THE LANCET

Supplementary appendix

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Supplement to: Braun F, Lütgehetmann M, Pfefferle S, et al. SARS-CoV-2 renal tropism associates with acute kidney injury. *Lancet* 2020; published online August 17. http://dx.doi.org/10.1016/S0140-6736(20)31759-1.

Materials and methods:

Autopsies
All autopsies were performed at the Department of Legal Medicine of the University Medical Center Hamburg-Eppendorf. The Ethics Committee of the Hamburg Chamber of Physicians was informed about the study (WF-051/20). The median post-mortem interval was 5 days. Clinical and laboratory information is provided in **Table S1**.

Clinical data
Principal comorbidities were assessed using the provided information by nursing homes, hospitals or private practices. Coronary heart disease, myocardial infarction and angina pectoris were grouped as one disease entity, while dementia and stroke were considered separate diseases. Hormonal abnormalities, diabetes and obesity were considered metabolic diseases. Liver diseases, malignancies and autoimmune diseases were considered other diseases. Acute kidney injury (AKI) was defined as the report of AKI, oliguria or the need for dialysis in the patient history as well as abnormalities in the bloodwork consistent with the KDIGO AKI definition. Of note, some patients died in nursing homes without full laboratory workup. For survival calculations, we estimated the interval between the first positive respiratory swab for SARS-CoV-2 (COVID-19 diagnosis) and date of death. Survival analysis was performed only within the first 21 days of clinical course as we detected imbalances in the number of patients from each group after this time point.

Quantitative RT-PCR for SARS-CoV-2
A full protocol has been previously reported[1]. Briefly, autopsy kidney samples were stored at 4°C in RNA later solution (ThermoFisher; AM7021-500ML). All tissue samples were grinded (Precellys 24, Bertin, Rockville, US) using 2ml tubes prefilled with ceramic beads (Precellys Lysing Kit) and 1ml RNA and DNA free PCR grade water. For RNA/DNA extraction, 200μl of the grinded tissue lysate was transferred to the MagnaPure96 (Roche, Mannheim, Germany).
For virus quantification, a previously published assay was adopted with modifications using chimeric 2'-O-methyl RNA bases at the penultimate base of both primers (mG and mC) to reduce primer dimer formation[2]. The forward primer, 5´-ACAGGTACGTTAATAGTTAATAGCmGT-3 (400nM end concentration), 5´TATTGCAGCAGTACGCACAmCA-3´ (400nM end concentration) and probe 5´-Fam-ACACTAGCC/ZEN/ATCCTTACTGCGCTTCG-Iowa Black FQ-3' (100nM end concentration) were used. Primer and probes were obtained from Integrated DNA Technologies (IDT, Leuven, Belgium). One-step RT-PCR (25μl volume) was run on the LightCycler480 system (Roche) using one step RNA control kit as master mix (Roche) and 5μl of eluate. Ct value for the target SARS-CoV-2 RNA (FAM) was determined using second derivative maximum method.
For quantification, standard in-vitro transcribed RNA (IVT-RNA) of the E gene of SARS-CoV-2 was used. The standard was obtained via the European Virus Archive[3]. Linear range of the assay is between 1×10^3 and 1×10^9 copies/ml. Quantitative β-globin PCR was performed with commercial TaqMan primer set (Thermo-Fischer, 401846) and Roche DNA control kit. PCR was run on the LightCycler480 system. The amount of DNA was normalized using human DNA standard (KR0454). SARS-CoV-2 RNA levels in tissues were normalized to β-globin DNA to adjust for differences in tissue input.

Cell culture and virus isolation
Vero cells (ATCC® CRL-1586), which are kidney tubular epithelial cells retrieved from an African green monkey, were cultivated and stained with an antibody against non-structural protein 3 (nsp3; Rockland, 100-401-A52)[4]. For virus isolation, kidney tissues were homogenized as described[5] and 250μl of the homogenized tissue solution were used for infection. Virus growth was confirmed by RT-PCR of cell culture supernatants[2].

