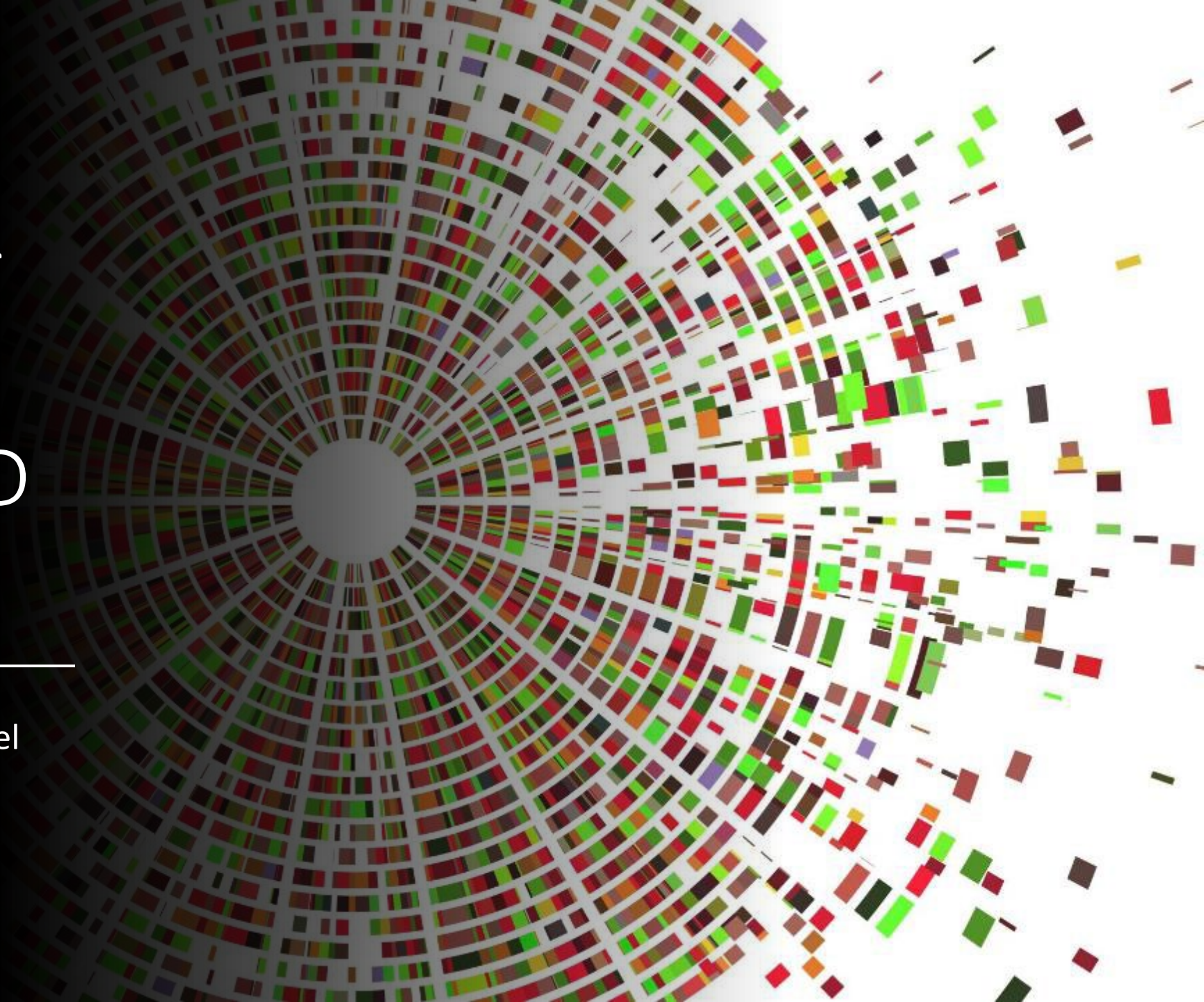
Emergence of SARS-CoV-2 and the COVID Pandemic

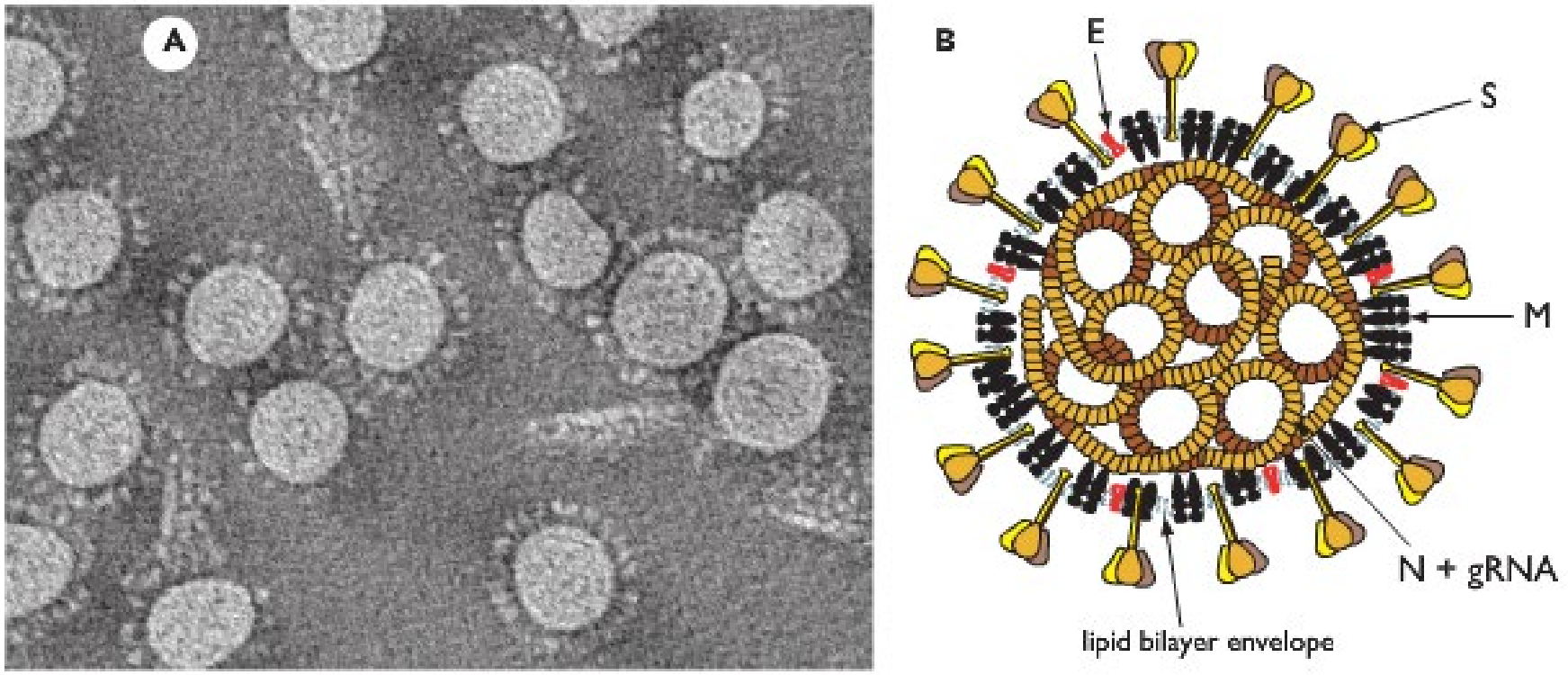
Debra Bramblett PhD and Michael Woods PhD



Learning Objectives

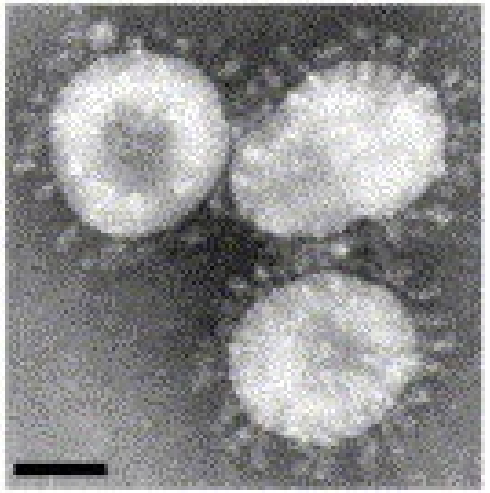
- By the end of this module, you should be able to:
- Describe the common molecular characteristics of Coronaviruses
- Compare the emergence of SARS-CoV2 to SARS-CoV and MERS-CoV
- Explain SARS-CoV replication and the role of the viral S protein and its cognate receptor there in.

