syndrome, a higher proportion has been reported in middle-aged men with a history of alcoholism, being the main risk factor that was associated with hyposplenism giving greater susceptibility to infections caused by encapsulated germs;[8] other known risk factors are splenectomy, diabetes mellitus, cirrhosis, and immunosuppression.[9-11] On the other hand, it has been found in case series of patients with infective endocarditis secondary to *S. pneumoniae* that pneumonia was present in almost half of the cases (46%), and Osler's triad in about 26%.[12] In contrast to the previously mentioned, the patient presented was a young adult woman, with no history of alcoholism or other previous comorbidities, which differs from the profile described in the published literature.

The usual clinical presentation is initially given by upper respiratory compromise (otitis or sinusitis) or lower respiratory compromise as pneumonia, with subsequent hematogenous dissemination to the central nervous system and heart.[13] In the case described, the chronology was not clear at all since the respiratory and neurological symptoms began almost simultaneously. The evolution of Austrian syndrome is usually rapid and aggressive,[7] a behavior like the one described on admission, and mortality rates reported up to 32%.[14]

Each component of the syndrome has important characteristics to highlight; in pneumonia, most of the patients manifest fever, chills, cough with purulent expectoration, and pleuritic pain with imaging evidence of non-segmental homogeneous consolidations.[14] However, this patient had left inferior pneumonia and left otomastoiditis, with a non-etiological determination as a pitfall but with a good response at those levels with therapy established. Otherwise, regarding infective endocarditis, only 1.2% present with Austrian syndrome,[11] and in these patients the left native valvular apparatus is more frequently affected, being the aortic valve more commonly compromised up to 49.3% of cases, without ostium compromise been reported.[8,12]

The penicillin resistance in *S. pneumoniae* strains has been increasing worldwide, in Colombia has been reported

MAIN POINTS

- Austrian syndrome does not seem to only affect immunocompromised people, and this could also suggest possible individual susceptibilities not identified.
- Penicillin-resistant pneumococcal disease is an incident problem found in community settings; a good prognosis is supported by appropriate antimicrobial stewardship despite the aggressive course reported in literature.
- Serotypes included in available vaccines could be responsible for disseminated and aggressive presentations such as Austrian syndrome in unvaccinated people. Vaccination programs must be carried out effectively so that they can have a better impact in prognosis.

intermediate resistance of 11% and total resistance of 4%.[15] Notwithstanding, in penicillin-resistant pneumococcal meningitis, the treatment of choice according to Infectious Diseases Society of America are vancomycin plus ceftriaxone when the susceptibility tests to ceftriaxone are unavailable and the minimum inhibitory concentration is >0.1 µg/mL. The role of systemic steroid in meningitis has been reported in terms of survival and neurological complications like hearing loss.[16]

Finally, vaccination against pneumococcus has decreased mortality secondary to this germ; however, these aggressive entities have also been described in serotypes not included in available vaccines with an increased probability of antimicrobial resistance, being pneumonia the usually route of entrance to subsequent dissemination.[10] The responsible serotype of this infection was 19A, a serotype included in the 13-valent pneumococcal conjugate vaccine (PCV13) and 23-valent pneumococcal polysaccharide vaccine (PPSV-23), which we did not know if they had been applied to the patient; these data support the importance of improving population vaccination strategies to avoid exposing them to risk of complications like this. This is the first case described in Colombia of an Austrian syndrome due to a resistant pneumococcus in a young adult patient without underlying risk comorbidities, and because its infrequent presentation, knowledge of the entity becomes more relevant given the high mortality described, the risk of valvular complications and neurological sequelae.

In conclusion, Austrian syndrome is an unusual form of invasive pneumococcal disease, usually conditioned by medical history of risks that increase the probability of dissemination at presentation. Additional features to highlight are the unusual location of vegetation, the fast resolution time of systemic compromise with satisfactory course despite aggressive course at debut, and the importance of vaccination to prevent the aggressive course of these infections.

Informed Consent: Written informed consent was obtained from the patient who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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Declaration of Interests: The authors have no conflict of interest to declare.

