

Supplementary Material

Visualising SARS-CoV-2 infection of the lung in deceased COVID-19 patients

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Table S2: Demographics, medical history, course and management of SARS-CoV-2 infection, and viral RNA assays on lung tissues samples of the COVID-19 cases

case ID	age	sex	body mass index	smoking history; pack years	pre-COVID-19 respiratory disease	pre-COVID-19 immunosuppressive drugs	active malignancy	treatment malignancy	comorbidities	vaccination	length hospital stay (days)	length ICU stay (days)	respiratory support	days intubated	pharmacological COVID-19 treatment	cause of death	serology anti-N IgG	time of death	presumptive duration of illness (days)	diagnosis-to-death interval (days)	result last qRT-PCR before death	interval last qRT-PCR to death (days)	origin last sample for qRT-PCR	postmortem tissue sampling	postmortem interval	abundance RNA scope V puncta	SARS-CoV-2 qRT-PCR tissue
1	71	male	25.5	former; 39	no	anti-CD20 + anti-CD37 and entansine conjugate	diffuse large B-cell lymphoma	anti-CD20 + anti-CD37 and entansine conjugate; prophylactic acyclovir	peripheral artery disease	pre-vaccination	13	12	mechanical ventilation	12	hydroxychloroquine	respiratory failure	negative	March 2020	16	13	positive	11	upper respiratory tract	bedside	15 min	+++	not reported
2	76	male	not reported	former	chronic obstructive pulmonary disease	no	prostate carcinoma	hormonal therapy with androgen receptor antagonist (Bicalutamide)	no	pre-vaccination	11	11	mechanical ventilation, proning	10	hydroxychloroquine	respiratory failure	positive	April 2020	18	11	positive	8	lower respiratory tract	bedside	15 min	0	negative
3	60	male	25.3	former	no	methylprednisolone	no	not applicable	arterial hypertension, polymyalgia rheumatica	pre-vaccination	26	23	mechanical ventilation, proning, extracorporeal membrane oxygenation	21	hydroxychloroquine, steroids, IL receptor modulator	COVID-19 sequelae	positive	May 2020	28	26	negative	3	lower respiratory tract	autopsy	11 h	+	negative
4	67	male	not reported	no	no	No	no	not applicable	no	pre-vaccination	44	44	mechanical ventilation, high-flow nasal cannula	19 and 2	hydroxychloroquine, steroids, IL receptor modulator	COVID-19 sequelae	positive	May 2020	51	44	negative	1	lower respiratory tract	autopsy	19 h	+	negative
5	72	female	27.7	no	no	hydrocortisone	no	not applicable	granulomatosis with polyangiitis, chronic kidney disease, adrenal insufficiency	pre-vaccination	45	45	mechanical ventilation, proning	44	hydroxychloroquine, steroids	COVID-19 sequelae	positive	May 2020	56	45	positive	5	lower respiratory tract	autopsy	41 h	++	not reported
6	89	male	29	active	no	methylprednisolone	no	not applicable	arterial hypertension, type 2 diabetes mellitus, chronic kidney disease, coronary artery disease, rheumatoid arthritis	pre-vaccination	5	3	non-invasive ventilation, proning	0	steroids	respiratory failure	negative	October 2020	9	5	positive	5	upper respiratory tract	bedside	35 min	+++	positive
7	75	male	27.3	former; 40	no	no	metastasized prostate carcinoma	hormonal therapy with enzalutamide and leuprorelide	type 1 diabetes mellitus, coronary artery disease	pre-vaccination	17	14	mechanical ventilation, proning	5	steroids, IL receptor modulator	respiratory failure	positive	October 2020	20	17	positive	5	lower respiratory tract	bedside	15 min	++	positive
8	90	male	27	no	no	no	no	not applicable	type 2 diabetes mellitus, coronary artery disease, heart failure	pre-vaccination	5	0	high-flow nasal cannula	0	steroids, remdesivir	respiratory failure	negative	October 2020	8	8	positive	8	upper respiratory tract	bedside	45 min	+++	positive
9	69	male	not reported	former; 60	no	no	no	not applicable	arterial hypertension, type 2 diabetes mellitus, chronic kidney disease, coronary artery disease	pre-vaccination	22	14	mechanical ventilation, proning	10	steroids	COVID-19 sequelae	positive	November 2020	29	22	positive	12	lower respiratory tract	bedside	55 min	0	positive
