JOURNAL OF
CONTEMPORARY MEDICINE

DOI:10.16899/jcm.1166228
J Contemp Med 2022;12(5):640-646

Original Article / Orijinal Araştırma

# How Secure was Convalescent Plasma Administration to Non-severe COVID-19 Cases with Lymphopenia?

## Lenfopenik Olan Hafif COVID-19 Vakalarında İmmun Plazma Tedavisi Ne Kadar Güvenliydi?

**Hilal Akay Çizmecioğlu[1], Aysel Oğuz[1], Mevlüt Hakan Göktepe[1], Pınar Diydem Yılmaz[2], Ahmet Emre Hatır[3], Ahmet Çizmecioğlu[4]**

[1]Necmettin Erbakan University School of Medicine, Department of Internal Medicine, Konya, Turkey
[2]Necmettin Erbakan University School of Medicine, Department of Radiology, Konya, Turkey
[3]Necmettin Erbakan University School of Medicine, Department of Family Medicine, Konya, Turkey
[4]Selcuk University School of Medicine, Department of Internal Medicine, Konya, Turkey

## Abstract

**Aim:** Many treatment methods have endeavored during the Coronavirus Disease of 2019 (COVID-19) pandemic. Particularly before the vaccines came into use, the medical world gained adequate experience with convalescent plasma (CP) administration, which was ignored after preventive remedies. In this study, we compared the clinical conditions and treatments during the infection with pulmonary fibrosis after recovery.

**Material and Method:** This prospective, cross-sectional study was conducted with COVID-19 patients. The patients were divided into two groups according to the severity of the disease. Sixty of them were reevaluated regarding pulmonary fibrosis via high-resolution computed tomography performed in the 6th month after recovery.

**Results:** A total of 60 patients (mean age=54.05±9.16) participated in this study. Both severe and non-severe groups were equal in the number of patients. There was no difference between the groups in the evaluation of fibrosis scores. However, in those with pulmonary fibrosis, age, CURB-65 scores, and D-dimer levels were found to be higher, whereas hematocrit levels were lower. In lymphopenic patients, almost 95% of those who underwent CP treatment had fibrosis (p=0.013). This fibrosis formation was more prominent in the non-severe group (p=0.028). Comparable fibrosis increation persisted in diabetics.

**Conclusion**: Based on the results, the pulmonary involvement of COVID-19 may form persistent fibrosis after recovery. The accuracy of administering CP treatment in non-severe patients with lymphopenia should be reviewed, as it might increase pulmonary fibrosis.

**Keywords:** COVID-19, Convalescent Plasma, Lymphopenia, Post-COVID syndrome, Pulmonary fibrosis

## Öz

**Amaç:** 2019 Koronavirüs Hastalığı (COVID-19) pandemisi sırasında birçok tedavi yöntemi denenmiştir. Tıp dünyası, hastalık önleyici tedavilerin (özellikle aşıların) kullanıma girmesinden sonra göz ardı edilen immun plazma (İP) uygulamasında yeterli deneyime sahip olmuştur. Bu çalışmada, iyileşme sonrası pulmoner fibrozis ile enfeksiyon sırasındaki klinik süreçleri ve tedavileri karşılaştırdık.

**Gereç ve Yöntem:** Bu prospektif, kesitsel çalışma COVID-19 hastaları ile yapılmıştır. Hastalar hastalık şiddetine göre iki gruba ayrıldı. Bunlardan altmış tanesi, iyileşme sonrası 6. ayda çekilen yüksek çözünürlüklü bilgisayarlı tomografi ile pulmoner fibrozis açısından yeniden değerlendirildi.

**Bulgular:** Bu çalışmaya toplam 60 hasta (ortalama yaş=54.05±9.16) katıldı. Hem şiddetli hem de olmayan gruplarda hasta sayısı eşitti. Fibrozis skorlarının değerlendirilmesinde gruplar arasında fark yoktu. Ancak pulmoner fibrozisi olanlarda yaş, CURB-65 skorları ve D-dimer seviyeleri daha yüksek, hematokrit seviyeleri daha düşük bulundu. Lenfopenik hastalarda, İP tedavisi görenlerin yaklaşık %95'inde fibrozis vardı (p=0.013). Bu fibrozis oluşumu, şiddetli olmayan grupta daha belirgindi (p=0.028). Benzer fibrozis artışı diyabetiklerde sebat etti.

**Sonuç:** Sonuçlara göre, COVID-19'un pulmoner tutulumu iyileşme sonrası kalıcı fibrozis oluşturabilir. Pulmoner fibrozisi artırabileceğinden, lenfopenisi olan hafif vakalarda İP uygulanmasının doğruluğu gözden geçirilmelidir.

