

ORIGINAL RESEARCH

Biomarkers of Fibrosis in Patients with COVID-19 One Year After Hospital Discharge: A Prospective Cohort Study

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Abstract

Beyond the acute infection of coronavirus disease (COVID-19), concern has arisen about long-term effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The aim of our study was to analyze if there is any biomarker of fibrogenesis in patients with COVID-19 pneumonia capable of predicting post-COVID-19 pulmonary sequelae. We conducted a multicenter, prospective, observational cohort study of patients admitted to a hospital with bilateral COVID-19 pneumonia. We classified patients into two groups according to severity, and blood sampling to measure matrix metalloproteinase 1 (MMP-1), MMP-7, periostin, and VEGF and respiratory function tests and high-resolution computed tomography were performed at 2 and 12 months after hospital discharge. A total of 135 patients were evaluated at 12 months. Their median age was 61 (interquartile range, 19) years, and 58.5% were men. We found between-group differences in age, radiological involvement, length of hospital

stay, and inflammatory laboratory parameters. Differences were found between 2 and 12 months in all functional tests, including improvements in predicted forced vital capacity (98.0% vs. 103.9%; $P = 0.001$) and $DL_{CO} < 80\%$ (60.9% vs. 39.7%; $P = 0.001$). At 12 months, 63% of patients had complete high-resolution computed tomography resolution, but fibrotic changes persisted in 29.4%. Biomarker analysis demonstrated differences at 2 months in periostin (0.8893 vs. 1.437 ng/ml; $P < 0.001$) and MMP-7 (8.7249 vs. 15.2181 ng/ml; $P < 0.001$). No differences were found at 12 months. In multivariable analysis, only 2-month periostin was associated with 12-month fibrotic changes (odds ratio, 1.0013; 95% confidence interval, 1.0006–1.00231; $P = 0.003$) and 12-month DL_{CO} impairment (odds ratio, 1.0006; 95% confidence interval, 1.0000–1.0013; $P = 0.047$). Our data suggest that early periostin postdischarge could predict the presence of fibrotic pulmonary changes.

Keywords: COVID-19; pulmonary sequelae; fibrosis biomarkers; pulmonary fibrosis

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A complete list of COVID-FIBROTIC study team members may be found before the beginning of the REFERENCES.

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To date, more than 600 million cases of coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection have been reported (1), and 10–20% of these cases developed pneumonia (2). Moreover, the increasing number of cases has brought a parallel rise in the number of survivors (3, 4) and heightened concern about the long-term effects of COVID-19 (5), particularly regarding pulmonary sequelae. A recently published systematic review found that during the 12 months after COVID-19 pneumonia, up to 29% of patients developed fibrotic changes in their chest radiographs, and almost 40% persisted with abnormal DL_{CO} levels 1 year after infection (6), similar to results seen elsewhere (7, 8).

Fibrotic sequelae have been described in patients after acute respiratory distress syndrome (9), but recent indicate that these changes may appear even in patients with COVID-19 who have mild disease (10). This implies that the virus itself could have a profibrotic action (11), like human immunodeficiency virus, cytomegalovirus, Epstein-Barr virus, or many other viruses that have been described as triggers of pulmonary fibrosis (12). For all these reasons, it seems necessary to detect those patients most susceptible to developing sequelae, because the long-term consequences are unknown, and biomarkers are biomolecules capable of predicting the incidence or outcome of diseases (13).

In this sense, multiple blood molecules related to the pathogenesis of fibrosis have been identified in idiopathic pulmonary fibrosis (IPF) (14). Some of them have been investigated for their role as objective predictors to determine disease progression (15). Furthermore, some COVID-19 studies have shown the elevation of fibrotic markers during the acute (16) and subacute phases of infection (17). The aim of the present study was to detect whether any biomarker pathophysiologically related to pulmonary fibrosis could be useful to detect those patients at risk of presenting fibrotic sequelae 12 months after hospital discharge.

