Methods

SARS-CoV-2 infection was defined as a suggestive clinical presentation and a positive nucleic acid test (rt-PCR) on nasopharyngeal and oropharyngeal swabs.

The electronic medical records of all patients were retrospectively reviewed. Personal protection equipment was used and all safety measures recommended by the WHO were strictly followed. Postmortem biopsies were taken from lung, liver, kidney and heart tissue. In one case a sample from small bowel was also available. We used the patients' last imaging studies as a reference. Thoracic approach: Lung samples (n=10) were obtained through a minimal (5cm) incision in fifth intercostal space (ICS) between the anterior and midaxillary lines in first 5 patients; a bilateral incision (5cm) in third ICS at the midclavicular line was performed in the subsequent 5 cases. Myocardial biopsies (n=3) were obtained through the left mini-thoracotomy. Abdominal approach: In one case, a medial abdominal incision (10cm) was performed in order to obtain liver and small bowel biopsies. The rest of samples (heart (n=4), liver (n=8) and kidney (n=4)) were obtained with BioPince™ Full Core Biopsy Instrument kit. All instruments used were appropriately disinfected in order to avoid contamination in subsequent RT-PCR analyses.

Tissues were immediately fixed in neutral buffered formalin for over 24 h, and then routinely processed under standard biosafety measures. All samples were then paraffin-embedded and 4 micron sections were stained with H&E. Additionally, in a representative section of each lung a immunohistochemical (IHC) panel with TTF-1, CD68, CD3, CD20, CD4, and CD8 antibodies (Dako-OMNI platform) was performed following manufacturer recommendations. Five pathologists reviewed all cases separately and the histological and IHC findings were agreed upon. RNA was extracted from four to eight 5-micron sections from formalin fixed paraffin embedded (FFPE) tissue from each of all available organs from each patient using RNeasy® FFPE Kit. All procedures were performed on FFP3 cabinet. Pathologists and technicians were adequately protected with approved PPE.

Depending on the size of the samples in the paraffin block, viral RNA was extracted from four to eight 5-micron sections of FFPE tissues. RNA extraction from biopsies was performed using the QIAamp Viral RNA Mini Kit (Qiagen) and the identification of SARS-CoV-2 transcripts encoding nucleocapsid (N) and an envelope protein (E) was performed using a commercial kit (SARS-CoV-2 Real Time PCR Kit, Vircell), both according to manufacturer recommendations, at the Microbiology Laboratory of the Clinica Universidad de Navarra. Samples with amplification of both targets with Ct values below 35 were considered positive for SARS-COV-2. We selected a threshold of 35 in both genes based on analogy with nasopharyngeal-swab standards.

Results

Clinicoradiological characteristics of the series (n=10) are summarized on supplementary files, table 1. The age range was between 68 and 92 years, and half were women. Most cases presented fever (n=8) and dyspnea (n=6) at admission. High blood pressure (HBP) was present in most patients (n=9). Obesity and type 2 diabetes mellitus (DM2) were present in 5 and 4 cases, respectively. Other cardiovascular disorders such as heart failure (HF, n=3), ischemic cardiomyopathy (IC, n=2), and atrial fibrillation (A-fib, n=3) was seen in a total of 5 patients. Three patients suffered from chronic kidney disease and one had received a kidney transplant (patient 8). One patient had previous history of respiratory disease with a diagnosis of chronic obstructive pulmonary disease (COPD). Despite uncertainty on efficacy, all patients received treatment for SARS-CoV-2 including antiviral therapy. They also received supplementary oxygenation as needed, including orotracheal intubation (n=4) in the intensive care unit (ICU), and treatment for their underlying diseases. Heparin was utilized in all patients during admission. Respiratory parameters in the 48 hours pre-mortem were collected in ICU patients (Table 2). All of them required high positive end expiratory pressure (PEEP >10cmH_2O) and in most cases (n=3) presented low peak pressure with normal dynamic compliance. The duration of the clinical course from the onset of symptoms of SARS-CoV-2 to death ranged from 8 to 25 days.

Table 1 shows the most critical levels of the relevant clinical laboratory parameters during the clinical course. The lowest lymphocyte count and the highest Troponin T, D-Dimer, C reactive protein (CRP), pro-BNP and ferritin levels were recorded. CRP and Ferritin were elevated in all patients. Lymphopenia was present in 9 cases. NT pro-BNP (n=10) and Troponin T (n=7) were elevated in most patients. All patients presented high D-Dimer levels.

Chest computerized tomography (CT) findings and images are shown in supplementary table 1 and Figure 1, respectively. In all patients, the main CT finding was ground-glass opacity (GGO), with or without consolidations. In our sample, both lungs were involved, except for case 2, who only showed abnormalities in the right lung. Pleural effusion (n=7) and air bronchogram (n=6) were also common findings in most patients. Crazy paving pattern (CPp), pericardial effusion or lymphadenopathies were not frequently observed (Figure 1).

