Post-COVID-19 respiratory sequelae two years after hospitalization: an ambidirectional study

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The Lancet Regional Health - Americas 2024;33: 100733

Published Online xxx https://doi.org/10.1016/j.lana.2024.100733

Summary

Background COVID-19 lung sequelae can impact the course of patient lives. We investigated the evolution of pulmonary abnormalities in post-COVID-19 patients 18–24 months after hospital discharge.

Methods A cohort of COVID-19 patients admitted to the Hospital das Clínicas da Faculdade de Medicina da USP in São Paulo, Brazil, between March and August of 2020, were followed-up 6–12 months after hospital discharge. A subset of patients with pulmonary involvement and chest computed tomography (CT) scans were eligible to participate in this second follow-up (18–24 months). Data was analyzed in an ambidirectional manner, including retrospective data from the hospitalization, and from the first follow-up (6–12 months after discharge), and compared with the prospective data collected in this new follow-up.

Findings From 348 patients eligible, 237 (68%) participated in this follow-up. Among participants, 139 (58%) patients presented ground-glass opacities and reticulations, and 80 (33%) presented fibrotic-like lesions (traction bronchiectasis and architectural distortion). Five (2%) patients improved compared to the 6-12-month assessment, but 20 (25%) of 80 presented worsening of lung abnormalities. For those with relevant assessments on both occasions, comparing the CT findings between this follow-up with the previous assessment, there was an increase in patients with architectural distortion (43 [21%] of 204 *vs* 57 [28%] of 204, p = 0.0093), as well as in traction bronchiectasis (55 [27%] of 204 *vs* 69 [34%] of 204, p = 0.0043). Patients presented a persistent functional impairment with demonstrated restrictive pattern in both follow-ups (87 [42%] of 207 *vs* 91 [44%] of 207, p = 0.76), as well as for the reduced diffusion capacity (88 [42%] of 208 *vs* 87 [42%] of 208, p = 1.0). Length of hospitalization (OR 1.04 [1.01–1.07], p = 0.0040), invasive mechanical ventilation (OR 3.11 [1.3–7.5] p = 0.011), patient's age (OR 1.03 [1.01–1.06] p = 0.0074 were consistent predictors for development of fibrotic-like lung lesions in post-COVID-19 patients.

Interpretation Post-COVID-19 lung sequelae can persist and progress after hospital discharge, suggesting airways involvement and formation of new fibrotic-like lesions, mainly in patients who were in intensive care unit (ICU).

Funding São Paulo Research Foundation (22/01769-5) and Instituto Todos pela Saúde (C1721).

Keywords: Ambidirectional cohort; COVID-19; PASC; Fibrosis; Small airways disease; Lung CT; Pulmonary function

Introduction

The COVID-19 pandemic challenged the health systems worldwide. Although vaccination drastically reduced the mortality globally, the post-COVID-19 diseases and sequelae can cause long-lasting physical, mental and cognitive effects. Some post-COVID-19 symptoms have been observed in high frequencies,[1] which include fatigue, muscle weakness, smell and taste disorder,

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Research in context

Evidence before this study

Our group searched literature (PubMed) for studies detailing prospective COVID-19 two-year follow-ups reporting respiratory assessments. We found only nine studies published in 2022 and 2023 and confirming at least dyspnea investigation (search performed from 15th of June 2022 to 8th November of 2023). Then, we narrowed our search for prospective cohort studies that included pulmonary function tests and chest computed tomography assessments after two-year of hospital discharge. We used the following search terms: ("post-COVID-19") AND ("prospective cohort" OR "follow-up") AND ("Dyspnea" OR "mMRC") AND ("PFTs" OR "pulmonary function tests") AND ("CT" OR "computed tomography") AND ("2-year" OR "two-year"). We concluded our literature search with only four studies matching all predefined criteria (dyspnea assessment, pulmonary function tests and chest CT investigations in patients after two-years of hospital discharge and recovery from severe COVID-19). After this systematic search, we concluded that this is the largest two-year ambidirectional post-COVID-19 cohort study (n = 237) focused on the evolution of the pulmonary abnormalities.

Added value of this study

This cohort study involving post-COVID-19 patients is the only two-year follow-up research performed in a South American country. Most of the COVID-19 studies report lung sequelae by less precise methods than computed tomography (CT), and when CT was applied, they evaluated very few patients in their cohort. Most of these studies did not report a simultaneous pulmonary function examination to investigate the extent of the physiological impact caused by the lung abnormalities. Among few studies found reporting the pulmonary health of patients two-years after severe COVID-19, our study has the largest number of participants with face-to-face examination, and pulmonary function tests followed by chest computed tomography investigations. Most of patients in our study presented an intensive care unit (ICU) history during the pandemic, and evidence of pulmonary involvement in the 6-12-month assessment. The literature reports that severe COVID-19 and prolonged hospitalization are linked to lung sequelae, suggesting that long-term follow-ups were required for a more profound understanding of this population. We found a persistent lung functional impairment with demonstrated restrictive pattern and reduced lung diffusion linked to non-fibrotic-like lung lesions or fibrotic-like lung lesions18-24 months after hospital discharge. Strikingly, some patients worsened compared to the previous follow-up, revealing that even after recovery from severe COVID-19 hospitalization, pulmonary sequelae can progress towards fibrotic-like lung lesions over the years. Therefore, this study raised substantial information to the current knowledge about post-COVID-19 lung sequelae at the long-term.

Implications of all the available evidence

Consecutive follow-ups reveal the long-term impact of severe COVID-19 disease in the population, pointing to important pulmonary clinical findings such as, persistent functional restriction, impaired diffusion, small airways involvement and development of new fibrotic-like lesions. Future studies should address if these lung lesions are fibrotic indeed, and if there are other susceptibility factors associated to the pathological progression. Therefore, a consolidation of lung permanent sequelae predictors will guide future clinical decisions and shape the strategy for the long-term management of post-COVID-19 chronic patients.

dizziness, chest pain, myalgia, and others.[2–4] The pulmonary involvement has been highlighted as a major concern in the post-COVID-19, with a potential risk of becoming permanent and a lifelong burden.[5,6] Several studies attributed a post-COVID evolving lung disease to a continuous inflammatory cascade that may ultimately result in fibrosis and architectural distortion.[7–10] Therefore, understanding the key drivers that predispose patients to develop lung sequelae is yet to be elucidated.

Millions of severe cases were registered worldwide during the COVID-19 pandemic, leading to a high concern about the number of individuals that could have significant pulmonary sequelae. Cohort studies carried out from 6 up to 24 months after hospital discharge evaluated the long-term pulmonary involvement in patients that recovered from severe COVID-19. Those studies demonstrated that survivors with late pulmonary involvement and imaging abnormalities, such as ground-glass opacities and architectural changes, presented decreased exercise capacity, hypoxia, reduced lung diffusing capacity for carbon monoxide, and restrictive pattern in pulmonary function tests.[6,8,11–13] However, long-term prospective cohort studies are still scarce.[14–18] Therefore, this is a limitation for understanding the late outcomes for chronic patients.

In Brazil, the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) is the biggest university hospital linked to the national health system (SUS), and it is located in the metropolitan region of São Paulo (~23 million inhabitants). The hospital became a reference in the management of severe COVID-19.[19] In this context, our team designed in 2020 a prospective follow-up study, 6–12 months after hospital discharge, to monitor COVID-19 survivors.[11,14] This study found that 82% of the patients presented lung sequelae in the CT imaging, which impacted the pulmonary function tests.[14]

Long-term assessments are essential to monitor lung disease progression, resolution, and their causes. Therefore, the aim of this ambidirectional study was to investigate the evolution of post-COVID-19 patients with pulmonary involvement, 18–24 months after hospital discharge.

Methods

Study design

This ambidirectional cohort study included patients aged ≥18 years who were admitted to the ward and/or ICU of HCFMUSP with positive RT-PCR for SARS-CoV-2, from March 30th to August 31st of 2020. Patients who performed chest CT scan in the first follow-up 6–12 months after hospital admission, were eligible to participate in this second follow-up study at 18–24 months after hospital discharge (n = 348). Excluding patients due to death (n = 11) and due to contact loss or no interest in continuing the follow-up (n = 100), 237 patients participated in this new follow-up. This study was part of a large medical assistance protocol implemented during the COVID-19 pandemic and described elsewhere.[19] The study was approved by the Research Ethics Committee of our institution (No. 31942020.0.000.0068). The written informed consent was signed by all patients. The STROBE statement can be found annexed to the Supplementary Information File.