**Anahtar Kelimeler:** COVID-19, Konvalesan Plazma, Lenfopeni, Post-COVID sendrom, Pulmoner fibroz

**Corresponding *(İletişim)*:** Ahmet Çizmecioğlu, Selcuk University School of Medicine, Department of Internal Medicine, Konya, Turkey
**E-mail *(E-posta)*:** mdahmet2002@gmail.com
**Received *(Geliş Tarihi)*:** 24.08.2022 **Accepted *(Kabul Tarihi)*:** 31.08.2022

## INTRODUCTION

Once preventive approaches and vaccination studies continue in the fight against Coronavirus Disease of 2019 (COVID-19), the biggest epidemic of the last decade, the long-term effects of the disease are now being investigated. Recovery from COVID-19 causes increasing concern globally, as systemic sequelae, particularly in the respiratory system, have been detected in some patients who have achieved microbiological normalization. Although most patients recover completely within a few weeks, some of them, including those infected with mild mutations, continue to experience "long-track" symptoms or post-COVID syndrome after recovery.[1]

The long-term complications of COVID-19 have not been adequately known. Since the clinical and radiologic features of severe acute respiratory syndrome (SARS) pneumonia in 2003 and Middle East Respiratory Syndrome (MERS) pneumonia in 2012 are similar to COVID-19 pneumonia, the predictability of the risk of disease progression may be similar.[2] In patients followed up after SARS-CoV and MERS-CoV, 25% to 35% of survivors experience persistent abnormalities in pulmonary functions and changes in imaging modalities consistent with pulmonary fibrosis.[3,4]

In cohort studies, some COVID-19 survivors developed fibrotic pulmonary remodeling-induced restrictive lung abnormalities associated with impaired exercise tolerance and poor quality of life during follow-up.[3,4]

This pulmonary fibrosis in COVID-19 is related to lung damage by both viral and immune-mediated mechanisms. It has long been known that cytokines play a prominent role in the immune response to viral infections. However, tissue, and organ damage may occur with the development of an excessive inflammatory response. Most COVID-19 patients with critical illness develop pneumonia and hyperinflammation, possibly due to a macrophage activation syndrome called a 'cytokine storm'. Several studies have shown that cytokine storm is associated with increased interleukin (IL)-1B, IL-2, IL-6, IL-17, IL-8, tumor necrosis factor, and monocyte chemoattractant protein. Consequently, lung fibrosis occurs as a secondary manifestation associated with the progression of the pathologic inflammatory response.[5]

More up-to-date data are now obtainable in the analysis of predictive complications or morbidities rather than in the disease process of COVID-19. Our study, therefore, aimed to compare the characteristics of COVID-19 patients, their types of pneumonia, and treatment modalities with the 6th-month pulmonary parenchyma status after recovery.

## MATERIAL AND METHOD

### Study Design

This prospective, cross-sectional study was conducted with COVID-19 patients between October 2020 and November 2021. The study protocol was approved by the Selcuk University School of Medicine Ethics Committee (Date: 04.12.2020, Decision No: 2020/2916) and supported by the current university's scientific research project under grant number 211518008. Informed consent forms were obtained from all patients prior to the study. Among the COVID-19 patients diagnosed via polymerase chain reaction (PCR) and followed-up in the internal medicine clinics, patients with pulmonary involvement in computed tomography at diagnosis were reevaluated regarding pulmonary fibrosis six months after discharge.

All patients were divided into subgroups according to their clinical and (Computed Tomography) CT involvement. Accordingly, patients with clinics (headache, cough, fever, sore throat, diarrhea, anosmia) and minimal abnormalities on a CT were rated as non-severe. Those with critical clinics (dyspnea, oxygen saturation [$SpO_2$] ≤93%, tachypnea [respiratory frequency ≥30 breaths/min], arterial partial oxygen pressure to inspired oxygen ratio [$PaO_2/FiO_2$] <300 mmHg, and pulmonary involvement >50% within 24–48 h) were rated as severe.[6]

Patients were also classified based on CT involvement scores (CT-IS) at the diagnosis time CTs and the fibrosis scores in their CT evaluations six months later.[7,8] Patients' demographic characteristics and initial laboratory test results (prominently for CURB-65) assigning the COVID-19 severity were noted.[9] Accordingly, the CURB-65 is a standardized severity score to predict 30-day mortality for community-acquired pneumonia concerning five variables (state of consciousness, serum urea level, respiratory rate, blood pressure, and age).[9]

### Patient Selection

The patient group featured patients between 18 and 65 years of age who had a positive PCR test result. Those with comorbidities (active malignancy, chronic pulmonary, renal, or cardiac disease, rheumatic disease, cerebral vascular event), smoking, or consuming alcohol, or a COVID-19 diagnosis not verified with a PCR test were excluded from the study. All patients' laboratory analyzes were taken prior to their treatment.

### Diagnosed Tests and Parameters

All samples were swabbed from the sectional upper respiratory tract (nose and throat). The COVID-19 diagnosis was performed with a Bio Speedy Bioeksen COVID-19 RT-qPCR diagnostic kit (Istanbul, Turkey). The CT scans were performed using the Somatom Drive 2×128 Dual Source CT scanner (Siemens Healthineers, Erlangen, Germany) in the Radiology Department. The CT scanner's portal rotation time is 0.28 ms, and the detector collimation is 0.5×256. The tube voltage and current were adjusted to the varying patient body mass index. (100-120 kV, and 280-300 mA). No contrast material was used in the CT scan procedure. Cases with an absolute lymphocyte count below $1\times10^9$/L were considered lymphopenic.[10]

