EUROPEAN RESPIRATORY journal
FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original research article

Clinical, radiological, and pathological findings in patients with persistent lung disease following SARS-CoV-2 infection

Claudia Ravaglia, Claudio Doglioni, Marco Chilosi, Sara Piciucchi, Alessandra Dubini, Giulio Rossi, Federica Pedica, Silvia Puglisi, Luca Donati, Sara Tomassetti, Venerino Poletti

Please cite this article as: Ravaglia C, Doglioni C, Chilosi M, *et al*. Clinical, radiological, and pathological findings in patients with persistent lung disease following SARS-CoV-2 infection. *Eur Respir J* 2022; in press (https://doi.org/10.1183/13993003.02411-2021).

Clinical, radiological, and pathological findings in patients with persistent lung disease following SARS-CoV-2 infection

Claudia Ravaglia[1], Claudio Doglioni[2], Marco Chilosi[3], Sara Piciucchi[4], Alessandra Dubini[5], Giulio Rossi[6], Federica Pedica[7], Silvia Puglisi[1], Luca Donati[8], Sara Tomassetti[9], Venerino Poletti[1,10,11]

Affiliations:

[1] Department of Thoracic Diseases, G.B. Morgagni Hospital/University of Bologna, Forlì, Italy

[2] Department of Pathology, University Vita-Salute, Milan and San Raffaele Scientific Institute. Milan, Italy

[3] Department of Pathology, Pederzoli Hospital, Peschiera del Garda, Verona, Italy

[4] Department of Radiology, G.B. Morgagni Hospital/University of Bologna, Forlì, Italy

[5] Department of Pathology, G.B. Morgagni Hospital/University of Bologna, Forlì, Italy

[6] Department of Pathology, Fondazione Poliambulanza Istituto Ospedaliero Multispecialistico, Brescia, Italy

[7] Department of Pathology, San Raffaele Scientific Institute. Milan, Italy

[8] Biostatistics and Clinical Trial Unit, Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori"-IRST S.r.l., IRCCS

[9] Department of Experimental and Clinical Medicine, Careggi University Hospital, Firenze, Italy

[10] DIMES, University of Bologna, Italy

[11] Department of Respiratory Diseases and Allergy, Aarhus University Hospital, Aarhus, Denmark

Corresponding Author

Claudia Ravaglia, Member of European Reference Network LUNG

Department of Thoracic Diseases, G.B. Morgagni Hospital/University of Bologna, Forlì, Italy

via C. Forlanini 34, 47121 Forlì, Italy

tel: +39 324 5470059; E-mail: claudiaravaglia79@gmail.com ; venerino.poletti@gmail.com

ABSTRACT

Several patients experience pulmonary sequelae after Sars-Cov-2 infection, ranging from self-limited abnormalities to major lung diseases. Morphological analysis of lung tissue may help in understanding pathogenic mechanisms and provide consistent personalized management. Aim of the study was to ascertain morphologic and immuno-molecular features of lung tissue. Transbronchial lung cryobiopsy was carried out in patients with persistent symptoms and computed tomography suggestive of residual lung disease after recovery from Sars-CoV-2 infection. 164 patients were referred for suspected pulmonary sequelae after COVID-19; 10 patients with parenchymal lung disease extent > 5% underwent lung biopsy. Histological pattern was not homogeneous, as three different case clusters could be evidenced, which were mirrored in clinical and radiological features: cluster one ("chronic fibrosing") characterized by post-infection progression of pre-existing interstitial pneumonias; cluster two ("acute/subacute injury") characterized by different types and grades of lung injury, ranging from organizing pneumonia and fibrosing NSIP to diffuse alveolar damage; cluster three ("vascular changes") characterized by diffuse vascular increase, dilatation and distortion (capillaries and venules) within otherwise normal parenchyma. Clusters two and three were characterized by immunophenotypical changes similar to those observed in early/mild covid-19 pneumonias (abnormal expression of STAT3 in hyperplastic pneumocytes and PD-L1, IDO and STAT3 in endothelial cells). This is the first study correlating histological/immunohistochemical patterns with clinical and radiological pictures of patients with post-COVID lung disease. Different phenotypes with potential different underlying pathogenic mechanisms have been identified.

INTRODUCTION

As COVID-19 pandemic has progressed, some patients are experiencing prolonged multi-organ symptoms and complications (long COVID or post-COVID syndrome) [1-13]. The implications and consequences of such ongoing clinical manifestations are a growing health concern. Evidence of residual organ damage following COVID-19 infection applies especially to pulmonary sequelae, with a spectrum ranging from self-limited abnormalities to a clinical profile of major lung diseases, with variable extent of significant inflammatory and/or fibrotic abnormalities. The histological evaluation of lung tissue in this late phase may help reveal peculiar morpho-phenotypical changes which, together with future studies examining patterns evolution, may help in better understanding pathogenic mechanisms and provide consistent personalized therapy. Here, we examine the morphologic and immuno-molecular features of transbronchial lung cryobiopsies (TBLC) performed in patients with persistent lung disease after recovery from Sars-CoV-2 infection and the potential explanation of the observed lung abnormalities.

METHODS

Study design and patient selection

We conducted a comparative, prospective, multicentric, investigator-initiated and observational study, from January 1st 2021 through March 31st 2021, with the aim to evaluate histological profile and immunohistochemical/molecular features of lung tissues in patients with persistent lung involvement on HRCT (high resolution computed tomography) scan and persistent symptoms (respiratory and/or systemic) after recovery from Sars-CoV-2 infection. Eligible subjects were aged between 18 and 80 years, had a history of previous molecular confirmed diagnosis of COVID-19 related pneumonia, with recovery at least 30 days before, persistent lung involvement on HRCT scan analysis > 5%, persistent symptoms (respiratory and/or systemic) and no contraindications for lung biopsy. Recovery was defined according to national directives updated in December 2020 by the presence of two consecutive negative tests for Sars-CoV-2 by rRT-PCR in 24 hours associated with possible improvement/stabilization of symptoms. Key exclusion criteria were severely compromised lung function or resting hypoxaemia, high bleeding risk, pulmonary hypertension assessed by echocardiography and severe comorbidities (details are reported in the online data supplement); finally, patients who could not provide consent or refused biopsy were excluded. This study was in accordance with regulations issued

by the Helsinki Declaration; the protocol was approved by the Institutional Review Board (IRB) of the *Area Vasta Romagna Ethical Committee* (Prot. 5285/2021) and patients have provided their informed consent. High Resolution Computed Tomography and Transbronchial Lung Cryobiopsy were performed as described previously and reported in supplemented data [14-17]. Morphologic examination was based on conventional hematoxylin-eosin stains and immunostaining with cytokeratin 7. Five dedicated pulmonary pathologists (A.D., G.R., V.P., M.C., and C.D.) blindly and independently reviewed the slides from all biopsies and quoted several morphological features involving different compartments constituting the lung parenchyma: epithelial and vascular components, alveolar and interstitial spaces, inflammatory cells. Divergent opinions were then overtly discussed and a final consensus was reached in all cases. All immunohistochemical tests were performed in the ULTRA Benchmark automated immunostainer (Ventana Medical Systems/Roche, Tucson, AZ) using standard procedures and reagents are described in Table S1. We have analyzed 15 control cases with diffuse parenchymal lung diseases as uninfected control specimens: three Cryptogenic Organizing Pneumonia (OP), three Non-Specific Interstitial Pneumonia (NSIP), two OP/NSIP, two Usual Interstitial Pneumonia (UIP), two fibrotic Hypersensitivity Pneumonitis (fHP), one idiopathic Acute Fibrinous and Organizing pneumonia (AFOP), one Sarcoidosis, one Smoking-Related Interstitial Fibrosis (SR-IF).

RESULTS

Clinical characteristics

164 patients were referred to our post-acute outpatient service for suspected pulmonary sequelae after COVID-19. In 141 patients, parenchymal lung disease was absent or < 5%, 13 patients had contraindications for lung biopsy or did not provide consent to the procedure; 10 patients underwent transbronchial lung cryobiopsy. Median age was 61 years (range, 38-76 years) and 78% were men. 7/10 patients had been hospitalized but no one had been intubated. Clinical characteristics are summarized in Table S2. Patients underwent bronchoscopy on average 3.5 months after recovery from Sars-CoV-2 infection. At the time of biopsy all cases resulted negative at Sars-Cov-2 molecular swab testing. Most patients reported fatigue and low grade fever, a minority reported joint and muscle pain and depression (Table S2). Median forced vital capacity (FVC) was 77% of predicted value (range 46-116); median carbon-monoxide diffusion

coefficient (DLCO) was 51% of predicted value (range 43-80). The most representative laboratory tests are shown in Table S3.