10	50	female	34.5	former; 10	no	no	no	not applicable	No	pre-vaccination	16	16	mechanical ventilation, proning, extracorporeal membrane oxygenation	14	steroids, remdesivir	COVID-19 sequelae	positive	November 2020	21	21	positive	5	lower respiratory tract	bedside	30 min	+	positive
11	82	female	26.9	no	chronic obstructive pulmonary disease	no	no	not applicable	type 2 diabetes mellitus, chronic kidney disease	pre-vaccination	10	9	mechanical ventilation, proning	8	steroids	respiratory failure	positive	November 2020	22	20	positive	8	lower respiratory tract	bedside	15 min	+	positive
12	68	male	29.4	active	chronic obstructive pulmonary disease	no	no	not applicable	with cardiomyopathy, peripheral artery disease	pre-vaccination	19	19	mechanical ventilation	17	steroids	COVID-19 sequelae	positive	November 2020	not reported	34	positive	11	lower respiratory tract	bedside	35 min	+	positive
13	87	female	40	no	no	no	no	not applicable	arterial hypertension, type 2 diabetes mellitus	pre-vaccination	4	0	high-flow nasal cannula	0	steroids, remdesivir	respiratory failure	not reported	November 2020	8	8	positive	8	upper respiratory tract	bedside	115 min	+++	positive
14	68	male	51.8	former; 15	no	no	no	not applicable	arterial hypertension, coronary artery disease	pre-vaccination	25	21	mechanical ventilation, proning, inhaled nitric oxide	10	steroids	COVID-19 sequelae	positive	November 2020	31	29	positive	9	lower respiratory tract	autopsy	19 h	++	positive
15	49	male	not reported	not reported	asthma	no	no	not applicable	no	pre-vaccination	32	32	mechanical ventilation, proning, extracorporeal membrane oxygenation	23	steroids	COVID-19 sequelae	positive	November 2020	39	32	negative	13	lower respiratory tract	autopsy	18 h	+	negative
16	75	male	not reported	not reported	no	no	no	not applicable	arterial hypertension, chronic kidney disease	not vaccinated	40	37	mechanical ventilation	37	steroids	COVID-19 sequelae	positive	December 2020	43	41	positive	10	lower respiratory tract	autopsy	19 h	0	positive
17	81	male	24.6	former	no	no	no	not applicable	coronary artery disease	not vaccinated	21	21	mechanical ventilation, proning	21	steroids	COVID-19 sequelae	positive	December 2020	not reported	21	positive	4	lower respiratory tract	autopsy	66 h	+	positive
18	75	male	not reported	not reported	no	no	no	not applicable	no	not vaccinated	51	47	mechanical ventilation, proning	47	steroids, remdesivir	COVID-19 sequelae	positive	December 2020	58	51	negative	2	upper respiratory tract	bedside	75 min	0	negative
19	59	male	40.8	no	chronic obstructive pulmonary disease, chemotherapy and lobectomy for lung spinocellular carcinoma	no	no	not applicable	arterial hypertension	not vaccinated	30	30	mechanical ventilation, proning, extracorporeal membrane oxygenation	26	steroids	COVID-19 sequelae	positive	December 2020	not reported	37	negative	1	upper respiratory tract	bedside	45 min	+	positive
20	82	male	not reported	no	no	no	no	not applicable	no	not vaccinated	23	23	mechanical ventilation, proning	11	steroids	respiratory failure	positive	December 2020	24	23	positive	11	lower respiratory tract	bedside	75 min	+	positive
21	47	male	29.9	no	no	high-dose methylprednisolone for brain oedema	brain oligodendroglioma	no	fatal brain oedema after stereotactic biopsy; arterial hypertension	not vaccinated	7	6	mechanical ventilation	6	none	brain tumour	negative	February 2021	not reported	2	positive	2	upper respiratory tract	bedside	45 min	+++	positive
22	84	male	not reported	no	no	no	no	not applicable	arterial hypertension	not vaccinated	17	13	non-invasive ventilation	0	steroids	respiratory failure	positive	February 2021	23	20	positive	20	upper respiratory tract	bedside	30 min	++	positive

qRT-PCR: quantitative reverse-transcription polymerase chain reaction; anti-N IgG: anti-nucleocapsid immunoglobulin G; IL: interleukin; COVID 21 was diagnosed with SARS-CoV-2 infection at an intensive care unit and was already intubated for brain oedema with intracranial hypertension as complication of an earlier brain tumour biopsy.