Molecular Virology of Coronavirus

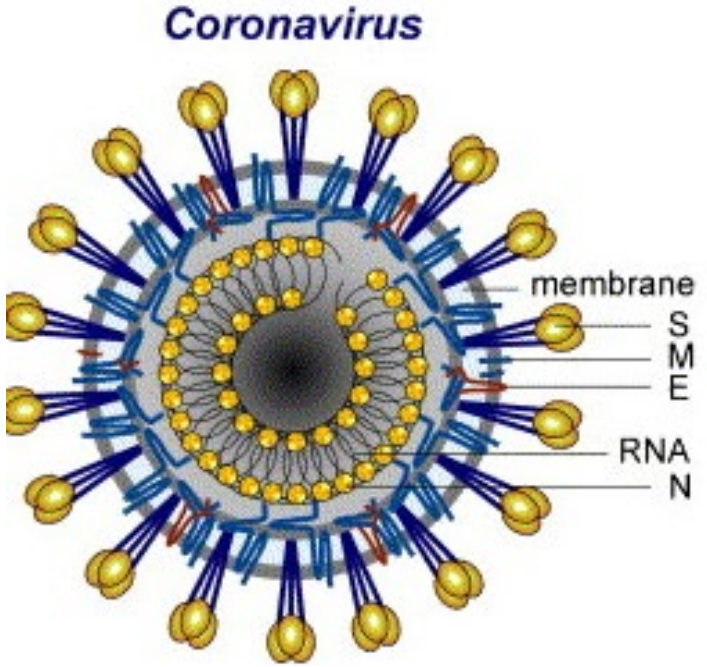


Fields Virology Sixth Edition Volume 1 Chapter 28 Figure 28.2

Coronavirus Structure

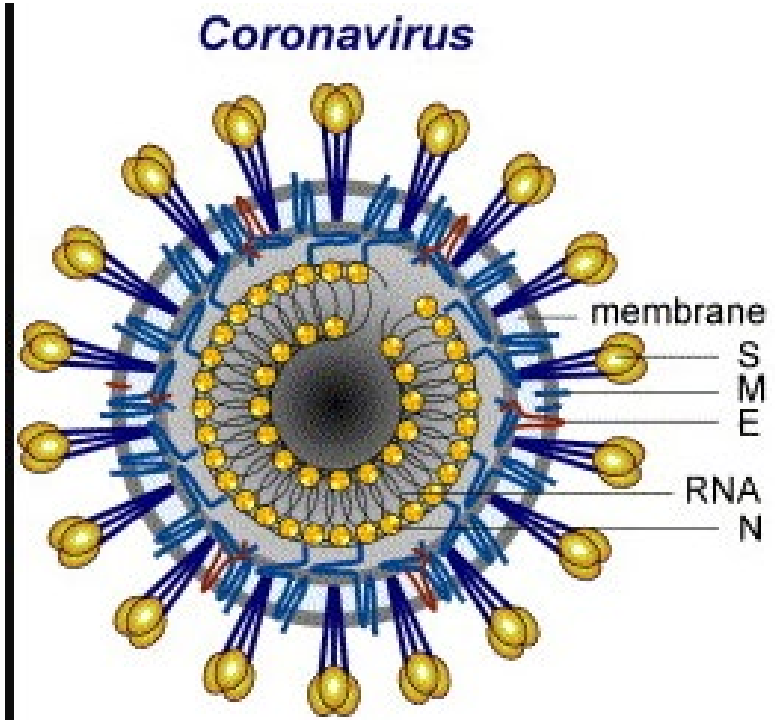


- Spherical virion (125nm) and a helical nucleocapsid
- Most notable common feature is a fringe of widely spaced, club-shaped spikes that project from the surface.
  - Described as giving the viral particle a solar corona
- Genome composition: ss (+) RNA of ~30kb
- Four main structural proteins:
  - S – spike protein, mediates cell entry
  - M- membrane protein
  - E- Envelope
  - N-nucleocapsid



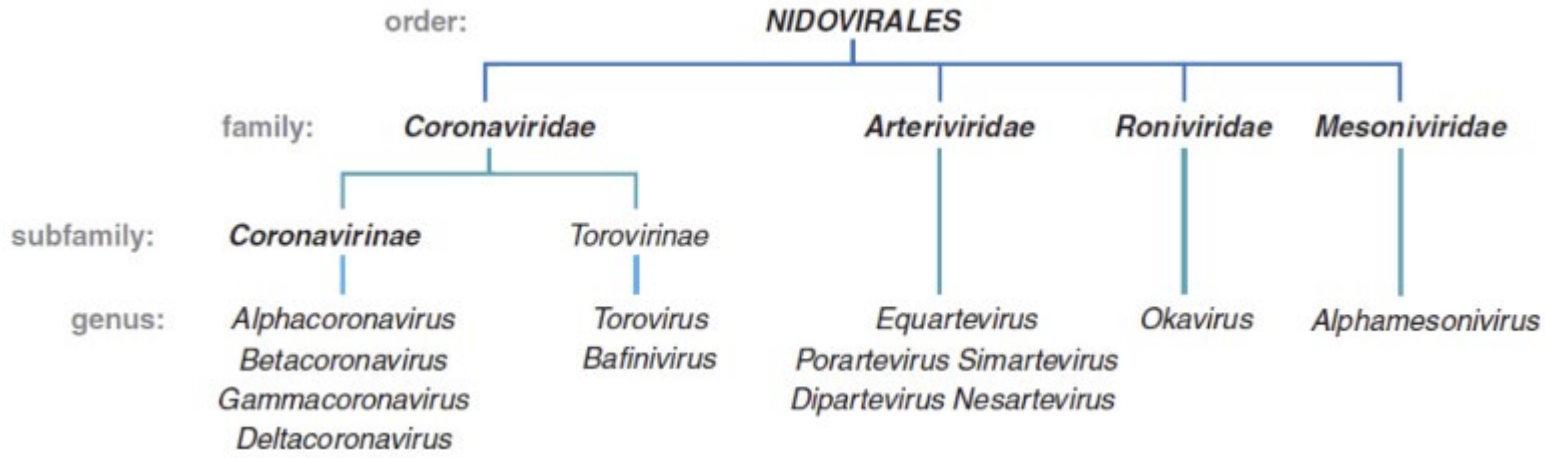
Coronavirus History

- Isolated in 1930's as causative agents of
  - Bronchitis in chickens
  - Gastroenteritis in pigs
  - Severe hepatitis and neurological diseases in mice
- Grouped together in the 1960s based on some common physical characteristics
- For about 40 years the *Coronavirus* studies focused on the economically significant respiratory and gastrointestinal diseases in domestic animals
  - **and it was recognized that Human coronavirus (HuCov) is responsible for a large fraction of the common cold.**



Coronavirus (CoV) Genogroups

- *Coronaviridae* is a genetically diverse family currently groups into four genogroups (alpha, beta, gamma and delta).
  - The replication of viral genomic RNA is inherently error –prone leading to the production of many species.
  - MERS and SARS are both part of the **Beta genogroup**
- Most CoV strains have <u>restricted host range</u> but the zoonotic CoVs have the ability to jump into new host species by acquiring mutations



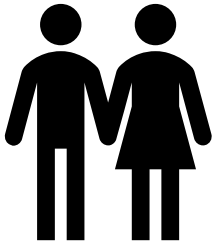
Emergence of Pathogenic zoonotic outbreaks of beta Coronaviruses

SARS-CoV

- Emergence of the **severe acute respiratory syndrome (SARS)** caused by SARS-CoV occurred in 2002
- **Illness:** High fever, chills and body aches; diarrhea in 10-20%; after 2-7 days dry, nonproductive cough leading to hypoxia in 10% of patients.
  - A total of **8,098** probable SARS cases were reported to the World Health Organization (WHO) from 29 countries, November 2002-July 2003
  - **774 died** (**case fatality rate 9.5%)** Only 8 cases of infection in the United states confirmed and 1 death.
- SARS related coronaviruses were detected in a variety of bats
- Transmission to humans occurred through Palm civets (a secondary animal reservoir) sold at wet markets in the Guangdon province

Horseshoe bat

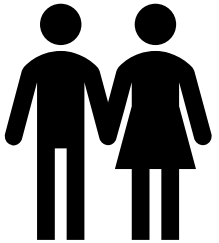
Palm civet



MERS-CoV

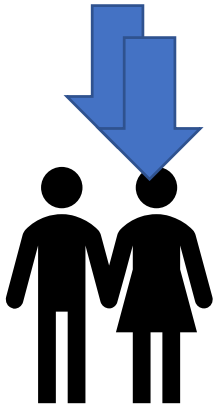
- In 2012 **Middle East Respiratory Syndrome** was first reported in Saudi Arabia, which spread to 27 other countries including the United States.
- This syndrome was caused by MERS-CoV .
- **Illness**: Fever, cough, shortness of breath
  - **2494** confirmed cases, **858** deaths, approximately **35% case fatality** rate
- Secondary animal reservoir is the **dromedary camel**
  - **Dromedary camels** in Africa and the Middle East appear to be the only host responsible for human infection
  - bats and alpacas can serve as potential reservoirs for MERS-CoV
- Human to Human transmission is possible, only by close unprotected contact.