High resolution computed tomography

Radiologic findings are summarized in Table 1. In a sub-group (cluster 1) , CT scan showed fibrotic appearances with some aspects of interstitial involvement, architectural distortion and traction bronchiectasis; in one of these patients, a previous CT scan had shown a pre-existing background of fibrotic interstitial lung abnormalities (ILA), which were stable and persistent after COVID-19 pneumonia (Figure 1), while in the second patient, acute consolidations masked and covered the possible presence of underlying pre-existing traction bronchiectasis.

In five cases (cluster 2), CT scan showed persistence of ground glass with perilobular pattern and consolidations as observed in organizing pneumonia (OP) or fibrotic NSIP (Figures 2-3); a common feature was residual vessel enlargement (gravity dependent perilobular pattern as assessed after prone positioning) as supposed venular dilatation in the peri-lobule [18]. Finally, third sub-group (cluster 3) was characterized by only mild residual radiological changes, with mild peripheral ground glass associated with perilobular pattern in 2/3. In a single case, features suggesting pleuro-parenchymal fibroelastosis in the upper lobes were observed.

Histological patterns and immunohistochemistry

Histological evaluation on H&E stained slides revealed significant modifications of the pulmonary structure in all ten investigated cases, matching the three-cluster separation. All cases were tested and resulted negative for Sars-Cov-2 [in situ hybridization in tissue (smFISH method, HuluFISH, Pixelbiotech Germany) and real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay in BAL (bronchoalveolar lavage)].

Cluster 1 ("pre-existing chronic fibrosing")

This cluster included two cases with features of either UIP (usual interstitial pneumonia), as defined by architectural distortion, spatial and temporal heterogeneity of scarring modifications, fibroblastic foci and microscopic honeycombing (Figure 4), or fibrosing smoking-related interstitial pneumonia, as defined by the accumulation of macrophages containing smoking-related light brown pigment in alveolar spaces and interstitial collagenous fibrosis (Table S4). The immunohistochemical analysis in the first case was consistent with UIP, including TUBB3

in myofibroblasts and focal p16 expression in pneumocytes [19]. The second case (showing pre-existing background of smoking related ILD at HRCT which persisted after COVID-19 pneumonia), showed histological features consistent with a smoking-related ILD, with also lymphoid nodules [20].

Cluster 2 ("*acute/subacute injury"*)

Cluster two was characterized by different types and grades of lung injury, ranging from organizing pneumonia or overlapping OP/NSIP to diffuse alveolar damage (DAD – proliferative phase, 2 cases) (Figure 4) [21]. These cases shared morphological and immunohistochemical similarities with cases of severe COVID-19 pneumonia previously described [16]. In the two cases consistent with DAD (proliferative/organizing without hjaline membranes), the interstitial spaces were diffusely thickened by dense fibrosis and aggregates of myofibroblasts (Figure 4). Residual alveolar spaces were lined by patchy collections of hyperplastic AECII, expressing pSTAT3 and Ki67. Four cases were characterized by the occurrence of endoalveolar Masson's bodies and various amounts of interstitial thickening (OP/NSIP) (Figure 4) (Table S4*). Inflammatory cells:* abundant immune infiltrates, composed mainly of lymphocytes were present in all cluster 2 cases, either diffusely distributed in interstitial spaces, or forming perivascular aggregates; lymphoid infiltrates were mainly composed of T and B cells; in T cells, CD4 cells and CD8 subsets were almost equally represented. Macrophages were less represented, neutrophils were rare and no eosinophils were observed. *Alveolar cells*: hyperplastic AECII were characterized by a high Ki67 index, and nuclear pSTAT3. TUBB3 staining was also expressed in alveolar cells, mainly in the cases with the most severe damage. *Vascular bed*: diffuse IDO, PD-L1 and pSTAT3 immunostaining was detected in the endothelial cells of both interstitial capillary vessels and venules, this representing the most relevant finding in this subgroup [22].

Cluster 3 ("vascular changes")

Cluster 3 was characterized by diffuse vascular increase, dilatation and distortion (both capillaries and venules) within an otherwise normal parenchyma (minimal patchy AECII hyperplasia in one case) (Table S4) (Figure 5). This pattern was comparable to that described in early-phase COVID-19 pneumonia with conserved parenchymal structure [16]. *Inflammatory cells*: a small number of macrophages and interstitial T lymphocytes were present, without obvious predominance of either CD4 or CD8. PD-L1 was expressed in alveolar macrophages (as may commonly be observed in alveolar macrophages in normal conditions), but no PD-L1

immunoreactivity was observed in the epithelial alveolar cells. *Vascular bed*: diffuse PD-L1 staining decorated the capillary lining along with IDO reactivity in endothelial cells (Figure 5). *Stromal/interstitial compartment*: no TUBB3 reactivity was observed. These cases shared similarities with some cases of early/mild COVID-19 pneumonia previously described [16], in particular the persistence of diffuse and strong expression of PD-L1 and indoleamine 2,3-dioxygenase (IDO) in endothelial cells of both interstitial capillaries and venules in post-acute phase cases. This peculiar expression profile may be ~~appears as strongly~~ more related with SARS-CoV-2 pneumonias, since these markers are not typically observed ~~absent~~ in lung tissue obtained in healthy subjects [23-24] and/or in control cases with diffuse parenchymal lung diseases as uninfected control specimens. These morphologic abnormalities were mirrored in BAL fluid (Table S3); in fact, all patients with lymphocytosis in BAL (> 20%) were characterized by organizing pneumonia in lung tissue, with abundant immune infiltrates, composed mainly of lymphocytes, located in perivascular and interstitial spaces, sometimes forming nodular aggregates.

DISCUSSION

To our knowledge, this is the first prospective analysis of morpho-phenotypical changes in patients with persistent symptoms and residual parenchymal lung disease after recovery from COVID-19. Most published studies have been small and mainly focused on clinical sequelae in patients admitted to hospital for COVID-19 [1,3-5,6,8,10]. Little is known of the extent and features of lung pathology in COVID-19 survivors, mostly those who had milder infections not requiring hospitalization. Three cases with post-COVID histologically confirmed OP have been published so far (two trans-bronchial lung biopsy and one surgical lung biopsy) [25,26] and eleven asymptomatic patients who had previously contracted COVID-19 underwent elective lung resections for other unrelated indications (recovered asymptomatic survivors were confirmed to have no discernible histopathological changes suggesting parenchymal damage) [27]. As is the case with other interstitial lung diseases (ILDs), lung biopsy may have a significant role in post-COVID-ILDs, in order to better define histological patterns for the purposes of management. The ILDs community has long been aware that fibrotic nonspecific interstitial pneumonia and organizing pneumonia progressing to fibrosis are not infrequently seen in survivors of DAD/acute lung injury [28]. Current algorithms mostly view post-COVID lung disease as a single entity; however, the existence of different COVID-induced pathways may underlie

separate post-COVID lung entities, which may require separate management. In this multidisciplinary study, we have identified three different subtypes or sub-groups (Table 2).

One group (cluster 1) was characterized by features suggesting the occurrence of interstitial lung disease before SARS-CoV2 infection. Both cases included were male and former smokers. Clinical behavior was characterized by persistent respiratory symptoms after COVID-19 pneumonia, shortness of breath on exertion and cough, with less frequent systemic symptoms (Table S2). One case, whose pattern was consistent with UIP, was characterized by fibrotic appearances on CT scan and lung tissue, architectural distortion, traction bronchiectasis, spatial and temporal heterogeneity of scarring modifications, and extended honeycombing (Figure 4 and Table 1). It is possible to argue that the acute consolidations in this case masked and covered the presence of underlying pre-existing fibrosing ILD. The second patient had a documented pre-existing background of fibrotic interstitial lung abnormalities (ILA), which were stable and persistent after COVID-19 pneumonia. Previous pulmonary function tests were normal (FVC 103% predicted, FEV1 104% predicted, DLCO 76% predicted). Lung cryobiopsy showed a pattern consistent with smoking-related interstitial fibrosis, SR-IF). We hypothesize that a number of post-covid-19 cases, characterized by radiological and/or pathological finding of fibrotic appearances might be actually more an expression of a pre-existing pulmonary interstitial disease (which may or may not be accelerated by the virus infection itself) than strictly related to the viral infection. Acceleration of pulmonary fibrosis by respiratory virus has been described in the bleomycin model of pulmonary fibrosis [29]; ~~i~~nterestingly, in these cases, foci of cells expressing the cell-senescence associated marker p16 were observed as in UIP/IPF [30-34].