Follow-up protocol

The modified Medical Research Council (mMRC) questionnaire for dyspnea scale was applied by teleconsultation. The in-person consultations included physical examination, evaluation of oxygen saturation (SpO2) with a pulse oximetry, which was measured at rest and after the 1-min sit and stand test. In addition, altered oximetry was diagnosed when resting SpO2 ≤ 90% and/or a decrease in SpO2 of ≥4%. In blood tests, the serum level of C-reactive protein (CRP) and D dimer were verified.[11,19] Chest CT scans (CT) were performed, and images were assessed by two thoracic radiologists, in a blind and independent manner. Patients were categorized according to their lung lesions and based on the following criteria: fibrotic-like lesions (traction bronchiectasis and architectural distortion) and non-fibrotic like lesions (ground-glass opacities and reticulations).[12] The lung lesion severity was quantified according to the following CT scores for each pulmonary lobe: 0, none; 1, <5%; 2, 5–25%; 3, 26–50%; 4, 51–75%; and 5, >75%. The total CT score was calculated based on the sum of the scores of the five lobes, ranging from 0 to 25.[11] Chest CT was not performed at the baseline, before the patient hospitalization discharge after recovery from severe COVID-19, so fibrosis-like lesions were identified only during the follow-ups. Pulmonary function tests were performed according to recommended standards.[20] The functional parameters evaluated comprehended pulmonary volume, airway flow and diffusion capacity. Restrictive pattern, obstructive pattern, and reduced lung diffusing capacity for carbon monoxide were defined as total lung capacity (TLC) < lower limit of normal (LLN), FEV_1/FVC ratio < lower limit of normal, and DLCO < lower limit of normal, respectively.[21–23]

Statistical analysis

HCFMUSP electronic medical records (EMR) were accessed to retrieve patients' hospitalization data. All clinical data were registered in the REDCap software (https://www.redcapbrasil.com.br/).

For the transversal analysis, the new collected data (18-24-month) were analyzed with the Mann–Whitney U test and the Student's t-test (independent T-test) for non-normally and normally distributed data respectively. For the longitudinal comparison, paired data, from the first (6-12-month) and the second follow-up (18-24-month) were analyzed with the Wilcoxon signed-rank test and the Student's t-test (paired T-test), for non-normally and normally distributed, respectively. Frequencies were used to describe the categorical variables. For the transversal analysis, independent frequencies were compared using the chi-square test applying the Yates' continuity correction or by doubling the exact one-tailed probability obtained from Fisher's exact test. For the longitudinal analysis of paired data, frequencies were compared using the exact form of McNemar's test. The employed software was IBM SPSS Statistics 29. Missing data were indirectly reported in the data tables by the total number of computed values (N) for each variable. Those patients were excluded from the analysis when necessary. A multiple logistic regression was performed to verify the impact of continuous and categorical variables in the development of lung fibrotic lesions in COVID-19 patients, from both infirmary and ICU care. Demographic and clinical variables were chosen following a previous publication from our group, containing data from the first follow-up (6–12 months after hospital discharge).[14] In detail, the selected data were patient's age, sex assigned at birth, length of hospitalization, CRP values at the first 72 h of hospitalization, use of vasoactive drug, need of invasive mechanical ventilation, and tracheostomy. Testing assumptions were performed in the IBM SPSS Statistics 29 and *Prism* (v9.5.1) software to check the numerical variables normality, linearity, homoscedasticity, and absence of multicollinearity (Supplementary Figures S1, S2 and S3).

Role of funding source

The funders of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Flow-chart, demographic and clinical features of the patients eligible and included in the study

The rationale behind the study and the selection of the participants is presented in the cohort flow-chart (Fig. 1). From the eligible patients (n = 348), 11 died and 100 were excluded due to no interest or to contact loss, therefore resulting in 237 (68%) patients in this second follow-up. The median elapsed time in days between the hospital admission and the follow-up exams was 762 days (IQR 731–802). The summary of examinations performed in both follow-ups (6–12 months and 18–24 months after hospital discharge) and further compared in this study are depicted in Fig. 2. Demographic and clinical variables were compared between participant patients (n = 237) and patients that refused to participate in this second follow-up (n = 100), but no statistical significance was found in most of the variables tested. The only significant observation was a higher inflammation during hospitalization in those patients who refused to participate in this follow-up (based on CRP-72 h median, 114 mg/l [IQR 61.2–209] *vs* 185 mg/l [IQR 98.4–266], p = 0.0029) (Table 1).

Further, we investigated the clinical variables obtained in the first follow-up (6–12 months) to elucidate if conditions of the patients at that time may have contributed to their refusal to participate in the second follow-up (18–24 months) (Supplementary Table S1). In this extended analysis, although no significant differences were observed in the oximetry measurements, dyspnea assessment or in the chest CT features, patients that refused to participate in the second follow-up presented some variables of the pulmonary function tests that differed significantly than those who continued their participation in the second follow-up. For instance, a significant lower median total lung capacity (% of predicted) (81, [IQR 73–91.8] *vs* 86, [IQR 77–94]) (p = 0.0073) was observed. A higher percentage of patients with a restrictive pattern was observed in those who refused to participate in the second follow-up (32 patients [55%] of 58 *vs* 93 patients [42%] of 219) (p = 0.11), as well as a significant higher median for the residual volume and total lung capacity ratio, RV/TLC (33.2, [IQR 28.2–38.1] *vs* 32, IQR [27.1–35.7]) (p = 0.020). These findings suggest that a portion of patients with worse conditions did not participate in the second follow up, leading to a potential bias in the cohort (Supplementary Table S1).

Regarding the 237 patients included in the second follow-up (18–24 months), the average age was 56.3 (SD 13.3) years, 112 (47%) were male, 125 (52%) were female, and 162 (68%) required ICU care after hospital admission. Among those in ICU care, 61 (38%) needed vasoactive drugs, 111 (68%) needed of invasive

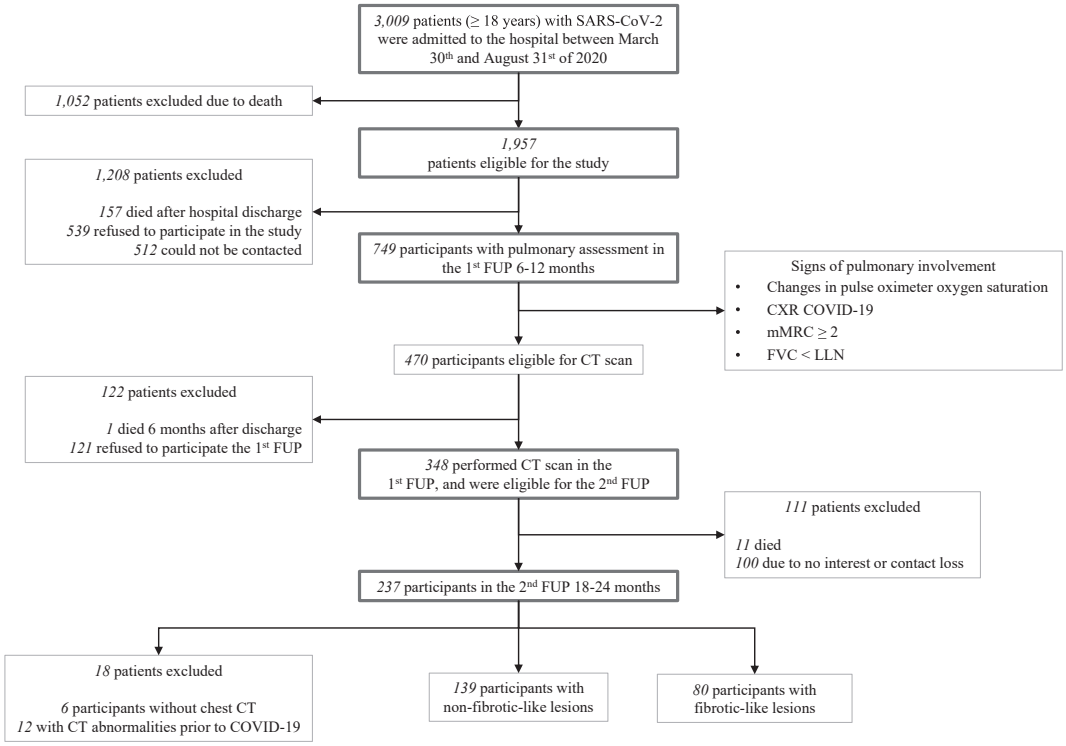

Fig. 1: Flow-chart for selecting participating patients in the 2nd follow-up (FUP). CT (computed tomography), CXR (chest X-ray), FVC (forced vital capacity), LLN (lower limit of normal), mMRC (modified Medical Research Council Dyspnea Score).