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Case Report

Granulomatous Mediastinitis: A Rare Cause of Pulmonary Hypertension

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Abstract

A rare case of a patient with chronic obstructive pulmonary disease who developed secondary anthracofibrosis to biomass exposure, fibrosing mediastinitis due to anthracotic enlarged lymph nodes in the mediastinum, and pulmonary hypertension because of compression of the lymph nodes on the pulmonary arteries is presented. This is a case report of a 71-year-old female patient who has been followed up with chronic obstructive pulmonary disease for 10 years, has no history of smoking, and has been exposed to biomass for many years. The patient, who had been hospitalized in various centers for the last 3 years due to progressive shortness of breath and dry cough, applied to us with dry cough and dyspnea complaints. On echocardiography, systolic pulmonary arterial pressure was found to be 59 mmHg. For the etiology of pulmonary hypertension, dual-energy thoracic computed tomography was performed with the suspicion of chronic thromboembolic pulmonary hypertension. No filling defect compatible with thromboembolism was detected. In right heart catheterization, mean pulmonary artery pressure was 27 mmHg, pulmonary capillary tip pressure was 7 mmHg, and pulmonary vascular resistance was 3.71 woods units. Endobronchial ultrasound was applied to the patient with the preliminary diagnoses of lymphoma, anthracosis, fibrosing mediastinitis, and infection. Widespread anthracosis was observed in all lobes and segments macroscopically. The lymph node in the subcarinal area was interpreted as anthracotic lymph node. Anthracosis is defined as black pigmentation involving the mucosal, and submucosal layers of the tracheobronchial tree and the lung parenchyma. If anthracosis is associated with luminal obliteration and/or mucosal proliferation causing obstruction, it is considered anthracofibrosis. In this case, we saw that secondary anthracofibrosis, fibrosing mediastinitis due to anthracotic enlarged lymph nodes in the mediastinum, and pulmonary hypertension may develop because of compression of the lymph nodes on the pulmonary arteries, and we wanted to draw attention to it was a rare case.

KEYWORDS: Anthracosis, anthracofibrosis, fibrosis mediastinitis, biomass exposure, pulmonary hypertension
Received: *October 9, 2022* ***Accepted:*** *March 7, 2023* ***Publication Date:*** *July 21, 2023*

INTRODUCTION

Anthracosis is an accumulation of carbon particles in mucosal and submucosal layers of the tracheobronchial tree causing black pigmentation. The most common reason is recurrent inhalation of coal dust particles, smoke, or exposure to air pollution. Bronchial destruction and deformity caused by anthracosis are called anthracofibrosis. It is associated with luminal obliteration and/or mucosal proliferation that causes obstruction. Carbon particles can also accumulate in lymph nodes and it is one of the common causes of enlarged mediastinal lymph nodes in developing countries.

Fibrous mediastinitis is a rare benign pathology characterized by intense fibrous tissue proliferation in the visceral mediastinum. Although its etiology is unclear, anthracosis might be one of the reasons for fibrous mediastinitis. Moreover, in fibrous mediastinitis, pulmonary hypertension may develop secondary to pulmonary vein/artery involvement. Here, we present a rare case of pulmonary hypertension (PH) secondary to anthracofibrosis.

CASE REPORT

A 71-year-old female presented with progressive dyspnea and dry cough. She has been hospitalized in various centers for the last 3 years for her progressive symptoms and has been treated for chronic obstructive pulmonary disease (COPD). Although she was a non-smoker, she has been exposed to biomass for 60 years as a turd fire. Two years ago, she was diagnosed with pulmonary thromboembolism by perfusion scan only and had 6 months of anticoagulant treatment. She was admitted to our center for unresolving symptoms.