The second cluster included patients with a more “auto-inflammatory” phenotype, with different clinical presentation, radiological features and immuno-morphological pattern. The inflammatory pathway may be triggered by the virus itself causing type 2 pneumocytes proliferation/activation, influx of inflammatory cells and vascular dysfunction. CT scan showed residual ground glass with perilobular pattern, sometimes consolidations, halo signs and reverse halo sign, with no fibrotic distortion; these changes can be included in a OP-like pattern (Table 1). All patients had different morphological types and grades of lung injury, ranging from organizing pneumonia to diffuse alveolar damage (DAD - proliferative phase) and had in common abundant immune infiltrates, composed mainly of lymphocytes, in the perivascular and interstitial spaces, sometimes forming nodular aggregates. In this cluster, patients had more frequently systemic symptoms, such as fever and fatigue, together with respiratory symptoms

(Table S2). Cluster two patients were treated with steroids following lung biopsy results (details are reported in Supplement). Patients with organizing pneumonia on lung biopsy experienced significant improvement in both symptoms and lung function impairment; three-months follow-up CT scan showed a significant reduction in ground glass areas and perilobular pattern in all cases. Patients with diffuse alveolar damage / proliferative phase features on lung biopsy showed moderate improvement, both clinical and functional, with complete resolution of fever and reduction of shortness of breath, although persistent on mild to moderate exertion; the three-months follow-up CT scan showed a slight reduction of the extension of parenchymal involvement.

Third subgroup included patients with persistent respiratory and systemic symptoms but minimal changes on CT scan. The histological picture was characterized by dilatation of the lumen of alveolar capillaries and venules and hyperplasia of capillaries within an otherwise normal or minimally abnormal parenchyma (minimal patchy AECII hyperplasia and heterogeneous interstitial thickening in one case). We considered this "pattern" as similar in some aspects to pulmonary capillary hemangiomatosis (hyperplastic interstitial capillaries along and in the alveolar walls, frequently dilated lumens of the capillaries and capillary congestion); however, we think they are clearly two distinct conditions. In fact, the capillary proliferation observed in cluster 3 cases was not typically centered around bronchovascular bundles and did not create any nodular appearance; endothelial cells were negative for proliferation cues (i.e. Ki-67) and vascular changes involved also post-capillary venules, which showed dilated and tortuous lumen, with thickened edematous walls and a patchy perivascular lymphocyte infiltrate, all changes that are not observed in capillary hemangiomatosis. In addition, hemosiderin-laden macrophages and intimal thickening of small muscular arteries were not observed. All these patients underwent echocardiography and pulmonary artery hypertension was excluded. Interestingly, the expression of molecules involved in immune derangement and vascular tone control (STAT3, IDO, PD-L1) were altered as in early phase COVID-19 pneumonia [16]. In order to assess whether these findings were related to Sars-CoV-2 infection, we have analyzed the control cases with diffuse parenchymal lung diseases as uninfected control specimens and we found that the endothelial expression of these markers was faint and/or restricted to rare cells.

One of the most relevant findings of our study was the persistent elevated expression of nuclear pSTAT3 in both alveolar epithelial cells and endothelial cells of interstitial capillary and venules months after the clearing of SARS-CoV-2 infection. Nuclear pSTAT3 was particularly

overexpressed in cluster 2 patients, characterized by more prominent systemic symptoms (mostly fever and fatigue) associated with persistence of ground glass opacities on CT scan, perilobular pattern and consolidations. These cases shared similarities with cases of severe COVID-19 pneumonia previously described [16], in which alveolar epithelial type 2 cells and endothelial cells expressed nuclear pSTAT3. A significant reduction in pSTAT3 after administration of Baricitinib in patients with severe Covid-19 pneumonia, preventing progression, with a safer and more favorable clinical outcome, has been previously demonstrated [35]. These results might suggest a potential rationale for the use of inhibitors of the Janus kinases JAK1 and JAK2 in a subgroup of patients with post-Covid persistent symptoms. All patients included in the study were found negative for Sars-CoV-2 in BAL, confirming the fact that pulmonary manifestations are not associated with the persistence of virus in the lung, but are related to pathogenic mechanisms irrespective of viral clearance and continuing after infections. We have observed that vascular abnormalities (disordered angiogenesis with luminal enlargement and dilated vessel wall, perivascular T-cell infiltration) are distinctly present from the beginning of COVID-19 pneumonia, can persist through progressive disease stages [16] and may linger also several weeks/months after infection. The co-regulatory ligand PD-L1 and the tolerogenic enzyme IDO are part of negative feed-back circuits that restrain immune responses and maintain peripheral tolerance [36-38] and are both implicated in defense mechanisms from lung injury [39-42]. Tryptophan is a substrate for IDO enzymatic activity, and abnormal levels of tryptophan metabolites have been demonstrated in Covid-19, with normalization at recovery [42-44]. As previously demonstrated, IDO is involved in the regulation of vascular remodeling and relaxation [24] and according to this study its potential role in reducing pulmonary vascular resistance may also be involved in long COVID or post-COVID syndrome. These observations might explain the persistence on CT scan, in some post-COVID cases, of areas of ground-glass attenuation, with or without a "crazy paving" pattern, associated with enlarged veins contained within ground-glass areas, partially disappearing with a change from the supine to the prone position ("venoplegic/hyperemic pattern") [42]. In some post-COVID cases of this series, we observed the persistence of these features on CT scan (Table 1), through progressive disease stages several weeks/months after infection.

This study has some limitations. Firstly, the baseline data of pulmonary function tests and chest CT scan were not available, the number of enrolled patients was limited and patients group was heterogeneous. Secondly, the short timeframe between the post-acute sampling and the acute

COVID-19 infection maybe a further issue of concern as it may not be representative of the long term abnormalities. In future, a larger sample would be needed and longer longitudinal observational studies will be critical to elucidate the health consequences attributable to COVID-19.

In conclusion, this is the first study showing correlation between histological patterns and immunohistochemistry with clinical and radiological pictures of patients with post-COVID syndrome, which identified different subtypes or phenotypes with potential different underlying pathogenic mechanisms (Table 2). Our results confirm that a multidisciplinary evaluation of these patients is needed (instead of a limited imagine evaluation) to identify key patient subgroups and their current optimal management. In particular, immune-histochemical evaluation of lung tissue may help revealing peculiar morphological and morpho-phenotypical changes of this new "Post-COVID/Long-COVID syndrome", but also help in better understanding of pathogenic mechanisms and lay the groundwork to efficiently conducting therapeutic intervention studies and designing future follow-up plans as previously described [17,45-48]. Although the pathogenic role of different cell types and the mechanisms leading to different disease endotypes are not fully understood, this heterogeneity is likely to reflect different COVID-induced phenotypes of interstitial lung disease, probably in predisposed subjects to abnormal wound healing. Post-COVID lung disease is not a single entity, but includes different subtypes or sub-groups [49], each of them potentially requiring separate and different management.

Contributors

CR, CD, MC and VP conceived of and designed the study; CR, CD and MC wrote the paper; CR, CD, MC, SP, AD, GR and VP contributed to data interpretation; all other authors commented on drafts of the paper and contributed to writing of the final version of the manuscript

Declaration of interests

We report no competing interests

Funding

Not applicable

Acknowledgments

We thank Fondazione Cariverona (ENACT Project) for the financial support and AMMP (Associazione Morgagni Malattie Polmonari) for patients support.

