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ORIGINAL ARTICLE / ÖZGÜN MAKALE

COVID-19 and pneumothorax, pneumomediastinum, subcutaneous emphysema: Analysis of risk factors

COVID-19 ve pnömotoraks, pnömomediastinum, subkütanöz amfizem: Risk faktörlerinin analizi

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ABSTRACT

Background: In this study, we aimed to analyze the risk factors of barotrauma in patients who were followed in the intensive care unit due to novel coronavirus disease 2019 (COVID-19) pneumonia.

Methods: Between March 2020 and January 2021, a total of 261 patients (155 males, 106 females; mean age: 63.3±15.3 years; range, 11 to 91 years) who were followed in the intensive care unit due to COVID-19 pneumonia and were diagnosed with pneumothorax, pneumomediastinum, and subcutaneous emphysema were retrospectively analyzed. Demographics data of the patients, past and current medical history, clinical management, patient progress, and survival data were obtained from medical records of our hospital.

Results: Twenty-seven of the patients were diagnosed with barotrauma. A total of 88.8% of the patients were followed with intubation. The development of pneumothorax, pneumomediastinum, and subcutaneous emphysema due to barotrauma was not dependent on sex, smoking/non-smoking status, using/not using corticosteroids, or comorbid diseases. There was a significant correlation between pneumothorax, pneumomediastinum, and subcutaneous emphysema development in intubated patients with different ventilator modes. Changing the ventilator mode from synchronized intermittent mandatory ventilation to airway pressure release ventilation increased the possibility of barotrauma by 15 times.

Conclusion: Despite all lung-protective applications, barotrauma is a common complication, particularly in mechanically ventilated patients who have COVID-19 pneumonia with severe acute respiratory distress syndrome. Mechanical ventilator pressure modes should be patient-specific and followed carefully and frequently for the risk of barotrauma.

Keywords: Barotrauma, COVID-19 pneumonia, pneumomediastinum, pneumothorax, subcutaneous emphysema.

ÖZ

Amaç: Bu çalışmada yeni koronavirüs hastalığı 2019 (COVID-19) pnömonisine bağlı yoğun bakım ünitesinde takip edilen hastalarda barotravmanın risk faktörleri incelendi.

Çalışma planı: Mart 2020 - Ocak 2021 tarihleri arasında COVID-19 pnömonisi nedeniyle yoğun bakım ünitesinde takip edilen ve pnömotoraks, pnömomediastinum ve subkutan amfizem tanısı konan toplam 261 hasta (155 erkek, 106 kadın; ort. yaş: 63.3±15.3 yıl; dağılım, 11-91 yıl) retrospektif olarak incelendi. Hastaların demografik verileri, geçmiş ve şimdiki tıbbi öyküleri, klinik tedavileri, hasta durumları ve sağkalım verileri hastanemizin tıbbi kayıtlarından elde edildi.

Bulgular: Hastaların 27'sine barotravma tanısı kondu. Hastaların toplam %88.8'i entübe olarak takip edildi. Barotravmaya bağlı pnömotoraks, pnömomediastinum ve subkutan amfizem cinsiyet, sigara kullanma/kullanmama durumu, kortikosteroid kullanma/kullanmama durumu ve eşlik eden hastalıklardan bağımsız idi. Farklı ventilatör modlarının kullanıldığı entübe hastalarda pnömotoraks, pnömomediastinum ve subkutan amfizem gelişimi arasında anlamlı bir ilişki izlendi. Ventilatör modunun senkronize aralıklı zorunlu ventilasyondan hava yolu basıncı serbestleştirme ventilasyona geçirilmesi, barotravma olasılığını 15 kat artırdı.

Sonuç: Akciğeri koruyan tüm uygulamalara rağmen, barotravma özellikle mekanik ventilasyonlu şiddetli akut solunum sıkıntısı sendromlu COVID-19 pnömoni hastalarında sık görülen bir komplikasyondur. Mekanik ventilatör basınç modları hastaya göre olmalıdır ve barotravma riski nedeniyle hastalar dikkatli ve sıklıkla takip edilmelidir.