She was investigated for COPD and chronic thromboembolic pulmonary hypertension (CTEPH). She had mild hypoxemia (pO_2: 68.4 mmHg, SaO_2: 95%). Pulmonary function test revealed irreversible mild obstruction (FEV_1: 1600 cc, 100%,

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FVC: 2590 cc, 132%, FEV_1/FVC: 62%). Diffusing capacity of the lung for carbon monoxide (DLCO) was 56%. In her echocardiography, ejection fraction was 61 and maximum systolic pulmonary arterial pressure (PAP) was reported as 59 mmHg over tricuspid regurgitation. The bilateral lower extremity venous Doppler ultrasound was normal, and she had no signs of deep venous thrombosis.

Dual-energy thorax computed tomography angiography (CTA) was performed for the etiology of PH, due to the suspicion of chronic thromboembolic disease. No filling defect compatible with thromboembolism was detected in dual-energy CTA. However, lobar and segmental branches of the pulmonary artery were surrounded by the soft tissue in the hilar region and at this level, their calibration was decreased significantly (compression-fibrotic process?). In these regions, nonsegmental perfusion defects in iodine maps were noticed (Figure 1A and 1B). Soft tissue increment with millimetric calcifications and bilateral hilar conglomerated lymph nodes were reported. In addition, nodular soft tissue structures cohering with interlobar and lobar lymph nodes were reported in both lungs. These findings were not compatible with CTEPH.

The patient underwent right heart catheterization; the mean PAP, the pulmonary capillary end pressure (wedge pressure), and the pulmonary vascular resistance (PVR) were recorded as 27 mmHg, 7 mmHg, and 3.71 woods, respectively.

Because of the conglomerated lymph nodes and the presence of suspicion of malignancy, the patient underwent a positron emission tomography (PET) scan. A PET scan revealed pathological increased 18F-fluorodeoxyglucose (FDG) uptake in bilateral mediastinal and hilar lymph nodes, concerning malignancy (SUV_{max}: 11.0) (Figure 2A and 2B).

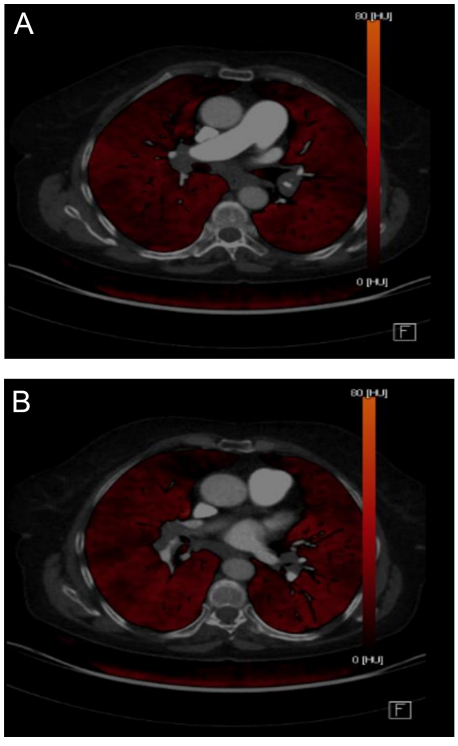


Figure 1. Nonsegmental perfusion defects in iodine maps.

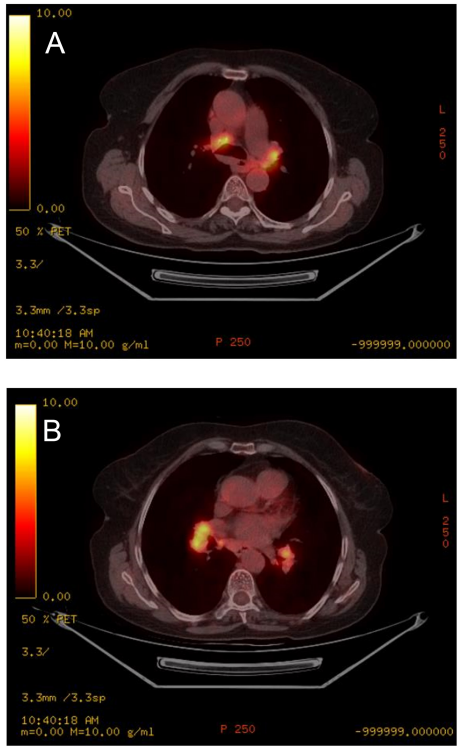


Figure 2. PET scan images.