REFERENCES

1. Ghosn J, Piroth L, Epaulard O, et al. Persistent COVID-19 symptoms are highly prevalent 6 months after hospitalization: results from a large prospective cohort. Clin Microbiol Infect 2021; 27: 1041.e1-1041.e4
2. Wells AU, Devaraj A, Desai SR. Interstitial Lung Disease after COVID-19 Infection: A Catalog of Uncertainties. Radiology 2021; 299: E216-E218
3. Han X, Fan Y, Alwalid O, et al. Six-month Follow-up Chest CT Findings after Severe COVID-19 Pneumonia. Radiology 2021; 299: E177-E186
4. Darcis G, Bouquegneau A, Maes N, et al. Long-term clinical follow up of patients suffering from moderate to severe COVID-19 infection: A monocentric prospective observational cohort study. Int J Infect Dis 2021; 109: 209–16
5. Nehme M, Braillard O, Chappuis F, et al. Prevalence of Symptoms More Than Seven Months After Diagnosis of Symptomatic COVID-19 in an Outpatient Setting. Ann Intern Med 2021: M21-0878
6. Fernández-de-Las-Peñas C, Palacios-Ceña D, Gómez-Mayordomo V, et al. Prevalence of post-COVID-19 symptoms in hospitalized and non-hospitalized COVID-19 survivors: A systematic review and meta-analysis. Eur J Intern Med 2021: S0953-6205 (21) 00208-9
7. Righi E, Mirandola M, Mazzaferri F, et al. Long-Term Patient-Centred Follow-up in a Prospective Cohort of Patients with COVID-19. Infect Dis Ther 2021: 1–12
8. Peghin M, Palese A, Venturini M, et al. Post-COVID-19 symptoms 6 months after acute infection among hospitalized and non-hospitalized patients. Clin Microbiol Infect 2021: S1198-743X (21) 00281-0

9. Nasserie T, Hittle M, Goodman SN. Assessment of the Frequency and Variety of Persistent Symptoms Among Patients With COVID-19: A Systematic Review. JAMA Netw Open 2021; 4: e2111417
10. Augustin M, Schommers P, Stecher M, et al. Post-COVID syndrome in non-hospitalised patients with COVID-19: a longitudinal prospective cohort study. Lancet Reg Health Eur 2021; 6: 100122
11. Ramos-Casals M, Brito-Zerón P, Mariette X. Systemic and organ-specific immune-related manifestations of COVID-19. Nat Rev Rheumatol 2021; 17: 315-332
12. Nalbandian A, Sehgal K, Gupta A, et al. Post-acute COVID-19 syndrome. Nat Med 2021; 27: 601-615
13. Montani D, Savale L, Beurnier A, et al. Multidisciplinary approach for post-acute COVID-19 syndrome: time to break down the walls. Eur Respir J 2021; 58: 2101090
14. Ravaglia C, Wells AU, Tomassetti S, et al. Diagnostic yield and risk/benefit analysis of trans-bronchial lung cryobiopsy in diffuse parenchymal lung diseases: a large cohort of 699 patients. BMC Pulm Med 2019; 19: 16
15. Casoni GL, Tomassetti S, Cavazza A, et al. Transbronchial lung cryobiopsy in the diagnosis of fibrotic interstitial lung diseases. PLoS One 2014; 9: e86716
16. Doglioni C, Ravaglia C, Chilosi M, et al. Covid-19 Interstitial Pneumonia: Histological and Immunohistochemical Features on Cryobiopsies. Respiration 2021; 100: 488-498
17. Chilosi M, Poletti V, Ravaglia C, et al. The pathogenic role of epithelial and endothelial cells in early-phase COVID-19 pneumonia: victims and partners in crime. Mod Pathol 2021; 34: 1444-1455
18. Piciucchi S. Awake prone positioning for COVID-19 acute respiratory failure: imaging and histological background. Lancet Respir Med, in press
19. Chilosi M, Marcolini L, Caliò A, Poletti V. Immunohistochemistry and Molecular Biology in Transbronchial Cryobiopsies. In: V P, editor. Transbronchial cryobiopsy in diffuse parenchymal lung disease: Springer; 2019. p. 81-102
20. Churg A, Muller NL, Wright JL. Respiratory bronchiolitis/interstitial lung disease: fibrosis, pulmonary function, and evolving concepts. Arch Pathol Lab Med 2010; 134: 27-32

21. Enomoto N, Sumikawa H, Sugiura H, et al. Clinical, radiological, and pathological evaluation of "NSIP with OP overlap" pattern compared with NSIP in patients with idiopathic interstitial pneumonias. Respir Med 2020; 174: 106201
22. Vabret N, Britton GJ, Gruber C, et al. Immunology of COVID-19: Current State of the Science. Immunity 2020; 52: 910-941
23. Bonavente FM, Soto JA, Pizarro-Ortega MS, et al. Contribution of IDO to human respiratory syncytial virus infection. J Leukoc Biol 2019; 106: 933-942
24. Xiao Y, Christou H, Liu L, et al. Endothelial indoleamine 2,3-dioxygenase protects against development of pulmonary hypertension. Am J Respir Crit Care Med 2013; 188: 482-491
25. Kanaoka K, Minami S, Ihara S, et al. Secondary organizing pneumonia after coronavirus disease 2019: Two cases. Respir Med Case Rep 2021; 32: 101356
26. In-Gyu B, Kyung-Wook H, Jung Wook Y, et al. Persistent pneumonic consolidations due to secondary organizing pneumonia in a patient recovering from COVID-19 pneumonia: a case report, 2020, pp. 1–9, https://doi. org/10.21203/rs.3.rs-37580/v1
27. Diaz A, Bujnowski D, McMullen P, et al. Pulmonary parenchymal changes in COVID-19 survivors. Ann Thorac Surg 2021: S0003-4975 (21) 01307-2
28. Nicholson AG, Osborn M, Devaraj A, Wells AU. COVID-19 related lung pathology: old patterns in new clothing? Histopathology 2020; 77: 169-172
29. Wang L, Cheng W, Zhang Z. Respiratory syncytial virus infection accelerates lung fibrosis through the unfolded protein response in a bleomycin-induced pulmonary fibrosis animal model. Mol Med Rep 2017; 16: 310-316
30. Chilosi M, Carloni A, Rossi A, Poletti V. Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. Transl Res 2013; 162: 156-73
31. Renzoni EA, Poletti V, Mackintosh JA. Disease pathology in fibrotic interstitial lung disease: is it all about usual interstitial pneumonia? Lancet 2021; 398: 1437-1449
32. Chilosi M, Caliò A, Rossi A, et al. Epithelial to mesenchymal transition-related proteins ZEB1, β-catenin, and β-tubulin-III in idiopathic pulmonary fibrosis. Mod Pathol 2017; 30: 26-38
33. Wu K, Kamimoto K, Zhang Y, et al. Basal-epithelial stem cells cross an alarmin checkpoint for post-viral lung disease. J Clin Invest 2021: 149336

34. Bui LT, Winters NI, Chung MI, et al. Chronic lung diseases are associated with gene expression programs favoring SARS-CoV-2 entry and severity. Nat Commun 2021; 12: 4314
35. Bronte V, Ugel S, Tinazzi E, et al. Baricitinib restrains the immune dysregulation in patients with severe COVID-19. J Clin Invest 2020; 130: 6409-6416
36. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515: 568-571
37. Mondanelli G, Ugel S, Grohmann U, Bronte V. The immune regulation in cancer by the amino acid metabolizing enzymes ARG and IDO. Curr Opin Pharmacol 2017; 35: 30-39
38. Platten M, von Knebel Doeberitz N, Oezen I, et al. Cancer Immunotherapy by Targeting IDO1/TDO and Their Downstream Effectors. Front Immunol 2015; 5: 673
39. Monaghan SF, Thakkar RK, Heffernan DS, et al. Mechanisms of indirect acute lung injury: a novel role for the coinhibitory receptor, programmed death-1. Ann Surg 2012; 255: 158-164
40. Liu H, Liu L, Visner GA. Nonviral gene delivery with indoleamine 2,3-dioxygenase targeting pulmonary endothelium protects against ischemia-reperfusion injury. Am J Transplant 2007; 7: 2291-2300
41. Lomas-Neira J, Monaghan SF, Huang X, et al. Novel Role for PD-1: PD-L1 as Mediator of Pulmonary Vascular Endothelial Cell Functions in Pathogenesis of Indirect ARDS in Mice. Front Immunol 2018; 9: 3030
42. Piciucchi S, Ravaglia C, Vizzuso A, et al. Reversibility of venous dilatation and parenchymal changes density in Sars-Cov-2 pneumonia: toward the definition of a peculiar pattern. Pulmonology 2021; 27: 353-357
43. Ansone L, Briviba M, Silamikelis I, Terentjeva A, Perkons I, Birzniece L, Rovite V, Rozentale B, Viksna L, Kolesova O, Klavins K, Klovins J. Amino Acid Metabolism is Significantly Altered at the Time of Admission in Hospital for Severe COVID-19 Patients: Findings from Longitudinal Targeted Metabolomics Analysis. Microbiol Spectr. 2021 Dec 8: e0033821
44. Guo L, Schurink B, Roos E, Nossent EJ, Duitman JW, et al. Indoleamine 2,3-dioxygenase (IDO)-1 and IDO-2 activity and severe course of COVID-19. J Pathol 2021 Dec 3. doi: 10.1002/path.5842.