Role of the funding source
No funding source had a role in data collection, analysis, interpretation, writing of the manuscript or the decision to submit.

Table S1. General clinical data for all 63 autopsy cases.

Patient no.	Sex	Age	Place of death	PMI (days)	Principal Comorbidities	Respiratory Tract	CVD	Kidney	Brain	Metabolism	Other	AKI during COVID-19	Creatinine at admission ($\mu mol/l$)	BUN at admission (mmol/l)	eGFR at admission (CKD-Epi - ml/min/1,73m^2)	Blood pressure Medication
1	male	80	NW	4	4	1	2	0	1	0	0	y	267,03	n.a.	19	Ramipril, Bisoprolol, Torsemide
2	female	85	NW	4	5	0	2	1	0	1	1	y	106,10	7,00	30	Ramipril, Metoprolol, Torsemide
3	male	86	NH	5	5	1	2	1	1	0	0	n.a.	n.a.	n.a.	n.a.	Torsemide, Propranolol, Spironolactone
4	male	94	OH	23	6	1	3	1	1	0	0	n.a.	n.a.	n.a.	n.a.	Bisoprolol, Ramipril
5	male	75	ICU	7	3	0	1	0	0	1	1	y	123,79	8,51	n.a.	n.a.
6	male	79	ICU	4	2	0	2	0	0	0	0	y	81,35	2,28	78	n.a.
7	female	86	NH	3	6	0	2	1	1	0	2	n.a.	n.a.	n.a.	n.a.	Urapidil, Amlodipine, Ramipril
8	female	91	NW	5	3	0	1	1	1	0	0	n.a.	87,54	10,16	53	n.a.
9	male	85	NW	7	4	1	2	0	1	0	0	n	70,74	n.a.	68	n.a.
10	female	84	ICU	3	3	1	0	0	0	0	2	n	45,98	3,12	8	Torsemide
11	male	83	NW	9	3	0	3	0	0	0	0	y	n.a.	n.a.	n.a.	HCT, Bioprolol, Candesartan, Amlodipine
12	male	86	ICU	5	5	0	2	1	1	0	1	y	123,79	n.a.	47	Bisoprolol, Torsemide
13	male	71	ICU	2	7	1	3	0	1	1	1	y	452,71	15,23	10	n.a.
14	male	84	ICU	4	3	0	2	0	0	1	0	y	n.a.	n.a.	n.a.	n.a.
15	male	81	ICU	1	3	0	1	0	0	0	2	y	154,74	4,49	36	Ramipril, Torsemide
16	female	88	NW	4	6	1	1	0	2	0	2	y	n.a.	n.a.	n.a.	n.a.
17	female	75	NW	12	6	1	4	1	0	0	0	y	50,40	2,50	91	n.a.
18	male	79	OH	6	7	1	3	1	1	1	0	n.a.	919,57	20,68	4	Metoprolol, Enalapril, Amlodipine, Doxazosin
19	female	74	NW	5	6	1	3	0	1	1	1	y	97,26	n.a.	50	n.a.
20	male	90	NW	3	6	0	4	1	0	0	1	n	97,26	7,34	67	n.a.
21	male	87	NW	5	5	1	1	1	2	0	0	y	114,95	n.a.	50	n.a.
22	female	74	ICU	8	4	1	1	1	1	0	0	y	185,68	n.a.	30	Amlodipine, Ramipril, Torsemide
23	female	92	NH	0	3	0	0	1	1	0	1	n.a.	n.a.	n.a.	n.a.	n.a.
24	male	85	NH	9	8	1	3	1	1	0	2	n.a.	n.a.	n.a.	n.a.	Torsemide, Amlodipine, Cadesartan, Xipamide
25	male	83	ER	2	1	0	1	0	0	0	0	y	548,20	n.a.	8	n.a.
26	female	50	ICU	4	5	1	1	0	0	0	3	y	106,10	10,51	55	n.a.
27	male	71	ICU	5	4	0	2	1	0	1	0	y	n.a.	n.a.	n.a.	n.a.