Anahtar sözcükler: Barotravma, COVID-19 pnömonisi, pnömomediastinum, pnömotoraks, subkutan amfizem.

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Novel coronavirus disease 2019 (COVID-19) has quickly spread worldwide since 2019. Globally, more than 8.5 million individuals were infected and over 0.4 million people died.[1,2]

In these patients, acute respiratory distress syndrome (ARDS) due to COVID-19 pneumonia is the main independent risk factor both indication for mechanical ventilation and barotrauma. These patients have a high mortality rate and older age, smoking, comorbid diseases (such as diabetes mellitus [DM], chronic obstructive pulmonary disease [COPD], coronary artery disease [CAD], chronic renal failure [CRF], and accompanied malignity) may increase the hospitalization time, mechanical ventilation time, mortality and complication rate.[3-5]

Barotrauma is the most common respiratory complication in patients with COVID-19 pneumonia under mechanical ventilation. Barotrauma includes pneumothorax (Px), pneumomediastinum (PMD), subcutaneous emphysema (SCE). The incidence of Px has been reported in 1% of those requiring hospital admission; however, its rate is reported as 15% in ventilated COVID-19 patients.[6,7]

The incidence of barotrauma in patients with chronic lung diseases, older age, smoking and comorbid diseases are higher than other patients. In the present study, we aimed to evaluate the risk factors causing the development of Px, PMD, and SCE and to investigate the effects of these complications regarding the mortality and morbidity in COVID-19-positive patients.

PATIENTS AND METHODS

This single-center, retrospective study was conducted at Ondokuz Mayıs University Faculty of Medicine, Department of Thoracic Surgery between March 2020 and January 2021. The patients who were hospitalized in the intensive care unit (ICU) due to the diagnosis of COVID-19 pneumonia and were diagnosed with Px, PMD, and SCE were

Table 1. Demographics of patients with barotrauma

	n	Median	Range
Type of barotrauma	27		
Px	19		
Right	13		
Left	3		
Bilateral	2		
HydroPx	1		
PMD	2		
SCE	3		
PMD+SCE	3		
SCE+Px	2		
Mode of ventilation			
Spontaneous	3		
Intubated	24		
SIMV	9		
APRV	5		
SIMV → APRV	10		
Time of intubation (days)		12.03	3-46
Methods of diagnosis			
Physical examination	27		
Chest X-ray	27		
Thorax CT	8		
Thoracentesis	2		
Incidental	0		
Type of management			
Chest drain	19		
Transcutaneous mediastinal air drainage	2		
Transcutaneous subcutaneus air drainage	3		

Px: Pneumothorax; PMD: Pneumomediastinum; SCE: Subcutaneous emphysema; SIMV: Synchronized intermittent mandatory ventilation; APRV: Airway pressure release ventilation; CT: Computed tomography.

included. A total of 26,058 patients were admitted to the COVID-19 outpatient clinic in our hospital. Of these, 5,138 were found to be positive for COVID-19 and 3,156 of the patients were treated in the hospital. Finally, 261 patients (155 males, 106 females; mean age: 63.3±15.3 years; range, 11 to 91 years) who were followed in the ICU were included.

Demographics data of the patients, past and current medical history (comorbidities, smoking status, laboratory tests results (C-reactive protein [CRP], ferritin and D-dimer), radiological findings (chest X-ray and thoracic computed tomography [CT]), clinical management, patient progress, and survival features were obtained from the medical records of our hospital.

In all of the patients, Px, PMD, and SCE were diagnosed by using chest X-ray and/or thoracic CT, if possible. If not due to unstable clinical status of the patients, Px, PMD, and SCE were diagnosed according to physical examination findings. Chest tube drainage was applied to all patients with Px in chest X-ray. Transcutaneous air drainage was applied in the patients who had extensive PMD and SCE. In patients who had moderate SCE, air was drained via an intravenous catheter which was placed in the subcutaneous area.