### Statistical Analysis

All data were analyzed using SPSS version 18.0 (SPSS, Inc., Chicago, IL). In descriptive analyses, frequency data were given using numbers (n) and percent (%), and numerical data were given using mean±standard deviation, median (1st quartile-3rd quarter), and minimum-maximum. The Chi-square ($\chi 2$) and Fisher Exact tests compared categorical data. Kolmogorov-Smirnov and Shapiro Wilk tests examined the compliance of numerical data with normal distribution. The distribution of normally distributed numerical data in two independent groups was evaluated with the Independent Sample's T-test, and the distribution of normally distributed numerical data in more than two groups was evaluated with the One-Way ANOVA test. Tukey or Tamhane Post Hoc analysis was used for the variables whose ANOVA test was significant. The non-normally distributed numerical data distribution in two independent groups was analyzed with the Mann-Whitney U test. The distribution of numerical data that were not normally distributed in more than two groups was evaluated with the Kruskal-Wallis test. The post hoc analysis of the significant data with the Kruskal Wallis test was performed with the Mann-Whitney U test, and Dunn Bonferroni correction was made. The relationship between two numerical variables was analyzed by Pearson Correlation analysis for non-skewed data and Spearman Correlation analysis for the skewed data. The results were evaluated at the 95% confidence interval, a significance level of $p<0.05$.

## RESULTS

In this study, 60 patients who met the inclusion criteria were evaluated. The mean age of all patients was 54.05±9.16. Pneumonia diagnoses were confirmed radiologically in all patients. Relatedly, there were 30 patients in each group (non-severe and severe) assembled to disease severity. The demographic features, major clinical complaints, and vital signs of the patients were summarized in **Table 1**. Overall, the highest recorded CURB-65 score was "3". In addition, the length of hospitalization was about 11 days for all patients. Eight patients' management continued in the intensive care unit (ICU), and the mean stay in ICU was 9.0±4.62 days.

The data about treatment regimens were as follows: all patients received favipiravir-based treatment and antibiotic support. 95% of them (n=57) were initiated with low molecular weight heparin, 10% of them (n=6) were taken hydroxychloroquine, and 75 of them (n=45) were administered with methylprednisolone. An additional pulse steroid (1 mg/kg) was needed for 12 (26.6%) of those who received steroids. Thirty-five patients (n=58.3%) received convalescent plasma (CP), 4 patients (n=6.7%) received tocilizumab, and 2 patients (n=3.3%) received intravenous immunoglobulin (IVIG). Twenty-nine (82.9%) of those who received CP treatment were already under corticosteroid treatment.

**Table 1. Patients characteristics and vital differences according to disease severity**

| | All groups | Non-Severe | Severe | p value |
|---|---|---|---|---|
| Gender, F*/M† | 25 / 35 | 11 /19 | 14 / 16 | 0.432 |
| Age, (year) | 54.05±9.16 | 52.46±9.75 | 53.63±8.68 | 0.625 |
| BMI ‡, (%) | 30.19±4.29 | 29.76±4.76 | 30.61±3.81 | 0.181 |
| Hypertension, n (%) | 21 (35) | 8 (26.7) | 13 (43.3) | 0.176 |
| Diabetes Mellitus, n (%) | 16 (26.7) | 7 (23.3) | 9 (30) | 0.559 |
| Hospitalization day | 10 (8-13.75) | 10 (8-13) | 10 (6.75-14.5) | 0.744 |
| **Symptoms** | | | | |
| Fever, n | 27 (45) | 16 (53.3) | 11 (36.7) | 0.194 |
| Dispnea, n (%) | 47 (78.3) | 20 (66.7) | 27 (90) | 0.028 |
| Sour throat, n (%) | 5 (11.7) | 3 (10) | 2 (6.7) | 0.999 |
| Cough, n (%) | 43 (71.7) | 24 (80) | 19 (63.3) | 0.152 |
| Astenia, n (%) | 40 (66.7) | 22 (73.3) | 18 (60) | 0.273 |
| Pain, n (%) | 30 (50) | 17 (56.7) | 13 (43.3) | 0.302 |
| Artralgia, n (%) | 26 (43.3) | 16 (53.3) | 10 (33.3) | 0.118 |
| Taste loss, n (%) | 22 (36.7) | 10 (33.3) | 12 (40) | 0.592 |
| Loss of appetite, n(%) | 21 (35) | 10 (33.3) | 11 (36.7) | 0.787 |
| Chilling, n (%) | 9 (15) | 4 (13.3) | 5 (16.7) | 0.999 |
| Nausea, Vomiting, n(%) | 9 (15) | 5 (16.7) | 4 (13.3) | 0.999 |
| Diarrhea, n (%) | 7 (11.7) | 4 (13.3) | 3 (10) | 0.999 |
| **Vital findings** | | | | |
| Blood pressure, mmHg | | | | |
| Sistolic | 128.4±15.75 | 128.76±11.95 | 128.03±9.02 | 0.859 |
| Diastolic | 73.38±9.0 | 73.40±8.26 | 73.36±9.82 | 0.989 |
| Pulse, bpm | 93.11±13.79 | 92.6±10.22 | 93.63±16.8 | 0.775 |
| Saturation, (%) | 85.96±7.54 | 91.33±2.78 | 80.60±6.96 | 0.001 |
| Need for $O_2$ | 51 (85) | 21 (70) | 30 (100) | 0.002 |
| HFO§ need, n (%) | 17 (28.3) | 2 (6.7) | 15 (50) | 0.001 |
| CURB-65¶, n (%) | | | | |
| 0 | 32 (58.3) | 18 (60) | 17 (56.7) | 0.794 |
| 1 | 16 (26.7) | 8 (26.7) | 8 (26.7) | |
| 2 | 8 (13.3) | 4 (13.3) | 4 (13.3) | |
| 3 | 1 (1.7) | 0 (0) | 1 (3.3) | |

p values are the comparison of non-severe and severe groups, (The independent t-Test, Chi-Squared test or Mann Whitney U test); *, Female; †, Male; ‡, Body mass index; §, High-flow Oxygen; ¶, Confusion, uremia, respiratory rate, blood pressure, age > 65 years.