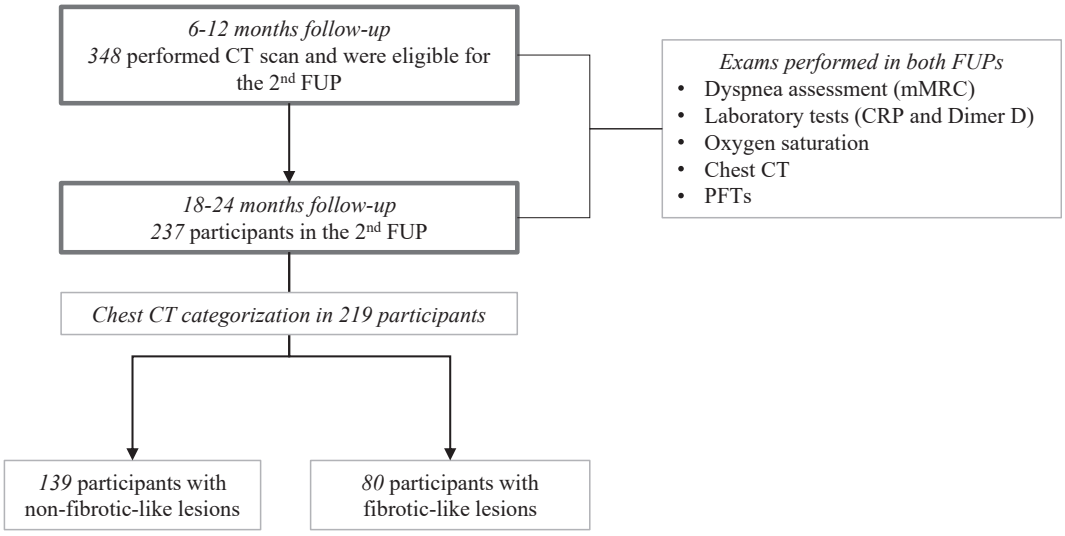

Fig. 2: Summarized flow-chart for the 1st and the 2nd follow-up (FUP), including the number of participants and the exams performed in each phase. CXR (Chest X-Ray), CT (Computed tomography), mMRC (modified Medical Research Council Dyspnea Score), CRP (C-Reactive Protein), PFTs (Pulmonary Function Tests).

Variables	Eligible Patients (n = 348)*	Patients at 18–24 Mo FUP (n = 237)	Refusing patients (n = 100)	p value
Demographics				
Age, (SD), n–years	55.9 (13.4), 348	56.3 (13.3), 237	54.8 (13.9), 100	0.43
Male, n/N (%)	161/348 (46)	112/237 (47)	41/100 (41)	0.35
BMI, median (IQR), n–kg/m^2	31.6 (28.0–36.0), 348	32.1 (28.7–36.9), 237	30.7 (27.7–35.3), 100	0.16
Comorbidities				
Hypertension, n/N (%)	215/348 (62)	143/237 (60)	65/100 (65)	0.49
COPD, n/N (%)	32/348 (9)	23/237 (10)	6/100 (6)	0.37
Diabetes, n/N (%)	142/348 (41)	98/237 (41)	41/100 (41)	1.0
Smoking History, n/N (%)	139/348 (40)	101/237 (43)	36/100 (36)	0.28
Hospitalization				
Length of stay, median (IQR), n–days	16 (8–28), 348	16 (8–28), 237	17.5 (9–30), 100	0.28
ICU care during hospitalization, n/N (%)	237/348 (68)	162/237 (68)	69/100 (69)	1.0
CRP 72 h, median (IQR), n–mg/l	126 (64.3–215), 297	114 (61.2–209), 228	185 (98.4–266), 69	0.0029
Characteristics in ICU				
ICU length of stay, median (IQR), n–days	11 (6–20), 237	11 (6.2–19.8), 162	12 (7–25), 69	0.43
SAPS 3 at admission, (SD), n	58.8 (13.8), 230	58.2 (13.6), 159	59.6 (14.5), 66	0.41
CRP 72 h, median (IQR), n–mg/l	150 (71.6–244), 228	138 (66.3–242), 154	185 (98.4–266), 69	0.066
D Dimer 72 h, median (IQR), n–ng/ml	1650 (898–4657), 224	1564 (879–3560), 151	2254 (1077–7246), 68	0.032
Dialysis, n/N (%)	13/237 (5)	9/162 (6)	4/69 (6)	1.0
VAD, n/N (%)	86/237 (36)	61/162 (38)	22/69 (32)	0.49
IMV during hospitalization, n/N (%)	163/237 (69)	111/162 (68)	48/69 (70)	0.99
Tracheostomy, n/N (%)	23/237 (10)	15/162 (9)	8/69 (12)	0.74

Values are presented as median (IQR), n/N (%) or mean (SD). (*) this n number includes 11 patients who died prior to follow-up and includes 18 patients who were excluded from analysis (see Fig. 1). Statistical comparisons were made between patients of this follow-up (n = 237) and those who refused to participate (n = 100). Abbreviations: BMI: Body Mass Index, COPD: Chronic Obstructive Pulmonary Disease, CRP: C-Reactive protein, CT: computed tomography, DLCO: diffusion capacity for carbon monoxide, FUP: follow-up, FVC: Forced vital capacity, ICU: Intensive care unit, IMV: Invasive mechanical ventilation, IQR: Interquartile range, LLN: Lower limit of normal, Mo: months, SAPS3: Simplified Acute Physiology Score III, SD: standard deviation, TLC: Total Lung Capacity, VAD: Vasoactive Drug. The criteria and the definition of eligible patients, participants and those who refused to participate can be found in the study flow-chart (Fig. 1).

Table 1: **Demographic and clinical comparisons of patients in the second follow-up at 18–24 months after hospital discharge against patients that refused to continue their participation in the study.**

mechanical ventilation and 15 (9%) needed tracheostomy (Table 1). Patients were treated with chloroquine or hydroxychloroquine, dexamethasone, methylprednisolone, prednisone, antibiotics, and anticoagulants. In detail, 236 patients (99%) of 237 received anticoagulants, 50 (21%) of 237 received antiplatelet agents, 223 (94%) of 237 received antibiotics, six (2%) of 237 received chloroquine or hydroxychloroquine, 66 (28%) of 237 received NSAIDs drugs, 58 (24%) of 237 received angiotensin-II receptor antagonists and 160 (67%) of 237 received corticosteroids.

Demographic and clinical comparison between patients with non- and fibrotic-like lesions

Demographic and clinical variables of hospitalized COVID-19 patients were compared to identify differences between those patients who developed non-fibrotic lung lesions and those with fibrotic-like lung lesions 18–24 months after hospital discharge (Table 2). A lower body mass index (BMI) (30.5 kg/m^2, [IQR 28.8–35]) ($p = 0.021$), a higher duration of hospitalization (28 days, [IQR 18–42.8]) ($p < 0.0001$) and of ICU care (15 days, [IQR 10–30]) ($p < 0.0001$), as well as a higher level of inflammation (based on CRP-72 h, 145 mg/l, [IQR 70.9–227]) ($p = 0.059$) were identified in the fibrotic-like group. The percentage of ICU hospitalized patients was significantly higher in the fibrotic-like group (73 patients [91%] of 80) ($p < 0.0001$) as well. Additionally, 59 (81%) of 73 ICU patients who developed fibrotic-like lesions needed invasive mechanical ventilation, and 13 (18%) of those patients needed tracheostomy. The need for invasive mechanical ventilation and/or tracheostomy were significantly different between the groups of fibrotic-like and the non-fibrotic-like patients ($p = 0.013$ and $p = 0.0034$, respectively).