The patient underwent endobronchial ultrasound (EBUS) which revealed mucosal anthracosis (Figure 3A). Endobronchial ultrasound-guided transbronchial needle aspiration of the paratracheal lymph node (Figure 3B) showed a large amount of anthracotic pigment on microscopic

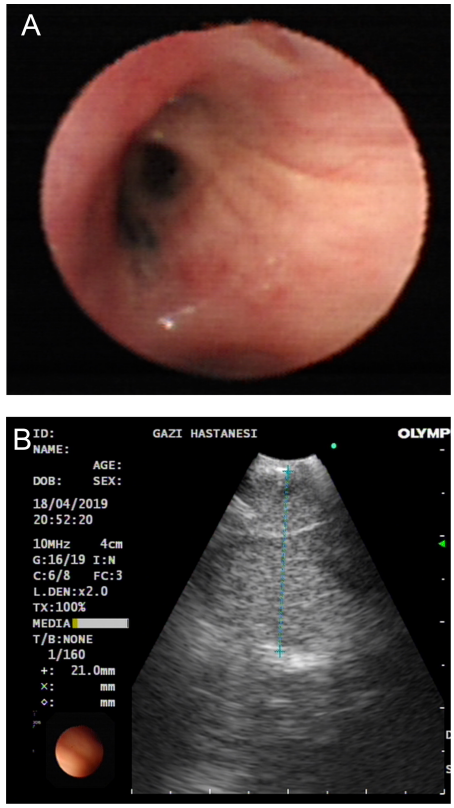


Figure 3. (A) Mucosal anthracosis. (B) EBUS-guided transbronchial needle aspiration of the paratracheal lymph node.

examination, there were no findings for specific granulomatous infection or lymphoprolipheratif disease, confirming the diagnosis of nodal anthracosis. She also had no signs of any granulomatous infection and acute phase parameters were normal.

The patient has been diagnosed as;

1. Anthracofibrosis secondary to biomass exposure
2. Fibrous mediastinitis due to anthracotic enlarged lymph nodes in the mediastinum
3. Pulmonary hypertension and perfusion defects as a result of lymph nodes pressing on the pulmonary arteries (fibrous mediastinitis).

Pulmonary arterial hypertension targeted therapies were not considered since there was no evidence that she would benefit from them. The patient was followed up with symptomatic treatment and inhaled a long-acting antimuscarinic agent for her mild COPD.

DISCUSSION

Here we presented a rare case of PH caused by fibrosing mediastinitis due to anthracofibrosis in whom all other causes of PH were excluded by detailed investigations. One might define anthracosis as infiltration of mucosal and submucosal layers of the tracheobronchial tree and black pigmentation, which includes the lung parenchyma. It is frequently found randomly in the bronchoscopic assessment of patients. If anthracosis is associated with luminal obliteration and/or mucosal proliferation that causes obstruction, it is identified as anthracofibrosis. Anthracofibrosis was first defined by Chung et al[1] in 1998. It should be considered in patients with COPD and asthma, usually middle-aged and old-aged women, who do not respond to the usual treatments, who have been living in a rural area, and who are non-non-smokers with a history of biomass exposure like turd fire as in our case.[2] Its pathogenesis includes indoor and outdoor air pollution, hereditary qualities, biomass history, chronic inflammatory conditions, or chronic infections. It was shown to be related to tuberculosis, COPD, pneumonia, asthma, sarcoidosis, and PH.