28	male	61	NH	14	3	0	1	0	2	0	0	n.a.	n.a.	n.a.	n.a.	n.a.
29	female	78	ICU	0	5	0	3	1	0	0	1	y	190,10	n.a.	n.a.	n.a.
30	male	90	NW	5	4	0	2	1	0	0	1	y	n.a.	n.a.	n.a.	n.a.
31	female	87	NH	0	3	1	1	0	1	0	0	n.a.	n.a.	n.a.	n.a.	Carvedilol, Losartan, Amlodipine, Torsemide
32	male	88	NW	5	3	1	1	1	0	0	0	y	389,05	44,86	11	n.a.
33	female	85	NW	0	5	0	3	1	0	0	1	n.a.	254,65	17,18	14	n.a.
34	female	91	NH	7	6	1	2	1	1	0	1	n.a.	n.a.	n.a.	n.a.	Torsemide, Spironolactone
35	male	94	NH	17	6	0	2	1	2	1	0	n.a.	n.a.	n.a.	n.a.	Torsemide
36	male	78	NW	6	4	0	1	0	1	1	1	n.a.	n.a.	n.a.	n.a.	n.a.
37	male	32	NW	0	1	0	0	0	0	0	1	n	31,83	1,73	165	Torsemide
38	female	71	ICU	0	2	0	0	1	0	0	1	n	667,57	0,83	46	n.a.
39	male	61	ICU	4	5	1	2	0	0	1	1	y	105,22	3,12	66	Bisoprolol, Ramipril, Amlodipine
40	female	59	ICU	0	1	0	0	0	0	0	1	n.a.	58,36	2,00	97	n.a.
41	female	89	NW	5	6	0	2	1	1	2	0	y	150,31	n.a.	26	n.a.
42	female	78	NH	17	2	1	1	0	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.
43	female	87	NH	7	4	0	2	1	1	0	0	n.a.	n.a.	n.a.	n.a.	none
44	female	88	NH	5	7	1	2	1	1	1	1	n.a.	n.a.	n.a.	n.a.	Amlodipine, Ramipril, Torsemide
45	female	87	NW	10	6	1	2	0	1	1	1	y	102,57	3,34	43	Metoprolol, Ramipril, Torsemide
46	female	76	OH	0	2	1	1	0	0	0	0	n.a.	n.a.	n.a.	n.a.	Metoprolol, Ramipril
47	male	55	NW	8	3	0	1	1	1	0	0	n	110,53	n.a.	65	none
48	female	84	NW	8	5	0	1	1	1	0	2	y	61,89	n.a.	81	n.a.
49	male	89	NW	3	4	0	2	0	1	0	1	y	97,26	8,00	60	Candesartan, Bisoprolol
50	male	48	NW	7	4	0	2	0	0	0	2	y	62,78	n.a.	111	n.a.
51	male	90	NW	16	5	1	2	1	1	0	0	n	n.a.	n.a.	n.a.	n.a.
52	female	72	ICU	2	2	0	0	0	0	1	1	y	70,74	2,33	74	n.a.
53	female	83	ICU	0	2	1	0	0	0	0	1	y	92,84	5,15	49	n.a.
54	female	80	NH	10	3	0	1	0	1	1	0	n.a.	n.a.	n.a.	n.a.	Torsemide, Ramipril
55	male	56	NW	3	4	0	1	1	1	1	0	n.a.	857,67	19,01	8	n.a.
56	male	84	NW	9	4	0	2	1	0	1	0	y	247,58	13,17	20	Metoprolol
57	male	71	ICU	9	3	0	2	0	1	0	0	n.a.	150,31	9,67	40	n.a.
58	male	86	OH	0	3	0	1	1	0	1	0	n.a.	n.a.	n.a.	n.a.	n.a.
59	male	75	ICU	4	4	1	2	1	0	0	0	y	203,37	26,02	26	n.a.
60	male	74	OH	9	3	1	2	0	0	0	0	n.a.	n.a.	n.a.	n.a.	n.a.
61	male	51	NW	8	2	0	0	0	1	0	1	y	n.a.	n.a.	n.a.	n.a.
62	male	58	ICU	2	3	0	0	0	1	1	1	y	150,31	17,51	44	n.a.
63	female	88	OH	0	4	0	2	1	1	0	0	n.a.	n.a.	n.a.	n.a.	Metoprolol, Ramipril, Torsemide

