

THE LANCET

Respiratory Medicine

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Supplementary Appendix:

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Supplementary Methods:

Fluorescence imaging of DNA and RNA content. In order to assess relative distribution of dsDNA versus RNA in tissue sections, DRAQ5 and SYTO RNASelect staining was performed on adjacent tissue sections. DRAQ5 (DR51000, Biostatus, Ltd.) is a far-red fluorescent, small molecule anthraquinone derivative that binds stoichiometrically and with high affinity to dsDNA in live or fixed cells, with negligible binding to RNA. SYTO RNASelect (S32703, Thermo Fisher Scientific) is a green-fluorescent cell-permeant nucleic acid stain that selectively binds RNA. Unstained FFPE sections were deparaffinized in xylene (196279, Fisher Scientific), and then rehydrated in a series of ethanol/PBS dilutions. Hydrated tissue sections were then stained with SYTO RNASelect (50 μ M) for 10 minutes, rinsed in deionized water, followed by DRAQ5 (50 μ M) for 10 minutes, and a final rinse in deionized water. Stained sections were then lightly air-dried then immediately cleared with xylene, and mounted with Canada balsam mounting medium (IS5015, Aldon Corporation). Slides were scanned for fluorescence in a Zeiss AxioScan Z1 whole slide scanner at \times 20 magnification, using 488 nm excitation for SYTO RNASelect, and 639 nm excitation for DRAQ5.

Supplementary Tables:

Table S1: Patient Medical History

Patient (age)	Medical History	Home Medications	Additional Hospital Treatment	Reported Days Ill Prior to Admission	Time on Ventilator	Time from Admission to Death
Patient 1 (44)	HTN	Allopurinol	Azithromycin	3 days	6 days	8 days
	DM2	Amlodipine	Cefepime			
	CKD 3	Aspirin 325mg	Vancomycin			
		Atorvastatin				
		Hydrochlorothiazide				
		Insulin glargine 44U				
		Lisinopril				
Patient 2 (44)	DM2	Lisinopril	Azithromycin	6 days	2 days	5 days
	HTN	Glyburide	Cefepime			
	CHF	Metformin	Vancomycin			
		Metronidazole				
		Fluticasone				
		Aspirin 81mg				
Patient 3 (63)	HTN	Amlodipin	Azithromycin	7 days	25 days	25 days
	Thyroidectomy	Aspirin 81mg	Cefepime			
		Cyclobenzapine	Vancomycin			
		Levothyroxine	Dexamethasone			
		Lisinopril-hydrochlorothiazide				
		Oxybutynin				
Patient 4 (76)	HTN	Amlodipine	Azithromycin	3 days	6 days	6 days

	DM2 Rheumatoid arthritis	Losartan Duloxetine Insulin Methotrexate	Cefepime Vancomycin			
Patient 5 (68)	DM2	N/A	Dextrose Hydroxychloroquine Insulin Pantoprazole	1 day	9 days	9 days
Patient 6 (78)	N/A	N/A	Insulin Phenylephrine	7 days	0 days	0 days
Patient 7 (53)	HTN Polymyositis OSA	Alendronate Amlodipine Coreg Citalopram Ergocalciferol Losartan Methotrexate Omeprazole Prednisone Pregabalin Trazodone	Enoxaparin Cefepime Levophed	0 days	1 day	1 day
Patient 8 (60)	COPD HTN Atrial fibrillation Post-intubation cardiac arrest	Aspirin Brimonidine Dorzolamide Ergocalciferol	Furosemide Acetaminophen Albuterol Amiodarone	3 days	14 days	14 days

		Flucanazole Ketorolac Lisinopril Metoprolol Pantoprazole Pravastatin	Aspirin Fentanyl Enoxaparin Ipratropium Hydroxychloroquine			
Patient 9 (66)	HTN	N/A	Azithromycin Ceftriaxone Enoxaparin Pantoprazole Propofol Norephinephrine Cisatracurium	2 days	12 days	12 days
Patient 10 (78)	ESRD DM2	N/A	Vancomycin Clopidogrel Pantoprazole	2 days	2 days	2 days