Pulmonary function tests, dyspnea, laboratorial and tomographic assessment in patients who developed lung sequelae

Dyspnea score, laboratorial exams, pulmonary function tests, and tomographic abnormalities are shown in Table 3 and Supplementary Table S1. Altered oximetry was observed in 10 (4%) of 237 patients and dyspnea (mMRC ≥2) in 104 (44%) of 237 patients. Both variables were not significantly different between non-fibrotic and fibrotic-like patients ($p = 0.078$ and $p = 1.0$ respectively). The median CT score 14 (IQR 11–17) and the prevalence of CT score ≥7 was observed in 75 (96%) of 78 patients with fibrotic-like lesions. In

Variables	Non-fibrotic like lesions (n = 139)	Fibrotic-like lesions (n = 80)	p value
Demographics			
Age, (SD), n–years	54.9 (13.0), 139	58.4 (12.9), 80	0.058
Male, n/N (%)	61/139 (44)	41/80 (51)	0.36
BMI, median (IQR), n–kg/m^2	33.5 (29–38.4), 139	30.5 (28.8–35), 80	0.021
Comorbidities			
Hypertension, n/N (%)	85/139 (61)	48/80 (60)	0.98
COPD, n/N (%)	15/139 (11)	4/80 (5)	0.22
Diabetes, n/N (%)	53/139 (38)	37/80 (46)	0.30
Smoking History, n/N (%)	59/139 (42)	33/80 (41)	0.97
Hospitalization			
Length of stay, median (IQR), n–days	11 (6.5–19), 139	28 (18–42.8), 80	<0.0001
ICU care during hospitalization, n/N (%)	77/139 (55)	73/80 (91)	<0.0001
CRP 72 h, median (IQR), n–mg/l	97.2 (57–177), 135	145 (70.9–227), 75	0.059
Characteristics in ICU			
ICU length of stay, median (IQR), n–days	8 (50–13), 77	15 (10–30), 73	<0.0001
SAPS 3 at admission, (SD), n	56.8 (13.4), 77	59.6 (13.8), 70	0.20
CRP 72 h, median (IQR), n–mg/l	115 (63.9–219), 75	145 (70.9–241), 68	0.73
D Dimer 72 h, median (IQR), n–ng/ml	1410 (815–3265), 72	1729 (952–3581), 68	0.19
Dialysis, n/N (%)	6/77 (8)	3/73 (4)	0.54
VAD, n/N (%)	27/77 (35)	28/73 (38)	0.80
IMV during hospitalization, n/N (%)	47/77 (61)	59/73 (81)	0.013
Tracheostomy, n/N (%)	2/77 (3)	13/73 (18)	0.0034

Values are presented as median (IQR), n/N (%) or mean (SD). Abbreviations: BMI: Body Mass Index, COPD: Chronic Obstructive Pulmonary Disease, CRP: C-Reactive protein, CT: computed tomography, FUP: follow-up, ICU: Intensive care unit, IQR: Interquartile range, IMV: Invasive mechanical ventilation, Mo: months, mMRC: modified medical research council for dyspnea scale, SAPS3: Simplified Acute Physiology Score III, SD: standard deviation, VAD: Vasoactive Drug.

Table 2: **Demographic and clinical comparisons between patients with lung lesions diagnosed in the second follow-up of post COVID-19 lung sequelae cohort 18–24 months after hospital discharge.**

Variable	Patients at 18–24 m FUP (n = 237)*	Non-fibrotic like lesions (n = 139)	Fibrotic-like lesions (n = 80)	p value
Altered Oximetry, n/N (%)	10/237 (4)	7/139 (5)	0	0.078
Dyspnea score (mMRC) ≥ 2, n/N (%)	104/237 (44)	62/139 (45)	35/80 (44)	1.0
CRP, median (IQR), n–mg/l	4.3 (1.9–9.9), 237	5.1 (1.9–11.2), 139	3.2 (1.6–7.9), 80	0.051
D Dimer, median (IQR), n–ng/ml	451 (311–809), 235	490 (344–850), 121	532 (402–952), 67	0.27
Computed tomography (CT)				
CT Score, median (IQR), n	6 (2–12), 211	3 (0–6), 133	14 (11–17), 78	<0.0001
CT Score ≥7, n/N (%)	105/211 (50)	30/133 (23)	75/78 (96)	<0.0001
Pulmonary function tests (PFT)				
FVC, (SD), n–% of predicted	83.0 (14.0), 224	83.5 (14.2), 131	82.6 (13.6), 77	0.65
FVC < LLN, n/N (%)	92/224 (41)	55/131 (42)	31/77 (40)	0.92
FEV_1, (SD), n–% of predicted	84.5 (16.5), 224	83.9 (16.4), 131	86.7 (15.7), 77	0.22
FEV_1, < LLN, n/N (%)	74/224 (33)	49/131 (37)	18/77 (23)	0.053
FEV_1/FVC, median (IQR), n	82.2 (78.1–85.9), 224	81.7 (78.1–84.6), 131	83.8 (79.7–87.8), 77	0.0005
FEV_1/FVC < LLN, n/N (%) (Obstructive pattern)	21/224 (9)	13/131 (10)	4/77 (5)	0.34
TLC, median (IQR), n–% of predicted	84 (77–92), 223	86 (78.5–93), 131	82.5 (75–90), 76	0.0064
TLC < LLN, n/N (%) (Restrictive pattern)	100/224 (45)	63/130 (48)	28/78 (36)	0.10
RV, median (IQR), n–% of predicted	81 (72–94), 223	83 (74–96.5), 131	76 (67.8–87), 76	0.0005
RV/TLC, median (IQR), n	33.6 (30.2–36.4), 224	33.8 (28.3–39.3), 131	33.1 (28–38), 76	0.33
DLCO, median (IQR), n–% of predicted	82 (71–94.3), 220	84 (74.3–97.8), 130	78 (68.5–86), 75	0.0083
DLCO < LLN, n/N (%) (Impaired diffusion)	93/220 (42)	50/130 (38)	36/75 (48)	0.23

Values are presented as median (IQR), n/N (%) or mean (SD). (*) this n number includes 18 patients who were excluded from analysis (see Fig. 1). Statistical comparisons were made between non-fibrotic-like patients (*n = 139*) and fibrotic-like patients (*n = 80*). Abbreviations: CT: computed tomography, DLCO: diffusion capacity for carbon monoxide, FEV: forced expiratory volume, FUP: follow-up, FVC: forced vital capacity, IQR: Interquartile range, LLN: Lower limit of normal, mMRC: modified medical research council for dyspnea scale, Mo: months, PFT: pulmonary function test, RV: residual volume, SD: standard deviation, TLC: total lung capacity. Altered oximetry: resting SpO2 ≤ 90% and/or a decrease in SpO2 of ≥4%.

Table 3: **Comparison between pulmonary function of patients with lung alterations and fibrosis diagnosed in second follow-up of post COVID-19 lung sequelae cohort after 18–24 months after hospital discharge.**

patients with non-fibrotic-like lesions, the median CT score was three (IQR 0–6) and only 30 (23%) of 133 patients presented CT score ≥7. Both variables were significantly higher in patients with fibrotic-like lung lesions compared to those with non-fibrotic lesions ($p < 0.0001$ on both analyses). Regarding pulmonary function tests, the prevalence of obstructive pattern was found in 21 (9%) of 224 patients, restrictive pattern in 100 (45%) of 224 patients, and reduced lung diffusing capacity for carbon monoxide in 93 (42%) of 220 patients. Patients with fibrotic-like lesions had a median predicted total lung capacity of 82.5 (IQR 75–90), compared to 86 (IQR 78.5–93) in patients with non-fibrotic lesions ($p = 0.0064$). A lower median predicted lung diffusing capacity for carbon monoxide was also found in fibrotic-like patients (78, [IQR 68.5–86]) compared to non-fibrotic-like (84, [IQR 74.3–97.8]) ($p = 0.0083$) (Table 3). Although not significant between the groups, 36 (48%) of 75 patients with fibrotic-like lesions presented reduced lung diffusing capacity for carbon monoxide, compared to 50 (30%) of 130 patients with non-fibrotic-like lesions (DLCO < lower limit of normal, Table 3). Additionally, the mean alveolar volume found in patients with fibrotic-like lesions (3.9, [SD 1.0]) was significantly lower than those with non-fibrotic-like lesions (4.3, [SD 0.9]) ($p = 0.0046$) (Supplementary Table S2).