The outcomes for lobar involvements performed at diagnosis time CTs, the fibrosis scores in the (high-resolution CT) HRCT performed at the sixth month, and the classification of notable prognostic laboratory results according to disease severity are given in **Table 2**.

Overall, age ($p=0.016$, $\eta^2=0.098$), CURB-65 scores ($p=0.012$, $\eta^2=0.108$), and D-Dimer levels ($p=0.018$, $\eta^2=0.095$) were found to be high, while mg ($p=0.033$, $\eta^2=0.077$) and hematocrit (Hct) ($p=0.028$, $\eta^2=0.081$) levels were lower in patients with fibrosis (**Figure 1**). In addition, those received high-flow oxygen (HFO) support had higher fibrosis scores ($p=0.002$, $\eta^2=0.155$). Intriguingly, the involvement in all lobes or the involvement severity did not associate with fibrosis ($p>0.05$); however, a Mann–Whitney U test found that fibrosis was associated with at least 50% involvement of the pulmonary parenchyma ($p=0.047$, $\eta^2=0.067$). Our study found no overall effect of disease severity, symptoms, and gender on fibrosis.

**Table 2. Pulmonary involvements and prominent prognostic laboratory results of the groups.**

| | All groups (n=60) | Non-Severe (n=30) | Severe (n=30) | p value |
|---|---|---|---|---|
| **Radiology** | | | | |
| RUL*, n (%) | 48 (80) | 21 (70) | 27 (90) | 0.053 |
| RML†, n (%) | 55 (91.7) | 28 (93.3) | 27 (90) | 0.999 |
| RLL‡, n (%) | 60 (100) | 30 (100) | 30 (100) | NA‡† |
| LUL§, n (%) | 45 (75) | 20 (66.7) | 25 (83.3) | 0.136 |
| LLL¶, n (%) | 60 (100) | 30 (100) | 30 (100) | NA‡† |
| Over 50%, n (%) | 21 (35) | 5 (16.7) | 16 (53.3) | 0.003 |
| All lobes, n (%) | 37 (61.7) | 15 (50) | 22 (73.3) | 0.063 |
| CT-IS** | 15.3±3.28 | 14.0±2.13 | 16.6±3.72 | 0.002 |
| CT*† findings | | | | |
| Mild | 10 (16.7) | 9 (30) | 1 (3.3) | |
| Moderate | 26 (43.3) | 13 (43.3) | 13 (43.3) | 0.001 |
| Severe | 24 (40) | 8 (26.7) | 16 (53.3) | |
| Fibrosis score | 2 (0-6) | 1 (0-5) | 3 (0-7.5) | 0.176 |
| Fibrosis, n (%) | 43 (72) | 21 (70) | 22 (73.3) | 0.774 |
| **Laboratory** | | | | |
| WBC*‡, $\times10^9$/L | 6.88 (4.95-9.04) | 5.84 (4.22-8.05) | 7.79 (6.03-10.05) | 0.009 |
| ANC*§, $\times10^9$/L | 5.22 (3.57-7.45) | 4.62 (2.67-6.68) | 6.03 (4.34-8.30) | 0.013 |
| ALC*¶, $\times10^9$/L | 1.09 (0.66-1.54) | 1.04 (0.52-1.52) | 1.09 (0.75-1.68) | 0.325 |
| Hemoglobin, gr/L | 13.49±1.96 | 13.4±1.63 | 13.53±2.27 | 0.721 |
| Platelet, $\times10^9$/L | 204.9±77.88 | 182.3±66.59 | 227.5±82.76 | 0.023 |
| ESR†*, mm/h | 41 (26-68) | 37.5 (24.5-53.7) | 50.5 (26-72.25) | 0.284 |
| Ferritine, ng/mL | 405 (213-874) | 324 (120-494) | 704 (341-1342) | 0.001 |
| Creatinine, mg/dL | 0.97 (0.78-1.16) | 1.02 (0.87-1.17) | 0.87 (0.71-1.18) | 0.133 |
| Uric acid, mg/dL | 4.55 (3.5-6.25) | 5.05 (3.65-6.5) | 4.35 (3.32-5.3) | 0.078 |
| LDH††, U/L | 349.5 (301-432.7) | 325 (289.7- 387.7) | 385 (331.7- 460) | 0.016 |
| CPK†‡, U/L | 103 (54-212.75) | 126 (60.75- 229.3) | 92.5 (51.3-175.2) | 0.211 |
| Albumin, g/L | 38.91±3.51 | 39.85±3.1 | 37.97±3.7 | 0.037 |
| ALT†§, U/L | 30.15 (18.8-50.65) | 30.4 (19.02- 49.12) | 25.75 (18.4-53.6) | 0.853 |
| AST†¶, U/L | 33.30 (25.4-50.72) | 37.1 (27.32- 53.77) | 29.3 (22.45-45.1) | 0.158 |
| INR‡* | 1.09±0.17 | 1.07±0.15 | 1.10±0.20 | 0.579 |
| Fibrinogen, mg/dL | 555.5 (456.5-657) | 491 (441.1- 606.8) | 584 (513- 667.7) | 0.056 |
| D-Dimer, ng/mL | 286.5 (197-446.7) | 240 (139- 333.5) | 438 (242- 800.2) | 0.001 |

