The University of Texas at Austin
Dell Medical School

THE EVOLVING INS AND OUTS OF COVID19

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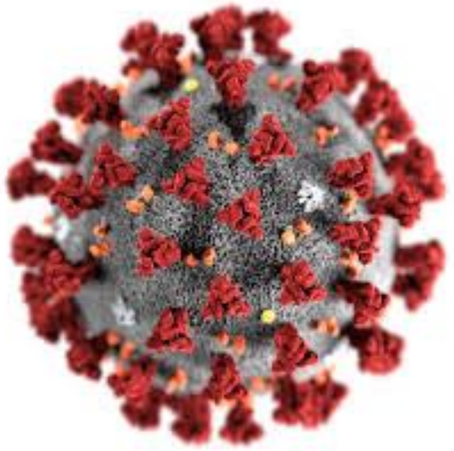
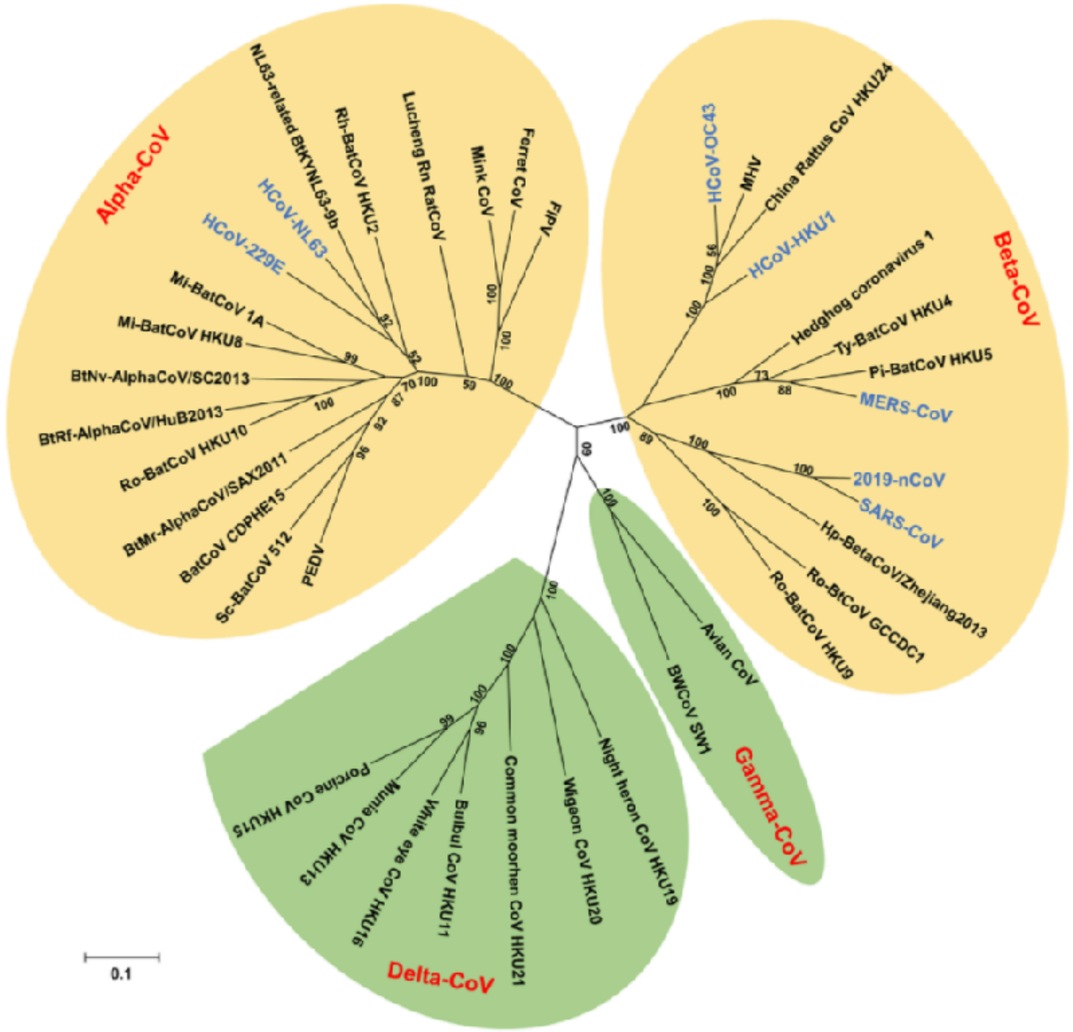


Coronaviruses Phylogeny

- **COVID-19**: Disease caused by SARS-Cov-2
- **SARS-Cov-2** is the 7th human coronavirus
- **Human coronaviruses**
  - 2019-nCoV, SARS-CoV, MERS- CoV, HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1
- **SARS-Cov-2** is the 3rd virus causing a severe clinical syndrome after SARS-CoV and Middle East Respiratory Syndrome (MERS)-CoV1



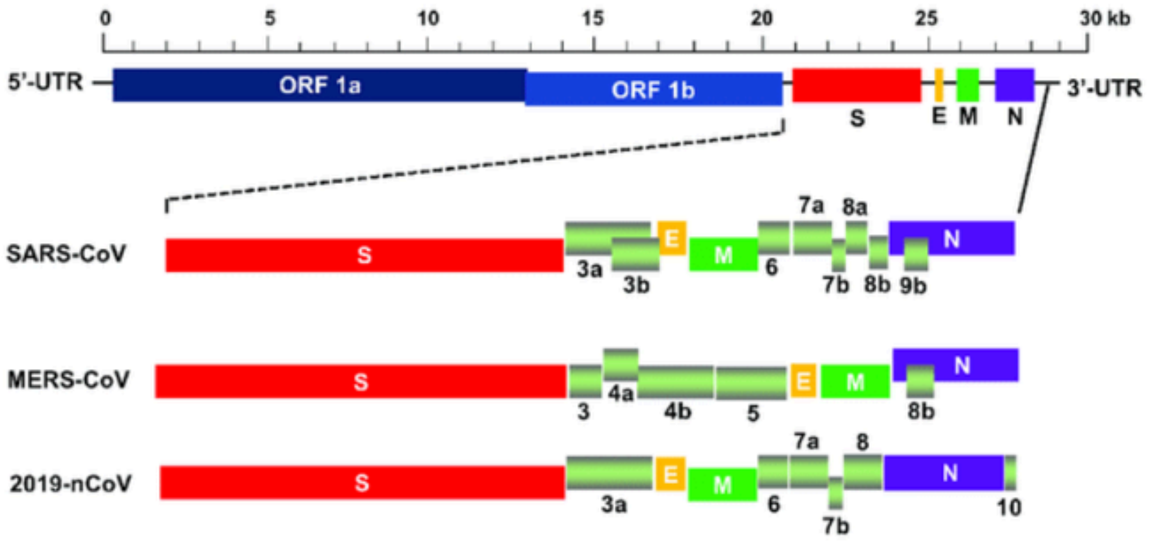
Wang et al, Frontiers in Microbiology, 2020

Human Coronaviruses account for 30% of all URIs

- **HCoV-229E**: medical student nasal secretions with respiratory disease (Hamre and Procknow, 1966)
- **HCoV-NL63**: infants with PNA/bronchiolitis (Fouchier et al., 2004)
- **HCoV-HKU1**: 71 yo M with PNA (Woo et al., 2005)
- **HCoV-OC43**: patients with URI (Tyrrell and Bynoe, 1965)

HCoV- 229E and HCoV-OC43 detected in the brain
(Arbour et al., 2000; Desforges et al., 2014).

SARS-Cov2 Structure and Homology to other Coronaviruses

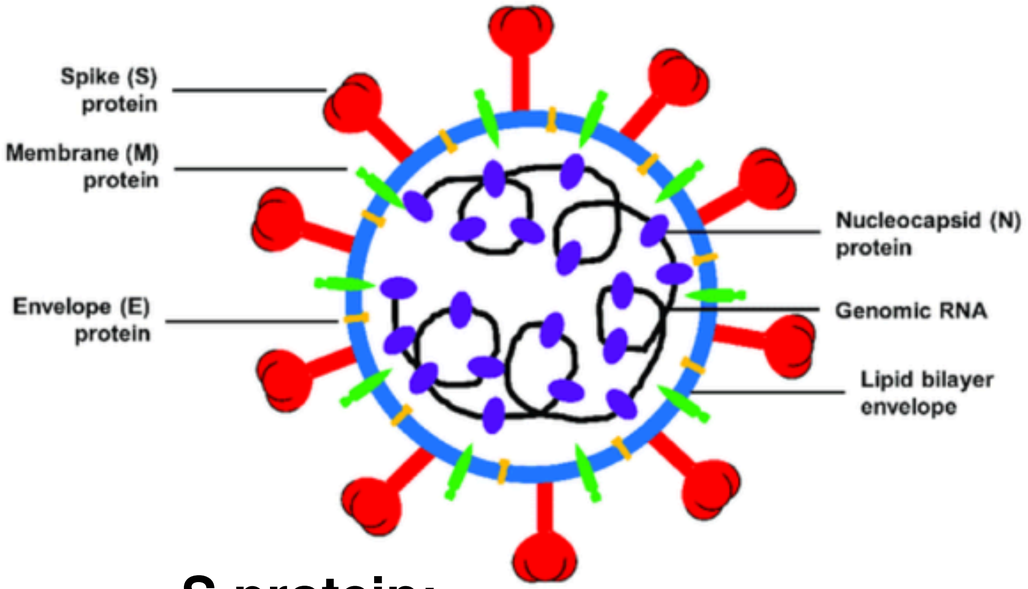


- Four structural proteins (S, E, M, and N)
- Six accessory proteins (3a, 6, 7a, 7b, 8, and 10)

Percentage sequence identity with SARS-CoV-2

	S protein	N protein	M protein	E protein
SARS-CoV	76.0%	90.6%	90.1%	94.7%
MERS-CoV	29.4%	45.9%	39.2%	34.1%

Ahmed et al, Viruses, 2020



Wang et al, Frontiers in Microbiology, 2020

S protein:

- S1 subunit, NTD, RBD, and S2 subunit
- Receptor binding and membrane fusion
- Major antigen for protective neutralizing antibodies

CORONAVIRUS

Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation

Daniel Wrapp[1]*, Nianshuang Wang[1]*, Kizzmekia S. Corbett[2], Jory A. Goldsmith[1], Ching-Lin Hsieh[1], Olubukola Abiona[2], Barney S. Graham[2], Jason S. McLellan[1]†