Impact of demographic and clinical variables on the development of late pulmonary fibrosis in post-COVID-19 patients

The variables that contributed to increase the risk for development of pulmonary fibrotic lesions in post-COVID-19 patients from infirmary and ICU care, at 18–24 months after hospital discharge were evaluated (n = 210) (Table 4). In total, 75 patients were in the fibrotic-like group and 135 in the non-fibrotic-like group. From 219 patients (100%) presenting non-fibrotic-like and fibrotic-like lesions, 9 (4%) were excluded from the analysis due to missing CRP-72 h values. A multiple logistic regression was employed to calculate the estimated odds ratio (OR) for lung fibrosis and its statistical significance (Table 4, Supplementary Figures S1 and S2). The area under the curve (AUC) of the predictive model was 0.81 (SE 0.029) (95% CI 0.75–0.87) (Table 4). Older patients, with a higher duration of hospitalization, and the need of invasive mechanical ventilation were more susceptible to develop lesions, (OR 1.03, 95% CI 1.01–1.06) ($p = 0.0074$); (OR 1.04, 95% CI 1.01–1.07) ($p = 0.0040$); and (OR 3.11, 95% CI 1.30–7.58) ($p = 0.011$), respectively.

n/N = 210/219	Estimate OR	95% CI	p value
Age (years)	1.03	1.01–1.06	0.0074
Sex (Male)	1.19	0.60–2.33	0.60
Hospitalization (days)	1.04	1.01–1.07	0.0040
CRP-72 h (mg/l)	1.00	0.99–1.00	0.32
VAD (yes)	0.71	0.29–1.64	0.43
IMV (yes)	3.11	1.30–7.58	0.011
Tracheostomy (yes)	1.97	0.36–15.33	0.45

n/N: number of patients included in the regression analysis out of a total of 219 patients categorized between fibrotic-like and non-fibrotic-like according to their chest CT features. CI: confidence interval. CRP-72 h: C-Reactive Protein. IMV: Invasive mechanical ventilation. OR: odds ratio. VAD: Vasoactive Drug. AUC: area under curve = 0.81, Standard Error: SE = 0.029, 95% CI = 0.75–0.87. 70.3% prediction power for fibrosis. Multicollinearity evaluation, testing assumptions and further details can be found in the supplementary material. Proportion of patients included in the repression analysis (n/N): 9 patients of 219 were removed from the analysis due to missing information about their CRP-72 h values, resulting in 210 patients analyzed.

Table 4: **Multiple logistic regression to investigate the impact of demographic and clinical variables on the development of lung fibrotic-like lesions in post COVID-19 patients 18–24 months after hospital discharge.**

The evolution of lung sequelae in post-COVID-19 patients after hospital discharge

The evolution of patients after COVID-19 hospitalization was assessed by comparing data collected from the cohort 6–12 months and 18–24 months after hospital discharge (Table 5 and Supplementary Table S3). In the second follow-up, 139 (58%) of 237 patients continued to present a degree of pulmonary involvement but without indications of fibrosis, 80 (33%) of 237 patients presented fibrotic-like lesions and 5 (2%) patients improved after the first-year assessment, regressing from fibrotic-like lesions to non-fibrotic lesions (Fig. 1). The percentage of patients with altered oximetry decreased significantly at 18–24 months, with 37 (17%) of 218 patients in the 6-12-month follow-up compared to 8 (4%) of 218 patients in this follow-up ($p < 0.0001$). Additionally, there was a decrease in the prevalence of mMRC dyspnea score ≥2 in the second follow-up (122 [51%] of 237 *vs* 104 [44%] of 237, $p = 0.067$). Considering the chest CT scans, the number of patients with traction bronchiectasis increased from 55 (27%) of 204 to 69 (34%) of 204 ($p = 0.0043$), as well as the architectural distortion from 43 (21%) of 204 to 57 (28%) of 204, ($p = 0.0093$), the mosaic attenuation pattern from 29 (14%) of 203 to 84 (41%) of 203 ($p < 0.0001$), and the bronchial wall thickening, from 44 (22%) of 204 to 108 (53%) of 204 ($p < 0.0001$). Regarding pulmonary function tests, although variations in the functional parameters were observed in the longitudinal comparison, they were not all significant. It is worth mentioning that there was no clinical improvement in the obstructive (17 [8%] of 215 *vs* 20 [9%] of 215) ($p = 0.45$) and restrictive pattern (87 [42%] of 207 *vs* 91 [44%] of 207) ($p = 0.76$), and lung diffusion (88 [42%] of 208 *vs* 87 [42%] of 208) ($p = 1.0$) in the second follow-up (Table 5).

Among those patients with CT abnormalities in the second follow-up (n = 80), 25% evolved to fibrotic-like lesions after the first follow-up (n = 20) (Table 6 and Supplementary Table S4). Comparing the demographic and hospitalization characteristics of patients that evolved to fibrotic-like lesions (n = 20) with patients that remained with non-fibrotic-like lesion patterns 18–24 months after the hospital discharge (n = 134) (non-categorized as fibrotic-like at any follow-up), only the median length of hospitalization and the percentage of patients under ICU care differed statistically (Table 6). In detail, the patients who developed fibrotic-like lesions after the first follow-up presented a median length of hospitalization of 21.5 days (IQR 16–32.5) and 95% received ICU care (19 of 20 patients). A lower median of 10 days (IQR 6.2–19) was observed for the patients that remained with a non-fibrotic-like lesions pattern (Table 6) ($p = 0.0041$), and only 54% (73 of 134 patients) received ICU care ($p = 0.0013$). Comparing the clinical characteristics obtained in the first follow-up (6–12 months) in both group of patients described above (Table 7), a higher percentage of non-fibrotic-like patients presented dyspnea (83 [62%] of 134 patients) compared to the patients that evolved to fibrotic-like-lesions after the first follow-up (five [25%] of 20) ($p = 0.0040$). On the other hand, significant differences were observed in the chest CT scans, such as higher median CT score for the new fibrotic-like patients (nine [IQR 7–12.5]) compared to the non-fibrotic-like patients (two [IQR 0–6]) ($p < 0.0001$), a higher percentage of the new fibrotic-like patients presented a CT score ≥7 (16 [84%] of 19) compared to the non-fibrotic-like patients (27 [21%] of 130) ($p < 0.0001$). Considering the qualifications of the tomographic lesions, the new fibrotic-like patients presented already at the 6–12 months follow-up, significantly higher percentages for several abnormal CT features (see Table 7 for the details) compared to the patients that did not evolve their pulmonary lesions. However, no clinically relevant differences were found after analyzing the pulmonary function tests comparing both groups (Table 7).

When analyzing longitudinally the group of patients that evolved to fibrotic-like lesions after the first follow up (Table 8), in the chest CT scans, the number of patients with CT score ≥7 was 16 (84%) of 19 in the first follow-up and increased to 17 (89%) of 19 patients in this second follow-up ($p = 1.0$) (Table 8). Notably, the number of patients with architectural distortions increased from one (5%) of 20 to 11 (55%) of 20 ($p = 0.0019$), as well as for traction bronchiectasis, which increased from zero of 20 patients to 17 (85%) of 20 ($p < 0.0001$). Analyzing the evolution of the pulmonary function of this subgroup, the number of patients with demonstrated restrictive pattern remained constant, seven (37%) of 19 in both follow-up assessments ($p = 1.0$). No significance was found when comparing