45. Oldani S, Ravaglia C, Bensai S, et al. Pathophysiology of light phenotype SARS-CoV-2 interstitial pneumonia: from histopathological features to clinical presentations. Pulmonology 2021 Mar 26: S2531-0437(21)00078-7
46. Vivarelli S, Falzone L, Torino F, et al. Immune-checkpoint inhibitors from cancer to COVID-19: A promising avenue for the treatment of patients with COVID-19 (Review). Int J Oncol 2021; 58: 145-157
47. Awadasseid A, Yin Q, Wu Y, et al. Potential protective role of the anti-PD-1 blockade against SARS-CoV-2 infection. Biomed Pharmacother 2021; 142: 111957
48. Loretelli C, Abdelsalam A, D'Addio F, et al. PD-1 blockade counteracts post-COVID-19 immune abnormalities and stimulates the anti-SARS-CoV-2 immune response. JCI Insight 2021; 6: e146701
49. Solomon JJ, Heyman B, Ko JP, et al. CT of Postacute Lung Complications of COVID-19. Radiology 2021: 211396

Tables

Table 1. Radiological and pathological findings in patients with persistent lung disease

	Acute phase HRCT	**Post-acute phase HRCT**	**Time from recovery (days)**	**histology**
Patient 1	Peripheral consolidation. Mild crazy paving (OP-like pattern)	Reticulation with some peri-lobular pattern, traction bronchiectasis	100	AECII Hyperplasia, honeycombing, patchy fibrosis, fibroblastic foci, lymphoid nodules
Patient 2	Bilateral ground glass. Vessel enlargement ("Veno-plegic hyperhemic pattern"). Smoking related ILD (AEF + emphysema)	Mild peripheral ground glass, smoking-related ILD (AEF + emphysema)	227	AECII Hyperplasia, interstitial fibrosis, perivascular fibrosis, lymphoid nodules, macrophages containing light brown pigment
Patient 3	Not performed	Peripheral consolidation. Ground glass. Vessel enlargement (= "gravity dependent perilobular pattern")	32	AECII Hyperplasia, organizing pneumonia, vascular dilatation, perivascular fibrosis, perivascular lymphocytes, lymphoid nodules
Patient 4	Peripheral consolidation. Ground glass. Vessel enlargement ("gravity dependent perilobular pattern")	Scattered bronchial ectasis, no fibrotic distortion, mild peri-lobular pattern	65	Organizing pneumonia

Patient 5	Ground glass. Vessel enlargement. "Venoplegic hyperhemic pattern" in a background of ILA	Stable ILA. Mild ground glass attenuation with mild peri-lobular pattern	76	AECII Hyperplasia, perivascular lymphocytes, interstitial fibrosis
Patient 6	Ground glass. Vessel enlargement ("Venoplegic hyperhemic pattern")	Extensive consolidations, Halo sign, Reverse halo sign. Peri-lobular pattern	160	AECII Hyperplasia, organizing pneumonia, vascular dilatation, perivascular fibrosis, perivascular lymphocytes, interstitial fibrosis
Patient 7	Not performed	Ground glass. Vessel enlargement "Venoplegic hyperemic pattern" in a background of NSIP-OP	56	AECII Hyperplasia, perivascular lymphocytes, interstitial fibrosis
Patient 8	Peripheral consolidation. Ground glass. Vessel enlargement ("gravity dependent perilobular pattern")	Mild residual ground glass with perilobular pattern	123	vascular dilatation, perivascular fibrosis
Patient 9	Ground glass. Vessel enlargement ("Venoplegic hyperhemic pattern")	Mild residual ground glass with perilobular pattern	137	minimal patchy AECII, vascular dilatation, perivascular fibrosis
Patient 10	Not performed	PPFE, mild ground glass	44	vascular dilatation, perivascular fibrosis

Definition of abbreviations: HRCT = high resolution computed tomography; ILD = interstitial lung disease; AEF = airspace enlargement with fibrosis; ILA = interstitial lung abnormalities; OP = organizing pneumonia; PPFE = pleuro-parenchimal fibroelastosis; AECII = alveolar epithelial type 2 cells

Table 2. Identification of possible sub-groups (clusters) of patients with post-acute Covid-19.

	Clinical picture	**Radiological pattern**	**Histopathology**
Cluster 1	Respiratory symptoms (cough, dyspnea). No systemic symptoms. Compromised DLCO.	Interstitial lung disease. Lung fibrotic appearances with architectural distortion. Traction bronchiectasis.	UIP or fibrosing interstitial pneumonia. Spatial and temporal heterogeneity of scarring modifications. Fibroblast foci. Honeycombing.
Cluster 2	Respiratory symptoms (cough, dyspnea). Systemic symptoms (fever, fatigue). Compromised DLCO.	Peripheral consolidation. Ground glass. Peri-lobular pattern. Vessel enlargement. Reverse halo sign.	Lung injury Organizing pneumonia OP/NSIP Diffuse alveolar damage. No hyaline membranes. Lymphocytic inflammatory infiltrate
Cluster 3	Respiratory symptoms (cough, dyspnea). Systemic symptoms (fatigue, aches). Normal DLCO.	Mild residual lung disease. Mild ground glass. Peri-lobular pattern	Diffuse vascular increase, dilatation and distortion (capillaries and venules). Otherwise normal parenchyma.

Definition of abbreviations: DLCO = carbon-monoxide diffusion coefficient; UIP = usual interstitial pneumonia

Figures legend

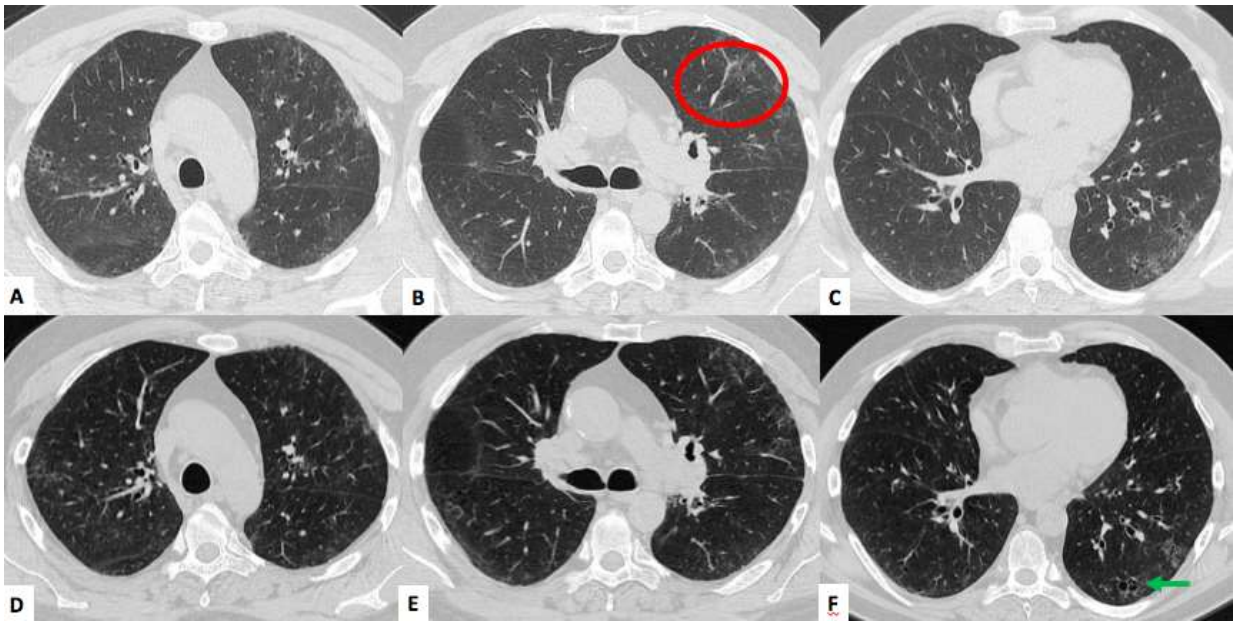

Figure 1

CT scan in a 60 y/o male, smoker. CT scan during acute infection (A-C) a mild peripheral ground glass is present in both upper lobes, mainly in the left hemithorax (red circle). Scattered areas of cystic changes suggestive of airspace enlargement with associated fibrosis (AEF) with mild architectural distortion is present bilaterally. CT scan performed four months later (D-F) shows reduction of ground glass attenuation, with residual ground glass attenuation and areas of AEF (F, green arrow).