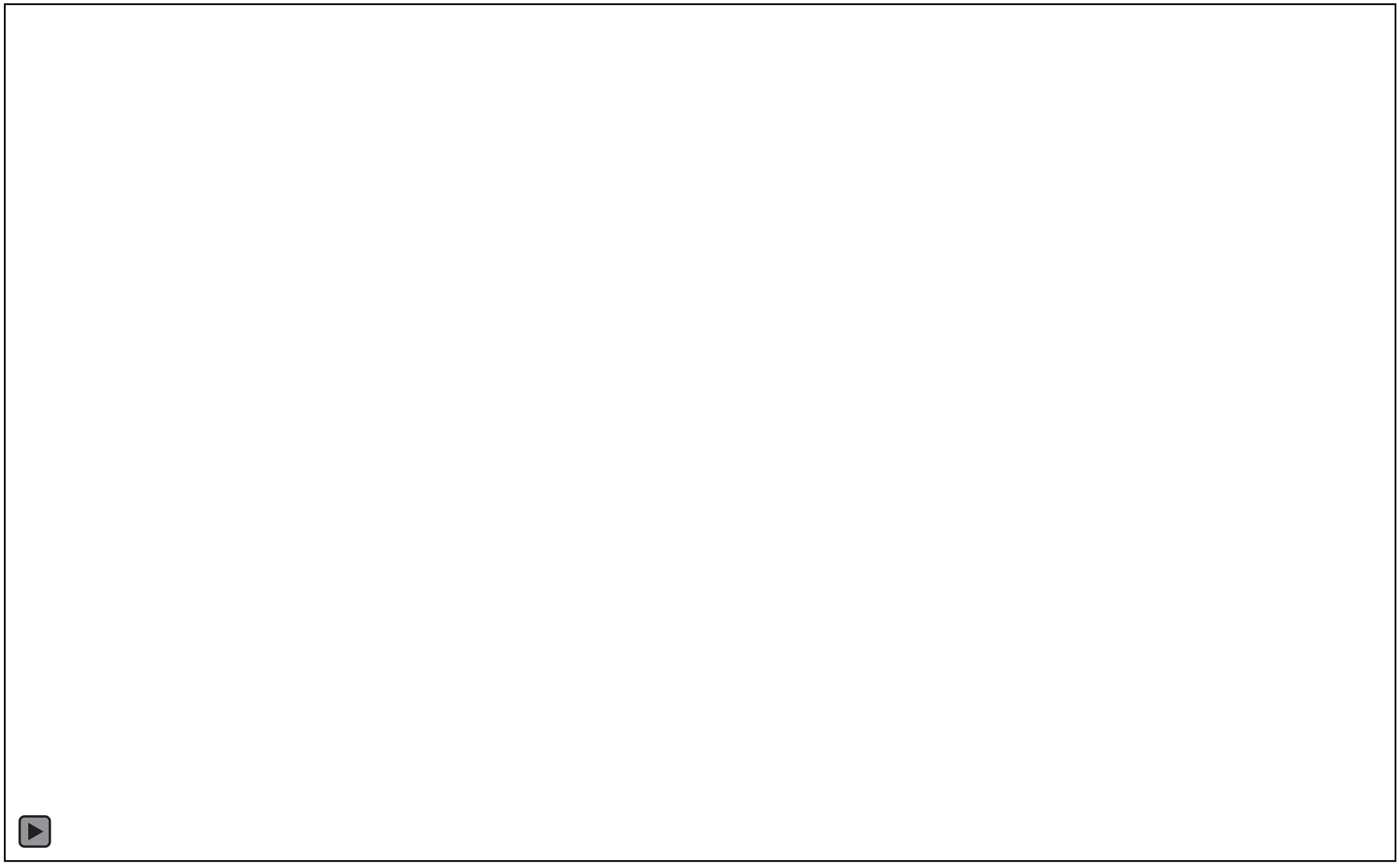
Taphozous perforatus bats from Saudi Arabia



SARS-CoV2

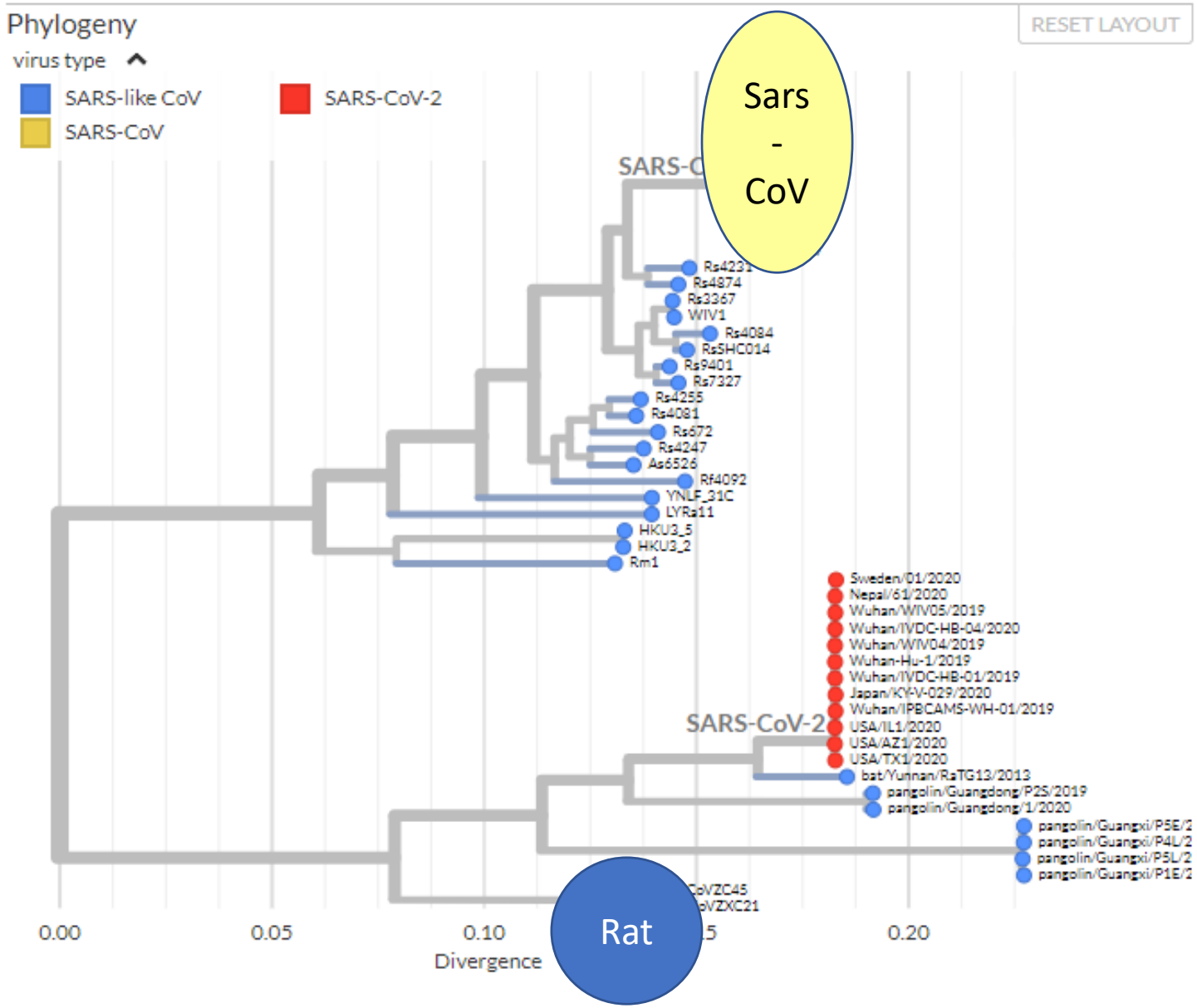
- In December 2019 a novel Coronavirus was identified in Wuhan China
  - Initially called 2019-nCOV and now called SARS-COV2
  - 45 initial cases where traced to people who visited wet markets in Wuhan or had close contact with a ill family member.
- Genetic sequence analysis supports that the most possible transmission chain is: **from bats to pangolins to humans**
- Spread quickly across the globe and as of 6/21/2020 :
  - 8,926,050 cases globally & 467,611 deaths
  - 2,278,588 cases in the US & 119,959 deaths
- As the virus has spread it has acquired mutations due to the error prone nature of the viral polymerase
- The random mutations can help to track the spread of the pathogen and learn about its transmission routes and dynamics.