p values are the comparison of non-severe and severe groups, (The independent t-Test, Chi-Squared test or Mann Whitney U test); *, Right upper lobe; †, Right middle lobe; ‡, Right lower lobe; §, Left upper lobe; ¶, Left lower lobe; **, Computed tomography involvement score; *†, Computed tomography; *‡, White blood cell; *§, Absolute neutrophil count; *¶, Absolute lymphocyte count; †*, Erythrocyte sedimentation rate; ††, Lactate dehydrogenase; †‡, Creatine phosphokinase; †§, Alanine transaminase; †¶, Aspartate aminotransferase; ‡*, International normalized ratio; ‡†, Not applicable.

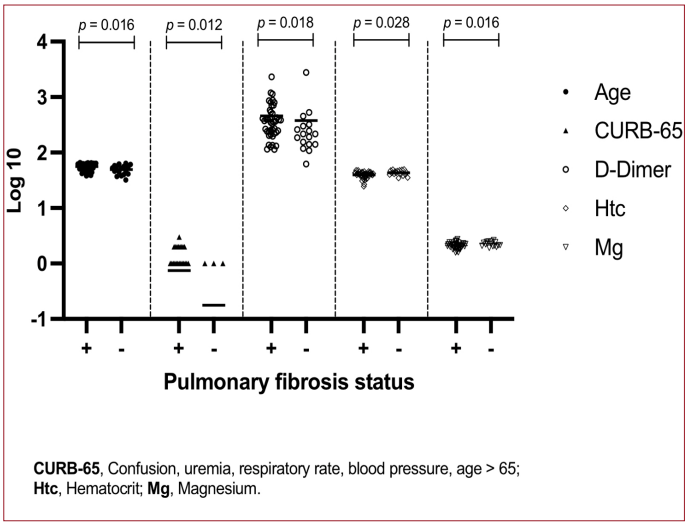


**Figure 1.** Essential patient characteristics and notable laboratory parameters associated with fibrosis status.

In comparing the fibrosis scores regarding disease severity, there were no differences in treatment management skills, pulmonary involvement, patient features, and clinics, other than receiving HFO (p=0.016, $\eta^2$=0.198). However, age (p=0.031, $\eta^2$=0.161), glucose (p=0.045, $\eta^2$=0.137), and urea (p=0.010, $\eta^2$=0.217) levels were higher in severe patients with fibrosis, whereas the mean fever was lower (p=0.040, $\eta^2$=0.148).

As lymphopenia was accepted as a holistic prognosis factor of COVID-19 pneumonia,[11,12] patient subgroups were rearranged according to lymphocyte state. The impact of IVIG or a Tocilizumab-based treatment on pulmonary fibrosis was not revealed (p>0.05) (**Figure 2a, 2b**). However, in patients with lymphopenia (n=28), fibrosis was encountered in 17 of 18 patients who were administered convalescent plasma (p=0.013) (**Figure 2b**). Furthermore, fibrosis scores were higher in the non-severe patients with lymphopenia administered with CP (p=0.028, $\eta^2$=0.343).

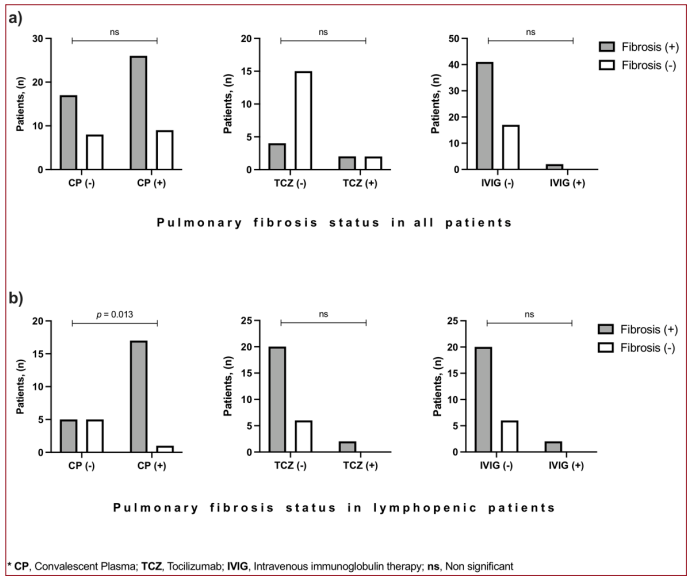


**Figure 2.** Treatment modalities impact on fibrosis scores, a) in all patients, and b) in patients with lymphopenia.

Hypoxemia ($sPO_2$ <94%), another determinant factor in COVID-19 prognosis, was crucial in producing fibrosis.[13] Among the hypoxic patients with fibrosis, age (p=0.005, $\eta^2$=0.153), CURB-65 scores (p=0.002, $\eta^2$=0.191), erythrocyte sedimentation rate (p=0.044, $\eta^2$=0.079), and D-Dimer levels (p=0.013, $\eta^2$=0.12) were higher, while hematocrit levels (p=0.012, $\eta^2$=0.123) and, therefore, hemoglobin (p=0.036, $\eta^2$=0.086) were lower.