Variable	6-12 Mo	18-24 Mo	p value
Altered Oximetry, n/N (%)	37/218 (17)	8/218 (4)	<0.0001
Dyspnea score (mMRC) ≥ 2, n/N (%)	122/237 (51)	104/237 (44)	0.067
CRP, median (IQR), n–mg/l	4.4 (2.2–8.4), 237	4.3 (1.9–9.9), 237	0.72
D Dimer, median (IQR), n–ng/ml	428 (240–694), 205	509 (352–867), 205	<0.0001
Computed tomography (CT)			
CT Score, median (IQR), n	6 (1–11.5), 207	6 (1.5–12), 207	0.22
CT Score ≥7, n/N (%)	100/207 (48)	101/207 (49)	1.0
At least one abnormal CT feature, n/N (%)	176/207 (85)	184/207 (89)	0.29
Consolidations, n/N (%)	1/204 (<1)	0	1.0
Ground-glass opacities, n/N (%)	151/203 (74)	153/203 (75)	0.83
Mosaic attenuation pattern, n/N (%)	29/203 (14)	84/203 (41)	<0.0001
Perilobular opacities, n/N (%)	29/204 (14)	9/204 (4)	<0.0001
Parenchymal bands, n/N (%)	127/204 (62)	128/204 (63)	1.0
Reticulations, n/N (%)	106/204 (52)	95/204 (47)	0.061
Architectural distortion, n/N (%)	43/204 (21)	57/204 (28)	0.0093
Traction bronchiectasis, n/N (%)	55/204 (27)	69/204 (34)	0.0043
Bronchial wall thickening, n/N (%)	44/204 (22)	108/204 (53)	<0.0001
Pneumatocele, n/N (%)	1/204 (<1)	1/204 (<1)	1.0
Pulmonary function tests (PFT)			
FVC, (SD), n–% of predicted	82.8 (14.6), 220	83.0 (14.0), 220	0.78
FVC < LLN, n/N (%)	87/215 (40)	86/215 (40)	1.0
FEV_1, (SD), n–% of predicted	84.9 (16.9), 220	84.5 (16.5), 220	0.18
FEV_1, < LLN, n/N (%)	65/215 (30)	71/215 (33)	0.36
FEV_1/FVC, median (IQR), n	80 (78–86), 220	82.2 (78.1–85.9), 220	<0.0001
FEV_1/FVC < LLN, n/N (%) (Obstructive pattern)	17/215 (8)	20/215 (9)	0.45
TLC, (SD), n–% of predicted	86.4 (12.9), 219	84.7 (11.4), 219	0.0016
TLC < LLN, n/N (%) (Restrictive pattern)	87/207 (42)	91/207 (44)	0.76
RV, median (IQR), n–% of predicted	84 (71–101), 219	81 (72–94), 219	0.013
RV/TLC, median (IQR), n	33.9 (29.1–40.2), 219	33.5 (28.3–39.2), 219	0.36
DLCO, median (IQR), n–% of predicted	81 (68–92), 216	82 (71–94.3), 216	0.023
DLCO < LLN, n/N (%) (Impaired diffusion)	88/208 (42)	87/208 (42)	1.0

Values are presented as median (IQR), n/N (%) or mean (SD). Abbreviations: CT: computed tomography, DLCO: diffusion capacity for carbon monoxide, FEV: forced expiratory volume, FUP: follow-up, FVC: forced vital capacity, IQR: Interquartile range, LLN: Lower limit of normal, Mo: months, mMRC: modified medical research council for dyspnea scale, PFT: pulmonary function test, RV: residual volume, SD: standard deviation, TLC: total lung capacity. Altered oximetry: resting SpO2 ≤ 90% and/or a decrease in SpO2 of ≥4%.

Table 5: **Data comparison between the first and the second follow-up of post COVID-19 lung sequelae cohort at 6–12 months and 18–24 months after hospital discharge.**

the evolution of the lung diffusing capacity for carbon monoxide in those patients. However, the values remained lower than the normal limit in nine (47%) of 19 patients in both follow-ups (Table 8 and Table S4). Inspecting the vaccination status of these patients and new occurrences of SARS-CoV-2 infection between the first and the second follow-ups, we found that 18 (100%) of 18 patients were vaccinated against COVID-19, with an average number of 4.0 (SD 1.1) doses per patient in the second follow-up. Only 4 (22%) of 18 patients presented new episodes of SARS-CoV-2 infection between the follow-ups.

Discussion

This ambidirectional study is among the longest and the largest designed to assess pulmonary abnormalities developed 18–24 months after hospital discharge due to severe COVID-19. Additional importance of this study is the inclusion of patients from a country with mixed ethnicities and socioeconomic backgrounds, both valuable information for future extrapolation studies. Participants were patients hospitalized at the HCFMUSP with indications of severe COVID-19 during the first wave of the pandemic, early in 2020 and not vaccinated. They were selected for the follow-ups based on their reported respiratory symptoms and confirmed pulmonary involvement on chest CT. We evaluated the results in a transversal (18–24 months) and longitudinal (6–12 months *vs* 18–24 months) manner and found a persistent functional impairment with demonstrated restrictive pattern, as well as progressing CT abnormalities pointing to evolving fibrotic-like lesions and small airways involvement 18–24 months after hospital discharge.

Variables	New fibrotic-like patients (n = 20)	Non-fibrotic-like patients (n = 134)	p value
Demographics			
Age, (SD), n–years	59.3 (14.9), 20	54.4 (12.9), 134	0.32
Male, n/N (%)	10/20 (50%)	60/134 (45%)	0.84
BMI, median (IQR), n–kg/m^2	30.5 (29–34.9), 20	33.3 (28.9–38.1), 134	0.34
Comorbidities			
Hypertension, n/N (%)	10/20 (50)	80/134 (60)	0.56
COPD, n/N (%)	1/20 (5)	14/134 (10)	0.78
Diabetes, n/N (%)	9/20 (45)	49/134 (37)	0.63
Smoking History, n/N (%)	9/20 (45)	56/134 (42)	0.97
Hospitalization			
Length of stay, median (IQR), n–days	21.5 (16–32.5), 20	10 (6.2–19), 134	0.0041
ICU care during hospitalization, n/N (%)	19/20 (95)	73/134 (54)	0.0013
CRP 72 h, median (IQR), n–mg/l	145 (76.4–257), 19	98.5 (57.5–184), 130	0.087
Characteristics in ICU			
ICU length of stay, median (IQR), n–days	13 (8–17), 19	8 (5–12), 73	0.058
SAPS 3 at admission, (SD), n	56.5 (13.8), 18	56.5 (13.6), 73	0.99
CRP 72 h, median (IQR), n–mg/l	169 (92.2–259), 18	120 (64.7–232), 71	0.38
D Dimer 72 h, median (IQR), n–ng/ml	993 (884–3653), 18	1426 (834–3265), 68	0.90
Dialysis, n/N (%)	5/19 (26)	9/73 (12)	0.24
VAD, n/N (%)	5/19 (26)	24/73 (33)	0.78
IMV during hospitalization, n/N (%)	13/19 (68)	45/73 (61)	0.78
Tracheostomy, n/N (%)	1/19 (5)	2/73 (3)	1.0

Values are presented as median (IQR), n/N (%) or mean (SD). Abbreviations: BMI: body mass index, CRP-72 h: C-Reactive Protein 72 h, CT: computed tomography, DLCO: diffusion capacity for carbon monoxide, FEF: forced expiratory flow, FEV: forced expiratory volume, FUP: follow-up, FVC: forced vital capacity, ICU: intensive care unit, IMV: invasive mechanical ventilation, IQR: Interquartile range, LLN: Lower limit of normal, Mo: months, PFT: pulmonary function test, RV: residual volume, SD: standard deviation, TLC: total lung capacity, VAD: vasoactive drug. Statistical comparisons of data obtained in the first follow-up (6–12 months) after hospital discharge.

Table 6: **Demographic and hospitalization characteristics of post COVID-19 patients that evolved to fibrotic-like lung lesions between the first and the second follow-up and patients that remained presenting lesions with non-fibrotic-like pattern in the second follow-up.**

Restrictive and obstructive ventilatory impairment were found in some post-COVID-19 patients after recovery from severe acute respiratory syndrome.[13,14,17,18] Patients presented significant improvements in the dyspnea score and the oximetry. Although there was an increase in the restrictive pattern, but not in the diffusion pattern in the follow-up, we considered that such variation was not clinically relevant. The fibrotic group had mild functional impairment 18–24 months after the first follow-up (6–12 months after hospital discharge), reinforcing the need of pulmonary function tests in the long-term. These pulmonary abnormalities are often related to the development of definitive airway and parenchymal lesions. However, it is scarce the number of studies that systematically evaluated the evolution and the linkage of clinical, functional, and tomographic features, with the perspective of improvement or worsening.