The size of lung biopsies ranged as follows: width (4.0 cm - 9.3 cm); Height (1 cm - 505 cm); and Depth (0.5 cm - 2 cm). The microscopic findings of the 10 lung samples studied are varied and are shown in supplementary table 2. In 6 out of 10 cases, common characteristics of clinical

ARDS, such as the presence of hyaline membranes, a characteristic of diffuse alveolar damage (DAD), were seen in different phases of progression (Fig 2). Moreover, marked type II pneumocyte hyperplasia and hypertrophy with reactive atypia and intraluminal foamy macrophages were noticeable in all cases. Some type II pneumocytes showed a hobnail arrangement and occasionally, binucleated type II pneumocytes were seen. In four cases, medium-size vessel thrombi were remarkable (Fig 2C). Capillary thrombi were not present in any case. In addition, mild chronic interstitial inflammation appeared in 6 out of 10 cases. Vascular smooth muscle hyperplasia was present in 5 out of 10 cases, with two of them showing marked serpiginous smooth muscle vessel walls.

Septal thickening with scant inflammatory cells and isolated foci of more striking alveolar septal fibroblast proliferation, lambertosis and focal intraalveolar edema was visible in 3 cases (supplementary table 2). Osseous metaplasia and squamous metaplasia of alveolar epithelial cells were noticed in 2 cases.

Seven heart biopsies were available. The only case that had a mild chronic inflammatory infiltrate was case number 8. (Fig 2E). The rest of the cases had no signs of inflammation. Cases 1 and 2 had myocardial hypertrophy, while case 5 showed ischemic myocardiocytes. The biopsy of case 4 confirmed the previous diagnosis of cardiac amyloidosis with the presence of an acellular eosinophilic deposit that was positive for Congo-Red staining

Nine liver biopsies were obtained. Normal hepatic parenchyma was evident in cases 3 and 6. Mild focal steatosis was present in cases 2, 7, and 8, and mild vascular congestion was seen in cases 5 and 7. Focal patchy necrosis (nutmeg liver) was observed in case 4. The only samples showing inflammation were from case 5, at a subcapsular level clinically related with peritonitis, and case 9, which presented mild portal inflammation. No small-vessel thrombi were observed in any of the biopsies.

Four kidney biopsies were obtained from the 10 cases. Only cases 4 and 5 presented vascular congestion, with case 4 also showing congestion of the glomerular capillaries. No capillary thrombi were observed. There were no signs of inflammation or fibrosis in any of the biopsies.

One biopsy of the small intestine was obtained from case 10. No signs of inflammation or thrombi of small vessels were evident.

It is remarkable that 9 patients had at least one organ with significant amount of SARS-COV-2 RNA, being most prevalent in lung tissue (N=8), followed by myocardial samples (N=7). RT-PCR for SARS-CoV2 was positive in all lung samples. The presence of Viral RNA was measured with

an E-gene CT count of 21-36 and a N-gene CT count of 17-34. RT-PCR for SARS-CoV2 was performed in all 7 cardiac samples. Viral RNA was measured with an E-gene CT count of 30-37 and a N-gene CT count of 30-40. RT-PCR for SARS-CoV2 was positive in all samples but one. Viral RNA was measured with an E-gene CT count of 31-36 and a N-gene CT count of 30-36. RT-PCR for SARS-CoV2 was positive in all kidney samples. Viral RNA was measured with an E-gene CT count of 32-36 and a N-gene CT count of 31-40.

	Age	Sex	Medical history	CT findings	Symptoms	Lymphocytes (10^6/L)	CRP (mg/dl)	Ferritin (ng/ml)	D-dimer (ng/ml)	TroponinT (ng/L)	Pro-BNP (pg/ml)	Tx	ICU (days)	BCRSS	DCC
						NV: 1.2 - 4	**NV:** ≤ 0.5	**NV:** 15 – 150	NV: 150 - 500	NV ≤ 14	NV ≤ 222				
Patient 1	76	F	HBP, COPD RA	GGO, CONS AB, PE	Fever Dyspnea	720	7	775	1010	39	1547	HC, AZM, HEP	No	4	10
Patient 2	89	F	AD	GGO, CONS AB, CPp, PE	Fever Dyspnea Cough	550	15.6	543	2090	445	35000	HC, AZM, HEP	No	4	10
Patient 3	79	M	HBP, DM2, AD, HF	GGO, CPp, AB, PE	Dyspnea	330	35.6	7590	33600	407	16000	HC, AZM, LPV/r, HEP	No	4	8
Patient 4	75	F	HBP, Obesity, AFib, RCM, Amyloidosis	CONS, AB, PE	Fever Dyspnea	240	2.7	238	1150	340	21200	HC, AZM, CCS, HEP	No	4	13
Patient 5	68	M	HBP, DM2, Obesity, DLP	GGO, CPp, CONS, AB	Fever Dyspnea Myalgia	100	51.7	2084	2530	18	2770	HC, AZM, CCS, LPV/r, Betaferon HEP	Yes (12)	5	13
Patient 6	81	F	HBP, Obesity, AFib, DLP, CKD	GGO, PE	Fever Cough	2500	25.6	5080	37220	197	3150	HC, AZM, CCS, LPV/r Tocilizumab HEP	Yes (5)	5	21
Patient 7	79	M	HBP, COPD, DM2, DLP, CKD, HF, IC, ICD,CVA Paraplegia	GGO, AB, CONS, PE	Fever Dyspnea Cough	480	21.5	1140	4390	163.5	29160	HC, AZM, HEP	No	4	8
Patient 8	71	F	HBP, CKD, KT, FL, Obesity	GGO, CONS, AB, PE	Fever Cough	190	13.8	1836	1530	6052	2030	HC, AZM, LPV/r, CCS. HEP	Yes (6)	8	15
Patient 9	76	M	HBP	GGO, CPp	Fever Cough	200	12	2200	3240	650	728	HC, LPV/r, CCS, HEP	Yes (18)	5	25
Patient 10	92	M	HBP, DM2, DLP, Obesity, AFib, HF, IC	GGO	Cough	270	27	1440	2030	24	3350	HC, AZM, CCS, HEP	No	4	14