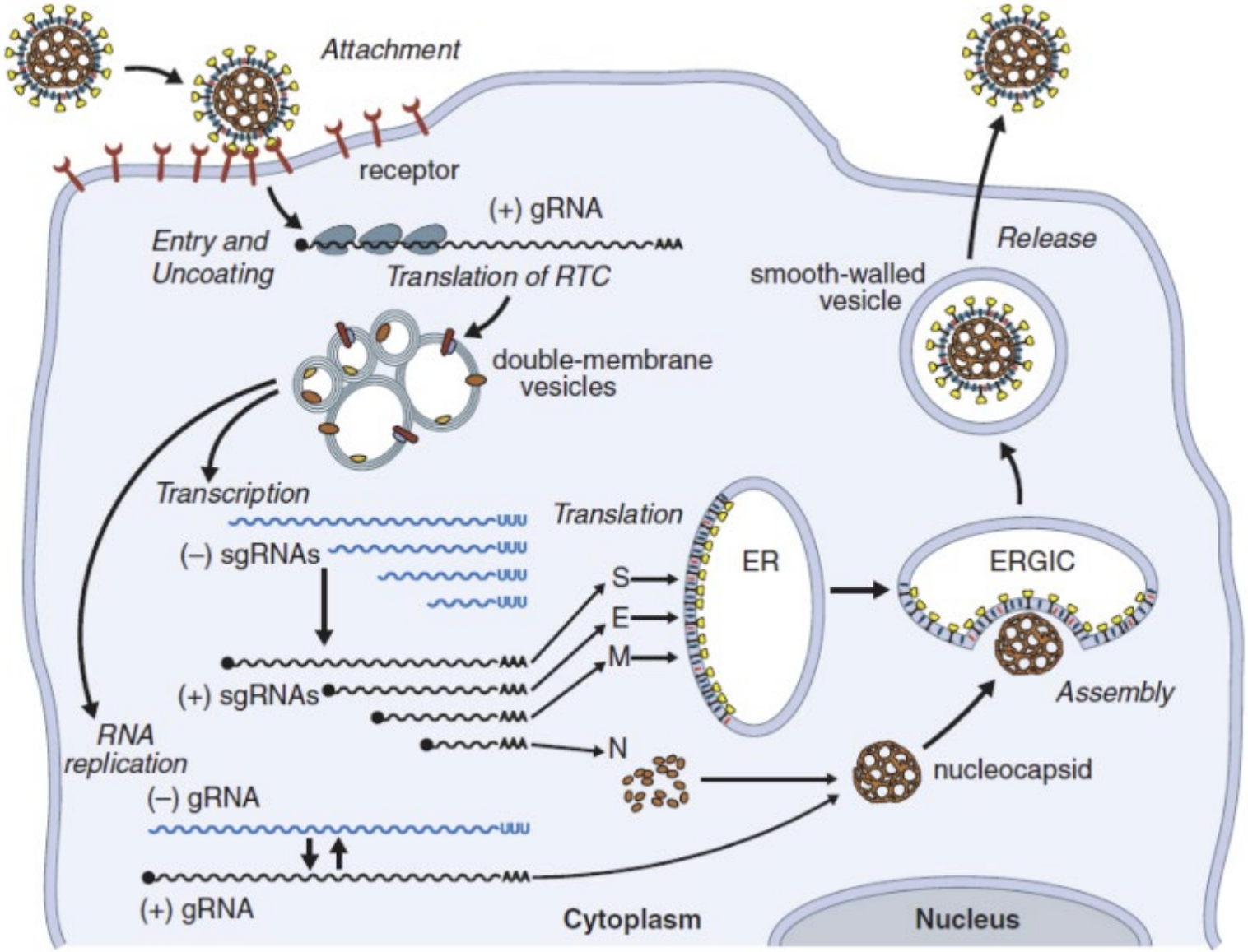




https://nextstrain.org/ncov/global?animate=2019-12-10,2020-05-30,0,0,30000&p=full

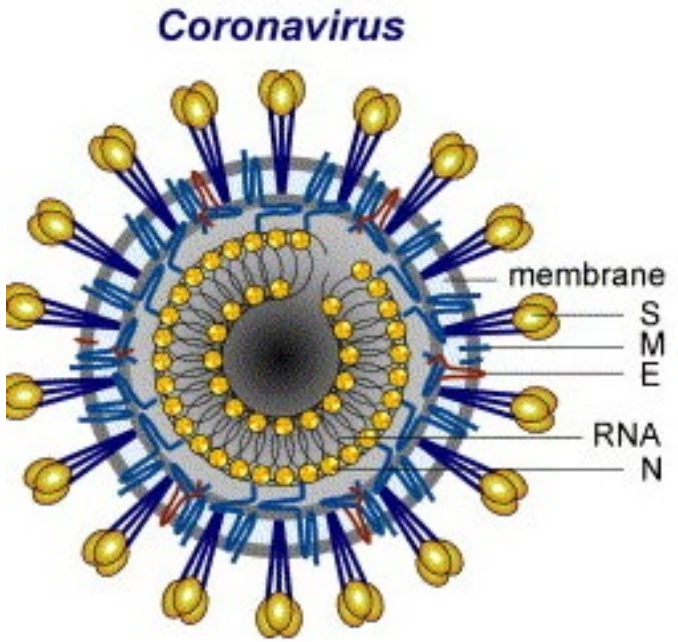
- Full genomic sequences from patients suggested that SARS-Cov-2 have 70% amino acid homology to SARS-CoV and 96% homology to a known bat coV



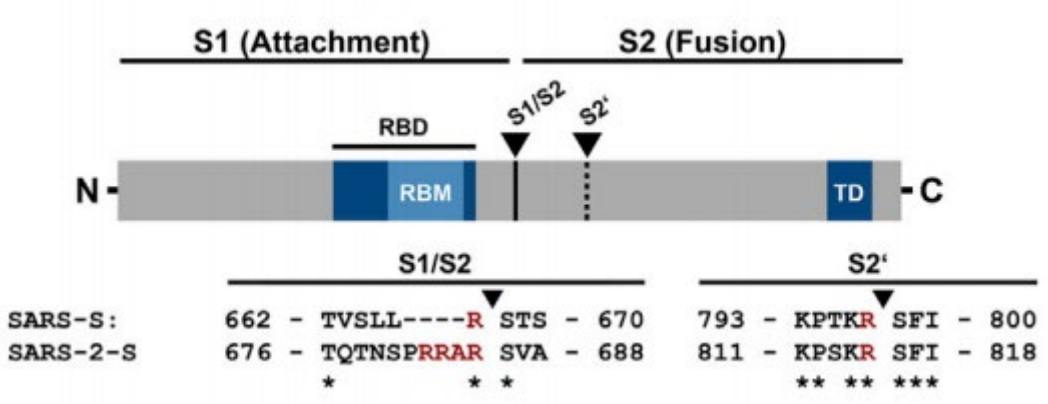

Attachment
receptor
(+) gRNA
Entry and Uncoating
Translation of RTC
double-membrane vesicles
Transcription
(–) sgRNAs
(+) sgRNAs
RNA replication
(–) gRNA
(+) gRNA
Translation
S
E
M
N
ER
ERGIC
Assembly
nucleocapsid
smooth-walled vesicle
Release
Cytoplasm
Nucleus

The viral surface protein S and its cognate receptor

- Spike glycoprotein S of all coronaviruses facilitates entry into the cell.
- S protein consist of a receptor-binding subunit S1 and a membrane-fusion subunit S2
- SARS-CoV S and SARS-CoV19 S bind to angiotensin-converting enzyme 2 (ACE2) as the entry receptor.
- S must be “primed” by a cellular protease to allow fusion of the viral envelope with the host cell envelope



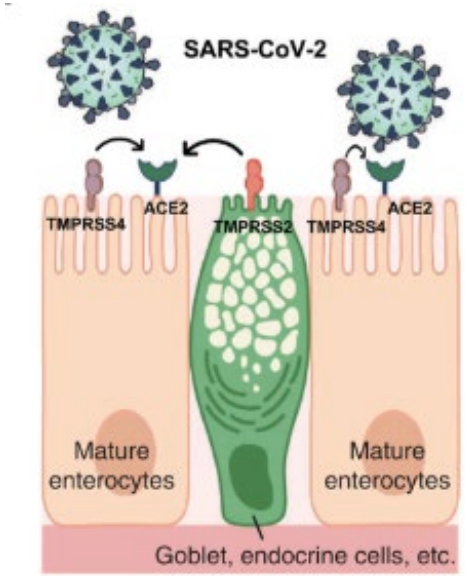
Processing of SARS-COV2 Receptor S

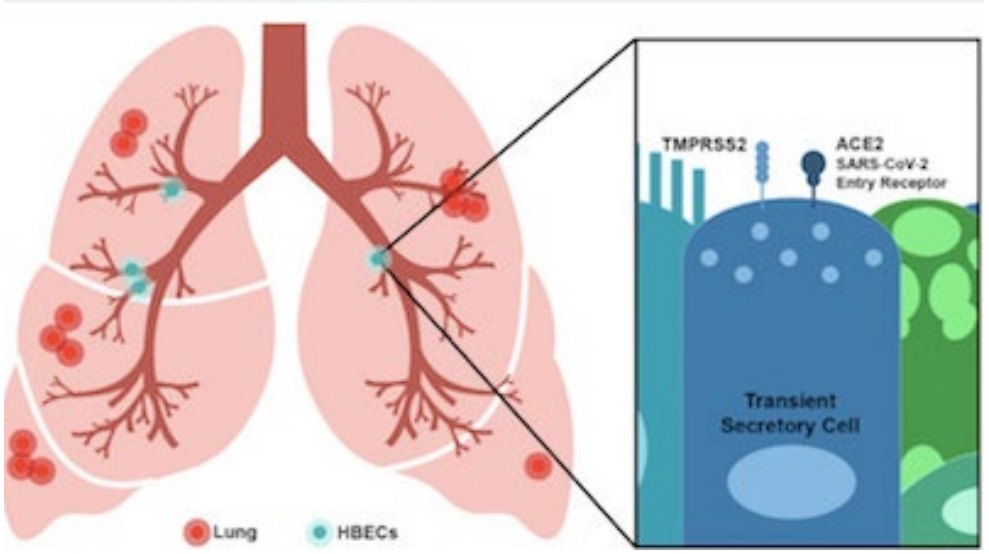


- S1 binds to a cellular receptor while S2 facilitates fusion of the viral membrane with a cellular membrane protein (Hoffman 2020).
- S is processed by a plasma membrane–associated type II transmembrane serine protease, TMPRSS2, before membrane fusion.

The SARS-COV2 Receptor and Targets

- cognate receptor, angiotensin-converting enzyme 2 (ACE2) similar to the first SARS-CoV virus.
- Uses TMPRPSS2 to enter Lung cell targets and to enter Enterocyte targets
- TMPRSS2 is blocked by a clinically proven protease inhibitor
  - camostat mesylate, which is active against TMPRSS2 (Kawase et al., 2012), blocks SARS-2-S-driven entry
- Evidence suggests that Convalescent SARS patients exhibit a neutralizing antibody directed against the S protein and it inhibits viral entry.
- Therefore anti-S antibodies raised against SARS-CoV2 S protein during infection or vaccination might be protective.