Legend: PMI: post-mortem interval, CVD: cardiovascular disease, AKI: acute kidney injury, BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate (ml/min/1,73m^2), NW: normal hospital ward, NH: nursing home, OH: own home, ICU: intensive care unit, ER: emergency room, n.a.: not available, y: yes, n: no, HCT: Hydrochlorothiazide

Table S2. General clinical data for all 39 cases with defined kidney clinical status.

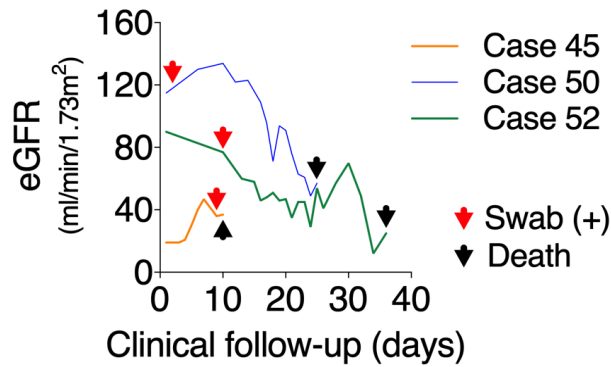| Group: renal SARS-CoV-2 tropism / AKI during COVID-19	Patient no.	Sex	Age	Principal Comorbidities	Creatinine at admission (μmol/l)	BUN at admission (mmol/l)	eGFR at admission (CKD-Epi - ml/min/1,73m^2)	Blood pressure Medication
no / no	37	male	32	1	31,83	1,73	165	Torsemide
no / no	38	female	71	2	667,57	0,83	46	n.a.
no / no	47	male	55	3	110,53	n.a.	65	none
no / no	51	male	90	5	n.a.	n.a.	n.a.	n.a.
no / yes	5	male	75	3	123,79	8,51	n.a.	n.a.
no / yes	6	male	79	2	81,35	2,28	78	n.a.
no / yes	14	male	84	3	n.a.	n.a.	n.a.	n.a.
no / yes	17	female	75	6	50,40	2,50	91	n.a.
no / yes	27	male	71	4	n.a.	n.a.	n.a.	n.a.
no / yes	29	male	78	5	190,10	n.a.	n.a.	n.a.
no / yes	39	male	61	5	105,22	3,12	66	Bisoprolol, Ramipril, Amlodipine
no / yes	59	male	75	4	203,37	26,02	26	n.a.
no / yes	62	male	58	3	150,31	17,51	44	n.a.
yes / no	9	female	85	4	70,74	n.a.	68	n.a.
yes / no	10	female	84	3	45,98	3,12	8	Torsemide
yes / no	20	male	90	6	97,26	7,34	67	n.a.
yes / yes	1	male	80	4	267,03	n.a.	19	Ramipril, Bisoprolol, Torsemide
yes / yes	2	female	85	5	106,10	7,00	30	Ramipril, Metoprolol, Torsemide
yes / yes	11	male	83	3	n.a.	n.a.	n.a.	HCT, Bioprolol, Candesartan, Amlodipine
yes / yes	12	male	86	5	123,79	n.a.	47	Bisoprolol, Torsemide
yes / yes	13	male	71	7	452,71	15,23	10	n.a.
yes / yes	15	male	81	3	154,74	4,49	36	Ramipril, Torsemide
yes / yes	16	female	88	6	n.a.	n.a.	n.a.	n.a.
yes / yes	19	female	74	6	97,26	n.a.	50	n.a.
yes / yes	21	male	87	5	114,95	n.a.	50	n.a.
yes / yes	22	male	74	4	185,68	n.a.	30	n.a.
yes / yes	25	male	83	1	548,20	n.a.	8	n.a.
yes / yes	26	female	50	5	106,10	10,51	55	n.a.
yes / yes	30	male	90	4	n.a.	n.a.	n.a.	n.a.
yes / yes	32	male	88	3	389,05	44,86	11	n.a.
yes / yes	41	female	89	6	150,31	n.a.	26	n.a.
yes / yes	45	female	87	6	102,57	3,34	43	Metoprolol, Ramipril, Torsemide
yes / yes	48	female	84	5	61,89	n.a.	81	n.a.
yes / yes	49	male	89	4	97,26	8,00	60	Candesartan, Bisoprolol
yes / yes	50	male	48	4	62,78	n.a.	111	n.a.
yes / yes	52	female	72	2	70,74	2,33	74	n.a.
yes / yes	53	female	83	2	92,84	5,15	49	n.a.
yes / yes	56	male	84	4	247,58	13,17	20	Metoprolol
yes / yes	61	male	51	2	n.a.	n.a.	n.a.	n.a.