Methods

Ethics, Study Design, and Participants

This study is based on a subcohort of the COVID-FIBROTIC study (Study of the

Appearance of Lung Fibrotic Changes Associated with SARS-CoV-2 Infection; www.clinicaltrials.gov identifier: NCT04409275; June 1, 2020), a prospective, observational, multicenter study aimed at evaluating pulmonary sequelae in patients admitted for bilateral COVID-19 pneumonia. Our substudy cohort consisted of patients from five of these centers. Center selection was based on the availability of and logistics required to send biobank samples. All patients were informed of the study procedures and gave their consent (see the data supplement). This study was performed in accordance with the Declaration of Helsinki. This human study was approved by the research ethics committee of the Hospital Clinic, Biomedical Research Institute, Valencia, Spain. All adult participants provided written informed consent to participate in this study.

Procedures

All included patients were scheduled for a first and second visit at 2 and 12 months after hospital discharge, respectively. During visits, baseline data and current clinical status were collected, including dyspnea graded according to the modified Medical Research Council dyspnea scale. At 2 months, all patients underwent blood sampling, pulmonary function tests, chest radiography, and high-resolution computed tomography (HRCT). At 12 months, all patients underwent pulmonary function tests and blood analysis. According to protocol, only patients with unresolved HRCT scans at 2 months underwent repeat HRCT scans.

Patients were classified into two groups (World Health Organization [WHO] scale) (18) according to the maximum respiratory support required during hospitalization:

1. Group 1: hospitalized mild disease (WHO scale 4): acute respiratory failure with conventional supplemental oxygen
2. Group 2: hospitalized severe and critical disease (WHO scale 5–7):
 - a. WHO scale 5: acute respiratory failure with noninvasive mechanical ventilation (non-IMV) or high-flow nasal oxygen cannula
 - b. WHO scale 6: intubation and IMV
 - c. WHO scale 7: IMV and additional organ support

Blood Sampling

All participants underwent blood collection with samples split into two aliquots. Serum biomarker samples from participating centers were stored in the biobank until analysis. The biomarkers analyzed at 2 months were matrix metalloproteinases (MMPs): MMP-1 (pg/ml) and MMP-7 (pg/ml), periostin (ng/ml), and vascular endothelial growth factor (pg/ml). At 12 months, we analyzed only the previously significant biomarkers: periostin and MMP-7 (adding the MMP-1 results because they shared the same blood kit test) (data supplement).

Functional Assessment

Pulmonary function tests were performed in all patients (Masterscreen, Jaeger). The protocol included forced spirometry with forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), and FEV₁/FVC; static lung volumes by body plethysmography with total lung capacity and residual volume; DL_{CO}; and 6-minute-walk test. All procedures were performed according to American Thoracic Society and European Respiratory Society guidelines and recommendations (19–22).

Radiological Assessment

Pulmonary damage on simple chest radiographs was quantified by modifying the Radiographic Assessment of Lung Edema (RALE) score to COVID-19 based on 8 points (23). HRCT images were evaluated by experienced chest radiologists following the Fleischner Society glossary of terms (24). Fibrosis-like changes were defined as the presence of traction bronchiectasis, parenchymal bands, and/or a reticular pattern (25, 26) (data supplement). The statistical analysis is described in the data supplement.

Results

We recruited 202 patients from the COVID-FIBROTIC cohort (17) for this subcohort, of whom 135 patients were finally analyzed at 12 months (Figure 1). The mean age was 61 (interquartile range, 19) years, and 58.5% were men. Comparing groups 1 and 2, we found differences in age (57.3 vs. 63.7 yr; $P = 0.005$), admission RALE score (3 vs. 4.3; $P < 0.001$), peak RALE score (4.2 vs. 6.7; $P < 0.001$), length of hospital stay (9.6 vs. 29.5; $P < 0.001$), and all laboratory parameters related to severity (lower