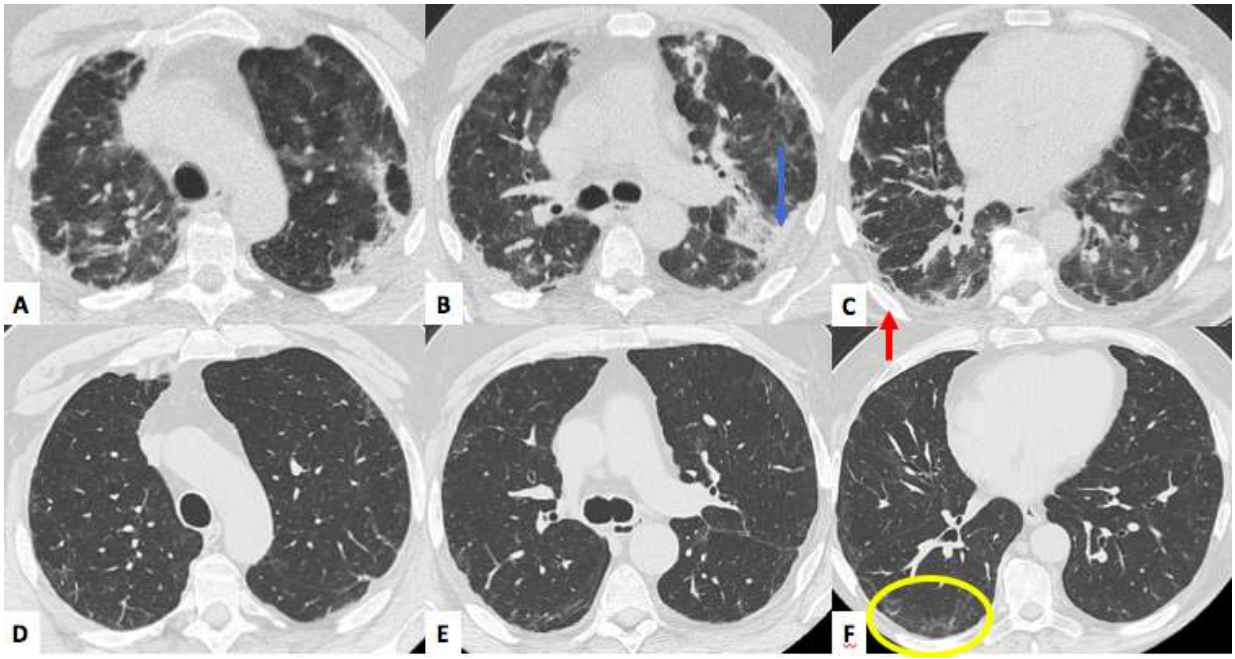

Figure 2

Male, 62 y/o, with acute Covid infection characterized by bilateral, peripheral consolidations (A, B, blue arrow) and perilobular pattern (C, red arrow). Two months later (D-F) CT scan shows mild peripheral reticulation and minimal perilobular pattern, mainly in the right lower lobe (F, yellow circle).

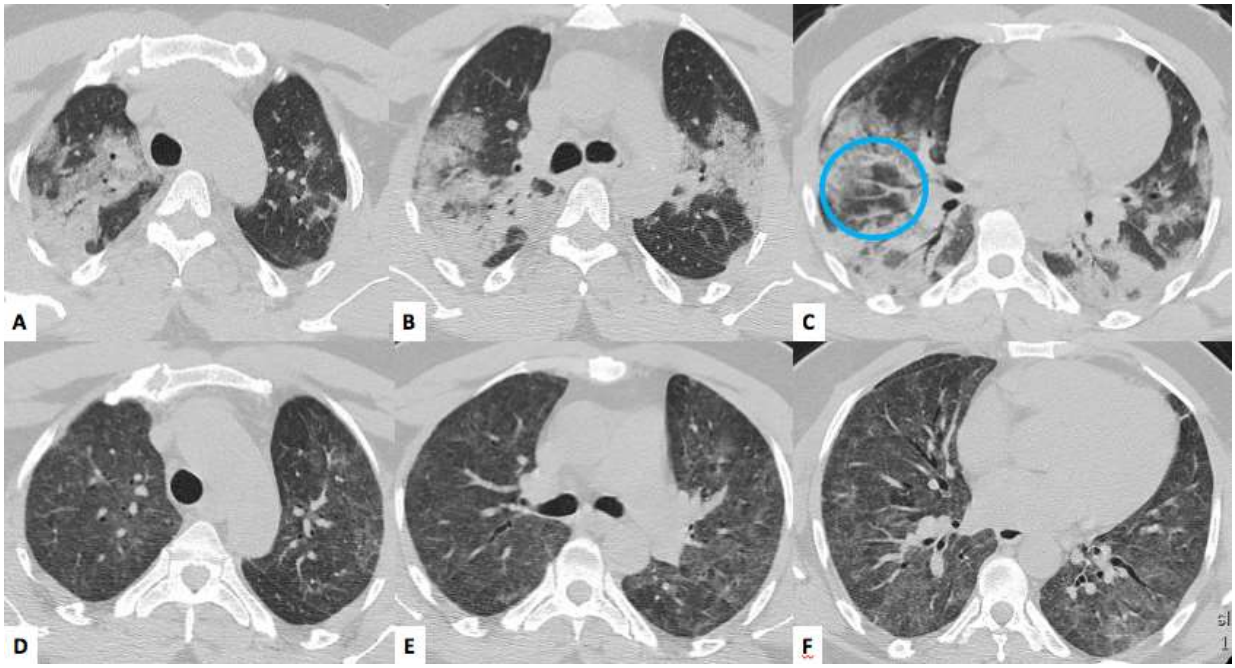

Figure 3

Male 50 y/o, with Covid-related acute pneumonia (A-C) with extensive, peribronchovascular consolidations, in both lungs (A-C) and lobular sparing in both lower lobes. Moderate ground glass attenuation is present in both upper lobes associated with vessel enlargement (C, blue circle). Two months later, a mild, diffuse ground glass attenuation is present in both lungs associated with central bronchiectasis in middle lobe and in both lower lobes.