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 21.0 software (IBM Corp., Armonk, NY, USA). The normality assumption of continuous data was examined with the Kolmogorov-Smirnov test and it was determined that the data did not provide the normality assumption. Therefore, the Mann-Whitney U test was used in comparison of the groups. In determining the dependencies between the groups, the chi-square test was used for those who provided the assumptions, and the chi-square Fisher exact test was used for those who did not provide the assumptions. Descriptive data were expressed in median (min-max) or number and frequency. The logistic regression analysis was performed to determine the effects of ventilator modes including Synchronized intermittent mandatory ventilation (SIMV), airway pressure release ventilation (APRV) and from SIMV to APRV) on barotrauma. A p value of <0.05 was considered statistically significant.

RESULTS

Of the patients, 204 were intubated and 57 were non-intubated. All of patients' demographic features, past and current medical features are shown in Table 1. The median intubation time was 8.85 (range, 3 to 46) days. The most common comorbid

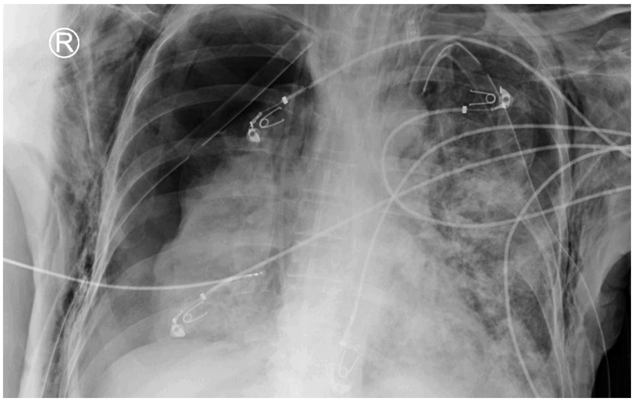

Figure 1. Bilateral Px: Mode: After from SIMV to APRV.

Px: Pneumothorax; SIMV: Synchronized intermittent mandatory ventilation; APRV: Airway pressure release ventilation.

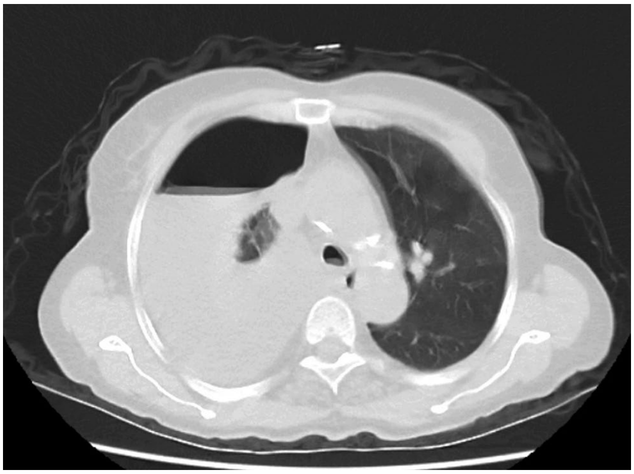

Figure 2. Right hydropneumothorax: Mode: APRV.

APRV: Airway pressure release ventilation.

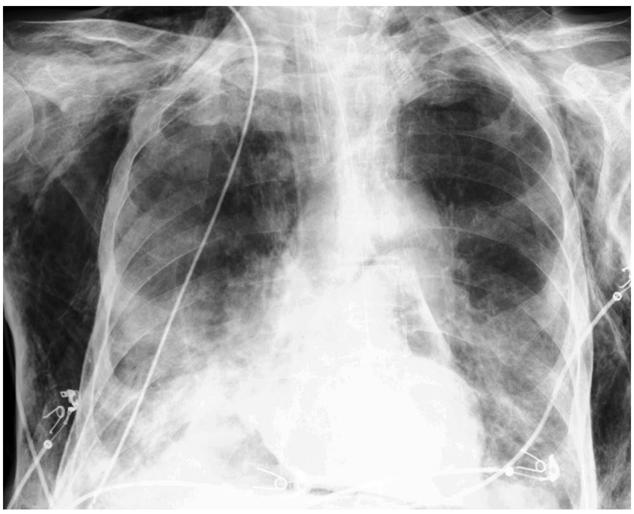

Figure 3. Extensive PMD and SCE were treated via transcutaneous air drainage: Mode: SIMV with high PEEP.