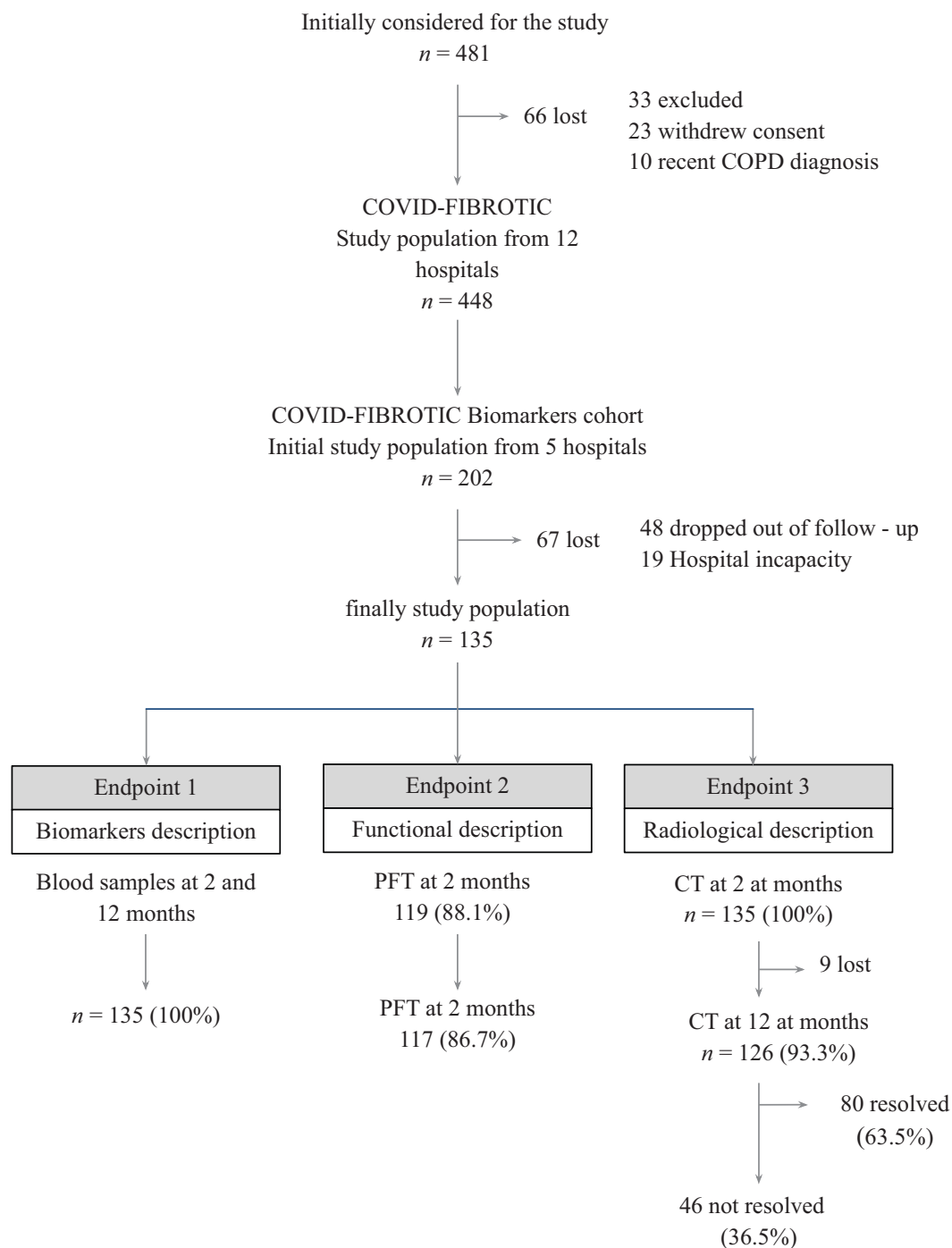


Figure 1. Flowchart of patients included in the COVID-FIBROTIC (Study of the Appearance of Lung Fibrotic Changes Associated with SARS-CoV-2 Infection) biomarker cohort. COPD = chronic obstructive pulmonary disease; CT = computed tomography; PFT = pulmonary function test.

lymphocyte count and higher peak concentrations of lactate dehydrogenase, C-reactive protein, ferritin, fibrinogen, and D-dimer) (Table 1).