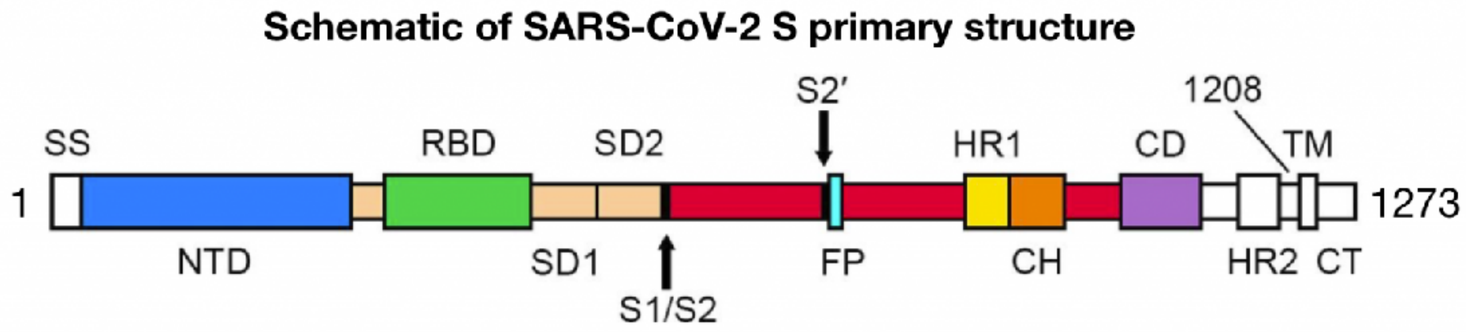
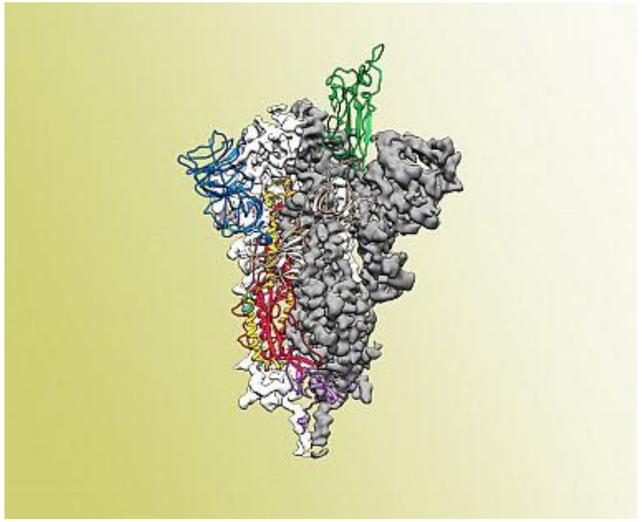


Figure adapted from Wrapp et al. 2020



Cryo-electron microscopy structure of 2019-nCoV S

Dr. Jason McLellan's lab at the University of Texas at Austin

ACE2 binds to the SARS-CoV-2 receptor-binding domain with ~10- to 20-fold higher affinity than ACE2 binding to SARS-CoV S.

Wrapp *et al.*, *Science* **367**, 1260–1263 (2020)

Received: 17 January 2020 | Accepted: 21 January 2020

DOI: 10.1002/jmv.25682

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Cross-species transmission of the newly identified coronavirus 2019-nCoV

Wei Ji[1] | Wei Wang[2] | Xiaofang Zhao[3] | Junjie Zai[4] | Xingguang Li[5]

" 2019-nCoV has most similar genetic information with bat coronovirus and **most similar codon usage bias with snake** "

Cold Spring Harbor Laboratory
bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

120 comments

Uncanny similarity of unique inserts in the 2019-nCoV spike protein to HIV-1 gp120 and Gag

Prashant Pradhan, Ashutosh Kumar Pandey, Akhilesh Mishra, Parul Gupta, Praveen Kumar Tripathi, Manoj Balakrishnan Menon, James Gomes, Perumal Vivekanandan, Bishwajit Kundu

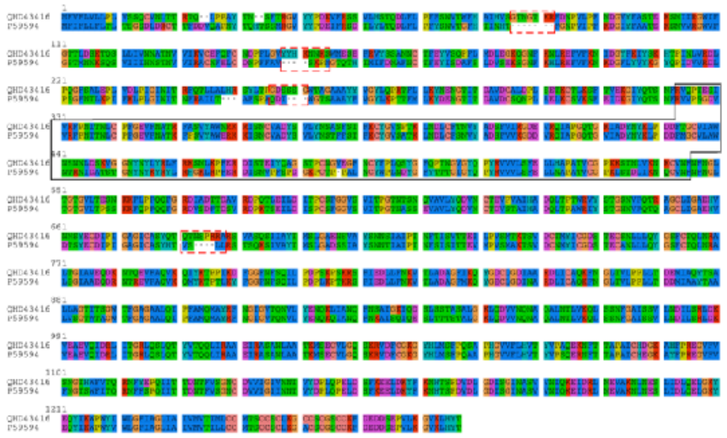
doi: https://doi.org/10.1101/2020.01.30.927871

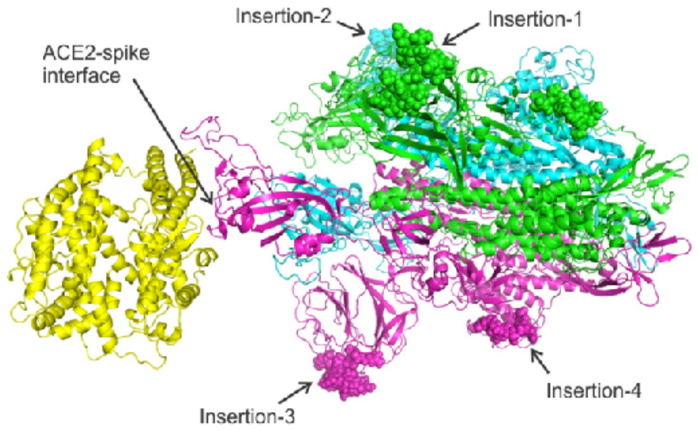
“insertions shared “uncanny similarity” to Human Immunodeficiency Virus 1 (HIV-1) proteins but not to other coronaviruses.”

Abstract

This paper has been withdrawn by its authors. They intend to revise it in response to comments received from the research community on their technical approach and their interpretation of the results. If you have any questions, please contact the corresponding author.

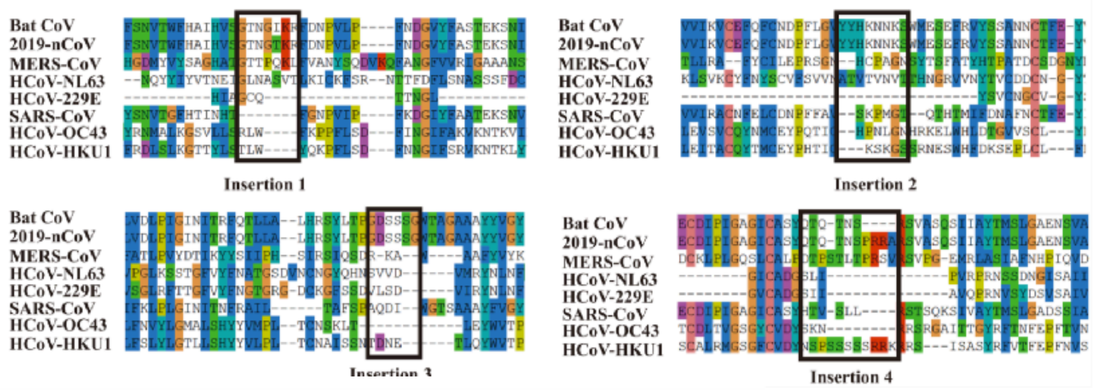
SARS CoV2 Animal Reservoirs: Bat > Snake

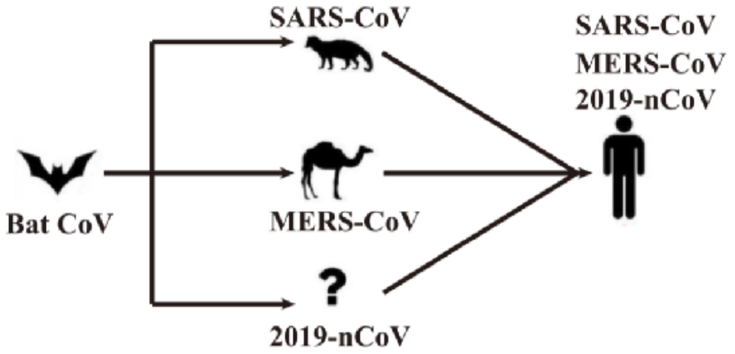




All four insertions are located outside the RBD, in contrast to the original conclusion made by Pradhan et al. which stated that the insertions are located on the interface with ACE2.

- All four insertion fragments found in other viruses.
- HIV-1 protein is among the top BLAST hits for **only one** of the four insertion fragments
- **Three of the four insertion fragments** are found in **bat** coronavirus RaTG13.





Intermediate Host likely mammal or bird and not snake

What is the Intermediate Host for SARS-Cov-2?