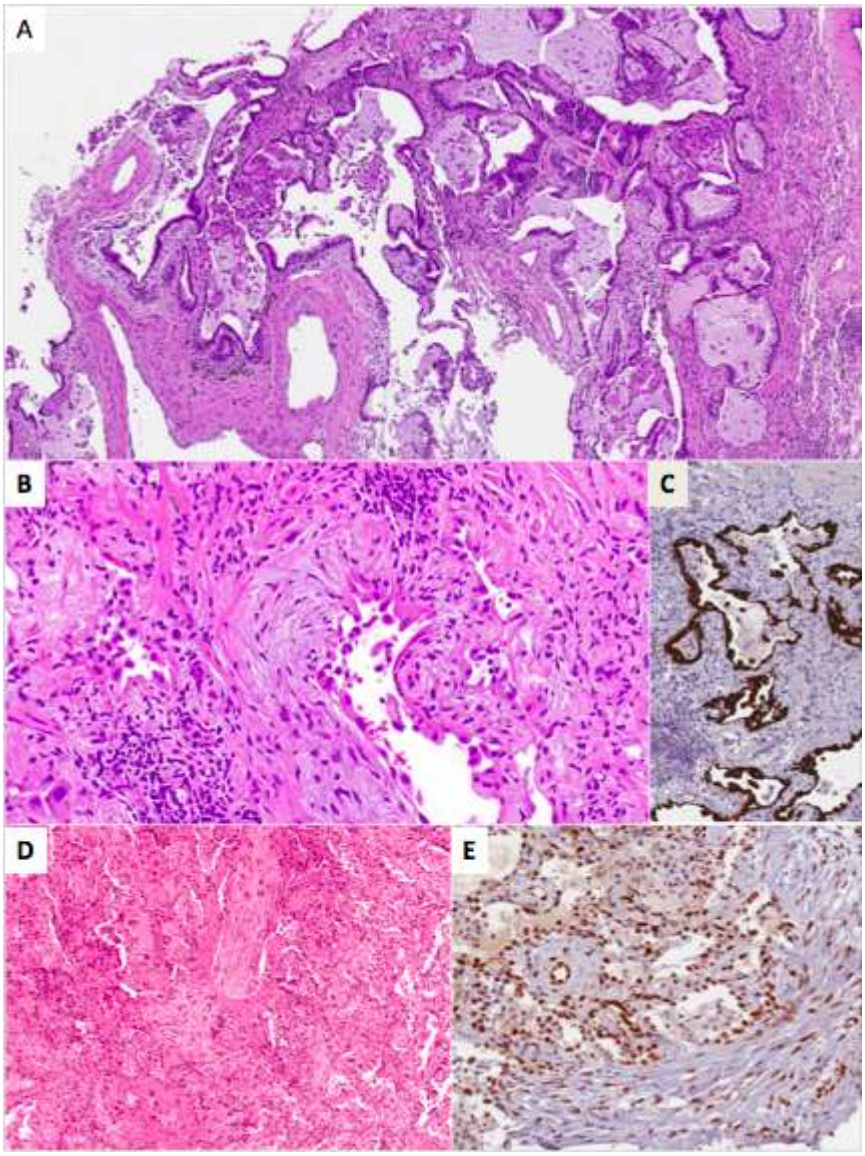

Figure 4

Cluster 1. A case with UIP pattern: architectural distortion, spatial and temporal heterogeneity of scarring modifications, and microscopic honeycombing. H&E (a); Cluster 2. A case with morphological evidence of ongoing interstitial fibrosis and extended AECII hyperplasia as observed in diffuse alveolar damage (DAD), proliferative phase. H&E (b). Cytokeratin-7 immunostaining of epithelial cells, at low magnification, showing the severe effacement of parenchymal structure. (c); Cluster 2. A case with organizing pneumonia pattern. H&E (d). Most epithelial cells and endothelial cells express nuclear pSTAT3 (e).

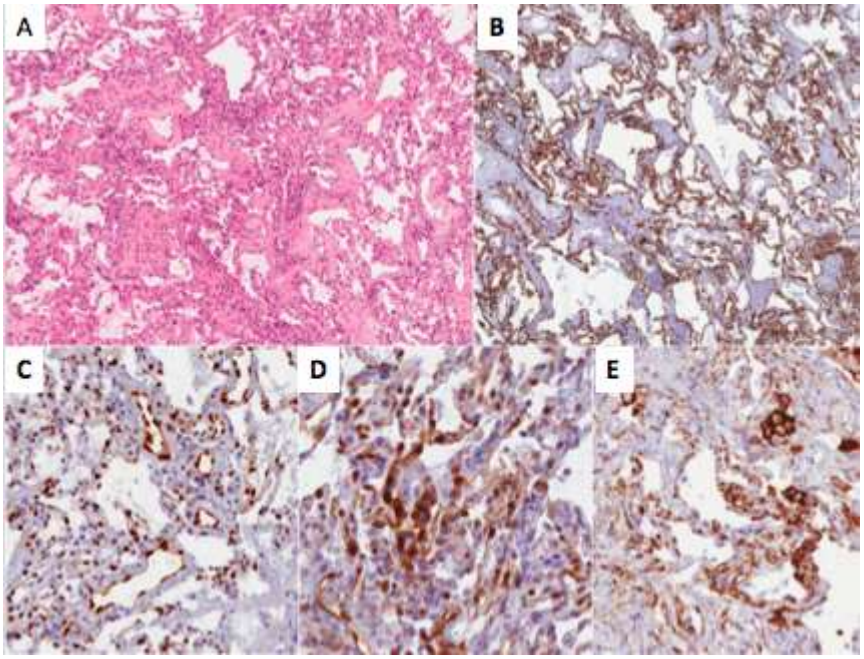

Figure 5

Cluster 3. A case with diffuse vascular increase, dilatation and distortion (both capillaries and venules) within an otherwise normal parenchyma. H&E (a); Cytokeratin-7 immunostaining (b); diffuse and strong endothelial expression of pSTAT3 (c), IDO (d) and PD-L1 (e).

Materials and methods

Patients selection

Patients were offered a comprehensive medical assessment with detailed history and physical examination: data on all clinical characteristics were collected and information about specific symptoms potentially correlated with COVID-19 were obtained. Before medical visit, all patients underwent lung function respiratory tests (forced vital capacity - FVC, diffusion capacity of the lung for carbon monoxide - DLCO, six-minute walking test - 6MWT) and chest high resolution computed tomography (HRCT). Key exclusion criteria were severely compromised lung function (FVC ≤ 50% or DLCO ≤ 40% of predicted value) or resting hypoxaemia (SpO2 <90% on room air), high bleeding risk (abnormal coagulation parameters or platelets <100x109/L or anti-platelet agents), pulmonary hypertension (estimated right ventricular systolic pressure > 50 mmHg on echocardiogram and/or right ventricular dysfunction), severe comorbidities (including ischaemic heart disease, severe obesity, uncontrolled severe hypertension or heart failure, severe heart valves disease, myocarditis, acute pulmonary embolus/thrombus/venous thromboembolic disorders, mental impairment, current infective illness)

Computed tomography images

Chest computed tomography had to be no more than two weeks older than the date of the bronchoscopy. Scans were viewed in the lung window settings (width 1000-1500 Hounsfield Units, HU; level 700 to -550 HU). Prevalent features were listed, including peri-lobular changes, ground glass opacities, consolidations, traction bronchiectasis, reticulation, honey-combing, nodules, crazy paving; distinct patterns were described when possible (fibrotic or non-fibrotic), such as UIP pattern (usual interstitial pneumonia), NSIP (non-specific interstitial pneumonia), HP (hypersensitivity pneumonitis), indeterminate for UIP pattern, OP-like (organizing pneumonia), etc.

Bronchoscopic procedure

Lung samples were obtained by trans-bronchial lung cryobiopsy. Cryobiopsy was performed with rigid technique: patients were intubated with rigid bronchoscope and tissue biopsy was carried out by using the flexible bronchoscope inserted through the rigid tube. 1,7 or 1,9 mm probes were used. The cryobiopsy site was decided taking into consideration the HRCT scan appearance of each case and the biopsies were targeted at specific areas of the scan in accordance with the dedicated radiologist. Fluoroscopy guidance was always used.

Prophylactic endobronchial Fogarty balloon was always placed in the lobar bronchus near the biopsy segment and inflated after each biopsy, then immediately deflated in case of no hemorrhage. Patients were deeply sedated (propofol and remifentanil) and spontaneous breathing was maintained during the procedure. During the broncoscopic procedure, bronchoalveolar lavage (BAL) was also performed in all patients. Blood examinations included blood count, coagulation profile, serum biochemical tests (including renal and liver function, lactate dehydrogenase, and electrolytes), interleukin-6 (IL-6), serum ferritin, and D-dimer.

Histological patterns and immunohistochemistry

All specimens were fixed in 10% buffered formalin and routinely paraffin-embedded. Morphologic examination was based on conventional hematoxylin-eosin stains and immunostaining with cytokeratin 7. The modifications were defined according to histological patterns. Immunohistochemical markers were applied, in order to better recognize and characterize different cell types and phenotypes within the pulmonary microenvironment. All immunohistochemical tests were performed in the ULTRA Benchmark 141 automated immunostainer (Ventana Medical Systems/Roche, Tucson, AZ) using standard procedures and reagents are described in Table S1

Results

None of the patients were immunocompromised before contracting COVID-19; none had received corticosteroids since hospital discharge or acute phase recovery.

Cluster two patients were treated with steroids following lung biopsy results. Prednisone was started at initial dose of 0.5 mg/kg per day given as a single oral dose in the morning for four weeks; if patient was stable or improved, prednisone was gradually tapered for the ensuing four to six weeks and after three to six months, the dose was gradually tapered to zero. Patients with organizing pneumonia on lung biopsy experienced significant improvement in both symptoms and lung function impairment; DLCO improved from 50%, 43% and 44% of predicted value to 69%, 72% and 62% respectively after three months; three-months follow-up CT scan showed a significant reduction in ground glass areas and perilobular pattern in all cases. Patients with diffuse alveolar damage / proliferative phase features on lung biopsy showed moderate improvement, both clinical and functional, with complete resolution of fever and reduction of shortness of breath, although persistent on mild to moderate exertion; the three-months follow-up CT scan showed a slight reduction of the extension of parenchymal involvement. There is still insufficient data about treatment of patients with clinical, radiological and morphological picture compatible with post-COVID-19 organizing pneumonia.

Complications

Pneumothorax was observed in two cases that could be attributed to cryobiopsy procedures; it spontaneously recovered with no need of drainage and was not associated with any longer-term consequences for the patients in both cases. There were no cases of bleeding nor other procedure-related adverse events.