Respiratory function tests showed a significant improvement in all predicted pulmonary function values over time: FVC

(98 vs. 103.9; $P < 0.001$), FEV_1 (98.2 vs. 103.2; $P < 0.001$), FEV_1/FVC (80.5 vs. 79.3; $P = 0.006$), DL_{CO} (74.7 vs. 84.1; $P < 0.001$), $DL_{CO} < 80\%$ (60.9% vs. 39.7%; $P < 0.001$), and total lung capacity (92.4 vs. 94.4; $P = 0.02$). Clinically significant dyspnea (modified Medical Research Council dyspnea scale

score, ≥ 2) was also reduced (20.5 vs. 6.6; $P = 0.002$) (Table 2). No changes over time were found in 6-minute-walk test results.

Radiological findings revealed that the number of “normal HRCT scans” increased over time (46.7% vs. 63.5%; $P < 0.001$), whereas the percentage of patients with

Table 1. Study Patient Characteristics

	Group 1 (n = 94)	Group 2 (n = 41)	Total (N = 135)	P Value
Age, yr	59 (21)	65 (13)	61 (19)	0.005
Male sex	55 (58.5%)	28 (68.3%)	83 (61.5%)	0.378
BMI, kg/m ²	27.7 (4.4)	27 (3)	27.5 (4)	0.362
Never smoker	57 (60.6%)	27 (65.9%)	84 (62.2%)	0.695
Comorbidities				
Pulmonary disease*	16 (17%)	7 (17.1%)	23 (17%)	1.00
Hypertension	34 (36.2%)	13 (31.7%)	47 (34.8%)	0.761
Diabetes	20 (21.3%)	8 (19.5%)	28 (20.7%)	0.999
Cardiovascular disease	13 (13.8%)	3 (7.3%)	16 (11.9%)	0.431
Admission RALE score	3 (1.5)	4.3 (1.6)	3.4 (1.6)	0.001
Peak RALE score	4.2 (2)	6.7 (1.2)	5 (2.1)	0.001
Length of hospital stay, d	9.6 (6)	29.5 (17.6)	15.8 (14.3)	0.001
Laboratory findings [†]				
Lymphocytes, ×10 ⁹ /L	1 (0.5)	0.7 (0.5)	0.9 (0.5)	0.004
LDH, U/L	529.2 (200.1)	897.1 (405.2)	643.6 (326.8)	0.001
C-reactive protein, mg/L	61.9 (74.9)	196 (152.3)	103.9 (121.9)	0.001
Ferritin, ng/ml	922.9 (843.2)	1,628.2 (1,278)	1,164.4 (1,062)	0.001
Fibrinogen, g/L	6.6 (1.6)	8 (2.1)	7 (1.9)	0.001
D-dimer, ng/ml	1,355.7 (3,697)	8,247.4 (10,083.1)	3,498.5 (7,117.7)	0.001

Definition of abbreviations: BMI = body mass index; LDH = lactate dehydrogenase; RALE = Radiographic Assessment of Lung Edema.

Age (years) is expressed as mean (interquartile range). The remaining data are expressed as count (percent) or mean (SD). Group 1: hospitalized mild disease. Group 2: hospitalized severe and critical disease. Mean comparison was carried out using Student's *t* test in the presence of normality and, if otherwise, using the Mann-Whitney *U* test. For quality variables, comparison of percentages between groups was carried out using Fisher's exact test for dichotomous variables or the chi-square test for contingency tables with more than two categories.