To the best of our knowledge, there are only four studies that assessed functional and radiological findings in a two-year follow-up after COVID-19.[6,18,24,25] Li D., and collaborators identified only the lung residual volume below 80% as a significant alteration in 142 patients two years after recovery from COVID-19.[24] Huang et al. described that 66 (28%) patients presented functional impairment in a two-year follow-up after COVID-19, evidenced by reduced total lung capacity, residual volume and lung diffusing capacity for carbon monoxide.[18] Although they reported similar findings to our results, there are some points to be noted. They assessed only 51 patients with ICU admission, and included in the same group, patients with need of oxygen supply by nasal cannula, non-invasive mechanical ventilation, and invasive mechanical ventilation. Additionally, they did not differentiate non-fibrotic from fibrotic patients. Han and collaborators, in a two-year post-COVID-19 follow-up, evaluated 144 patients but only 56 with a degree of pulmonary involvement and only 22 admitted at ICU.[6] In their study, potential fibrotic patients had higher severity of respiratory symptoms, including dyspnea and reduced lung diffusing capacity for carbon monoxide, when compared to patients without fibrotic lesions. However, no other significant differences in pulmonary function tests were identified.[6] A post-COVID-19 prospective study carried on in Spain, by González J. et al. investigated the 24-month pulmonary health outcomes in a cohort of discharged ICU patients (n = 109).[25] The authors found in the longitudinal analysis, after comparing with results obtained from 3, 6 and 12

Variable	New fibrotic-like patients (n = 20)	Non-fibrotic-like patients (n = 134)	p value
Altered Oximetry, n/N (%)	2/19 (11)	23/122 (19)	0.57
Dyspnea score (mMRC) ≥ 2, n/N (%)	5/20 (25)	83/134 (62)	0.0040
CRP, median (IQR), n–mg/l	6.1 (4.8–8.1), 20	4.1 (2.2–8.4), 134	0.21
D Dimer, median (IQR), n–ng/ml	335 (215–458), 20	374 (241–735), 133	0.18
Computed tomography (CT)			
CT Score, median (IQR), n	9 (7–12.5), 19	2 (0–6), 131	<0.0001
CT Score ≥7, n/N (%)	16/19 (84)	27/130 (21)	<0.0001
At least one abnormal CT feature, n/N (%)	20/20 (100)	102/134 (76)	0.031
Consolidations, n/N (%)	1/20 (5)	0	0.26
Ground-glass opacities, n/N (%)	20/20 (100)	83/134 (62)	0 .0018
Mosaic attenuation pattern, n/N (%)	7/20 (35)	16/134 (12)	0 .018
Perilobular opacities, n/N (%)	4/20 (20)	2/134 (1)	0.0054
Parenchymal bands, n/N (%)	18/20 (90)	60/134 (45)	0.00040
Reticulations, n/N (%)	17/20 (85)	36/134 (27)	<0.0001
Architectural distortion, n/N (%)	1/20 (5)	0	0.26
Traction bronchiectasis, n/N (%)	0	0	–
Bronchial wall thickening, n/N (%)	4/20 (20)	36/134 (27)	0.70
Pneumatocele, n/N (%)	0	1/134 (<1)	1.0
Pulmonary function tests (PFT)			
FVC, (SD), n–% of predicted	84.9 (17.6), 19	83.2 (13.9), 126	0.62
FVC < LLN, n/N (%)	7/19 (37)	53/126 (42)	0 85
FEV_1, (SD), n–% of predicted	87.1 (20.1), 19	84.0 (15.6), 126	0.43
FEV_1, < LLN, n/N (%)	5/19 (26)	40/126 (32)	0.83
FEV_1/FVC, median (IQR), n	83 (77.9–87.6), 19	80.3 (78.6–82), 126	<0.0001
FEV_1/FVC < LLN, n/N (%) (Obstructive pattern)	1/19 (5)	11/126 (9)	1.0
TLC, (SD), n–% of predicted	84.7 (11.8), 19	88.3 (12.7), 126	0.24
TLC < LLN, n/N (%) (Restrictive pattern)	7/19 (37)	49/126 (39)	1.0
RV, median (IQR), n–% of predicted	84 (73.5–92.5), 19	87 (76.3–103), 126	0.15
RV/TLC, median (IQR), n	35.1 (30–38.1), 19	31.3 (24.9–34.9), 126	0.25
DLCO, median (IQR), n–% of predicted	75 (65.5–89.8), 19	83 (72–94), 124	0.22
DLCO < LLN, n/N (%) (Impaired diffusion)	9/19 (47)	49/124 (40)	0.69

Values are presented as median (IQR), n/N (%) or mean SD. Abbreviations: CT: computed tomography, DLCO: diffusion capacity for carbon monoxide, FEV: forced expiratory volume, FUP: follow-up, FVC: forced vital capacity, IQR: Interquartile range, LLN: Lower limit of normal, Mo: months, mMRC: modified medical research council for dyspnea scale, PFT: pulmonary function test, RV: residual volume, SD: standard deviation, TLC: total lung capacity. Altered oximetry: resting SpO2 ≤ 90% and/or a decrease in SpO2 of ≥4%. (–) p value could not be calculated due to constant variable. Statistical comparisons of data obtained in the first follow-up (6–12 months) after hospital discharge. (–) p value could not be calculated as the table is degenerate.

Table 7: **Clinical characteristics at the 6–12 months after hospital discharge of post COVID-19 patients that evolved to fibrotic-like lung lesions between the first and the second follow-up and patients that remained presenting lesions with non-fibrotic-like pattern in the second follow-up.**

months after hospital discharge, a progressive recovery of patient's lung function and exercise capacity. As evidenced in our study, González J. et al. also found high percentages of patients (45%) with impaired lung diffusion (reduced DLCO) despite improved lung function. Regarding the radiological findings, 54% still presented some type of pulmonary lesion after two years, being fibrotic lesions present in 12% of those patients. In addition, the authors highlight the role of the invasive mechanical ventilation for worsening the pulmonary health outcomes for post-COVID-19 patients (lowest lung diffusing capacity for carbon monoxide values and more than double in the frequency of patients with fibrotic pattern). In a similar analysis, we observed in the multiple logistic regression that invasive mechanical ventilation contributed for developing late fibrotic-like lesions in post-COVID-19 patients (OR 3.11, 95% CI 1.30–7.58).

Regarding the tomographic findings in our study, 20 (8%) of 237 patients with chest CT abnormalities in the 6-12-month follow-up, progressed to fibrotic lesions 18–24 months after hospital discharge. Although the CT score did not change in this second follow-up compared to the first follow-up, there was an increase in the number of patients with alterations suggestive of small airways involvement, such as mosaic attenuation and

Variable	6-12 Mo	18-24 Mo	p value
Altered Oximetry, n/N (%)	2/19 (11)	0	0.50
Dyspnea score (mMRC) ≥ 2, n/N (%)	5/20 (25)	9/20 (45)	0.22
CRP, median (IQR), n–mg/l	6.1 (4.8–8.1), 20	3.9 (1.9–8.8), 20	0.52
D Dimer, median (IQR), n–ng/ml	335 (215–458), 20	540 (261–824), 20	0.05
Computed tomography (CT)			
CT Score, median (IQR), n	9 (7–12.5), 19	11 (7–13), 19	0.037
CT Score ≥7, n/N (%)	16/19 (84)	17/19 (89)	1.0
At least one abnormal CT feature, n/N (%)	20/20 (100)	20/20 (100)	–
Consolidations, n/N (%)	1/20 (5)	0	1.0
Ground-glass opacities, n/N (%)	20/20 (100)	20/20 (100)	–
Mosaic attenuation pattern, n/N (%)	7/20 (35)	8/20 (40)	1.0
Perilobular opacities, n/N (%)	4/20 (20)	0	0.12
Parenchymal bands, n/N (%)	18/20 (90)	19/20 (95)	1.0
Reticulations, n/N (%)	17/20 (85)	19/20 (95)	0.62
Architectural distortion, n/N (%)	1/20 (5)	11/20 (55)	0 .0019
Traction bronchiectasis, n/N (%)	0	17/20 (85)	<0.0001
Bronchial wall thickening, n/N (%)	4/20 (20)	11/20 (55)	0.039
Pneumatocele, n/N (%)	0	0	–
Pulmonary function tests (PFT)			
FVC, (SD), n–% of predicted	84.9 (17.6), 19	82.4 (16.1), 19	0.17
FVC < LLN, n/N (%)	7/19 (37)	9/19 (47)	1.0
FEV_1, (SD), n–% of predicted	87.1 (20.1), 19	84.3 (19.0), 19	0.093
FEV_1, < LLN, n/N (%)	5/19 (26)	5/19 (26)	1.0
FEV_1/FVC, median (IQR), n	83 (77.9–87.6), 19	83 (77.4–87.1), 19	0.067
FEV_1/FVC < LLN, n/N (%) (Obstructive pattern)	1/19 (5)	2/19 (11)	1.0
TLC, (SD), n–% of predicted	84.7 (11.8), 19	83.6 (10.0), 19	0.14
TLC < LLN, n/N (%) (Restrictive pattern)	7/19 (37)	7/19 (37)	1.0
RV, median (IQR), n–% of predicted	84 (73.5–92.5), 19	82 (73.5–90.5), 19	0.74
RV/TLC, median (IQR), n	35.1 (30–38.1), 19	36 (31–38.6), 19	0.11
DLCO, median (IQR), n–% of predicted	75 (65.5–89.8), 19	77.5 (68.8–91.3), 19	0.21
DLCO < LLN, n/N (%) (Impaired diffusion)	9/19 (47)	9/19 (47)	1.0