Legend: AKI: acute kidney injury, BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate (ml/min/1,73m²), n.a.: not available, y: yes, n: no, HCT: Hydrochlorothiazide

Table S3. Definition of acute kidney injury in 32 cases

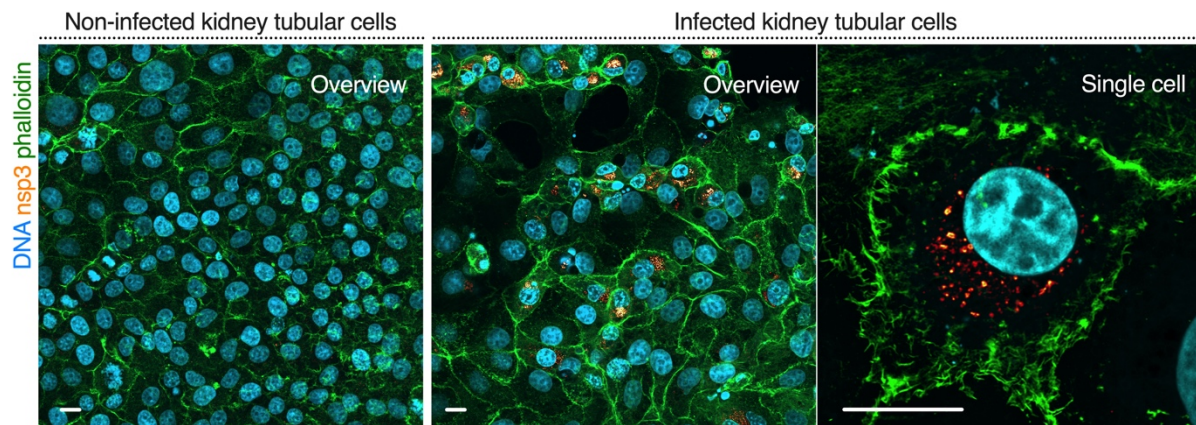| Patient no.	Comorbidities detail	AKI (history / bloodwork)	AKI term
1	Emphysema, aHT, AF, Dementia	history	acute kidney failure
2	AF, CI, CKD, Hypothyroidism, follicular Lymphoma	bloodwork	
5	aHT, Diabetes, Steatohepatitis	history	acute kidney failure, need for dialysis
6	AF, Cardiomyopathy	bloodwork	
11	aHT, MI, Cardiomyopathy	history	acute kidney failure
12	aHT, AF, MGN, Dementia, Monoclonal Gammopathy	history	acute kidney failure
13	Asbestosis, aHT, MI, AV-Block III, Stroke, Diabetes, Prostate Cancer	bloodwork	
14	aHT, CHD, Diabetes	history	decreasing diuresis, need for dialysis
15	CHD, Myelofibrosis, Steatohepatitis	history	acute kidney failure
16	Emphysema, aHT, Demetia, Stroke, Autoimmune hepatitis, Thyroid Cancer	history	acute kidney failure
17	COPD, MI, Cardiomyopathy, VHD, PAOD, CKD	bloodwork	
19	COPD, aHT, CHD, CI, Dementia, Diabetes, Steatohepatitis	history	decrease in GFR
21	Emphysema, Cardiomyopathy, CKD, Dementia, PD	history	acute kidney failure
22	Emphysema, MI, CKD, Dementia	history	acute kidney failure
25	Cardiomyopathy	history	acute kidney failure
26	COPD, aHT, chronic Pancreatitis, Gastric Ulcer, Cirrhosis	history	acute kidney failure
27	aHT, AF, CKD, Diabetes	bloodwork	
29	aHT, MI, AV-Block I, CKD, RA	history	acute kidney failure
30	CHD, AF, CKD, MDS	history	acute kidney failure
32	Emphysema, CHD, CKD	history	acute kidney failure
39	PF, aHT, Cardiomyopathy, Diabetes, Sarcoidosis	bloodwork	
41	aHT, Cardiomyopathy, CKD, angiopathic Encephalopathy, Diabetes, Hypothyroidism	history	acute kidney failure
45	Emphysema, AF, Cardiomyopathy, Stroke, Diabetes, Myelofibrosis	bloodwork	
48	aHT, CKD, PD, ALS, Breast Cancer	history	acute kidney failure
49	aHT, PAOD, Stroke, Sarcoidosis	history	acute kidney failure
50	CHD, Cardiomyopathy, AML, Steatohepatitis	bloodwork	
52	Diabetes, Myelofibrosis	bloodwork	
53	COPD, NHL	history	acute kidney failure
56	CHD, Cardiomyopathy, CKD, Diabetes	history	acute kidney failure
59	Emphysema, aHT, AF, CKD	history	acute kidney failure, need for dialysis
61	Epilepsy, Cirrhosis	history	acute kidney failure
62	Dementia, Obesity, Trisomy 21	bloodwork	