PMD: Pneumomediastinum; SCE: Subcutaneous emphysema; SIMV: Synchronized intermittent mandatory ventilation; PEEP: Positive end-expiratory pressure.

conditions included CAD (n=112), DM (n=86), and COPD (n=52). All patients were treated with antiviral treatment (favipiravir) and broad-spectrum antibiotherapy (piperacillin tazobactam -/+ levofloxacin) and low-molecular-weight heparin. If there was no contraindication, corticosteroid (prednisolone/dexamethasone dose 1 to 2 mg/kg) was administered to all of the patients. Twenty-seven of the patients were diagnosed with barotrauma (Px, PMD, and SCE) (Table 1).

Table 2. Relationship between barotrauma and variables

	Barotrauma (Px, ME, SCE)					
	n	%	n	%	χ^2	*p*
Sex					0.709	0.400
Female	93	39.7	13	48.1		
Male	141	60.3	14	51.9		
COPD					0.037	0.847
-	187	79.9	22	81.5		
+	47	20.1	5	18.5		
Obesity					0.164	0.564
-	221	94.4	26	96.3		
+	13	5.6	1	3.7		
Smoking					1.714	0.190
-	134	57.3	19	70.4		
+	100	42.7	8	29.6		
DM					0.150	0.698
-	156	66.7	19	70.4		
+	78	33.3	8	29.6		
Hypothyroidy					0.347	0.749
-	208	88.9	25	92.6		
+	26	11.1	2	7.4		
CAD					0.029	0.865
-	134	57.3	15	55.6		
+	100	42.7	12	44.4		
CRF					3.278	0.092
-	194	82.9	26	96.3		
+	40	17.1	1	3.7		
Malignancy					3.238	0.072
-	182	77.8	25	92.6		
+	52	22.2	2	7.4		
Superinfection or coinfection					0.379	0.538
-	201	85.9	22	81.5		
+	33	14.1	5	18.5		
Mode					32.878	<0.001
Non-intubated	54	23.1	3	11.1		
SIMV	127	54.3	9	33.3		
APRV	41	17.5	5	18.5		
From SIMV to APRV	12	5.1	10	37		
Corticosteroid					2.092	0.148
-	65	27.8	4	14.8		
+	169	72.2	23	85.2		
Survival					0.041	0.847
Exitus	214	91.5	25	92.6		
Alive	20	8.5	2	7.4		

COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; CAD: Coronary artery disease; CRF: Chronic renal failure; SIMV: Synchronized intermittent mandatory ventilation; APRV: Airway pressure release ventilation.

Table 3. Binary logistic regression analysis results

Variable	No	Total no	Prevalence (%)	B	SE	Wald	Sig.	Exp (B)	95% CI for Exp (B)	
Constant	-	-	-	-2.890	0.593	23.744	<0.001	0.056	-	-
Mod										
Non-intubated	3	57	5.26	-	-	23.737	<0.001	-	-	-
SIMV	9	136	6.62	0.243	0.686	0.126	0.723	1.276	0.332	4.895
APRV	5	46	10.87	0.786	0.759	1.073	0.300	2.195	0.496	9.719
SIMV to APRV	10	22	45.5	2.708	0.732	13.703	<0.001	15.000	3.576	62.922

B: Coefficients; SE: Standart error; Exp (B): Exponential, B coefficients; SIMV: Synchronized intermittent mandatory ventilation; APRV: Airway pressure release ventilation.

Of the patients with Px (n=18), 13 had right Px, three had left Px, and two had bilateral Px (Figure 1), while one patient had left hydropneumothorax (Figure 2). The chest tube placement was performed for all of the patients with Px.

Of the patients, three of them had SCE, two patients had PMD, three had SCE+PMD (Figure 3), and two patients had SCE+Px. In the patients who had extensive PMD and/or SCE, transcutaneous air drainage was applied for mediastinal emphysema. In patients who had moderate SCE, air was drained using an intravenous catheter that placed in the subcutaneous area. If the mediastinal and/or SCE did not disturb clinical status of the patients or not extensive, only conservative treatment was applied for both of them.