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- Mohd et al. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) origin and animal reservoir. Virology Journal (2016) 13:87 DOI 10.1186/s12985-016-0544-0
- Letko M et al **Adaptive Evolution of MERS-CoV to Species Variation in DPP4.** Cell Rep. 2018 Aug 14;24(7):1730-1737. doi: 10.1016/j.celrep.2018.07.045.
- Dong, R. et al Analysis of the Hosts and Transmission Paths of SARS-CoV-2 in the COVID-19 Outbreak Genes 2020, 11(6), 637; https://doi.org/10.3390/genes11060637
- COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU) https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6
- Fehr AR and Perman S. Methods Mol Biol 2015; 1282 1-23 doi:10.1007/978-1-4939-2438-7_1
- Hoffmann et al , SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181, 271–280.e8 (2020).
- Hoffmann et al., 2020, Molecular Cell 78, 779–784 May 21, 2020 https://doi.org/10.1016/j.molcel.2020.04.022ll
- Lukassen et al , 2020, EMBO J (2020) 39: e105114 https://doi.org/10.15252/embj.20105114
- https://nextstrain.org/ncov/global?animate=2019-12-10,2020-05-30,0,0,30000&p=full
- Fields Virology Sixth Edition Volume 1 Chapter 28 Figure 28.2

Pathogenesis of COVID-19
Still, Much to be Learned

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Learning objectives

- By the end of this module, you should be able to:
  - Discuss the primary pathological features of severe COVID-19 in the lungs
  - List the sites of extrapulmonary involvement in COVID-19
  - Describe the role of ACE2 in the pathogenesis of COVID-19

SARS-CoV-2 causes COVID-19, a respiratory disease with numerous extrapulmonary complications

- Primary signs/symptoms include cough, fever and shortness of breath
- Clear association with underlying co-morbidities
- Need for mechanical ventilation associated with high mortality rate

Table 1. Baseline Characteristics of Patients With Coronavirus Disease 2019 at Presentation

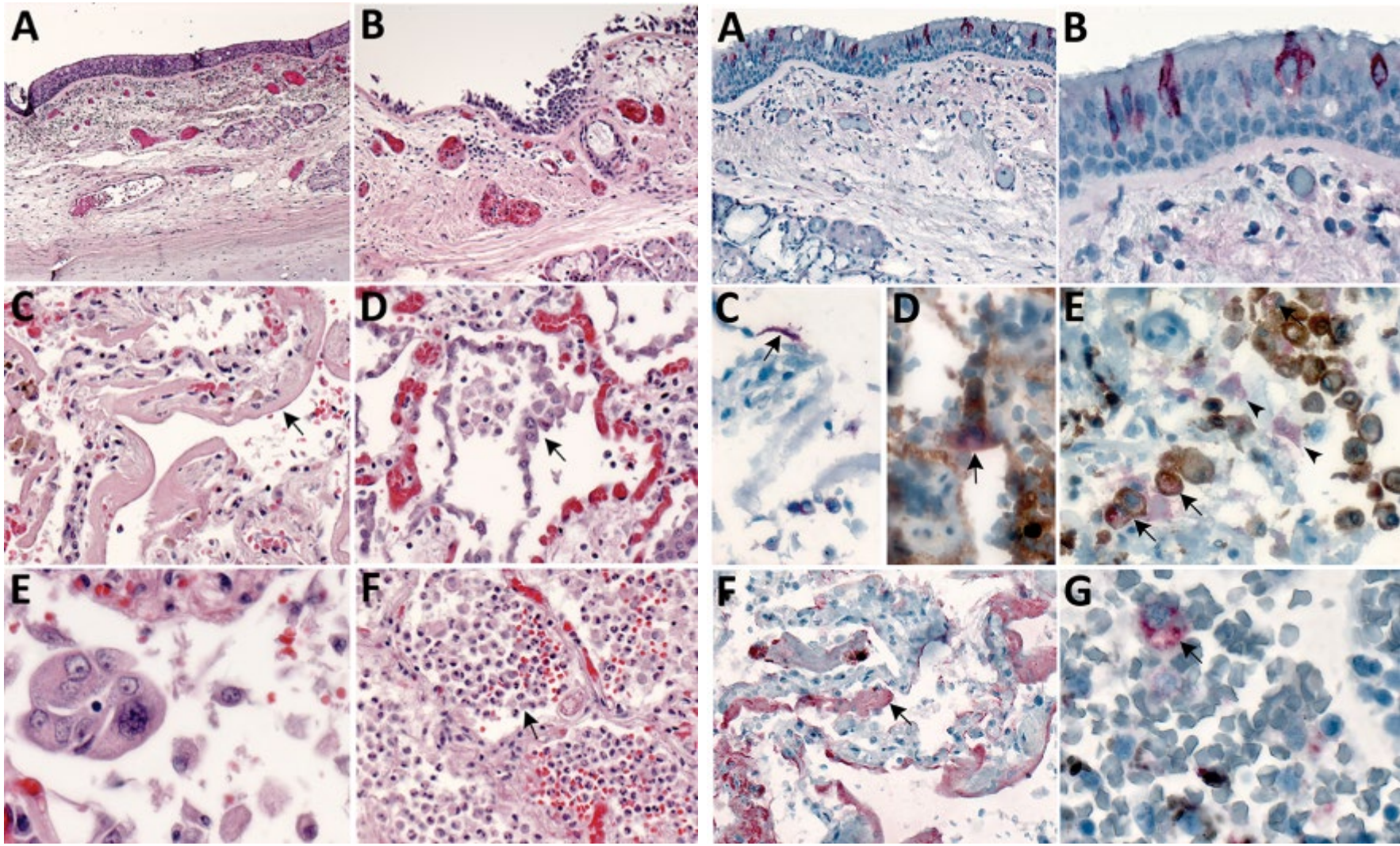
Characteristic	Patients, No. (%) All (N = 463)	Discharged home (n = 108)	Hospital admission (n = 355)	P value
Demographic characteristics				
Age, mean (SD), y	57.5 (16.8)	44.8 (15.1)	61.4 (15.4)	.005
Age >60 y	218 (47.1)	16 (14.8)	202 (56.9)	<.001
African American race	334 (72.1)	74 (68.5)	260 (73.2)	.34
Female	259 (55.9)	69 (63.9)	190 (53.5)	.06
Known exposure	124 (26.8)	31 (28.7)	93 (26.2)	.61
Prior emergency department visit in past 7 d	81 (17.5)	0	81 (22.8)	<.001
Symptoms				
Cough	347 (74.9)	87 (80.6)	260 (73.2)	.14
Nasal congestion	113 (24.5)	38 (35.2)	75 (21.2)	.003
Dyspnea	282 (60.9)	40 (37.0)	242 (68.2)	<.001
Fever	315 (68.0)	75 (69.4)	240 (67.6)	.72
Headache	74 (16.0)	28 (25.9)	46 (13.0)	.001
Myalgias	194 (42.0)	46 (42.6)	148 (41.8)	.89
Anorexia	100 (21.7)	10 (9.3)	90 (25.4)	<.001
Nausea	94 (20.4)	12 (11.1)	82 (23.2)	.007
Vomiting	53 (11.5)	8 (7.4)	45 (12.7)	.13
Diarrhea	100 (21.7)	14 (13.1)	86 (24.3)	.01
Comorbidities				
Asthma	73 (15.8)	20 (18.5)	53 (14.9)	.37
Chronic obstructive pulmonary disease	49 (10.6)	8 (7.4)	41 (11.6)	.22
Obstructive sleep apnea	57 (12.3)	8 (7.4)	49 (13.8)	.08
Diabetes	178 (38.4)	22 (20.4)	156 (43.4)	<.001
Hypertension	295 (63.7)	37 (34.3)	258 (72.7)	<.001
Coronary artery disease	59 (12.7)	3 (2.7)	56 (15.8)	<.001
Congestive heart failure	49 (10.6)	3 (2.8)	46 (13.3)	.001
Chronic kidney disease	182 (39.3)	21 (19.4)	161 (45.4)	<.001
End-stage renal disease	26 (5.6)	2 (1.9)	24 (6.8)	.06
Cancer	49 (10.6)	6 (5.6)	43 (12.3)	.05
Rheumatologic disease	10 (2.2)	1 (0.9)	9 (2.5)	.47
Solid organ transplant	11 (2.4)	3 (2.8)	8 (2.3)	.72
Body mass index, mean (SD)[a]	33.0 (8.5)	31.0 (7.3)	33.6 (8.7)	.01
Any obesity	262 (57.6)	52 (48.2)	210 (59.2)	.04
Severe obesity	89 (19.2)	14 (13.0)	75 (21.3)	.06
Tobacco use	160 (34.8)	23 (21.9)	137 (38.6)	.002
Vital signs, median (interquartile range)				
Lowest emergency department oxygen saturation as measured by pulse oximetry, %	94 (90-96)	98 (96-99)	93 (88-95)	<.001
Heart rate, beats/min	96 (84-109)	96 (84-106)	96 (83-109)	.71
Temperature, °F	99.0 (98.0-100.0)	99.0 (98.0-99.5)	99.0 (98.0-100.0)	.97
Respiratory rate, breaths/min	19 (18-22)	18 (17-18)	20 (18-22)	.02
Baseline chest radiograph findings				
Unilateral infiltrate	62 (13.4)	7 (11.9)	55 (16.5)	
Bilateral infiltrate	187 (40.3)	5 (8.5)	182 (54.7)	<.001
Normal	105 (22.7)	41 (69.5)	63 (18.9)	
Baseline laboratory values, median (interquartile range)				
White blood cell count, cells/μL	5.8 (4.2-7.5)	6.1 (3.8-8.6)	5.8 (4.3-7.5)	.03
Absolute lymphocyte count, cells/μL	0.8 (0.6-1.2)	1.0 (0.7-1.6)	0.8 (0.6-1.1)	.03
Creatinine, mg/dL	1.1 (0.84-1.54)	0.85 (0.69-1.18)	1.12 (0.85-1.61)	.001
Aspartate aminotransferase, IU/L	30 (26-55)	26 (24-58)	35 (27-55)	<.001
High-sensitivity cardiac troponin I >99th percentile	107 (23.1)	2 (1.9)	105 (29.6)	<.001

SI conversion factors: To convert degrees Fahrenheit to degrees Celsius, subtract 32 and multiply by .5556; white blood cell count to $\times 10^9$/L, multiply by .001; lymphocytes to $\times 10^9$/L, multiply by .001; creatinine to micromoles per liter, multiply by 88.4; aspartate aminotransferase to microkatals per liter, multiply by 0.0167.