Huanan market in Wuhan

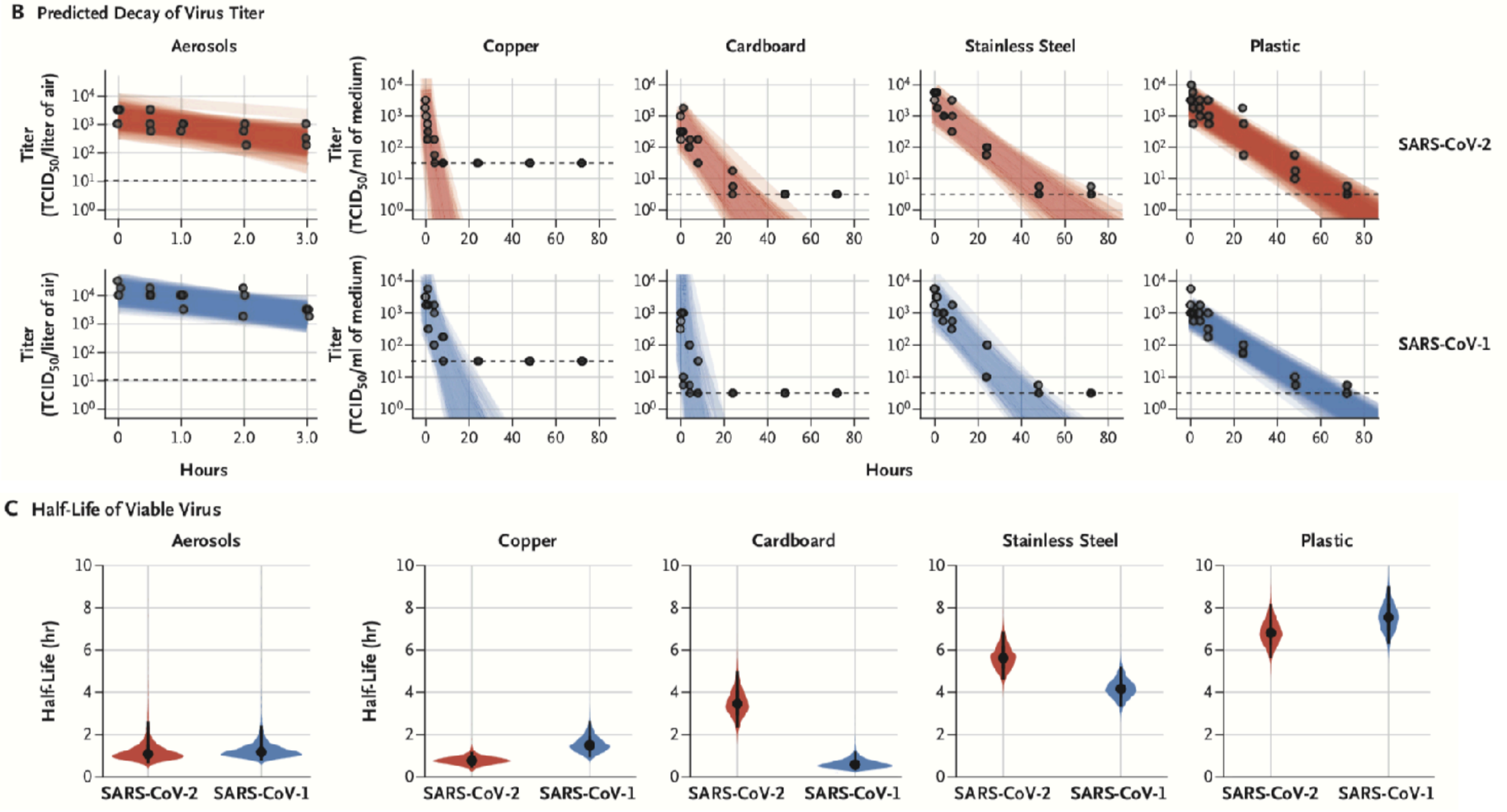
- **SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus**
  - natural selection in an animal host before zoonotic transfer
  - natural selection in humans following zoonotic transfer

RBD and burin S1/S2 cleavage site

Anderson et al, Nature Medicine, 2020

The NEW ENGLAND JOURNAL of MEDICINE

Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1



nature medicine

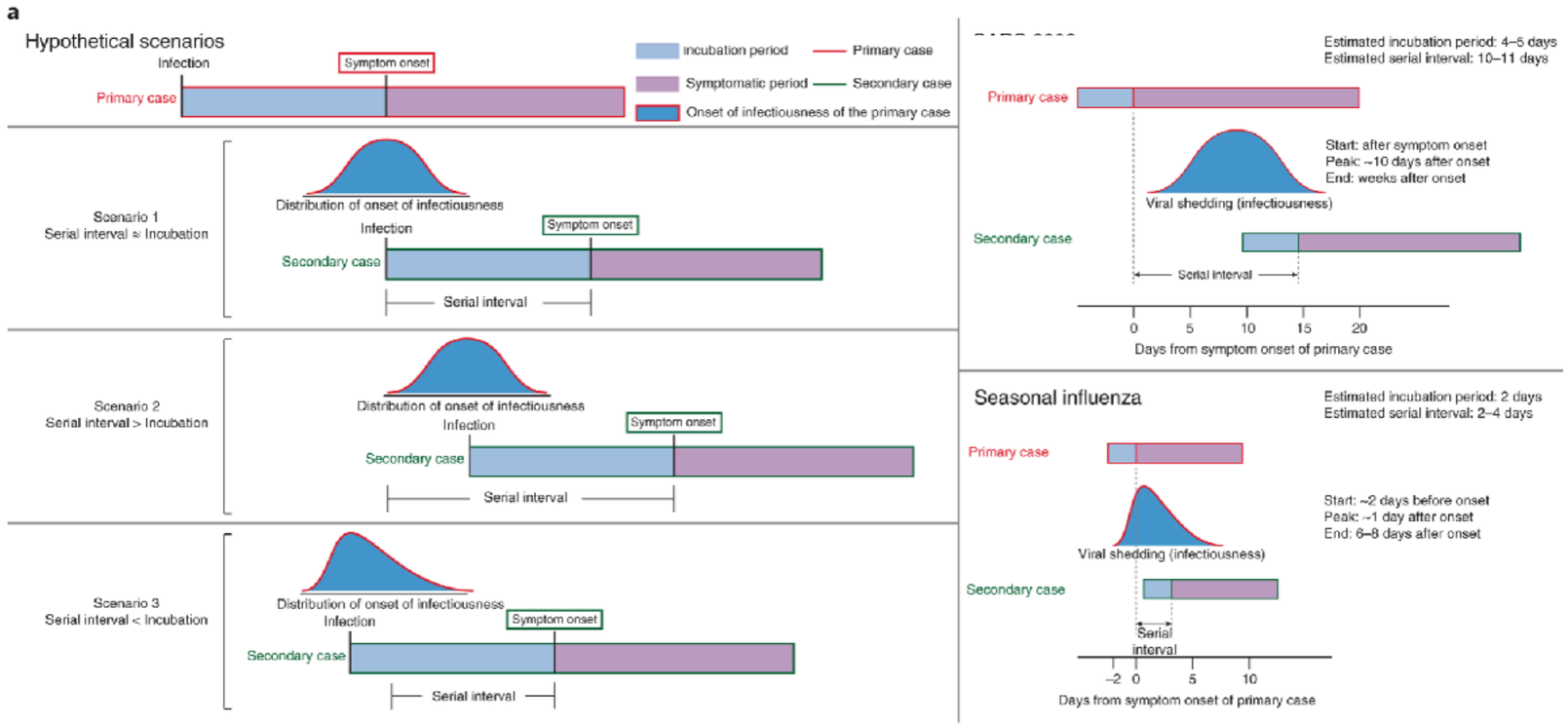
Brief Communication | Published: 15 April 2020

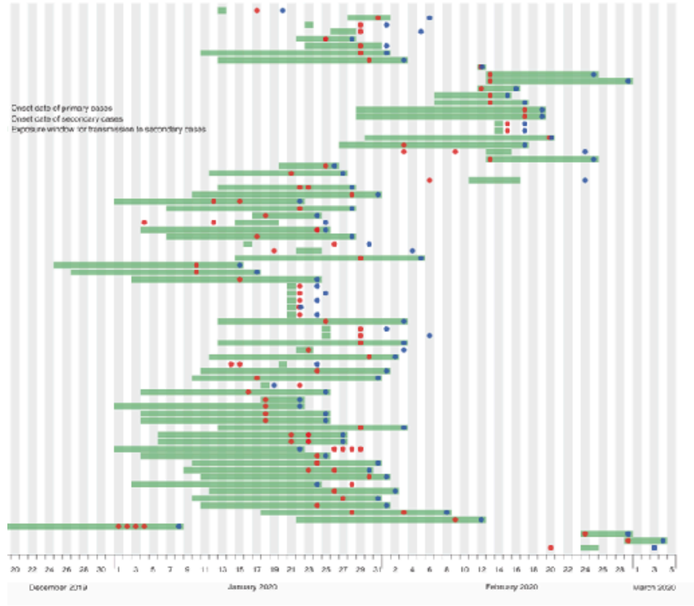
Temporal dynamics in viral shedding and transmissibility of COVID-19

Xi He, Eric H. Y. Lau ✉, Peng Wu, Xilong Deng, Jian Wang, Xinxin Hao, Yiu Chung Lau, Jessica Y. Wong, Yujuan Guan, Xinghua Tan, Xiaoneng Mo, Yanqing Chen, Baolin Liao, Weilie Chen, Fengyu Hu, Qing Zhang, Mingqiu Zhong, Yanrong Wu, Lingzhai Zhao, Fuchun Zhang, Benjamin J. Cowling, Fang Li & Gabriel M. Leung

Nature Medicine (2020) | Cite this article

Patients became infectious days before onset of symptoms





Non-Neurological Clinical Manifestations of COVID19

Symptoms

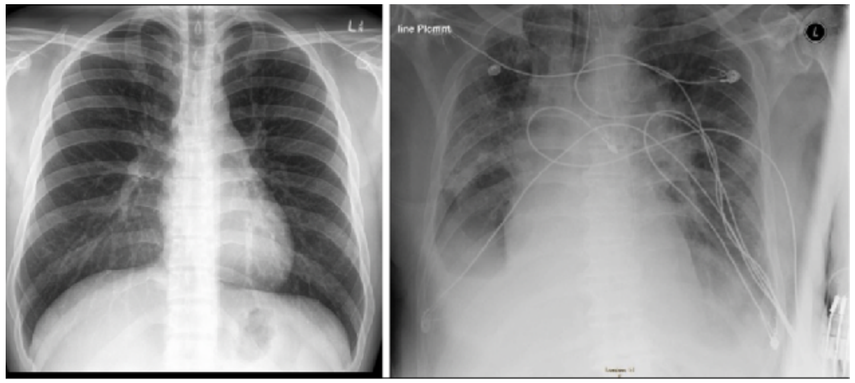
- Fever
- Cough/SOB
- Anorexia
- Diarrhea
- Throat pain
- Abdominal pain

Labs

- high neutrophil counts
- low lymphocyte counts
- high CRP/ddimer
- high LFTs
- abnormalities in renal fx
- high CK
- Cytokine storm:
  - high IL6, IL10, PD-1, GM-CSF secreting Th1-like cells and CD14+CD16+ monocytes

Imaging

- CXR:
  - Bilateral multifocal consolidations that may progress to involve entire lungs
  - pleural effusions
- Chest CT
  - Multifocal ground-glass opacities and consolidations