One detailed finding was about diabetics. All of the diabetic patients with the involvement of 50% of their pulmonary parenchyma (n=10) had higher fibrosis scores (p=0.035) (**Figure 3**).

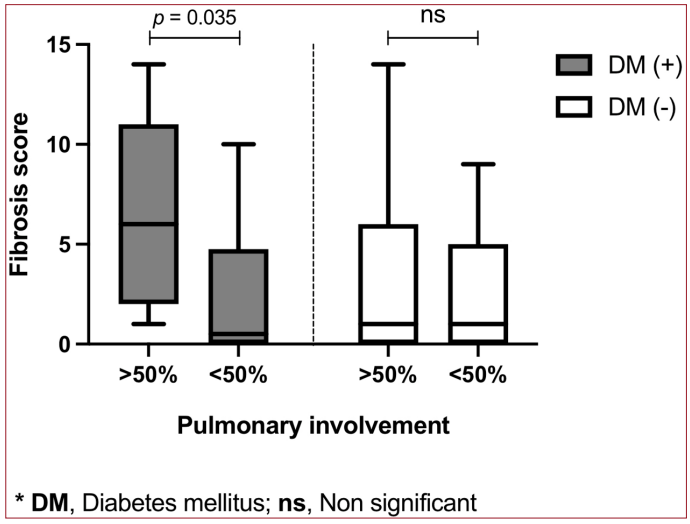


**Figure 3.** Comparison of fibrosis scores in diabetic and non-diabetic patients regarding pulmonary involvement rates.

Finally, notable correlations among the parameters of the study are given in **Table 3**.

**Table 3. Marked correlations between patient features.**

| | | | r†* | p†† |
|---|---|---|---|---|
| CURB-65* score | vs. | ALC¶ | -0.302 | 0.019 |
| | | Fibrosis score | 0.380 | 0.003 |
| | | Fibrosis in HRCT** | 0.329 | 0.010 |
| IgA† level | vs. | Diarrhea | 0.324 | 0.012 |
| RUL‡ involvement | vs. | $sPO_2$*† | -0.273 | 0.035 |
| | | ALC | 0.267 | 0.039 |
| | | Involvement in CT | 0.262 | 0.043 |
| LUL§ involvement | vs. | Dyspnea | 0.257 | 0.048 |
| | | Fever | -0.406 | 0.001 |
| | | CRP level | 0.386 | 0.002 |
| | | Involvement in CT | 0.378 | 0.003 |
| 50% involvement | vs. | $sPO_2$ | -0.366 | 0.004 |
| | | HFO*‡ treatment | 0.392 | 0.002 |
| | | CRP*§ level | 0.509 | 0.001 |
| | | Involvement in CT | 0.753 | 0.001 |
| | | Hypertension | 0.341 | 0.008 |
| | | Diabetes mellitus | 0.348 | 0.006 |
| All lobes involvement | vs. | HFO treatment | 0.268 | 0.039 |
| | | CRP level | 0.330 | 0.010 |
| | | Involvement in CT | 0.443 | 0.001 |
| Convelescant Plasma treatment | vs. | WBC*§ | -0.312 | 0.015 |
| | | ANC*¶ | -0.334 | 0.009 |
| | | Fibrinogen | -0.317 | 0.013 |

*, Confusion, uremia, respiratory rate, blood pressure, age > 65 years; †, Immunoglobulin A; ‡, Right upper lobe; §, Left upper lobe; ¶, Absolute lymphocyte count; **, High- resolution CT; *†, Blood $O_2$ saturation; *‡, High-flow Oxygen; *§, C-Reactive protein; *§, White blood cell; *¶, Absolute neutrophil count; †*, Correlation coefficient; ††, P value.

## DISCUSSION

Our study evaluated the pulmonary parenchyma status of patients with COVID-19 pneumonia six months after recovery. Per the results, fibrosis scores were higher in over 70% of the patients. Insistant fibrosis was detected in 43 of the 60 patients. It was observed that disease severity was not a worsening factor in the progression to fibrosis. Furthermore, the cumulative 50% of parenchymal involvement had the most significant effect on fibrosis, rather than individual lobe involvement. Fibrosis was high-towered in lymphopenic patients who received CP therapy. Finally, fibrosis was detected in all diabetic patients with advanced pulmonary involvement.

Several studies have revealed that the long-term consequences of COVID-19 infection include pulmonary fibrosis in a subset of patients with the potential for stationary or progressive disease.[14,15] The etiology of the pulmonary sequelae of COVID-19, pulmonary fibrosis, has not yet been fully elucidated and has been considered multifactorial.[16] Recent studies reported triggers such as a cytokine storm evoked by an improper inflammatory response, bacterial superinfections, thromboembolic state, and pulmonary involvements.[17-19] In this context, another debatable finding in our study was that fibrosis scores were highest in patients with cumulative 50% pulmonary involvement, rather than single or multiple whole lobe involvement. An inflammatory

process involving the entire lung, albeit partial, will either be more destructive or transform more fibrosis during remodeling. There seemed to be a dilemma here regarding steroids. As noted in our results, most patients who received CP therapy had already been administered corticosteroid therapy; however, the fibrosis inhibitory effect of steroids was not remarkable in our study.