[a] Body mass index is calculated as weight in kilograms divided by height in meters squared.

Suleyman G, Fadel RA, Malette KM, et al. Clinical Characteristics and Morbidity Associated With Coronavirus Disease 2019 in a Series of Patients in Metropolitan Detroit. JAMA Netw Open. 2020;3(6):e2012270. doi:10.1001/jamanetworkopen.2020.12270

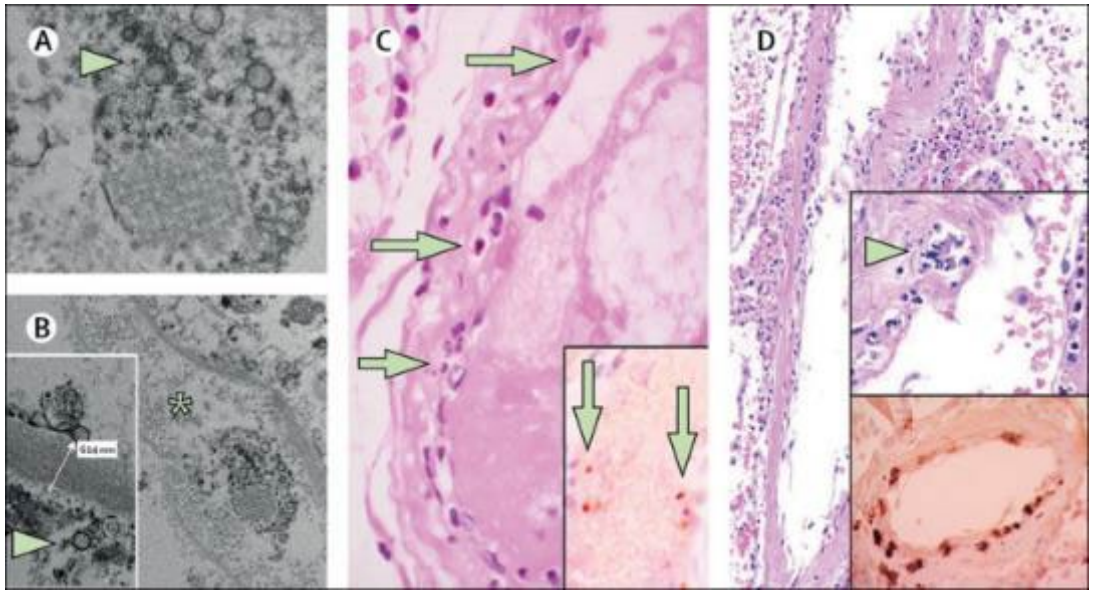
Most people who die from COVID-19 exhibit findings of diffuse alveolar damage (DAD), consistent with ARDS



Martines RB, Ritter JM, Matkovic E, Gary J, Bollweg BC, Bullock H, et al.; COVID-19 Pathology Working Group. Pathology and pathogenesis of fatal COVID-19 cases associated with SARS-CoV-2 pandemic. Emerg Infect Dis. 2020 Sep [access 23 June 2020]. https://doi.org/10.3201/eid2609.202095

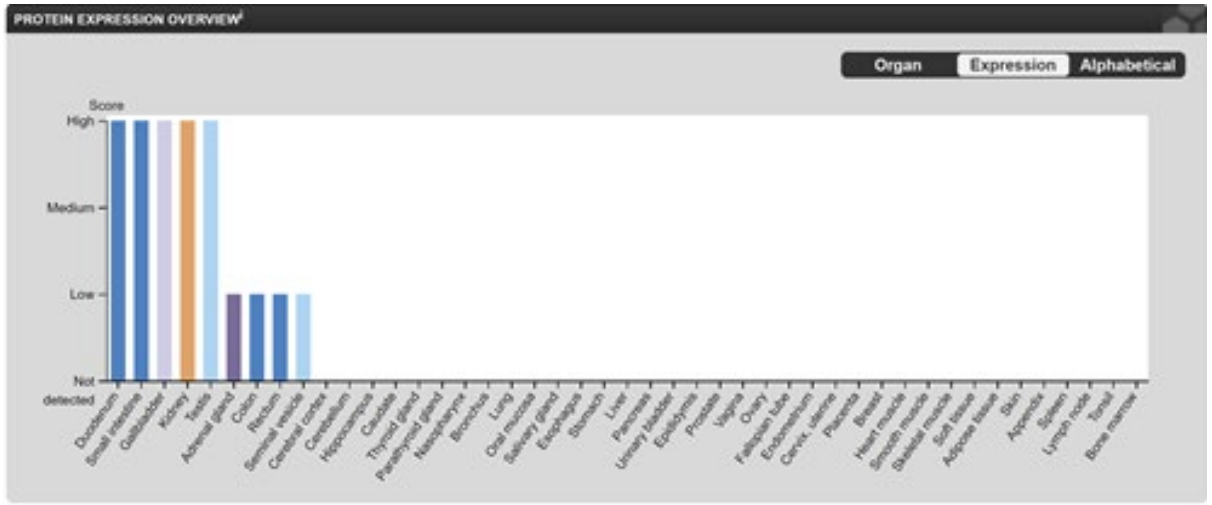
The viral receptor, ACE2, is predominantly expressed in the kidney and GI tract

SARS-CoV-2 infects endothelial cells in many tissues



Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395(10234):1417-1418. doi:10.1016/S0140-6736(20)30937-5

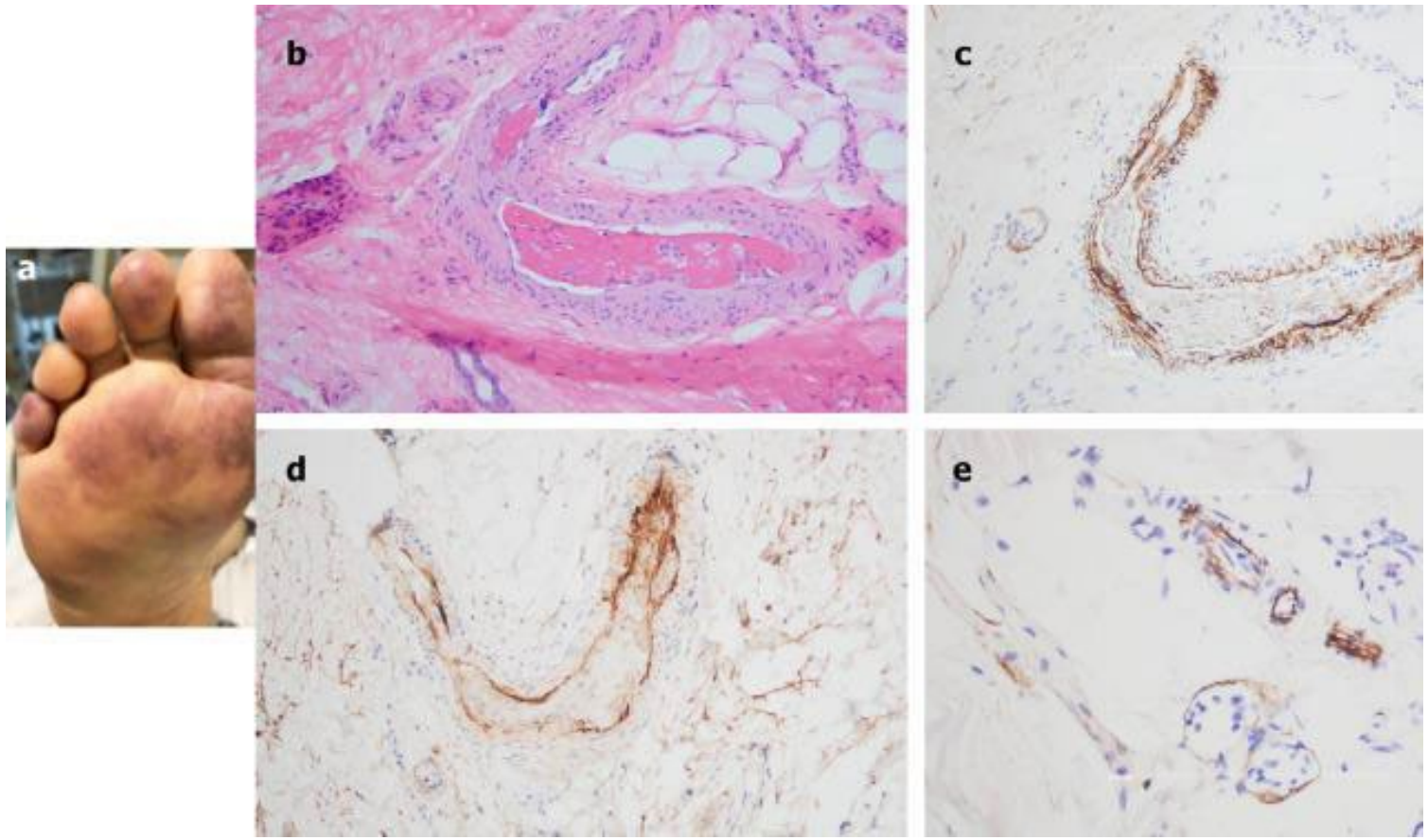
ACE2 Expression – The Human Protein Atlas



