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