Neurological Clinical Manifestations of COVID19

Symptoms

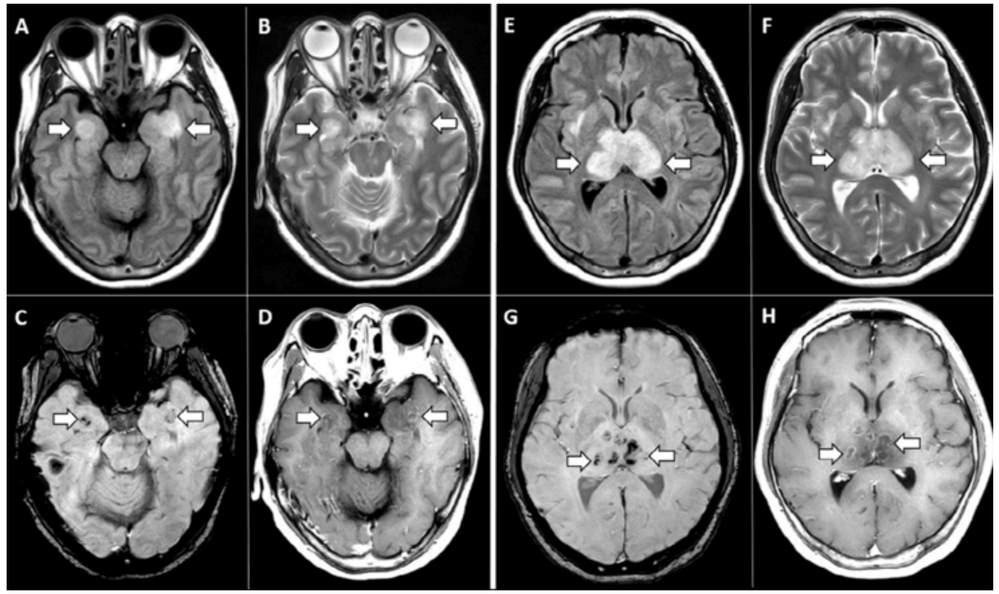
- Dizziness
- Headache
- AMS
- CVA
- Ataxia
- Seizure
- GBS
- Anosmia
- ageusia
- Skeletal muscle injury

Labs

- CSF with elevated protein, mild or no pleocytosis

Imaging

- Ischemic CVA
- Hemorrhagic CVA



Poyiadji et al, *COVID-19–associated Acute Hemorrhagic Necrotizing Encephalopathy,* Radiology, 2020

JAMA Neurology | Original Investigation

Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China

Ling Mao; Huijuan Jin; Mengdie Wang; Yu Hu; Shengcai Chen; Quanwei He; Jiang Chang; Candong Hong; Yifan Zhou; David Wang; Xiaoping Miao; Yanan Li, MD, PhD; Bo Hu, MD, PhD

214 COVID19 patients

36.4% had neurologic manifestations

Table 1. Clinical Characteristics of Patients With COVID-19

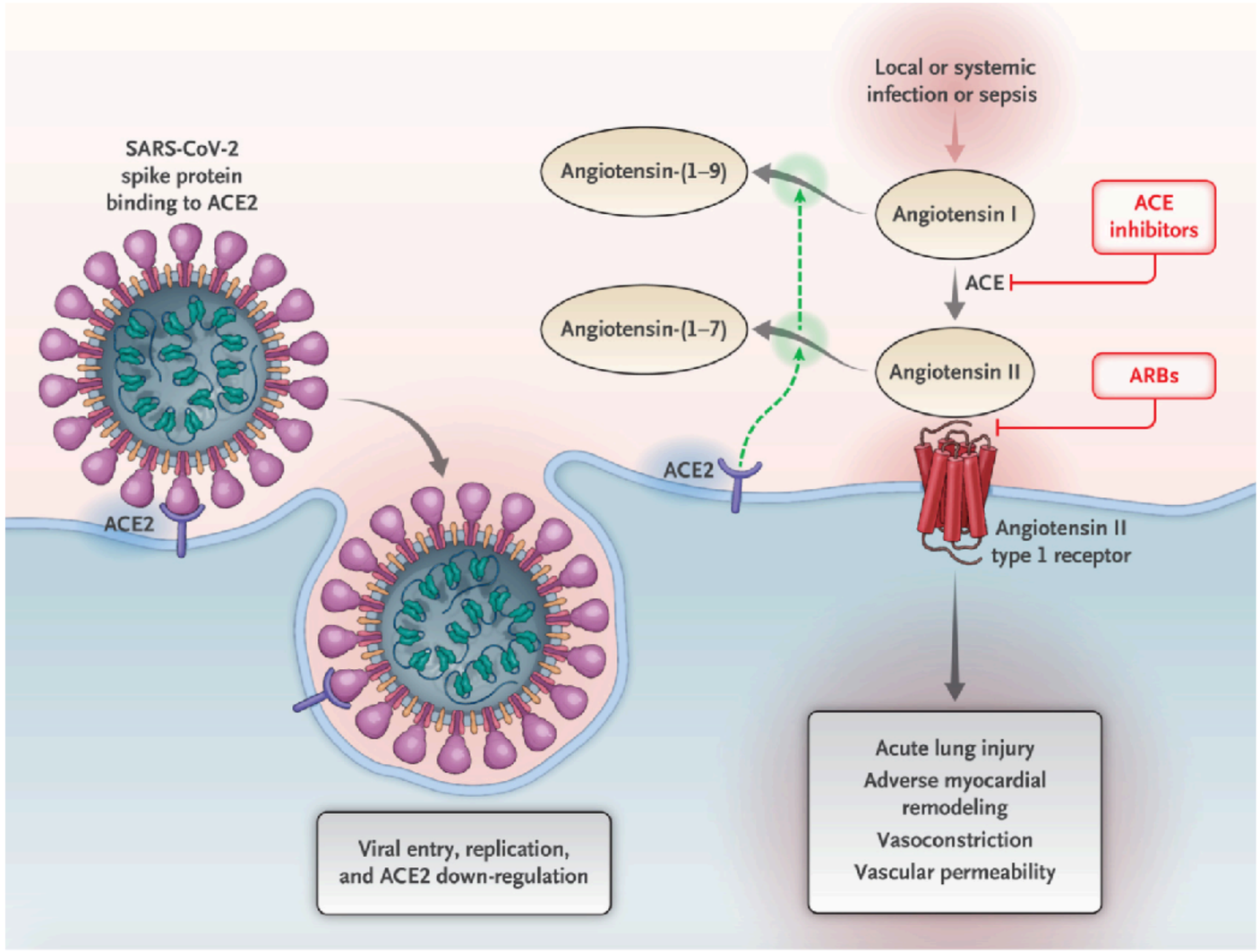
Characteristic	No. (%) Total (N = 214)	Severe (n = 88)	Nonsevere (n = 126)	*P* value[a]
Age, mean (SD), y	52.7 (15.5)	58.2 (15.0)	48.9 (14.7)	
Age, y				
<50	90 (42.1)	24 (27.3)	66 (52.4)	<.001
≥50	124 (57.9)	64 (72.7)	60 (47.6)	
Sex				
Female	127 (59.3)	44 (50.0)	83 (65.9)	.02
Male	87 (40.7)	44 (50.0)	43 (34.1)	
Comorbidities				
Any	83 (38.8)	42 (47.7)	41 (32.5)	.03
Hypertension	51 (23.8)	32 (36.4)	19 (15.1)	<.001
Diabetes	30 (14.0)	15 (17.0)	15 (11.9)	.29
Cardiac or cerebrovascular disease	15 (7.0)	7 (8.0)	8 (6.3)	.65
Malignancy	13 (6.1)	5 (5.7)	8 (6.3)	.84
Chronic kidney disease	6 (2.8)	2 (2.3)	4 (3.2)	.69
Typical symptoms				
Fever	132 (61.7)	40 (45.5)	92 (73.0)	<.001
Cough	107 (50.0)	30 (34.1)	77 (61.1)	<.001
Anorexia	68 (31.8)	21 (23.9)	47 (37.3)	.04
Diarrhea	41 (19.2)	13 (14.8)	28 (22.2)	.17
Throat pain	31 (14.5)	10 (11.4)	21 (16.7)	.28
Abdominal pain	10 (4.7)	6 (6.8)	4 (3.2)	.21
Nervous system symptoms				
Any	78 (36.4)	40 (45.5)	38 (30.2)	.02
CNS	53 (24.8)	27 (30.7)	26 (20.6)	.09
Dizziness	36 (16.8)	17 (19.3)	19 (15.1)	.42
Headache	28 (13.1)	15 (17.0)	13 (10.3)	.15
Impaired consciousness	16 (7.5)	13 (14.8)	3 (2.4)	<.001
Acute cerebrovascular disease	6 (2.8)	5 (5.7)	1 (0.8)	.03
Ataxia	1 (0.5)	1 (1.1)	0	NA
Seizure	1 (0.5)	1 (1.1)	0	NA
PNS	19 (8.9)	7 (8.0)	12 (9.5)	.69
Impairment				
Taste	12 (5.6)	3 (3.4)	9 (7.1)	.24
Smell	11 (5.1)	3 (3.4)	8 (6.3)	.34
Vision	3 (1.4)	2 (2.3)	1 (0.8)	.37
Nerve pain	5 (2.3)	4 (4.5)	1 (0.8)	.07
Skeletal muscle injury	23 (10.7)	17 (19.3)	6 (4.8)	<.001