Even in early pandemic days, many potential drug regiments have been revealed, and those with solid clinical evidence have taken part in the guidelines. Among the treatment regimens led by antiviral treatments, antibody-based treatments such as CP, IVIG, and monoclonal antibodies have also been performed adequately.[20] Much clinical experience has been reported regarding CP treatment, and its efficacy was increased when administered earlier.[21] In our study, we did not locate any adverse effects of CP treatment on fibrosis in general. However, we noticed that in lymphopenic patients, CP treatment seemed influential in the formation of fibrosis. Moreover, this effect was more apparent in non-severe patients.

One logical statement that can clarify this could be the following: CP contains antibodies developed via immunization against COVID-19.[22] Considering the available knowledge, these antibodies all had the Fc and Fab regions.[23] While the Fab region generates an immunological response through the complement pathway, the Fc region induces immunomodulation through the corresponding receptors on the macrophage.[24] Therefore, the prepared antibodies from the CP may have further induced or bi-directionally affected the immunomodulation of the macrophages in non-severe cases. Second, antigen-antibody complex formation can further increase macrophage activation. Thus, supernumerary macrophage-activated phagocytosis may occur due to the immune complexes formed rather than the self-antigenicity of COVID-19. Favoring a treatment that will accelerate or increase the immune complex formation in non-severe patients may have activated the macrophages earlier and more intensely. This impaired immune response is likelier to occur in lymphopenic patients.[11,25,26] As a result, early and prolonged macrophage activation may have caused the most fibrosis.

As the lungs are primarily affected in the disease progress, the autopsy series revealed that intense inflammation occurs in the lung tissue prior to death, particularly in the basement membrane.[27] COVID-19-induced lung damage was highly heterogeneous in postmortem lung tissue evaluations. Hence, fibrosis is inevitable on the inflammation site when healing is achieved in this damaging process involving all inflammatory cells.[27] Although none of our patients died in the severe group, our study found sequela pulmonary fibrosis in most cases. Intriguingly, disease severity did not affect the increase in fibrosis scores in lymphopenic and non-lymphopenic patients. The fact that fibrosis was detected frequently in the non-severe group may indicate that the inflammation in the lung tissue was at least as intense as in severe cases, even though there were still unknown aspects of the disease.

Studies have already united a consensus about the complications of diabetes mellitus in COVID-19 pandemia.[28] Due to the negative impacts of uncontrolled diabetes on vascular structure and immune response, COVID-19 has been quite mortal in diabetic patients.[29] Although there are determinations regarding the pathogenesis focused on microvascular immunothrombolysis,[30,31] unclear parts remain in the etiology. In line with the literature, fibrosis was detected in all the diabetics in our study. Remarkably, patients with the involvement of 50% of their parenchyma had higher fibrous scores than those with single lobe involvement. This may indicate that, in addition to detecting more airspace consolidation in diabetics,[32] vascular microemboli are highly involved in the pathogenesis.[33]

One criticism of this work on COVID-19-related pulmonary fibrosis is the sample size. The main reason for the limited number of patients is to perform a re-radiation test (HRCT) with the patient's consent after recovery, even if it is within the medical-indication coverage. Another point is to highlight fibrosis formation, even in non-severe patients; the number of patients with critical clinical states should be increased so that the discrepancy can be clearly understood.

## CONCLUSION

Overall, this study evaluated pulmonary fibrosis formation in the sixth month after recovery from COVID-19 pneumonia. The study confirmed that fibrosis in the pulmonary parenchyma persisted in most of the cases. One prominent finding was that CP treatment in non-severe patients with lymphopenia tended to formate more pulmonary fibrosis. Therefore, how accurate was CP administration in these patients? If this observation is to be moved forward, a better understanding of CP-related pulmonary fibrosis needs to be developed.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** The study was carried out with the permission of Selcuk University School of Medicine Ethics Committee (Date: 04.12.2020, Decision No: 2020/2916).

**Informed Consent:** All patients signed the free and informed consent form.

**Referee Evaluation Process:** Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

**Financial Disclosure:** This study was supported by Necmettin Erbakan University Research Fund (Project Number: 211518008).