In 25 patients receiving mechanical ventilation at the time of Px, SCE, PMD diagnosis, only three patients were breathing spontaneously. The median time on mechanical ventilation was 12.03 (range, 3 to 46) days. The average positive end-expiratory pressure (PEEP) was 3 to 10 cmH_2O. The average peak and plateau pressures were 20 to 29 cmH_2O and 12 to 29 cmH_2O, respectively.

In thoracic CT of all patients (except for Px, PMD, and SCE), there were moderate/diffuse bilateral ground-glass and consolidative opacities, and few patients had bronchiectasis and 52 patients had emphysematous changes.

All of patients were followed by daily chest X-ray. Of the patients with barotrauma, 25 died and two were discharged. The overall median time of hospitalization time for discharged patients was 11 (range, 3 to 48) days and 12.47 (range, 3 to 48) days for the non-survivors.

When the patients followed in the ICU who developed and did not develop barotrauma were compared in terms of comorbid disease, the development of barotrauma was not dependent on sex, smoking/non-smoking status, using/not using corticosteroids, comorbid diseases (CAD, CRF, COPD/asthma, pulmonary embolism, obesity, DM, hypothyroidism, malignancy, or superinfection).

Table 4. Findings of the difference between some variables and barotrauma

	Barotrauma	n	Mean	Median	Min-Max	U	*p*
Age	-	234	64.47	67.0	11.0-94.0	3071.0	0.813
	+	27	62.22	67.0	11.0-86.0		
C-reactive protein	-	199	159.77	133.0	3.0-501.0	2531.5	0.859
	+	26	153.98	162.5	6.0-355.0		
Ferritin	-	207	1,931.5	1,203.0	14.0-10,000.0	2783.0	0.972
	+	27	1,919.3	977.1	202.0-8,770.0		
D-dimer	-	204	4,723.5	3,412.5	24.0-10,000.0	2208.5	0.271
	+	25	5,482.5	5,240.0	63.0-10,000.0		
Hospitalization time	-	234	6.7	4.0	0.0-133	2034.5	0.002
	+	27	11.0	9.0	0.0-46.0		

In addition, the development of barotrauma with survival was not dependent (p>0.05). However, there was a significant relationship between Px, PMD, and SCE developing in intubated patients and ventilator modes (p<0.05) (Table 2). According to the findings, 23.1% of the patients who did not develop barotrauma were not intubated and 76.9% were intubated. In patients with barotrauma, 11.1% were not intubated and 88.8% were intubated. Logistic regression analysis was performed to determine the relationship between barotrauma and ventilator modes. The results are given in Table 3. Accordingly, changing the ventilator

Table 5. Evidence of dependencies between survival and other variables

	Survival					
	Exitus		Alive			
	n	%	n	%	χ^2	*p*
Sex					1.934	0.164
Female	94	39.3	12	54.5		
Male	145	60.7	10	45.5		
COPD					1.767	0.266
-	189	79.1	20	90.9		
+	50	20.9	2	9.1		
Obesity					0.032	0.859
-	226	94.6	21	95.5		
+	13	5.4	1	4.5		
Smoking					5.330	0.021
-	135	56.5	18	81.8		
+	104	43.5	4	18.2		
DM					1.136	0.286
-	158	66.1	17	77.3		
+	81	33.9	5	22.7		
Hypothyroidy					0.067	0.795
-	213	89.1	20	90.9		
+	26	10.9	2	9.1		
CAD					3.996	0.046
-	132	55.2	17	77.3		
+	107	44.8	5	22.7		
CRF					0.795	0.544
-	200	83.7	20	90.9		
+	39	16.3	2	9.1		
Malignancy					0.728	0.435
-	188	78.7	19	86.4		
+	51	21.3	3	13.6		
Superinfection or coinfection					0.016	0.898
-	204	85.4	19	86.4		
+	35	14.6	3	13.6		
Mode					39.049	<0.001
Non-intubated	41	17.2	16	72.7		
SIMV	134	56.1	2	9.1		
APRV	42	17.6	4	18.2		
SIMV to APRV	22	9.2	0	0		
Corticosteroid					0.009	0.926
-	63	26.4	6	27.3		
+	176	73.6	16	72.7		

COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; CAD: Coronary artery disease; CRF: Chronic renal failure; SIMV: Synchronized intermittent mandatory ventilation; APRV: Airway pressure release ventilation.