*Pulmonary disease: asthma, obstructive sleep apnea, others.

[†]All laboratory findings are peak values except for lymphocytes, for which the lowest value is given.

ground-glass opacities decreased (43.9% vs. 24.6%; $P < 0.001$). However, the percentage of patients who developed fibrosis-like changes from 2 to 12 months did not change significantly (25% vs. 29.4%; $P = 0.520$) (Table 2).

At 2 months, we found differences in severity (between groups 1 and 2) in periostin (0.8893 vs. 1.437 ng/ml; $P < 0.001$) and MMP-7 (8.7249 vs. 15.2181 ng/ml; $P < 0.001$). At 12 months, no between-group differences were found for any biomarker (Table 3).

To assess the ability of biomarkers to predict lung sequelae, we performed multivariable logistic regression. Analyzing the usefulness of 2-month biomarkers to predict 12-month fibrotic changes, only periostin was associated with outcome (odds ratio, 1.0014; 95% confidence interval, 1.0001–1.0023; $P = 0.003$) (Table 4). No biomarker obtained at 12 months was related to 12-month fibrotic changes (Table E1 in the data supplement). Regarding pulmonary function tests, periostin at 2 months was associated with DL_{CO} impairment at 12 months (odds ratio, 1.0006; 95% confidence interval, 1.0000–1.0013; $P = 0.047$) (Table 5). However, no effect was found at 12 months (Table E2).

Table 2. Pulmonary Function of Patients at Follow-Up

	First Follow-Up (2 mo)	Second Follow-Up (12 mo)	P Value
FVC, % pred	98 (17.8)	103.9 (15.1)	0.001
FEV ₁ , % pred	98.2 (15.8)	103.2 (14.2)	0.001
FEV ₁ /FVC, %	80.5 (5.7)	79.3 (4.9)	0.006
DL _{CO} , % pred	74.7 (19.3)	84.1 (16.1)	0.001
DL _{CO} <80%, pred	70 (60.9%)	46 (39.7%)	0.001
TLC, % pred	92.4 (14.8)	94.4 (11.9)	0.02
6MWT distance, m	554.5 (99.3)	554.8 (102)	0.12
mMRC ≥2	27 (20.5%)	8 (6.6%)	0.002
Normal HRCT pattern	63 (46.7%)	80 (63.5%)	0.001
Fibrosis-like changes*	33 (25%)	37 (29.4%)	0.52
GGO	58 (43.9%)	31 (24.6%)	0.001

Definition of abbreviations: 6MWT = 6-minute-walk test; FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; GGO = ground-glass opacity; HRCT = high-resolution computed tomography; mMRC = modified Medical Research Council; TLC = total lung capacity. Data are expressed as count (percent) or mean (SD). Pulmonary function tests (PFT) were registered at two moments in 117 patients. HRCT scans at 2 months, $N = 135$ patients. HRCT scans at 12 months, $N = 126$ patients. Comparison of pulmonary function between first and second follow-up visits was carried out using direct *t* test for quality variables and McNemar's test otherwise.

*Fibrosis-like changes were defined as the presence of traction bronchiectasis, reticular pattern, and/or parenchymal bands.

Discussion

In our multicenter, prospective, observational study, we analyzed blood biomarkers of fibrogenesis and their link to functional and radiological sequelae 12 months after bilateral COVID-19 pneumonia. The most common functional lung sequelae described 1 year after SARS-CoV-2 infection is diffusion impairment (8). In our cohort, although patients showed improvement in all functional values after