**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

## REFERENCES


1. Jiang DH, McCoy RG. Planning for the post-COVID syndrome: how payers can mitigate long-term complications of the pandemic. J Gen Intern Med 2020;35:3036-9.
2. Lewis KL, Helgeson SA, Tatari MM, et al. COVID-19 and the effects on pulmonary function following infection: A retrospective analysis. EClinicalMedicine 2021;39:101079.
3. Das KM, Lee EY, Singh R, et al. Follow-up chest radiographic findings in patients with MERS-CoV after recovery. Indian J Radiol Imaging 2017;27:342-9.
4. Hui DS, Wong KT, Ko FW, et al. The 1-year impact of severe acute respiratory syndrome on pulmonary function, exercise capacity, and quality of life in a cohort of survivors. Chest 2005;128:2247-61.
5. Crisan-Dabija R, Pavel CA, Popa IV, et al "A Chain Only as Strong as Its Weakest Link": An Up-to-Date Literature Review on the Bidirectional Interaction of Pulmonary Fibrosis and COVID-19. J Proteome Res 2020;19:4327-38.
6. COVID-19 Treatment Guidelines [homepage on the Internet]. NIH: [Cited 14 June 2022]. Available from: https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/
7. Tumsatan P, Wongwiwatchai J, Apinives C, et al. Mediastinal Lymphadenopathy in Patients with Systemic Sclerosis. J Med Assoc Thai 2016;99:348-53.
8. Malpani Dhoot N, Goenka U, Ghosh S, et al. Assigning computed tomography involvement score in COVID-19 patients: prognosis prediction and impact on management. BJR Open 2020;2:20200024.
9. Nguyen Y, Corre F, Honsel V, et al. Applicability of the CURB-65 pneumonia severity score for outpatient treatment of COVID-19. J Infect 2020;81:e96-e8.
10. Approach to the adult with lymphocytosis or lymphocytopenia [homepage on the Internet]. USA: [Cited 14 June 2022]. Available from: https://www.uptodate.com/contents/approach-to-the-adult-with-lymphocytosis-or-lymphocytopenia?search=lymphopenia/;
11. Mohamed Khosroshahi L, Rezaei N. Dysregulation of the immune response in coronavirus disease 2019. Cell Biol Int 2021;45:702-7.
12. Tan L, Wang Q, Zhang D, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther 2020;5:33.
13. Gattinoni L, Gattarello S, Steinberg I, et al. COVID-19 pneumonia: pathophysiology and management. Eur Respir Rev 2021;30.
14. Yu M, Liu Y, Xu D, et al. Prediction of the Development of Pulmonary Fibrosis Using Serial Thin-Section CT and Clinical Features in Patients Discharged after Treatment for COVID-19 Pneumonia. Korean J Radiol 2020;21:746-55.
15. Huang Y, Tan C, Wu J, et al. Impact of coronavirus disease 2019 on pulmonary function in early convalescence phase. Respir Res 2020;21:163.
16. Hama Amin BJ, Kakamad FH, Ahmed GS, et al. Post COVID-19 pulmonary fibrosis; a meta-analysis study. Ann Med Surg (Lond) 2022;77:103590.
17. Margaria JP, Moretta L, Alves-Filho JC, et al. PI3K Signaling in Mechanisms and Treatments of Pulmonary Fibrosis Following Sepsis and Acute Lung Injury. Biomedicines 2022;10.
18. Xiang M, Jing H, Wang C, et al. Persistent Lung Injury and Prothrombotic State in Long COVID. Front Immunol 2022;13:862522.
19. Phua J, Weng L, Ling L, et al. Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. Lancet Respir Med 2020;8:506-17.
20. Anti-SARS-CoV-2 Antibody Products. [homepage on the Internet]. NIH: [Cited 14 June 2022]. Available from: https://www.covid19treatmentguidelines.nih.gov/therapies/anti-sars-cov-2-antibody-products/;
21. Akay Cizmecioglu H, Goktepe MH, Demircioglu S, et al. Efficacy of convalescent plasma therapy in severe COVID-19 patients. Transfus Apher Sci 2021;60:103158.
22. Rojas M, Rodriguez Y, Monsalve DM, et al. Convalescent plasma in Covid-19: Possible mechanisms of action. Autoimmun Rev 2020;19:102554.
23. Nezlin R. Dynamic Aspects of the Immunoglobulin Structure. Immunol Invest 2019;48:771-80.
24. van Erp EA, Luytjes W, Ferwerda G, et al. Fc-Mediated Antibody Effector Functions During Respiratory Syncytial Virus Infection and Disease. Front Immunol 2019;10:548.
25. Moss P. The T cell immune response against SARS-CoV-2. Nat Immunol 2022;23:186-93.
26. Taeschler P, Adamo S, Deng Y, et al. T-cell recovery and evidence of persistent immune activation 12 months after severe COVID-19. Allergy 2022.
27. Valdebenito S, Bessis S, Annane D, et al. COVID-19 Lung Pathogenesis in SARS-CoV-2 Autopsy Cases. Front Immunol 2021;12:735922.
28. Lu X, Cui Z, Pan F, et al. Glycemic status affects the severity of coronavirus disease 2019 in patients with diabetes mellitus: an observational study of CT radiological manifestations using an artificial intelligence algorithm. Acta Diabetol 2021;58:575-86.
29. Mohammadi A, Balan I, Yadav S, et al. Post-COVID-19 Pulmonary Fibrosis. Cureus 2022;14:e22770.
30. van Dam LF, Kroft LJM, van der Wal LI, et al. Clinical and computed tomography characteristics of COVID-19 associated acute pulmonary embolism: A different phenotype of thrombotic disease? Thromb Res 2020;193:86-9.
31. Loo J, Spittle DA, Newnham M. COVID-19, immunothrombosis and venous thromboembolism: biological mechanisms. Thorax 2021;76:412-20.
32. Gangadharan S, Parker S, Ahmed FW. Chest radiological finding of COVID-19 in patients with and without diabetes mellitus: Differences in imaging finding. World J Radiol 2022;14:13-8.
33. Bhandari S, Rankawat G, Singh A, Gupta V, Kakkar S. Impact of glycemic control in diabetes mellitus on management of COVID-19 infection. Int J Diabetes Dev Ctries 2020:1-6.