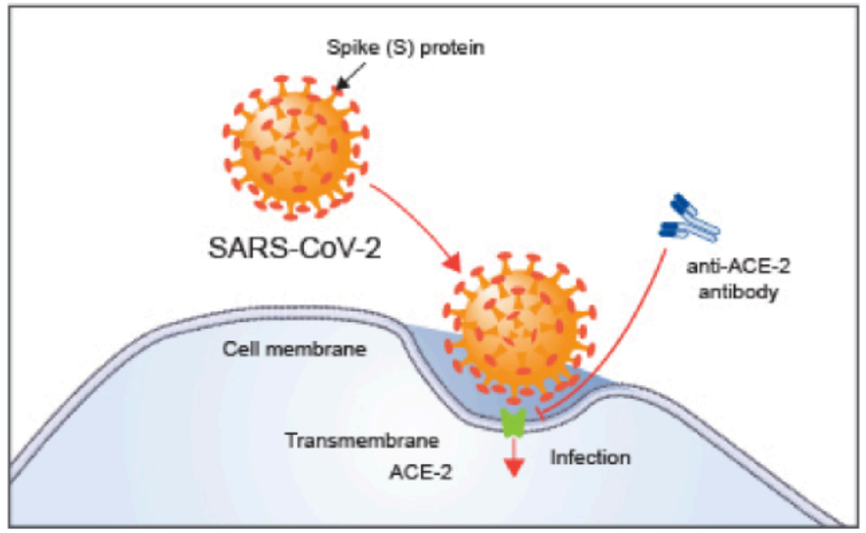
SARS-CoV vs SARS-CoV-2

Characteristic	SARS-CoV	SARS-CoV-2
Target receptor	ACE-2	ACE-2
N protein	IFN-γ inhibitor	Unknown
R0	0.4	1.4-2.5
Chest X-ray	Ground glass opacities	Bilateral, multilobar ground glass opacities
Chest CT-scan	Lobar consolidation	
	Nodular opacities	No nodular opacities
Prevention	Hand hygiene, cough etiquette	Possibly hand hygiene, cough etiquette
Transmission	Droplets	Droplets
	Contact with infected individuals	Contact with infected individuals, even asymptomatic ones
Case fatality rate (overall)	9.6%	2.3%

Ceccarelli et al, European Review for Medical and Pharmacological Sciences, 2020

ACE-2 Receptor and SARS-Cov-2 Cellular Entry

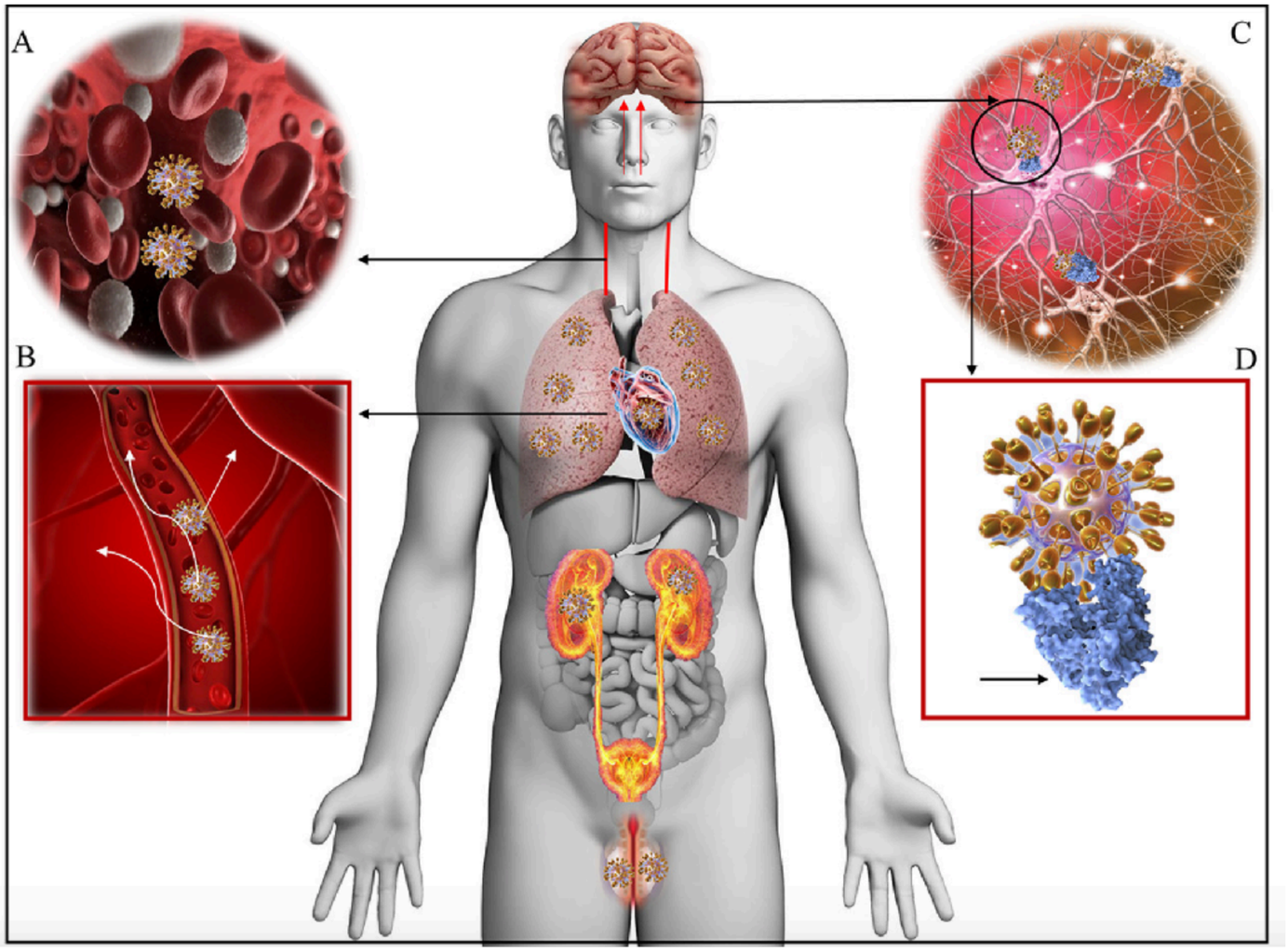




Clinical Trials

- Losartan (NCT04312009)
- Recombinant Human Angiotensin-converting Enzyme 2 (rhACE2) (NCT04287686)
- TMPRSS2 blocker (Camostat — upcoming clinical trial)

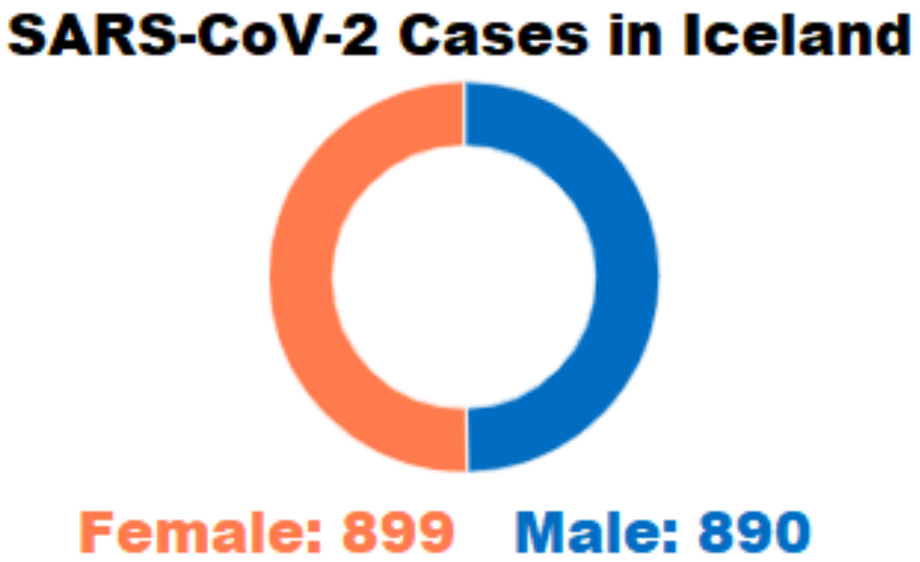
ACE-2 Receptor Distribution in the Body

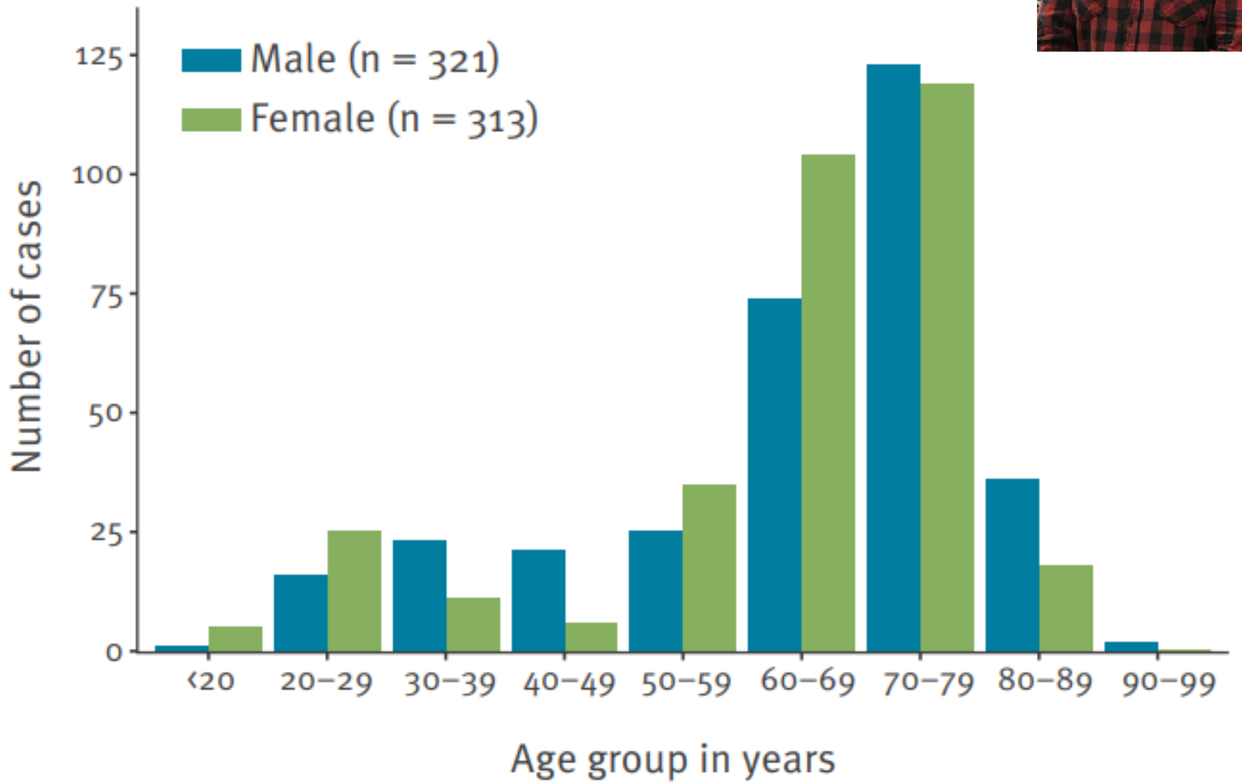


ACE2 Brain Distribution

- **Neurons** (Ding et al. J. Pathol. 2004; Gu et al. J. Exp. Med. 2005; Xu et al. Clin. Infect. Dis. 2005)
- **Motor cortex** (Doobay et al. Am. J. Physiol - Regul. Integr. Comp. Physiol. 2007)
- **Hypothalamus** (Chappell et al. J. Biol. Chem. 1989)
- **Thalamus and brainstem** (McCray, P. B., et al . J. Virol. 2007.)