Table 3. Biomarkers at 2 and 12 Months

Biomarkers	Group 1 (n = 94)	Group 2 (n = 41)	Total (N = 135)	P Value
2 mo				
Periostin, ng/ml	0.889 (0.386)	1.437 (0.918)	1.055 (0.647)	0.001
MMP-1, ng/ml	7.858 (8.569)	9.660 (8.140)	8.405 (8.452)	0.256
MMP-7, ng/ml	8.724 (5.798)	15.218 (10.856)	10.696 (8.216)	0.001
VEGF, pg/ml	203.2 (221.7)	258.9 (226.0)	220.1 (236.4)	0.209
12 mo				
Periostin, ng/ml	0.553 (0.256)	0.628 (0.306)	0.576 (0.273)	0.149
MMP-1, ng/ml	8.992 (7.562)	9.175 (7.934)	9.046 (7.645)	0.899
MMP-7, ng/ml	10.025 (5.851)	11.135 (4.601)	10.351 (5.520)	0.293

Definition of abbreviations: MMP = matrix metalloproteinase; VEGF = vascular endothelial growth factor.

Data are expressed as mean (SD). Group 1: mild disease. Group 2: moderate-severe disease. Mean comparison was carried out using the Student's *t* test.

1 year, $DL_{CO} < 80\%$ persisted in almost 40%, consistent with published data (27, 28). Regarding radiological sequelae, ground-glass opacity is the most frequent early radiological abnormality (29). However, inflammatory lesions tend to resolve over time (30), whereas fibrotic changes (25, 26) may persist in 21–29% of patients, according to two recent meta-analyses (6, 31).

In this study, we found differences between severity groups at 2 months in periostin and MMP-7. However, only 2-month periostin was able to predict the presence of radiological fibrotic sequelae and DL_{CO} impairment at 12 months. Periostin is a matricellular protein that exerts its function by binding to integrin molecules on the surface of target cells (32). It is therefore an essential protein for wound healing, stabilization of collagen cross-linking (33), and mediation into tissue remodeling (34). Measurement of baseline periostin levels in

patients with IPF has been proposed as a good biomarker of disease progression because there is an increase in plasma periostin concentrations in patients with progressive IPF, which reflects the presence of active fibrosis areas in the lungs (35, 36). As in IPF, SARS-CoV-2 induces direct damage on the alveolar epithelium, leading to the release of inflammatory mediators and disruption of the basement membrane by the MMPs to allow access of inflammatory cells to the focus of injury (17). Some studies have found elevated concentrations of periostin in the lungs and blood of patients with severe COVID-19. In a small sample of patients with severe COVID-19, Zeng and colleagues (37) demonstrated increased extracellular factors in BAL (including periostin) in comparison with patients with nonsevere COVID-19. Another study found that periostin blood concentrations obtained during admission were significantly higher

in patients with COVID-19 than in healthy patients and that periostin concentrations remained elevated 1 month after admission in patients with severe disease (38).

Regarding its value as a marker of pulmonary sequelae, we have shown an association between 2-month periostin concentrations and the presence of fibrotic sequelae in HRCT and DL_{CO} abnormalities at 12 months (Tables 4 and 5). This is consistent with our previous data: We found an association between periostin concentrations at 2 months and early fibrotic changes already visible on 2-month HRCT. We have also demonstrated this association with diffusion impairment at 6 months (17). Factors that may influence the presence of 1-year fibrotic sequelae, such as sex, age, and severity, have been noticed, but because of the different nature of variables (quantitative vs. qualitative), effect sizes are not comparable in a multivariable logistic regression with periostin (39). Moreover, we decided to include them merely because they could be potential confounding factors, but our interest is the effect of periostin.

There are currently no data on the evolution of periostin and other biomarkers 1 year after COVID-19 disease. Our hypothesis is that changes in periostin values are due to a temporal relationship with acute tissue damage caused by SARS-CoV-2. Although periostin concentrations remain elevated in the severe group at 2 months of disease, the acute infection has completely resolved at 1 year, and periostin values of group 2 have been reduced by more than one-half, equaling mild group periostin concentrations. However, tissue damage is already established, so the resulting fibrotic lesions would remain visible despite the normalization of the marker. In this study, no other biomarker demonstrated such a marked change comparable to that in periostin.