Legend: aHT: arterial hypertension, AF: atrial fibrillation, CI: cardiac insufficiency, CKD: chronic kidney disease, MI: myocardial infarction, MGN: membranous glomerulonephritis, CHD: coronary heart disease, COPD: chronic obstructive pulmonary disease, VHD: valvular heart disease, PAOD: peripheral arterial occlusive disease, PD: Parkinson's disease, RA: rheumatoid arthritis, MDS: myelodysplastic syndrome, PF: pulmonary fibrosis, ALS: amyotrophic lateral sclerosis, AML: acute myeloid leukemia, NHL: non-Hodgkin's lymphoma

Fig. S1. Renal function dynamics in selected patients with SARS-CoV-2 renal tropism

Legend: Three selected patients were followed up with serial bloodwork for a maximum of 7 days prior to COVID-19 diagnosis until death. All cases were SARS-CoV-2 PCR positive in the respiratory tract and the kidney. All three cases developed AKI in the context of COVID-19 infection. Case 45 suffered a first episode of AKI after orthopaedic surgery. Following a brief recovery period, the patient developed AKI and was diagnosed with COVID-19 before death.

Fig. S2. Viral replication detection in non-human primate kidney epithelial cells after infection with patient-derived SARS-CoV-2

Legend: Expression of non-structural protein 3 (nsp3) as an indicator of replicating patient-derived SARS-CoV-2 in kidney tubular epithelial cells from non-human primates (Vero cells). Scale bar 10μm

Supplementary References:

1. Puelles VG, et al. "Multi-organ and renal tropism of SARS-CoV-2". N Engl J Med 2020 DOI: 10.1056/NEJMc2011400.
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