Sex Differences in Covid-19 Epidemiology





49.7% male, 50.2% female

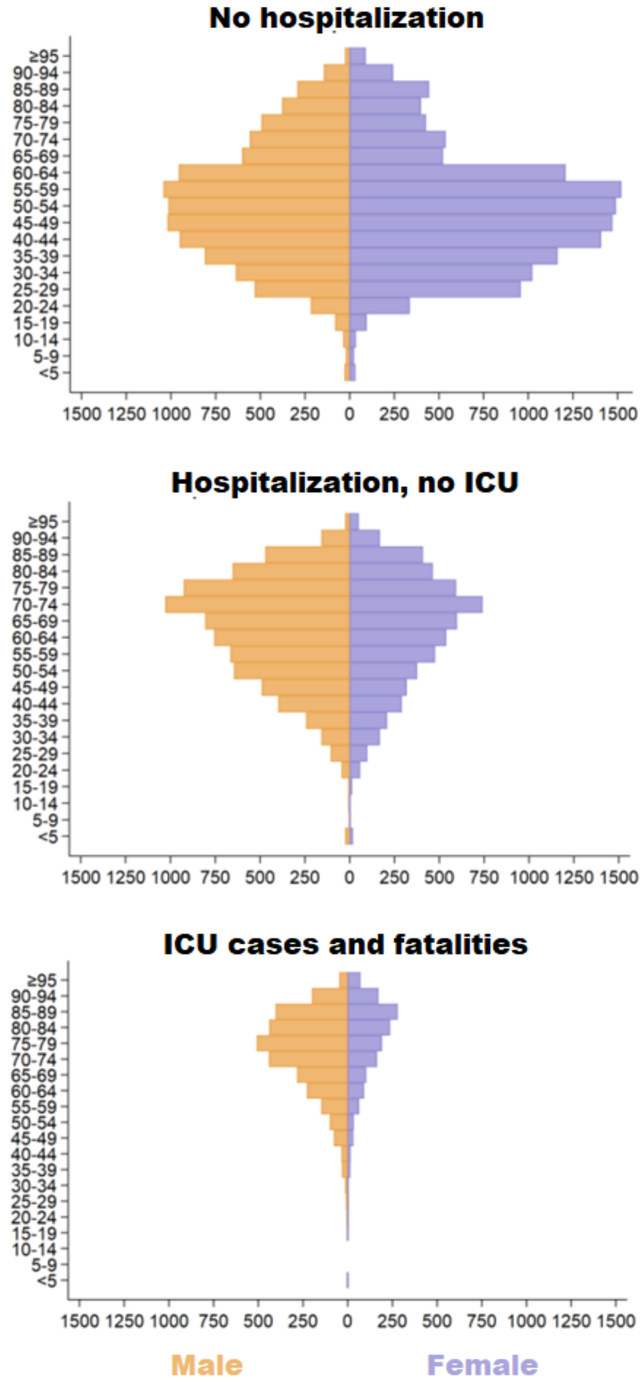
Iceland, 04/22/2020, Iceland Directorate of Health

50.6% male, 49.4% female

Diamond Princess, 03/12/2020, Mizumoto et al.

Slide Courtesy of Blaine Caslin

Sex Differences in Covid-19 Epidemiology

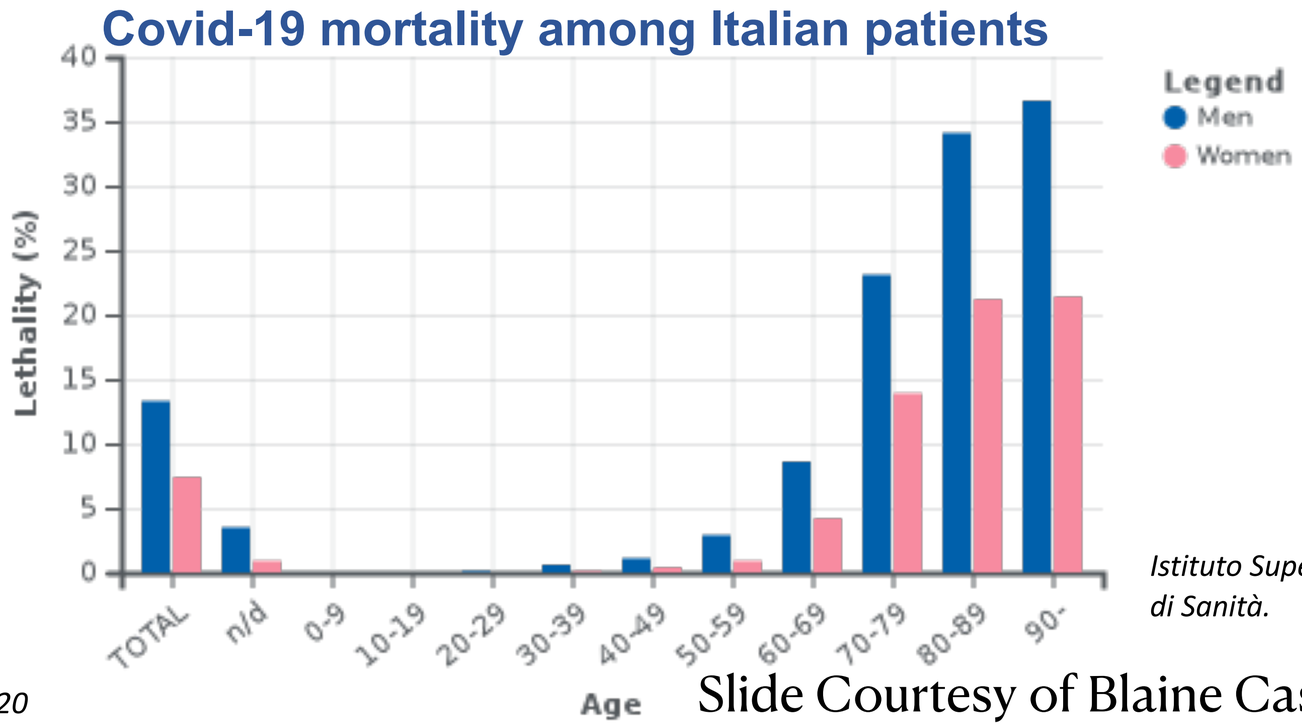


Spanish National Epidemiological Surveillance Network, updated 04/11/2020

Strong male bias in cases involving hospitalization, intensive care, and mortality

This pattern is seen in pediatric Covid-19 cases

- **56.6% male**, China, n=728 (Dong et al 2020)
- **57% male**, United States, n=149,760 (CDC)

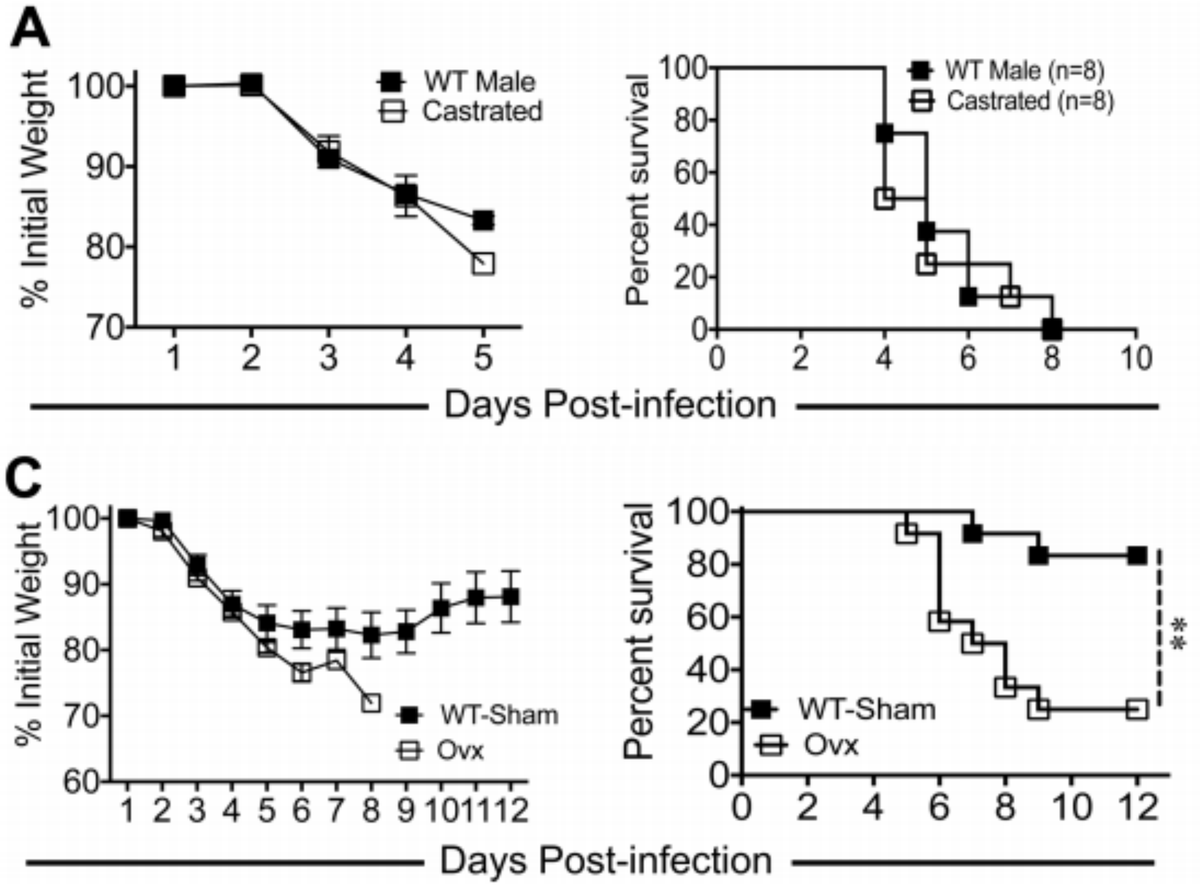


Istituto Superiore di Sanità.