Values are presented as median (IQR), n/N (%) or mean SD. Abbreviations: CT: computed tomography, DLCO: diffusion capacity for carbon monoxide, FEV: forced expiratory volume, FUP: follow-up, FVC: forced vital capacity, IQR: Interquartile range, LLN: Lower limit of normal, Mo: months, mMRC: modified medical research council for dyspnea scale, PFT: pulmonary function test, RV: residual volume, SD: standard deviation, TLC: total lung capacity. Altered oximetry: resting SpO2 ≤ 90% and/or a decrease in SpO2 of ≥4%. (–) p value could not be calculated due to constant variable. Statistical comparisons of data obtained in the first follow-up (6–12 months) after hospital discharge. (–) p value could not be calculated as the table is degenerate.

Table 8: **Longitudinal comparison of post COVID-19 patients that evolved to fibrotic-like lung lesions between the first and the second follow-up.**

bronchial wall thickening. Cho and collaborators found evidence of air trapping in a cohort of post-COVID-19 patients who remained symptomatic for more than 30 days following diagnosis for SARS-CoV-2. They observed a correlation with the residual volume and total lung capacity (RV/TLC ratio), and suggested occurrence of small airways disease.[26] However, the authors highlighted that long-term consequences are still unknown.

Predictive models were proposed to improve the knowledge about the prevalence and risk factors for COVID-19 sequelae, including pulmonary lesions.[11,27,28] Our analysis confirmed, higher length of hospitalization, need of invasive mechanical ventilation, and increased age were driving factors for development of late fibrotic-like lesions in patients with a previous identification of pulmonary involvement secondary to COVID-19. In comparison to our previous report 6-12-month after hospital discharge,[14] in this new analysis, increased age, but not CRP-72 h, improved the prediction of fibrotic-like lesions outcomes. Comorbidities are known risk factors that negatively impact a patient's lung health in COVID-19.[29] However, there was no association between comorbidities and the development of late lung fibrosis on post-COVID-19 patients in our study (data not shown). In addition to the predictive analysis, we surveyed the data of patients who evolved to fibrotic-like lesions after the first follow up (6–12 month) (n = 20), and we identified that a higher length of

hospitalization and need of ICU care were factors that differed this population from those patients who did not evolve to fibrotic-like lesions (n = 134). It is worth mentioning that these patients who worsened between the follow-ups already presented at the first follow-up several lung CT abnormalities (e.g. higher CT score) and in more abundance (e.g. higher percentages of ground-glass opacities, mosaic attenuation pattern, perilobular opacities, parenchymal bands, and reticulations) than those who did not evolved to fibrotic-like lesions after the first follow-up.

Among the strengths of this study, we highlight the largest cohort to analyze pulmonary function and radiological outcomes in post-COVID-19 patients, with most coming from ICU care. In addition, we identify and investigate in a longitudinal manner, the clinical and demographic profile of those patients who evolved to fibrotic-like lesions more than a year after hospitalization. In order to identify and understand what are the driving factors that contribute to the development of fibrosis in post-COVID-19 patients, we correlated some clinical and demographic observations to determine their magnitude of impact. Further, we believe this study will have a significant impact for understanding of how the pulmonary health of post-COVID-19 patients evolve at the long-term and provide guidance to the health systems in the identification of patient's profile that are susceptible to develop lung lesions years after the hospitalization and requiring specialized treatment. In addition to previous findings for H1N1[30] and other respiratory viruses responsible for causing severe illness, this type of long-term follow-up study addressing the history of COVID-19, will strengthen healthcare professionals and institutions expertise to deal with future respiratory viral pandemics at short and long-term.

The limitations of our study include the fact that 237 (68%) of 348 eligible patients participated in this new follow-up. In addition, when comparing characteristics of participants of both follow-ups (6–12 and 18–24 months after hospital discharge) with those who refused to participate in this second follow-up, we found a slightly bias in the cohort. Most of the patients who refused to participate in the second follow-up presented significant worse conditions at the time of the first follow-up as evidenced by the pulmonary function tests, such as a higher percentage of patients with a restrictive lung pattern. Among the participants of this study, although most of these patients presented a degree of pulmonary involvement, we have not addressed in this study the impact on the chronic symptoms and quality of life of those patients, but rather we focused on the pulmonary health. However, further research from our team will address this gap in a close future. In addition, for patients that either improved from the first follow-up or had progressed their pulmonary lesions, we do not have accurate information about how many of them received post-treatment such as rehabilitation. Other limitations include the fact that pulmonary lesions were defined based on CT scan images. Then, it is not possible to exclude that patients with ground-glass opacities and reticulations (non-fibrotic group) would have fibrosis whether lung biopsy would be performed. Moreover, for the predictive regression analysis, we cannot disregard that larger cohorts containing high levels of comorbidities can have an impact in the prediction of lung fibrosis. Certainly, missing values can reduce the power of any predictive analysis. Even though we reported a low percentage of missing values for the logistic regression (<5%), we cannot disregard this as another potential bias. A larger cohort, inclusion of participants ethnicity and verification of more clinical variables may be necessary to improve the statistical power for prediction of lung fibrosis in the Brazilian population. However, such studies are not yet available for comparison. In perspective, we believe future long-term pulmonary assessments and evaluation of prediction models consistency over-time will help designing a customized post-COVID-19 patient management and treatment.

Conclusions

This cohort study revealed that post-COVID-19 patients presenting persistent pulmonary involvement in previous follow-ups can evolve to late fibrosis-like lesions 18–24 months after hospital discharge. However, this evidence needs to be confirmed by histopathological analysis. Worsening of tomographic alterations, such as mosaic attenuation and bronchial wall thickening suggested development of small airways disease, which need to be further studied and may have an impact for the future management and treatment of those patients. Older patients, prolonged hospitalization, and the need of invasive mechanical ventilation were consistent predictors for pulmonary fibrosis in post-COVID-19 patients at the long-term. It is worth mentioning that a slight bias in the cohort was observed, and patients who refused to participate in this study already presented pulmonary restrictive conditions during the one-year follow-up. Then, we cannot disregard that those patients can potentially develop lung fibrotic-like lesions overtime as observed in this study, then increasing the number of patients with long-term pulmonary sequelae. Therefore, due to the non-homogenous evolution of chronic post-COVID-19 patients, it is essential to keep the long-term monitoring of their lung health.

Contributors

CRRC, CAL, LL, ML were responsible for the study conceptualization. CRRC, CAL, LL were responsible for the data curation. CRRC, CAL, LL, MG, RC, JS and MS performed the formal analysis. CRRC, CAL, LL, RC, JS, MS, CT, ML and BB were responsible for the data investigation and validation. CRRC, CAL, and LL were responsible for writing the manuscript. CC, CAL, LL, RC, JS, MS, CT, ML, PS, CN, MG, BB reviewed and edited the manuscript. CRRC and PS were responsible for acquiring funding for this study. All authors agreed to be accountable

for this work. The data was available to all authors, and they agreed in the decision to submit it for publication after the final revision.

Data sharing statement

Data will be made available with deidentified participants, as well as the data dictionary, as non-editable files shared via corresponding author.

Declaration of interests

We declare no competing interests.

Acknowledgements

We acknowledge the support from the HCFMUSP COVID-19 task force during the in-hospital data collection and during the setting-up of the follow-up assessments. We thank the São Paulo Research Foundation (FAPESP) (Grant number: 22/01769-5) and the Instituto Todos pela Saúde (ITpS–HCFMUSP Síndrome Pós Covid 19 – C1721) for funding the study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2024.100733.

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