Supplementary TABLE 1: Clinical, CT and laboratory findings. Medical history: High blood pressure (HBP); chronic obstructive pulmonary disease (COPD); rheumatoid arthritis (RA), Alzheimer´s disease (AD); diabetes mellitus type 2 (DM2); atrial fibrillation (AFib); restrictive cardiomyopathy (RCM); dyslipidemia (DLP); chronic kidney disease (CKD); heart failure (HF); **ischemic cardiomyopathy (IC)**; implantable cardioverter defibrillator (ICD); cerebrovascular accident (CVA); kidney transplantation (KT); follicular lymphoma (FL). **CT findings:** Ground-glass opacities (GGO); consolidation (CONS); "crazy paving" pattern (CPp); pleural effusion (PE); air bronchogram (AB). **C-Reactive protein (CRP). Treatment (Tx)**: Hydroxychloroquine (HC); Azithromycin (AZM); Lopinavir/ritonavir (LPV/r); Corticosteroids (CCS);Heparin (HEP). **Intensive unit care (ICU) days. Brescia-COVID Respiratory Severity Scale (BCRSS). Duration of clinical course (DCC).** Lab-test normal values (NV)

	Lung	Heart	Other organs	Positive SARS COV2 PCR
Patient 1	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia); Vacuolated macrophages; Mild inflammation; Loose fibrosis; Amylaceous bodies	Myocardial hypertrophy; No inflammatory infiltrate	NS	
Patient 2	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia; Hyaline membranes); Vacuolated macrophages; Vascular wall hyperplasia; Edema; Vascular congestion and hemorrhage	Myocardial hypertrophy; No inflammatory infiltrate	Liver: Mild steatosis Kidney: Normal	Lung Heart
Patient 3	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia; Hyaline membranes); Vacuolated macrophages; Vascular wall hyperplasia; Medium size-vessel thrombi; Osseous metaplasia; Squamous metaplasia.	NS	Liver: Normal	Lung
Patient 4	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia); Vacuolated macrophages; Vascular wall hyperplasia; Mild inflammation with multinucleated giant cells; Vascular congestion and focal hemorrhage.	Amiloid deposits (pre-existing)	Liver: Patchy hepatic necrosis due to congestion Kidney: Mild vascular congestion	Lung Heart Liver Kidney
Patient 5	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia); Vacuolated macrophages; Vascular wall hyperplasia; Mild inflammation; Partially organized pneumonia; Mesothelial hyperplasia; Loose fibrosis.	Ischemia; No inflammatory infiltrate	Liver: Subcapsular inflammatory cellular infiltration; Congestion Kidney: Mild vascular congestion	Lung
Patient 6	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia; Hyaline membranes); Vacuolated macrophages; Medium size-vessel thrombi; Interstitial pneumonia with mild inflammation; Squamous metaplasia; invasive aspergillosis.	NS	Liver: Normal	Lung
Patient 7	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia; Hyaline membranes); Vacuolated macrophages; Moderate inflammation; Vascular wall hyperplasia; Medium size-vessel thrombi; Focal edema.	NS	Liver Mild steatosis; Sinusoidal congestion	Lung
Patient 8	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia); Vacuolated macrophages; Hyaline membranes; Loose interstitial fibrosis	Focal inflammatory cellular infiltrates	Liver Mild steatosis	Lung Heart Liver
Patient 9	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia); Vacuolated macrophages; Acute superimposed pneumonia	No inflammatory infiltrate	Liver: Mild portal inflammation Kidney: Normal	Heart Liver Kidney

Patient 10	DAD (Type II pneumocyte hyperplasia and hypertrophy with reactive atypia; hyaline membranes); Vacuolated macrophages; Medium size-vessel thrombi; Moderate inflammation; Osseous metaplasia; Focal edema	Mild hemorrhage; Focal interstitial fibrosis; No inflammatory infiltrate	Liver: Mild portal inflammation and sinusoidal congestion Small bowel: Normal	Lung Heart Liver Small bowel

Supplementary Table 2. Summary of histopathological findings. Not sampled (NS).