Slide Courtesy of Blaine Caslin

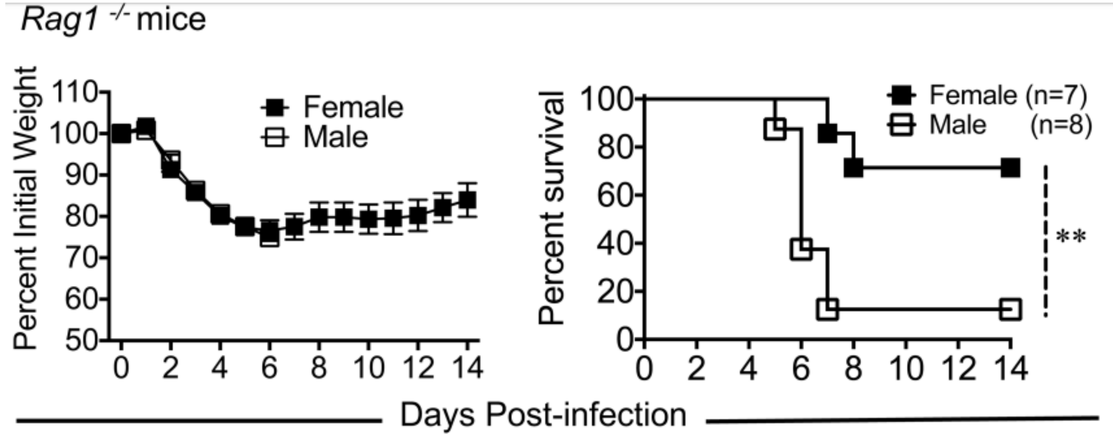
Sex Differences in Covid-19 Epidemiology

SARS-CoV-1 also targets ACE2 and showed similar sex bias



Ovariectomy removes female protective effect in animal model of SARS-CoV-1 infection

Female protection retained in *Rag1*$^{-/-}$ model



The Journal of Immunology

Sex-Based Differences in Susceptibility to Severe Acute Respiratory Syndrome Coronavirus Infection

Rudragouda Channappanavar,* Craig Fett,* Matthias Mack,† Patrick P. Ten Eyck,‡ David K. Meyerholz,§ and Stanley Perlman*

Slide Courtesy of Blaine Caslin

Lab Techniques 101

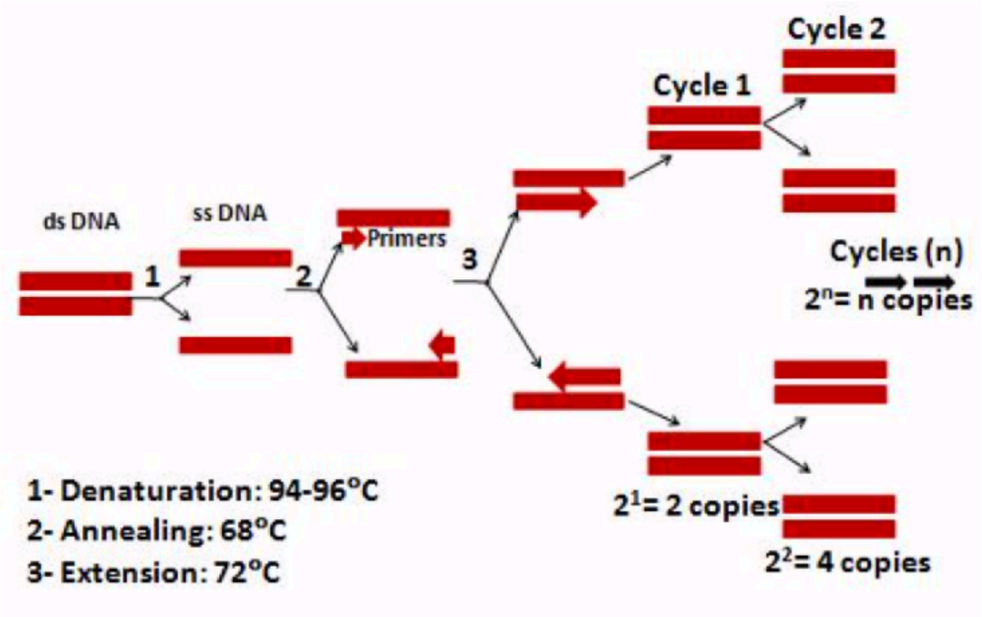
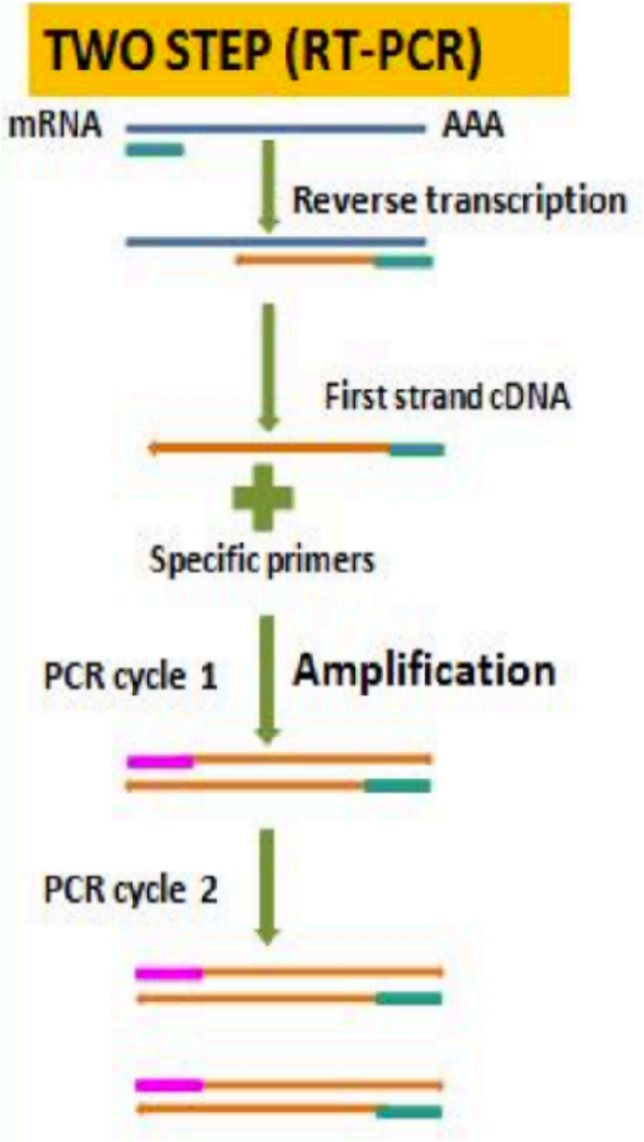


Fig. 1: Principle of PCR.



Sensitivity: correctly identify those with the disease

Specificity: correctly identify those without the disease

Singh et al, International Journal of Advanced Research in Biological Sciences, 2020

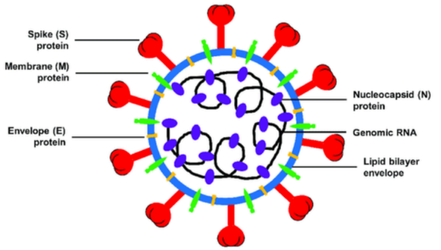
RT-PCR Test for COVID-19

LabCorp COVID-19 RT-PCR test EUA Summary

ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
COVID-19 RT-PCR TEST
(LABORATORY CORPORATION OF AMERICA)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

Primers to nucleocapsid gene (N) and RNase P (RP)



Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Caveats to Test interpretation

- Timing of infection
- Type of RT-PCR (ie which gene/gene region being amplified)
- Sample contamination
- Sensitivity/Specificity (currently 70-80% sensitivity)

New COVID-19 Diagnostic Tests (Acute Infection)

FDA has granted EUAs to several companies that are working on developing rapid testing technologies

ID NOW

Abbot

+ve result in 5 min

Rutgers Clinical Genomics Laboratory

Saliva test

LabCorp's RT-PCR Self Collection kit

CRISPR

Mammoth Biosciences and UCSF: DETECTR

Broad Institute: SHERLOCK

COVID-19 Diagnostic Tests (Past Infection)

FDA has granted EUAs to four companies for serological antibody test

- 120 antibody-testing developers with applications for EUA

Cellex IgM/IgG
94% sensitivity
96% specificity

Ortho Clinical Diagnostics
83% sensitivity
100% specificity

Chembio
87% sensitivity
93% specificity

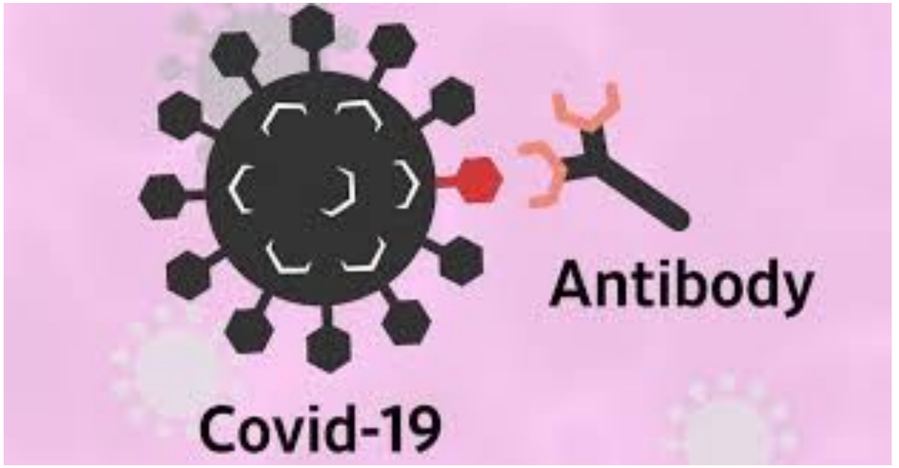
Mount Sinai
92.5% sensitivity
100% specificity

Serological Testing Considerations

- >90 companies in the market without FDA approval
- Type of antigen chosen for serological test
- Sensitivity and Specificity
- Unknowns about neutralizing antibodies for re-infection (ie long lasting immunity)
- Populations tested

USC-LA County Study: Early Results of Antibody Testing Suggest Number of COVID-19 Infections Far Exceeds Number of Confirmed Cases in Los Angeles County

- ~4.1% (approximately 221,000 to 442,000 adults) tested +ve for Abs against SARS-CoV2.
- estimate is 28 to 55 times higher than the 7,994 confirmed cases of COVID-19



COVID-19 Antibody Seroprevalence in Santa Clara County, California

Eran Bendavid[1], Bianca Mulaney[2], Neeraj Sood[3], Soleil Shah[2], Emilia Ling[2], Rebecca Bromley-Dulfano[2], Cara Lai[2], Zoe Weissberg[2], Rodrigo Saavedra-Walker[4], Jim Tedrow[5], Dona Tversky[6], Andrew Bogan[7], Thomas Kupiec[8], Daniel Eichner[9], Ribhav Gupta[10], John P.A. Ioannidis[1,10], Jay Bhattacharya[1]

- Prevalence rate of 2.49% (95CI 1.80-3.17%) to 4.16% (48,000 and 81,000)
- estimate is 85-fold more than the number of confirmed cases.