- SARS-CoV-2 infects human intestinal and kidney organoids in vitro
  - (Lamers et al., *Science* 01 May 2020:eabc1669 DOI: 10.1126/science.abc1669
  - Allison, S.J. SARS-CoV-2 infection of kidney organoids prevented with soluble human ACE2. Nat Rev Nephrol 16, 316 (2020). https://doi.org/10.1038/s41581-020-0291-8

Thrombosis may occur in COVID-19 patients

Magro C, Mulvey JJ, Berlin D, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases. *Transl Res*. 2020;220:1-13. doi:10.1016/j.trsl.2020.04.007

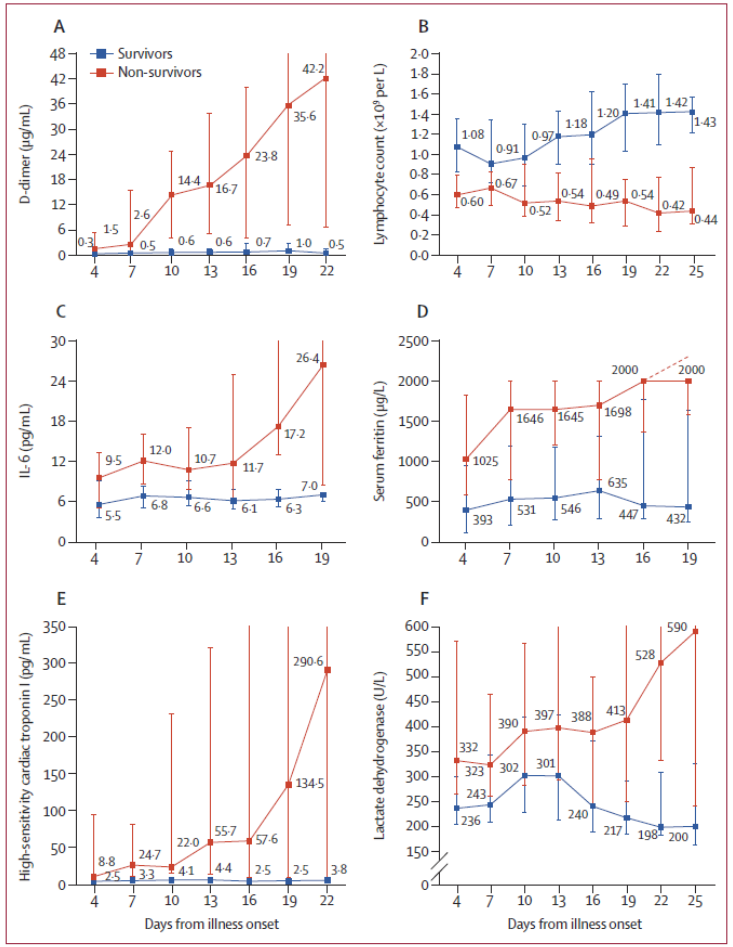


Baseline coagulation parameters	All patients (n = 150)
Platelet count (10^9/L)—normal range: 150–400.10^9/L	200 [152; 267]
aPTT—normal range: 0.7–1.2	1.2 [1.1; 1.3]
PT (%)—normal range: > 70%	84 [73; 91]
INR—normal range: 1.00–1.15	1.12 [1.05; 1.25]
D-dimers (mg/L)—normal range: < 0.5 mg/L	2.27 [1.16; 20]
Fibrinogen (g/L)—normal range: 2–4 g/L	6.99 [6.08; 7.73]
Antithrombin activity (%)—normal range: 50–150%	91 [78; 102]
Factor V (%)—normal range: > 70%	136 [115; 150]
Factor VIII (%)—normal range: 60–150%	341 [258; 416]
vWF activity (%)	328 [212; 342]
vWF antigen (%)—normal range: 50–150%	455 [350; 521]
Lupus anticoagulant[a]—n (%)	50/57 (87.7)
Screen patient (s)	68.6 [59.5; 85.4]
Screen ratio—normal range: < 1.2	1.63 [1.43; 2.04]
Confirm patient (s)	43.9 [40.9; 48.4]
Confirm ratio—normal range: < 1.2	1.25 [1.13; 1.46]
Screen/confirm ratio—normal range: < 1.2	1.4 [1.25; 1.48]

Helms J, Tacquard C, Severac F, et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med. 2020;46(6):1089-1098. doi:10.1007/s00134-020-06062-x

Thrombosis in COVID-19 is associated with systemic inflammation

Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study [published correction appears in Lancet. 2020 Mar 28;395(10229):1038] [published correction appears in Lancet. 2020 Mar 28;395(10229):1038]. Lancet. 2020;395(10229):1054-1062. doi:10.1016/S0140-6736(20)30566-3



	Total (n=191)	Non-survivor (n=54)	Survivor (n=137)	p value
(Continued from previous page)				
Anaemia	29 (15%)	14 (26%)	15 (11%)	0·0094
Platelet count, ×10⁹ per L	206·0 (155·0–262·0)	165·5 (107·0–229·0)	220·0 (168·0–271·0)	<0·0001
<100	13 (7%)	11 (20%)	2 (1%)	<0·0001
Albumin, g/L	32·3 (29·1–35·8)	29·1 (26·5–31·3)	33·6 (30·6–36·4)	<0·0001
ALT, U/L	30·0 (17·0–46·0)	40·0 (24·0–51·0)	27·0 (15·0–40·0)	0·0050
>40	59/189 (31%)	26 (48%)	33/135 (24%)	0·0015
Creatinine >133 μmol/L	8/186 (4%)	5 (9%)	3/132 (2%)	0·045
Lactate dehydrogenase, U/L	300·0 (234·0–407·0)	521·0 (363·0–669·0)	253·5 (219·0–318·0)	<0·0001
>245	123/184 (67%)	53 (98%)	70/130(54%)	<0·0001
Creatine kinase, U/L	21·5 (13·0–72·4)	39·0 (19·5–151·0)	18·0 (12·5–52·1)	0·0010
>185	22/168 (13%)	11/52 (21%)	11/116 (9%)	0·038
High-sensitivity cardiac troponin I, pg/mL	4·1 (2·0–14·1)	22·2 (5·6–83·1)	3·0 (1·1–5·5)	<0·0001
>28	24/145 (17%)	23/50 (46%)	1/95 (1%)	<0·0001
Prothrombin time, s	11·6 (10·6–13·0)	12·1 (11·2–13·7)	11·4 (10·4–12·6)	0·0004
<16	171/182 (94%)	47 (87%)	124/128 (97%)	0·016*
≥16	11/182 (6%)	7 (13%)	4/128 (3%)	..
D-dimer, μg/mL	0·8 (0·4–3·2)	5·2 (1·5–21·1)	0·6 (0·3–1·0)	<0·0001
≤0·5	55/172 (32%)	4 (7%)	51/118 (43%)	<0·0001*
>0·5 to ≤1	45/172 (26%)	6 (11%)	39/118 (33%)	..
>1	72/172 (42%)	44 (81%)	28/118 (24%)	..

Abnormal blood clots during infection are not a new phenomenon

A NOTE

ON THE

CAUSATION AND TREATMENT OF THROMBOSIS

OCCURRING IN

CONNECTION WITH TYPHOID FEVER

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We have recently, in the hope of learning something of the causes of the thrombosis which is met with in connection with typhoid fever, addressed ourselves to the task of making a series of comparative observations on the blood in (*a*) typhoid fever patients in the acute stage

Wright AE, Knapp HH. A Note on the Causation and Treatment of Thrombosis occurring in connection with Typhoid Fever. Med Chir Trans. 1903;86:1-19.