Table 6. Relationship between survival and acute phase reactants, age, length of hospital stay in COVID + patients

Survival	n	Mean	Median	Min-Max	U	*p*
Age						
Exitus	239	64.9	68.0	11.0-94.0	1912.5	0.034
Alive	22	56.4	61.5	15.0-82.0		
C-reactive protein					1598.5	0.029
Exitus	203	164.5	141.0	3.0-501.0		
Alive	22	108.9	97.8	22.0-266.0		
Ferritin					1624.0	0.017
Exitus	214	2,007.5	1,310.0	14.0-10,000.0		
Alive	22	1,176.7	635.3	117.0-8,879.0		
D-dimer					2089.5	0.742
Exitus	208	4,833.9	3,618.0	24.0-10,000.0		
Alive	21	4,532.4	3,166.1	588.0-10,000.0		
Hospitalization time					1630.5	0.003
Exitus	239	7.5	4.0	0.0-133.0		
Alive	22	3.7	0.0	0.0-20.0		

mode from SIMV to APRV increased the possibility of barotrauma by 15 times more than other patients. The obtained model made the correct prediction at the rate of 89.7%, and it was also significant according to the results of the Hosmer-Lemeshow test ($p>0.05$).

No statistically significant difference was found between patients with and without barotrauma in terms of age, CRP, ferritin, and D-dimer values ($p>0.05$). However, when the duration of hospitalization time was evaluated, the duration of hospitalization time was found to be significantly higher in patients with barotrauma compared to other patients ($p<0.05$) (Tables 4 and 5).

Survival was not dependent on sex, corticosteroid use, and comorbidities ($p>0.05$). However, survival was determined to be dependent on ventilator mode ($p<0.05$). Of the patients who died, 82.9% were intubated and 17.2% were followed in spontaneous breathing. Of the alive patients who were followed, 72.7% were in spontaneous breathing and 27.3% were intubated. A total of 56.5% of the non-survivors did not smoke, 43.5% smoked, while 81.8% of the survivors did not smoke and 18.2% smoked. There was a statistically significant dependence between smoking and survival ($p<0.05$). Also, 55.2% of the patients who died did not have CAD, 44.8% had CAD, 77.3% of the alive patients did not have CAD, and 22.7% had CAD ($p<0.05$) (Table 5). Age, CRP, and ferritin levels, and hospitalization time of the patients who died were found to be significantly higher than the survivors ($p<0.05$). However, no significant difference was found between the survivors and non-survivors in terms of D-dimer value (Table 6).

DISCUSSION

In patients who had COVID-19 pneumonia, the most frequent seen comorbidities are cardiovascular and cerebrovascular diseases, DM, hypertension, tumors, and obesity. Patients with comorbidities and also older patients have a higher mortality rate than the others.[7] In our study, none of the comorbid diseases were found to be a dependent risk factor for barotrauma. However, particularly in patients who had CAD, the mortality rate was higher than in other patients. Moreover, respiratory infection and pulmonary parenchymal damage are more severe in these patients. However, many of these patients (approximately 12 to 26) are followed in the ICU which require invasive mechanical ventilation due to ARDS. The basis of protective ventilation must be the reduction of tidal volume (6 mL/kg) and the airway plateau pressure below 30 cmH_2O.[8] Despite these preventive measures, the incidence of Px, PMD, and SCE is reported as 15% in patients who are followed with intubation due to COVID-19 pneumonia. Pulmonary barotrauma is a complication of positive pressure mechanical ventilation and these complications are associated with increased morbidity and mortality.[6] In our study, we found that the incidence of barotrauma in the patients who were followed in the ICU due to COVID-19 pneumonia was 10.3%. Moreover, while only two of our patients were surviving, the others died. According to this result, we believe that barotrauma increases mortality significantly.