*HTN = hypertension, DM2 = Type II diabetes mellitus, CKD (2/3) = chronic kidney disease (stage 2/3), OSA – obstructive sleep apnea, COPD = chronic obstructive pulmonary disease, ESRD = end-stage renal disease, N/A = unable to obtain prior medication history

Table S2: Patient Antemortem Laboratory Values

	<i>N</i> 1.8-8.00 (10 ³ /uL)	<i>L</i> 1.1-5.0 (10 ³ /uL)	<i>Pl</i> 130-400 (10 ³ /uL)	<i>PT/PTT</i> (10-13/24-37s)	<i>D-Dimer</i> (<250ng/mL)	<i>Fibrinogen</i> 200-600 (mg/dL)	<i>BNP</i> (<100pg/mL)	<i>Trop I</i> (<=0.08ng/mL)	<i>Glu (65-99mg/dL)</i>	<i>AST</i> (<45U/L)	<i>Cr</i> 0.7-1.4 (mg/dL)
Patient 1	20.20	0.4	288	13.8/38.4	1110	>700	628 (7 days before death)	up to 0.16 (7 days before death)	294	500	9.09
Patient 2	16.3	1.0	208	Not done	Not done	Not done	42 (3 days before death)	4.19 (3 days before death)	235	37	3.28
Patient 3	12.30	0.92	309	Not done	Not done	Not done	76 (13 days before death)	76 (12 days before death)	392	17	6.48
Patient 4	10.90	0.90	113	13.9 (no PTT)	249 (6 days before death)	Not done	76 (6 days before death)	<0.02 (6 days before death)	347	56	3.01
Patient 5	19.3	1.03	80	Not done	Not done	Not done	52 (9 days before death)	0.6 (9 days before death)	217	24	9.74
Patient 6	6.3	0.81	134	14.0/29.8	8197	Not done	378	0.03	575	39	1.26
Patient 7	4.0	2.70	128	11.5/29.4	47559	Not done	88	<0.02	121	357	0.43
Patient 8	6.8	0.4	290	9.9/24.3 (12 days before death)	*2.06 (ref: 0.19 - 0.5 ug/mL) *outside lab	Not done	1466 (4 days before death)	0.2 (4 days before death)	140	21	0.74
Patient 9	22.32	0.7	531	Not done	Not done	Not done	106 (12 days before death)	<0.02 (12 days before death)	204.	18	4.24
Patient 10	14.38	0.56	153	12.1/ND	10020 (2 days before death)	Not done	939 (2 days before death)	61.53	145	1771	10.2

N = neutrophils, *L* = lymphocytes, *P* = platelets, **Glu** = glucose, ND = not done; all values from within 24 hours of death unless otherwise noted

Table S3: BMI and Organ Weights

Patient #	BMI (kg/m ²)	Heart (g)* nl: 300 (270-360)	Left Lung (g) nl: 375 (325-480) ¹	Right Lung (g) nl: 450 (360-570) ¹
1	37.5	N/A	1050	1030
2	47.76	420	750	800
3	56.97	550	680	940
4	38.15	540	860	860
5	35.75	480	950	1140
6	33.1	370	660	670
7	28.5	420	300	305
8	44.8	450	890	1040
9	28.3	340	660	550
10	34.3	600	500	890

*Normal heart weight varies by gender and body length, and full normal values can be referenced in data presented in Finkbeiner et al., 2009.¹

N/A = not applicable, heart not removed.

Supplementary Figures:

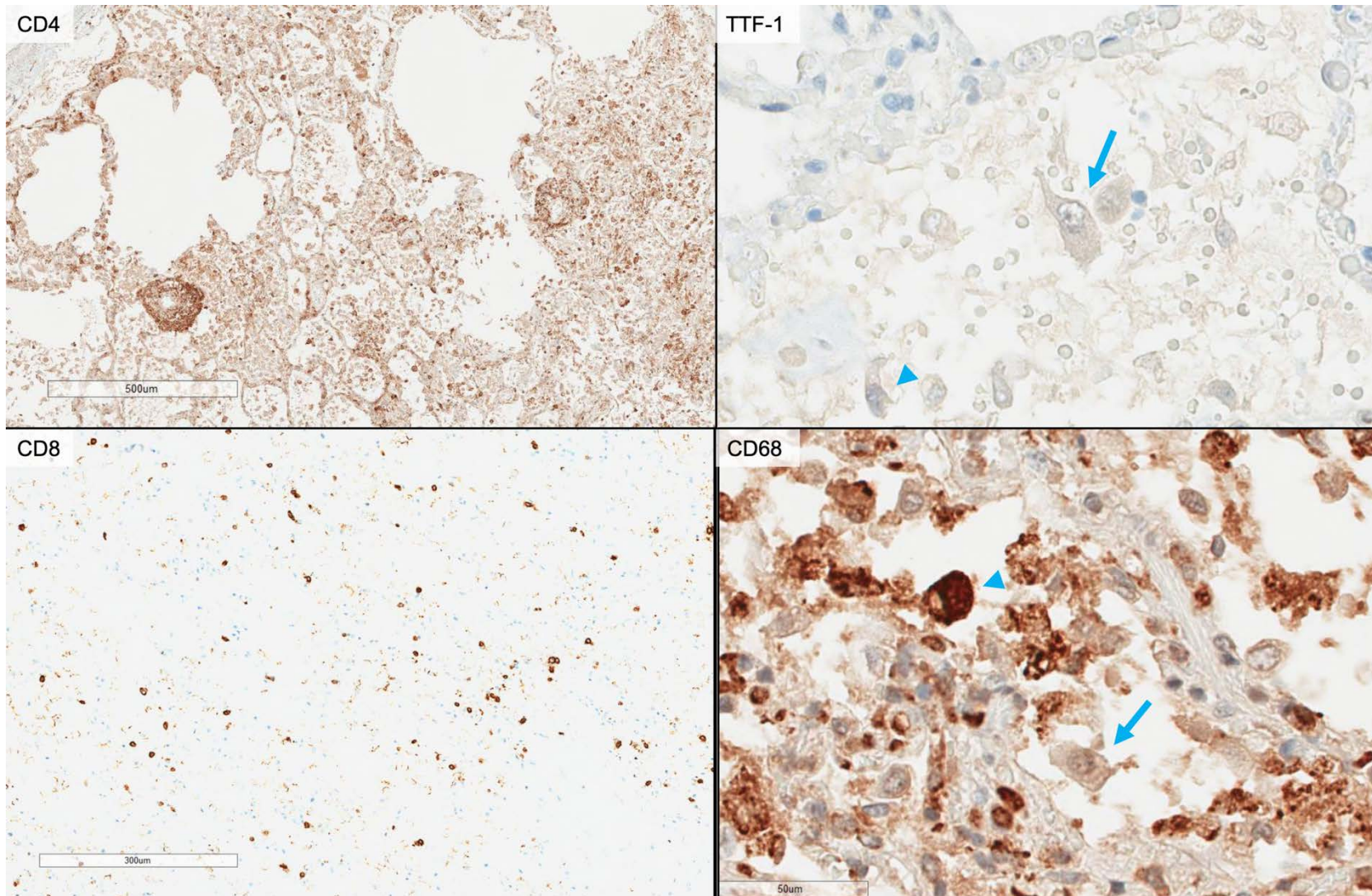


FIGURE S1: Immunostains of pulmonary tissue. CD4 and CD8 immunostains highlight lymphocytes, CD4+ cells being visibly more present than CD8+ cells around small airways. A TTF-1 stain shows faint staining of an enlarged, atypical pneumocyte within an alveolar space (blue arrow), with faint staining of additional desquamated pneumocytes (blue arrowhead). A CD68 immunostain highlights alveolar macrophage (blue arrowhead), with background staining of an enlarged pneumocyte (blue arrow) similar to that seen in attached alveolar pneumocytes.