Table S3: Demographics, medical history, virology, and data on lung samples of the control cases

case ID	age (years)	sex	body mass index	smoking	respiratory disease	immuno-compromised	comorbidities	procurement	date of death	cause of death	days intubated	reason decline of lungs	tissue samples	serology anti-N IgG	SARS-CoV-2 qRT-PCR tissue
1	56	male	27	no	no	no	arterial hypertension, dilated cardiomyopathy	donation after brain death	March 2019	bacterial meningitis	10	not reported	right lung	prepandemic	negative
2	68	male	25	no	no	no	coronary artery disease	donation after circulatory death	July 2019	hypoxic-ischemic encephalopathy	13	pneumonia left lower lobe	right lung	prepandemic	negative

Table S4: SARS-CoV-2 RNA assays on lung tissue samples

	case ID	diagnosis-to-death interval (days)	N1 qRT-PCR	N2 qRT-PCR	abundance RNAscope N puncta
COVID cases	21	2	33.1	32.5	+++
	6	5	22.8	22.9	+++
	8	8	29.0	28.2	+++
	13	8	31.9	31.0	+++
	2	11	negative	negative	0
	1	13	not reported	not reported	+++
	7	17	36.4	32.6	++
	11	20	34.8	34.5	+
	22	20	35.0	32.3	++
	10	21	36.5	36.0	+
	17	21	36.6	32.6	+
	9	22	34.6	32.8	0
	20	23	29.6	24.9	+
	3	26	negative	negative	+
	14	29	negative	31.8	++
	15	32	negative	negative	+
	12	34	negative	35.0	+
	19	37	35.6	30.2	+
16	41	negative	36.0	0	
4	44	negative	negative	+	
5	45	not reported	not reported	++	
18	51	negative	negative	0	
CONTROL cases	1	not applicable	negative	negative	0
	2	not applicable	negative	negative	0

qRT-PCR=quantitative reverse-transcription polymerase chain reaction.

The COVID cases are ranked according to the interval between the time of diagnosis of SARS-CoV-2 infection and the time of death. PCR was performed on cryopreserved lung samples from COVID and control cases. The abundance of RNAscope N puncta is scored in a semiquantified manner as “+++” for diffuse detection, “++” for focal detection, “+” for rare detection and “0” for no detection.

The values for the CDC N1 and N2 viral RNA primers represent the cycling threshold (Ct); a Ct-value ≥ 38 was considered as negative result.

Table S5: Histopathological analysis of H&E-stained sections of the COVID-19 cases

case ID	diagnosis-to-death interval (days)	abundance RNAscope N puncta	spatial distribution of histopathological injury					characteristics of histopathological injury							
			bronchiole	terminal bronchiole	centrilobular	mediolobular	panlobular	thrombus	early exudative phase			proliferative phase --- fibrotic phase			
									marked edema	neutrophil infiltrate	neutrophil & mononuclear infiltrate	mononuclear infiltrate with very early fibrosis	mild fibrosis with residual mononuclear infiltrate	end-stage fibrosis	
21	2	+++						recent		++	+				
6	5	+++													
8	8	+++							present	+	+	+	+		
13	8	+++						recent	present	++	+				
2	11	0													
1	13	+++									++	+			
7	17	++									+	+			
11	20	+						old			+	+	++		
22	20	++										++	++		
10	21	+						old			+	++	+		
17	21	+								+++					
9	22	0						old			++	++			
20	23	+									+	+++			
3	26	+								+++				+++	
14	29	++									+	++	++		
15	32	+										+++	+++		
12	34	+						old				+++	+++		
19	37	+										+++	+++		
16	41	0												+++	
4	44	+							present						
5	45	++						recent		+++					
18	51	0													+++

An H&E-stained lung tissue section of each case was interpreted blindly by a pathologist (Figure 2). The cases are ranked according to the increasing diagnosis-to-death interval. The distribution of pathological findings within the lung tissue is indicated with dark grey for histological structures that were predominantly affected and light grey for structures that were only affected to a limited extent. Inflammation and fibrosis are scored as '+++' for extensive, '++' for moderate, and '+' for limited signs of the specified characteristics of histopathological injury.