Anthracosis is also one of the common reasons for enlarged mediastinal lymph nodes in developing countries.[3] Anthracotic mediastinal lymph nodes often appear oval, with calcification. Sclerosing mediastinitis or mediastinal fibrosis terms are also used for "Fibrous mediastinitis." In addition, it has been defined an as "idiopathic fibro-inflammatory lesion." Even though the etiology has not been fully clarified, factors such as tuberculosis, aspergillosis, mucormycosis, blastomycosis, cryptococcosis, and anthracosis have been reported in the etiology of fibrous mediastinitis. In this case, all other causes were excluded, and athracosis was accepted as the etiology of fibrous mediastinitis. Furthermore, this chronic inflammatory process can cause compression in the vena cava superior, systemic veins, esophagus, tracheobronchial structures, pulmonary arteries, and veins. Besides, PH may develop secondary to pulmonary vein/artery involvement which is the situation in the case we presented.

Pulmonary hypertension is a progressive cardiopulmonary disease. Pulmonary hypertension is diagnosed with an increase in mean PAP ≥20 mmHg assessed by resting right heart catheterization.[4] Pulmonary artery pressure can increase due to precapillary or postcapillary diseases. Our patient had precapillary pulmonary arterial hypertension as she had increased PAP together with increased PVR, and normal wedge pressure. Pulmonary hypertension is classified into 5 main groups.[4] The PH in group V is also precapillary and is caused by unclear and/or multifactorial mechanisms. This group includes many conditions that may be complicated by complex and sometimes overlapping pulmonary vascular involvement.[4] Hematologic diseases such as chronic hemolytic anemia, myeloproliferative diseases, metabolic and systemic and diseases such as pulmonary Gaucher disease, sarcoidosis, Langerhans cell histiocytosis, neurofibromatosis, glycogen storage disease, chronic kidney failure, and complex congenital heart disease are among the diseases included in this group.[5] This group also lists tumor obstruction and fibrous mediastinitis.[4] Our patient's progressive dyspnea was thought to be caused by PH due to fibrous mediastinitis and mimicked pulmonary embolism before. Group IV PH (CTEPH) was ruled out in our case by dual-energy CTA which is mentioned as the new modality alternative perfusion imaging techniques in the current guideline.[4] No PH-directed therapy was initiated for the patient, as in group 5 PH, treating the underlying disorder remains the standard of care.[4]

Anthracotic enlarged mediastinal lymph nodes were investigated in other cases for malignancy and granulomatous infections.[3] Because of the pathological FDG uptake in PET scans, we wanted to rule out, especially malignancy or tuberculosis, no findings were noted for these possibilities in the histopathological investigation. Fluorodeoxyglucose can accumulate in inflammatory cells such as activated macrophages and neutrophils at the location of inflammation or infection. Fluorodeoxyglucose can also accumulate in granulomatous pathologies such as sarcoidosis and tuberculosis, which can cause false-positive PET scan results. Anthracotic particles can moreover act as an antigen and can stimulate macrophages. Some studies have reported increased FDG uptake in PET scans in anthracotic lymph nodes in the mediastinum.[5] In a study by Yilmaz Demirci et al[3], the mean SUVmax value was reported as 4.76 (1-16.8) in anthracosis sampled lymph nodes. As a result, if there is a history of exposure to hazards and noxious particles, the possibility of a benign condition ought to be considered when serious uptake in mediastinal and hilar lymph nodes is observed in a PET scan.[6]

EBUS–TBNA is considered a minimally invasive procedure that permits real-time assessment and biopsy of mediastinal lymph nodes.[5] Anthracosis is the most common source of endogenous particles in mediastinal lymph node samples and is mostly found within macrophages. Endobronchial ultrasound–TBNA is a useful procedure in the identification of anthracotic particles.[6] Kirchner et al[7] examined mediastinal lymph nodes with EBUS–TBNA. Then, the anatomical positions of these lymph nodes were analyzed by multislice computed thorax tomography, and they found that the foremost common location of anthracotic lymph nodes was the

subcarinal.[7] Yilmaz Demirci et al[3] evaluated 1138 mediastinal lymph nodes with EBUS–TBNA and reported that interlobar and subcarinal lymph nodes were the most frequently affected locations (34.3% and 36.8% respectively). It has been emphasized that anthracotic mediastinal lymph nodes most frequently have a well-defined oval shape with calcifications.