During the follow-up of our cohort, we detected 25% of patients who developed fibrotic changes after infection with COVID-19. However, these sequelae remained stable during the follow-up period of the study, presenting no evidence of pulmonary progressive fibrosing disease to date. However, we are completely unaware of the evolution of these alterations in the long term. Whether these fibrotic changes could trigger true progressive pulmonary fibrosis is unknown. Biomarkers such as periostin may hypothetically put us on the track to know

Table 4. Multivariable Logistic Regression to Predict Fibrosis-Like Changes at 12 Months from 2 Month Biomarkers

	Univariable Analysis		Multivariable Analysis	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Periostin, ng/ml	1.0016 (1.0008–1.0026)	<0.001	1.0014 (1.0001–1.0023)	0.003
MMP-1, ng/ml	1.0001 (1.0001–1.0001)	0.017	—	—
MMP-7, ng/ml	1.0001 (1.0000–1.0001)	0.001	—	—
VEGF, pg/ml	1.0009 (0.9993–1.0025)	0.248	—	—
Age, yr	1.0577 (1.0218–1.0995)	0.003	1.0543 (1.0122–1.1033)	0.015
Severity groups	6.6917 (2.9072–16.0560)	<0.001	3.2971 (1.2726–8.6155)	0.014
Sex	0.7765 (0.3372–1.7231)	0.541	—	—

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

The variables for the multivariable analysis were selected using the Akaike information criterion in a backward-forward stepwise procedure. All presented variables were candidates to be selected.

Table 5. Multivariable Logistic Regression to Predict DL_{CO} Impairment at 12 Months from 2 Month Biomarkers

	Univariable Analysis		Multivariable Analysis	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Periostin, ng/ml	1.0004 (0.9999–1.0011)	0.126	1.0006 (1.0000–1.0013)	0.047
MMP-1, ng/ml	1.0000 (0.9999–1.0000)	0.855	—	—
MMP-7, ng/ml	1.0000 (0.9999–1.0000)	0.104	—	—
VEGF, pg/ml	0.9995 (0.9978–1.0011)	0.566	—	—
Age, yr	1.0445 (1.0108–1.0827)	0.013	1.0437 (1.0086–1.0837)	0.0184
Severity groups	1.8276 (0.8118–4.1396)	0.145	—	—
Sex	2.9282 (1.3499–6.4923)	0.007	3.646 (1.579–8.771)	0.003

The variables for the multivariable analysis were selected using the Akaike information criterion in a backward-forward stepwise procedure. All presented variables were candidates to be selected.

which patients to follow in the long term because elevated periostin has been shown to be associated with the persistence of fibrotic sequelae at 12 months.

Our study has several limitations. The first of these is the patients lost to follow-up. Among the 202 available patients in the 2-month analysis, we were able to obtain a second blood sample from only 135 (66.8%) patients. The reasons were mainly COVID-19 lockdown restrictions and biobank logistics. Second, our selection was limited to well-known biomarkers of fibrogenesis, and many others fell beyond the scope of our study, such as osteopontin,

epidermal growth factor receptor, or IL-6. Third, computed tomography scans were assessed qualitatively, although semiquantitative analysis would have provided valuable information, especially on the evolution of the lesions. However, the extent of lesions was not systematically collected owing to logistical constraints because we were in the first COVID-19 wave. Finally, we are aware of a potential selection bias, because patients included in the study had had COVID-19 bilateral pneumonia, which could add to the difficulty of exploring results for infected patients who did not require hospital admission.

In conclusion, several biomarkers are associated with COVID-19 severity at 2 months after discharge; yet, only 2-month periostin concentrations could predict the presence of radiological and functional fibrotic pulmonary sequelae at 12 months. The importance of this association lies in its ability to detect patients potentially at risk of developing progressive fibrosis triggered by SARS-CoV-2 infection. Further studies are needed to evaluate fibrosis development after COVID-19 disease and the potential role of several other biomarkers. ■

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