In COVID-19 pneumonia, lung compliance is decreased in the affected lung areas. High positive pressure ventilation causes a risk of over insufflation of the relatively preserved parts of the lungs. Eventually, alveolar ruptures occur in distal parenchymal areas due to barotrauma.[9-11] Fifteen of our patients who developed barotrauma were followed in the pressure-controlled modes. In addition, in 10 patients who had severe COVID-19 pneumonia and barotrauma needed high ventilation pressures to regulate hypoxia and provide alveolar opening. Immediately afterwards, barotrauma occurred in these patients. All these results support that high pressure has a seriously damaging effect on the lung parenchyma and it facilitates barotrauma development in these patients.

Smoking is a risk factor for barotrauma in COVID-19 pneumonia patients. Cigarette smoke has the ability to induce a variety of patterns of lung injury. In the histopathological lung examination of these patients, many manifestations can be seen from reversible inflammation to irreversible emphysema or fibrosis. This causes fragility in the lung parenchyma. As a result, patients who have emphysema due to smoking become more susceptible to damage due to mechanical ventilation.[12] A total of 42% of our patients were heavy/moderate smokers and of 57% were never smokers. We found that many of our smoking patients had emphysema with varying degrees on their CT scans. Accordingly, there was no increase in the frequency of barotrauma in smoking patients compared to non-smoking patients; however, we considered that smoking was associated with the increased risk of mortality.

The most important factor for the need for intensive care of patients with COVID-19 pneumonia is poor clinical status and comorbid conditions. However, the most important factors for the severity and prognosis of the disease are laboratory parameters. In COVID-19 patients, CRP, ferritin, and D-dimer levels are increased. There is a strong correlation between CRP, ferritin, and abnormal coagulation parameters with the severity and prognosis of disease.[13-16] In our study, we could not find a correlation between serum CRP, ferritin, and D-dimer levels and the risk of barotrauma. However, we only found a significant correlation between CRP value and mortality.

The development of barotrauma can occur due to pulmonary parenchymal fragility specific to viral pneumonia. High positive pressure in mechanical ventilation can facilitate small parenchymal leaks in fragile parenchymal tissue. Increased subcutaneous, intrathoracic, and intramediastinal pressures result in decreased chest compliance.[8,17] In our study, in the patients whose ventilatory mode changes from SIMV to APRV had a significantly higher risk for barotrauma than the other intubated patients. It was thought that there was a direct association between the development of barotrauma and the applied mechanical ventilation parameters. High barotrauma rates in patients with COVID-19 infection on mechanical ventilation were associated with longer hospitalization time, longer intubation time, and higher mortality. In our study, the mortality rate in patients with barotrauma was 92%. Alive patients with barotrauma were followed in spontaneous breathing and were not intubated. In addition, hospitalization and intubation time were significantly higher in these patients than the other patients.

The most important limiting factor of our study was the patients with COVID 19 were being treated in the ICU instead of our clinic. This situation has difficulties in obtaining ventilator follow-up information of patients. All medical information of patients is recorded in the digital system of our hospital, except ventilator modes.

In conclusion, barotrauma is a common complication in mechanically ventilated COVID-19 patients. Although its diagnosis and treatment are easy, it is one of the important factors that increases the hospitalization and mechanical ventilation time and affects the prognosis of the disease adversely. Particularly in patients who have COVID-19 pneumonia with severe acute respiratory distress syndrome, care should be taken in terms of barotrauma during lung protective mechanical ventilation applications. Mechanical ventilator pressure modes should be patient-specific, but despite all protective measures, barotrauma can be an inevitable consequence in intubated COVID-19 patients. Regarding the all of these reasons, these patients should be followed carefully and frequently for the risk of barotrauma.

Ethics Committee Approval: This study was approved by Turkish Republic Health Ministry (Ref. no: 2021-01-06T10_26_46) and The Medical Research Ethics Committee (Ref. no: B.30.2.ODM.0.20.08/1485-55). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient Consent for Publication: A written informed consent was obtained from each patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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