Table S1. Immunohistochemical and FISH probes utilized in this study

Antibody	**Clone**	**Source**	**Target**
Cytokeratin 7	SP52	Ventana-Roche	Epithelial cells
Ki67	30-9	Ventana-Roche	AECII
Tubulin beta-3	TUJ1	Covance	Myofibroblast Activated AEII
CD4	SP35	Ventana-Roche	T cell
CD8	SP57	Ventana-Roche	T suppressor lymphocytes
IDO	D5J4E	Cell Signaling	Immune checkpoint inhibition
PD-L1	22C3	Agilent-Dako	Immune checkpoint inhibition
phospho-STAT3	D3A7	Cell Signaling	IL-6 pathway
Sars-Cov-2	smFISH, HuluFISH	Pixelbiotech Germany	Sars-CoV-2 virus

Table S2. Patients characteristics, comorbidities, symptoms, lung function

	Pat. 1	**Pat. 2**	**Pat. 3**	**Pat. 4**	**Pat. 5**	**Pat. 6**	**Pat. 7**	**Pat. 8**	**Pat. 9**	**Pat. 10**
Age (years)	76	60	62	50	63	63	73	49	47	38
Sex (M/F)	M	M	M	M	M	F	F	M	M	F
Smoke (Yes,No,ex)	ex	ex	no	no	no	no	ex	ex	no	no
Comorbidities	Hypertension	No	Hypertension	Hypertension	No	No	Hypertension	No	No	No
Prior imaging studies	no	fILA	no	no	no	no	no	no	no	no

Prior PFTs	no	normal	no	no	no	no	no	no	no	no
Hospital or Home (acute phase)	Hospital	Hospital	Hospital	Hospital	Hospital	Home	Hospital	Hospital	Home	Home
Respiratory Support (acute phase)	Oxygen	Oxygen	Oxygen	Oxygen NIV	Oxygen	No	Oxygen	Oxygen NIV	No	No
Treatment (acute phase)	Antivirals Steroids LMWH Canakinumab Antibiotics	Steroids LMWH Hydroxychloroquine Antibiotics	Steroids LMWH	Steroids LMWH	Antivirals Steroids LMWH	Antibiotics	Steroids LMWH Antibiotics	Steroids LMWH	LMWH Azithromycine	Steroids Azithromycine
Persistent symptoms	Cough Dyspnea	Cough Dyspnea	Cough Fatigue Fever	Cough Dyspnea Fatigue Fever	Cough Dyspnea	Dyspnea Fever	Dyspnea Fever	Cough Dyspnea Fatigue Aches	Cough Dyspnea Fatigue Aches Depression	Dyspnea Fatigue Aches Fever Depression
Time (days) *	100	227	32	65	76	160	56	123	137	44
FVC (%)	87	96	78	46	64	74	96	77	116	72
DLCO (%)	44	61	50	43	43	44	51	72	80	73

Definition of abbreviations: M = male; F = female; LMWH = low molecular weight heparin; NIV = non invasive ventilation; FVC = forced vital capacity; DLCO = carbon-monoxide diffusion coefficient; PFTs = pulmonary function tests; fILA = fibrotic interstitial lung abnormalities. *Time means interval in days between recovery (defined in the text) and bronchoscopy

Table S3. Laboratory findings and broncho-alveolar lavage data (BAL) of patients at the time of biopsy

	Pat.1	Pat.2	Pat.3	Pat.4	Pat.5	Pat.6	Pat.7	Pat.8	Pat.9	Pat.10
Blood lymphocytes (Nx10^9/L)	1,63	3,08	3,05	4,61	1,71	2,83	1,93	1,54	1,15	4,02
Blood D-dimer (ug/L)	620	532	1130	487	421	357	886	198	160	174
Ferritin (ug/L)	257	148	541	101	234	283	492	615	376	12
Blood LDH (U/L)	312	238	241	291	379	180	285	250	182	148
Auto-antibodies				anti-Ro 60			ANA 1/89 speckled			Mi-2beta
CRP (mg/L)	1	3	15,1	1,8	0,9	53	14,2	1	0,8	0,3
BAL total cells (10^6/L)	398	102	3	179	364	717	128	159	177	103
BAL lymphocytes (%)	15	7	Not evaluable	25	25	72	15	11	5	2
BAL neutrophils (%)	3	9	Not evaluable	3	6	3	7	19	3	2
BAL macrophages (%)	82	83	Not evaluable	71	69	25	75	70	92	96
BAL eosinophils (%)	0	1	Not evaluable	1	0	0	3	0	0	0
CD4+/CD8+ lymphocytes ratio	0,8	0,8	Not evaluable	2,5	1,4	0,6	0,2	0,6	Not evaluable	Not evaluable

Table S4. Histological patterns and immunohistochemistry

Cluster 1 (pre-existing chronic fibrosing)	Cluster 2 (acute/sub-acute injury)	Cluster 3 (minimal changes - vascular abnormalities)
Histological patterns		
-fibroblastic foci -microscopic honey combing -interstitial collagenous fibrosis -nodular aggregates of lymphocytes	-OP -alveolar cell hyperplasia -DAD (organization phase) -perivascular and interstitial lymphoid infiltrates	-diffuse dilatation of vascular lumina (both interstitial capillaries and post-capillary venules)
Immunohistochemistry		

Alveolar cells -focal p16 -no PD-L1 *Endothelial cells* -limited IDO -focal PD-L1	*Alveolar cells* -nuclear pSTAT3 -Ki67 -rare PD-L1 *Endothelial cells* -diffuse IDO (*) -diffuse PD-L1 (*) -diffuse nuclear pSTAT3 (*) (*) both interstitial capillary vessels and venules. *Stromal/interstitial compartment* -TUBB3 (areas of stromal remodeling)	*Alveolar cells* -no PD-L1 -no TUBB3 *Endothelial cells* -diffuse PD-L1 (*) -diffuse IDO (*) (*) both interstitial capillary vessels and venules. *Stromal/interstitial compartment* -no TUBB3

Abbreviations: FF = fibroblastic foci; HC = honeycombing; OP = organizing pneumonia; DAD = diffuse alveolar damage; TUBB = Tubulin-beta; IDO = indoleamine 2,3-dioxygenase-1

Table S5. Clinical and radiological features of patients with parenchymal lung disease on CT scan with extension > 5% but not undergoing lung biopsy (contraindications or not consenting)

	HRCT SCAN	**GENDER (M/F)**	**AGE (years)**	**SYMPTOMS**	**TIME FROM RECOVERY (days)**
Patient 1	Reticulation with some peri-lobular pattern, traction bronchiectasis	F	47	Persistent dyspnea on exertion, fatigue, cough Patient had been admitted with respiratory failure, intubated and ventilated	123
Patient 2	Peripheral consolidation. Ground glass. Vessel enlargement (= "gravity dependent perilobular pattern")	M	56	Persistent fatigue, diffuse muscle pain, memory loss, depression Patient had been admitted for respiratory failure, but not intubated	128
Patient 3	Ground glass attenuation, reticulation, peri-lobular pattern	F	78	Persistent dyspnea on exertion, fatigue, low grade fever Patient had been treated at home	92
Patient 4	Reticulation with some peri-lobular pattern, traction bronchiectasis	M	62	Persistent dyspnea on exertion, fatigue. Patient had been admitted with respiratory failure and intubated	94
Patient 5	Ground glass attenuation with peri-lobular pattern, reticulation and traction bronchiectasis	F	52	Persistent dyspnea on exertion, fatigue, cough, fever Patient had been admitted for respiratory failure, but not intubated	124
Patient 6	Reticulation	M	77	Persistent dyspnea and fatigue Patient had been admitted but not ventilated	68
Patient 7	Minimal changes (mild residual ground glass and gravity dependent perilobular pattern)	M	37	Fatigue, muscle pain, depression Patient had been treated at home	56

Patient 8	Peripheral consolidation. Ground glass. Perilobular pattern	F	59	Fatigue, dyspnea, joints pain Patient had been treated at home	115
Patient 9	Ground glass and gravity dependent perilobular patter	F	53	Fatigue. Alopecia. Dyspnea Patient had been treated at home	54
Patient 10	Reticulation with some peri-lobular pattern, traction bronchiectasis	M	52	Dyspnea. Fatigue Patient had been admitted for respiratory failure but not intubated	88
Patient 11	Ground glass, halo sign, reticulation	M	84	Fatigue. Dyspnea Patient had been admitted for respiratory failure but not intubated	99
Patient 12	Minimal changes (mild residual ground glass and gravity dependent perilobular pattern)	F	49	Fatigue. Dyspnea. Alopecia Patient had been treated at home	160
Patient 13	Reticulation with some peri-lobular pattern, traction bronchiectasis	M	76	Dyspnea. Cough Patient had been admitted for respiratory failure but not intubated	87