Proposed model for pro-thrombotic state in COVID-19

Magro et al., "Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: A report of five cases," *Translational Research*, June 2020, Vol. 220, pp. 1-13, doi: 10.1016/j.trsl.2020.04.007

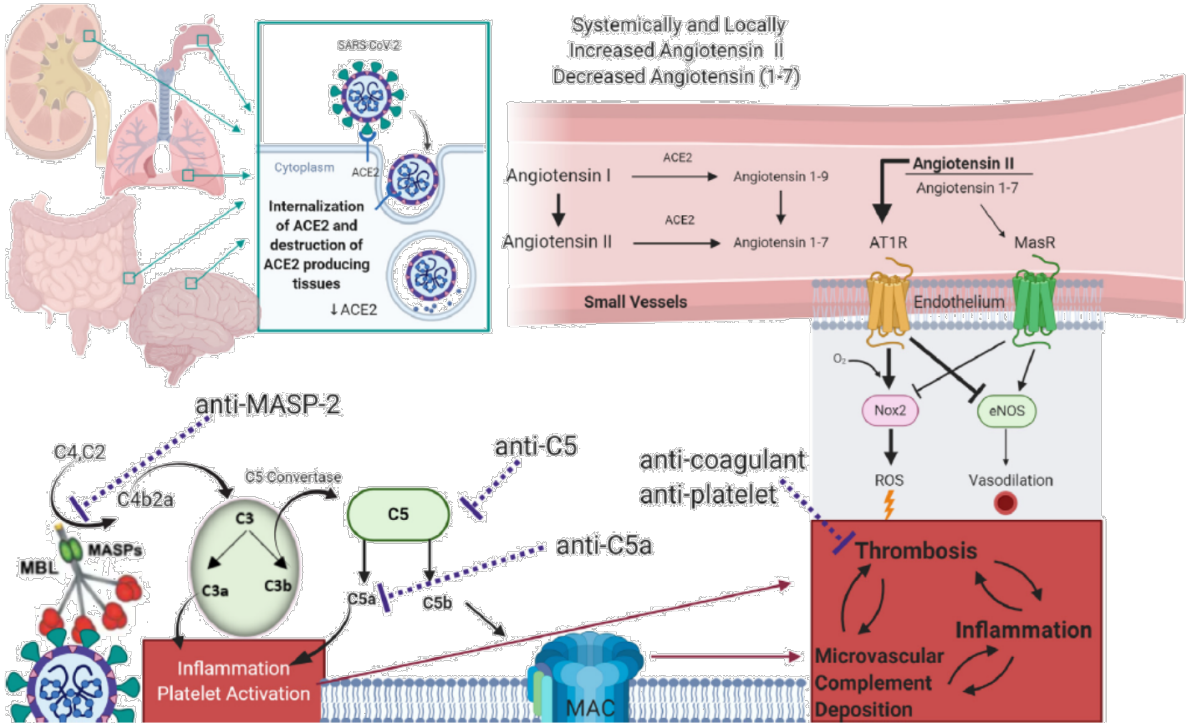
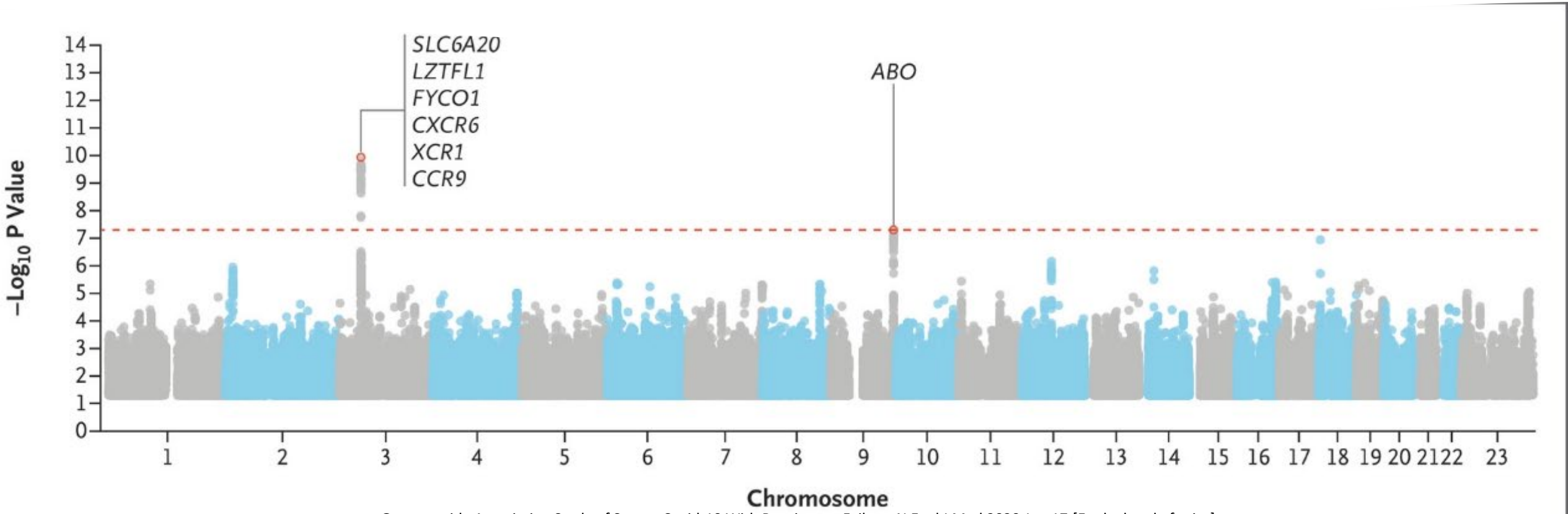


Fig 10. Model for AP and LP complement activation by SARS-CoV2, and its interaction with coagulation cascades.

- Angiotensin I and angiotensin II have been associated with inflammation, oxidative stress, and fibrosis, and ACE2 is involved in their deactivation
- Downregulation of ACE2 due to overwhelming coronavirus infection could interfere with ACE2 activity
- Resulting increases in angiotensin II could lead to reactive oxygen species formation and interference with antioxidant and vasodilatory signals such as NOX2 and eNOS, with further complement activation.
- The potential loss of auto-vasoconstriction and regulation of lung blood flow through injured vascular segments would also lead to increased shunting and severe hypoxemia.

There appears to be an underlying genetic susceptibility to COVID-19 respiratory failure

- Genome wide association studies identified two genetic loci associated with COVID-19 respiratory failure.
- At locus 3p21.31, the association signal spanned the genes SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6 and XCR1.
- The association signal at locus 9q34.2 coincided with the ABO blood group locus; in this cohort, a blood-group–specific analysis showed a higher risk in blood group A than in other blood groups (odds ratio, 1.45; 95% CI, 1.20 to 1.75; P=1.48×10−4) and a protective effect in blood group O as compared with other blood groups (odds ratio, 0.65; 95% CI, 0.53 to 0.79; P=1.06×10−5).

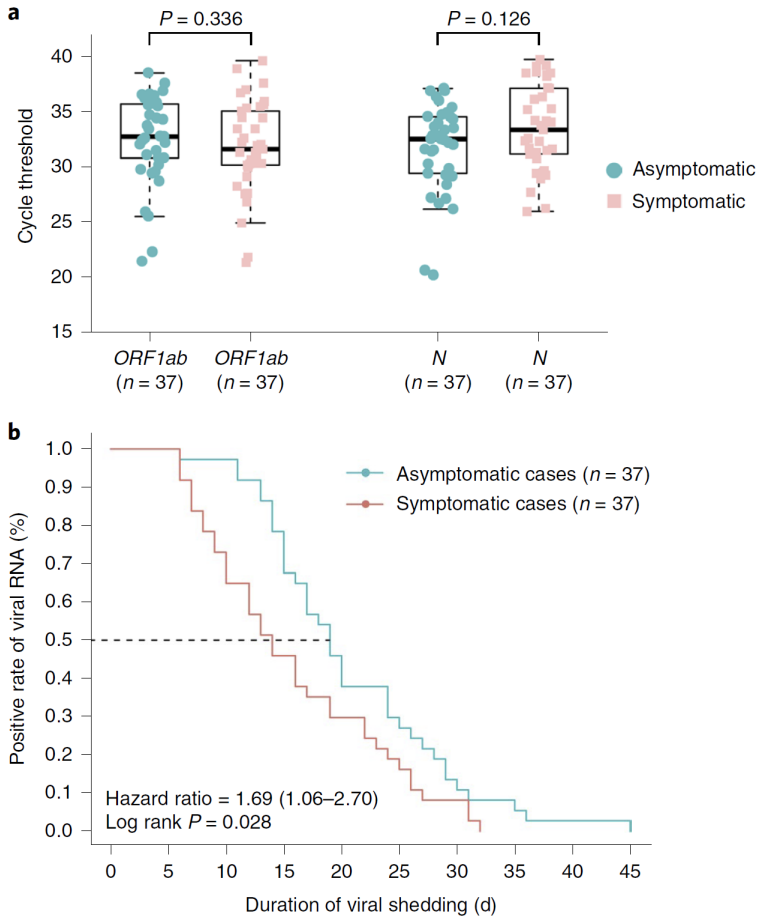


Genomewide Association Study of Severe Covid-19 With Respiratory Failure. N Engl J Med 2020;Jun 17:[Epub ahead of print]

Asymptomatic infections are a major contributor to spread

- Patients may still suffer lung damage

- Long, Q., Tang, X., Shi, Q. *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* (2020). https://doi.org/10.1038/s41591-020-0965-6



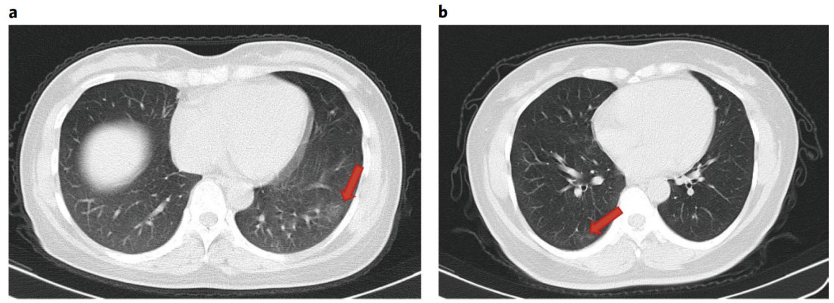


Fig. 1 | Chest CT scans from two asymptomatic patients. a, CT scan of a 45-year-old female showing focal ground-glass opacities in the lower lobe of the left lung (arrow). **b**, CT scan of a 50-year-old female showing ground-glass opacities and stripes coexisting in the lower lobe of the right lung (arrows).

Acquired immunity is less robust in asymptomatic patients; wanes in any case

- Long, Q., Tang, X., Shi, Q. *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* (2020). https://doi.org/10.1038/s41591-020-0965-6