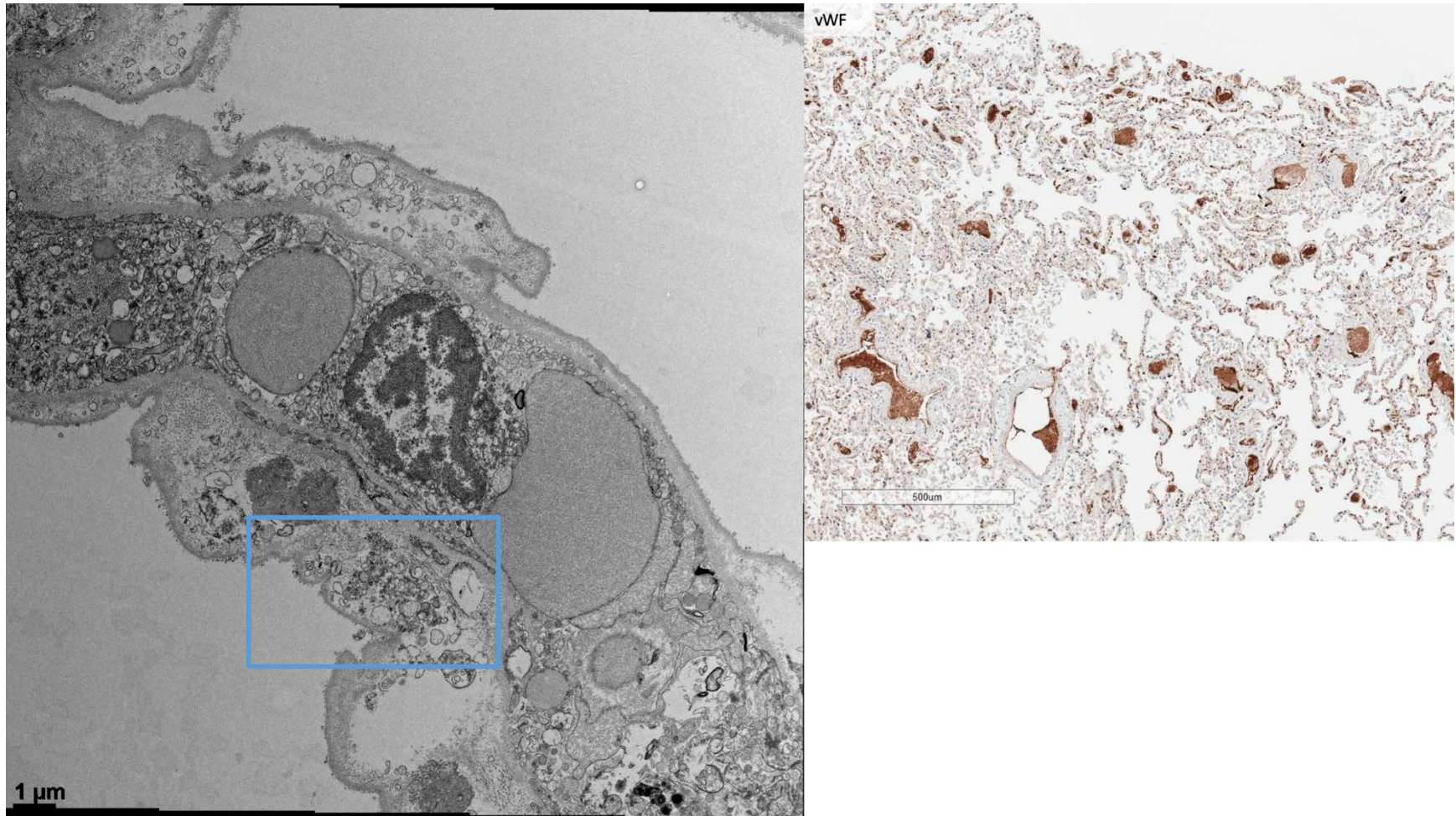


FIGURE S2: A) Electron microscopy showing an infected pneumocyte. The region in the blue box is presented in **Figure 4**. A Von Willebrand Factor immunostain is additionally shown, highlighting small arteries and veins with accumulated platelets and megakaryocytes.

Supplemental References:

- 1) Finkbeiner WE, Connolly AJ, Ursell PC, Davis RL. Autopsy Pathology: A Manual and Atlas E-Book. Elsevier Health Sciences, 2009.