In conclusion, PH caused by fibrosing mediastinitis due to anthracofibrosis is a very rare condition. Anthracofibrosis may moreover be kept in mind within the handle of differential diagnosis of PH and granulomatous illnesses.

Informed Consent: Written informed consent was obtained from the patient who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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Letter to the Editor

Lymphocytes and Eosinophils Associated with the Coronavirus Disease-2019 Severity

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Dear Editor,

We would like to share ideas on the publication "Are lymphocytes and eosinophils associated with the COVID-19 severity: A large, retrospective study."[1] The goal of Duman et al[1] was to look at the connection between the coronavirus disease-2019 (COVID-19) severity at hospital admission and demographics, lymphocytes, and eosinophils. According to Duman et al.[1] lymphocyte counts and percentages are rapid and accurate biomarkers for estimating the severity of COVID-2019 and may direct doctors toward appropriate management early.

We concur that the investigated laboratory parameter may offer some clinical advantages in the treatment of COVID-19. The pathogen variant, therapeutic therapy, and background health state are a few of the elements that mostly affect how severe COVID-19 is. The white cell count is a laboratory statistic that can be impacted by a number of variables. Eosinophil, for instance, may be impacted by an ongoing parasite infestation. The quality control process itself is a crucial element that may go unmentioned. The basic complete blood count values, which are derived from an automated hematology analyzer, provide the basis for the parameter. It is important to note the specification and quality control of the analysis performed using an automated hematology analyzer. There may be difference between different analyzers. Different hematological analyzers and other complicating personal illnesses can have an impact on the results.[2,3]

Declaration of Interests: The authors have no conflict of interest to declare.

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Author's Reply

RE: Lymphocytes and Eosinophils Associated with the Coronavirus Disease-2019 Severity

Dildar Duman[1], Zuhal Karakurt[2], Nagihan Durmuş Koçak[1], Nalan Adıgüzel[2], Tekin Yıldız[1], Gökay Güngör[2], Huriye Berk Takır[1], Fatma Merve Tepetam[3], Hakan Günen[1], On Behalf of the COVID-19 Interest Group

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Dear Editor,

We thank the authors for their valuable ideas[1] on our study.[2] Their main concerns are the white cell count is a laboratory statistic that can be impacted by a number of variables. Eosinophil, for instance, may be impacted by an ongoing parasite infestation. The quality control process itself is a crucial element that may go unmentioned.[3,4]

This is indeed true but as we mentioned in the study, data were obtained from the hospital's electronic database to prevent human errors. We have big data and even small differences can become significant with so many numbers. Our results were correlated with other publications, neglecting complicating illnesses. Hemogram values of all patients were studied in our hospital on admission. In our hospital, one laboratory and one system is used. Quality control is carried out periodically.[2]

Other concerns are that the basic complete blood count values, which are derived from an automated hematology analyzer, provide the basis for the parameter. It is important to note the specification and quality control of the analysis performed using an automated hematology analyzer. There may be differences between different analyzers. Different hematological analyzers and other complicating personal illnesses can have an impact on the results.[3,4]

In our study, as we stated in the material and methods section, a Coulter LH 780 Hematology Analyzer (Beckman Coulter Inc., Brea, Calif, USA) was used for complete blood counts (leukocyte, neutrophil, eosinophil, lymphocyte, and platelet) analysis. This same automated hematology analyzer was used for all patients in this study.[2]

Takubo et al[3] evaluated assays of the same fresh blood samples with 6 different types of reference automated hematology analyzers developed by the following manufacturers including Beckman Coulter. The complete blood counts and leukocyte differential counts were surveyed with a reference-automated hematology analyzer from each manufacturer and no large differences greater than 10% of mean value were observed with Beckman Coulter.[3,4]

In summary, we believe that our results are reliable, because the same automated hematology analyzer was used to evaluate complete blood count values of all patients. Quality control is carried out periodically. All blood samples were taken in our hospital on admission. Data were obtained from the hospital's electronic database neglecting human error. Hemogram values of a large population in this study correlate with other studies.

Declaration of Interests: The authors have no conflict of interest to declare.

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