Study Recruitment

A Stanford Professor's Wife Recruited People For His Coronavirus Study By Claiming It Would Reveal If They Could "Return To Work Without Fear"

"If you have antibodies against the virus, you are FREE from the danger of a) getting sick or b) spreading the virus. In China and U.K. they are asking for proof of immunity before returning to work. If you know any small business owners or employees that have been laid off, let them know -- they no longer need to quarantine and can return to work without fear."

NIH begins study to quantify undetected cases of coronavirus infection

ClinicalTrials.gov

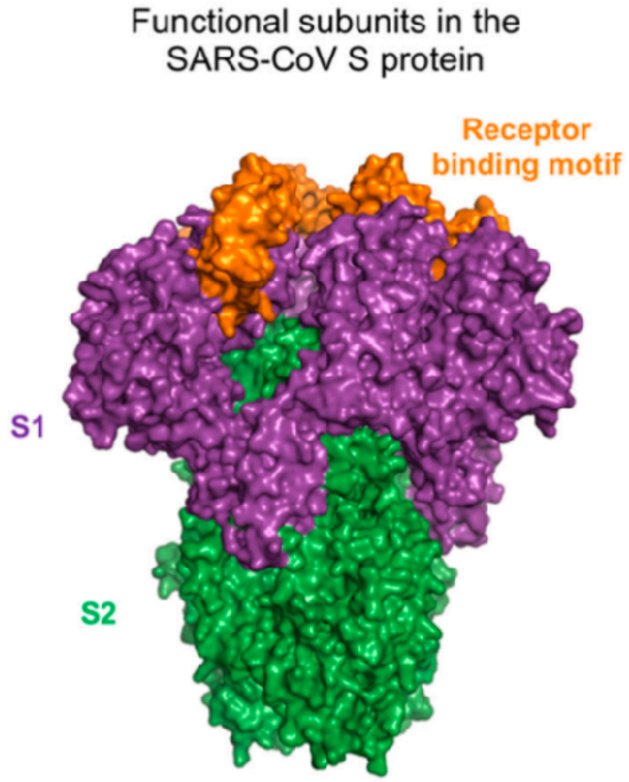
SARS-COV2 Pandemic Serosurvey and Blood Sampling

NCT04334954

- 10,000 individuals
- Healthy volunteers over the age of 18 from anywhere in the United States
- screened over the phone with a health assessment questionnaire
- at-home blood sampling: Neoteryx

Vaccine Design Considerations

- **Full-length S protein**
  - may have enhancement of viral infection in vitro (Kam et al., 2007; Jaume et al., 2012)
- **RBD**
  - critical neutralizing domain —> potent neutralizing antibodies
  - But — large genetic mismatches in known structural epitopes targeting this domain
- **S2 subunit**
  - epitopes less exposed
  - high homology across viral strains — can induce a strong immune response against divergent virus strains
- **S1 subunit**
  - alternative target for subunit vaccine
- **N Protein**
  - highly effective short term, not effective for long term immunity



- **Humoral responses**
- **T cell responses against S and N proteins**

Vaccines in clinical Trials

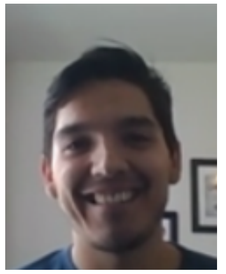
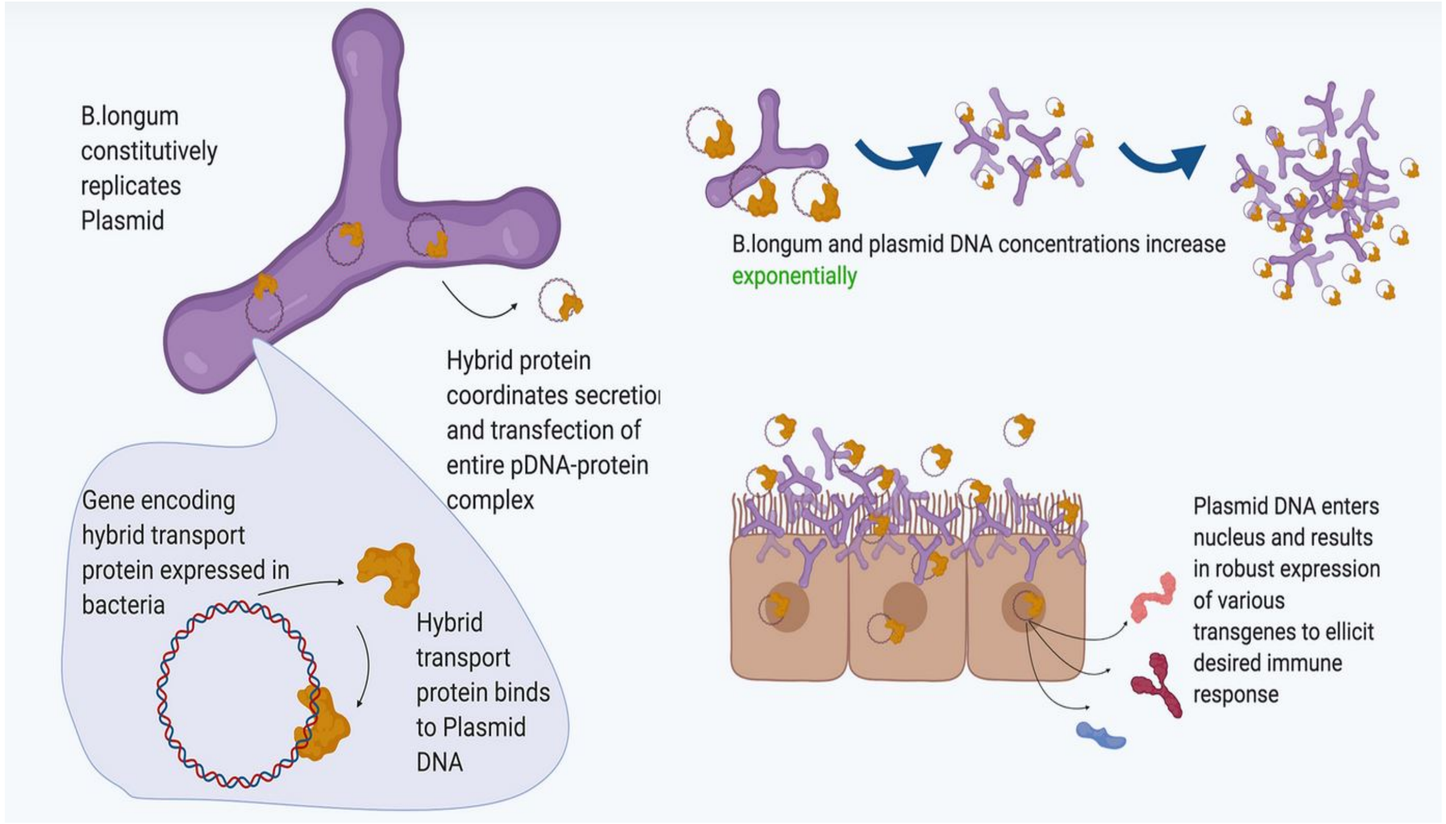


Table 1 – Summary of vaccine candidates currently in clinical trials.

Vaccine Candidate	Characteristics	Developer	StatusNTC
mRNA-1273	mRNA vaccine that encodes for a prefusion stabilized form of the spike (S) protein of the virus	Moderna	Phase 1 NCT04283461
INO-4800	Optimized DNA plasmid that encodes for spike (S) protein	Inovio Pharmaceuticals	Phase 1 NCT04336410
Ad5-nCoV	Adenovirus-based viral vector vaccine that expresses the spike (S) protein	CanSino Biologicals	Phase 1 NCT04313127
Pathogen-specific aAPC	Artificial antigen presenting cells (aAPCs) modified to present selected viral proteins	Shenzhen Geno-Immune Medical Institute	Phase 1 NCT04299724
LV-SMENP-DC	Uses lentiviral vector system to express viral proteins and immune modulatory genes to modify dendritic cells (DCs) and activate T cells	Shenzhen Geno-Immune Medical Institute	Phase 1 NCT04276896

bacTRL-Spike
(NCT04334980)

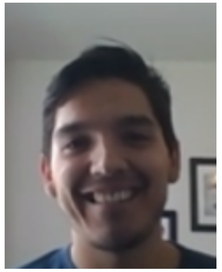
- *Bifidobacterium* probiotic engineered to carry DNA encoding the SARS-CoV-2 spike protein
- **Route**: Oral lyophilized gel-capsules
- "Durable multivalent antigen expression in the gastrointestinal lymphoid tissues initiate robust mucosal and systemic humoral and cell-mediated immunity, whereas expression of neutralizing nanobodies provide immediate passive immunity"
- Developed by **Symvivo** (trial to start in April 30)



Steps

1. Colonization of the gut
2. Binding to intestinal epithelial cells
3. Replication, secretion and transport of plasmid DNA molecules encoding antigenic transgenes and neutralizing nanobodies
4. Immune response

Slide Courtesy of Hector Fernandez



Clinical Trials in the US

- 178 registered studies with the National Library of Medicine in the US

Remdesivir	NCT04280705, NCT04292899, NCT04292730, WHO Solidarity, DisCoVeRy, and more.
Chloroquine and hydroxychloroquine	NCT04318444, NCT04325893, NCT04321278, NCT04341727, And more: Chloroquine and Hydroxychloroquine
IFN-beta with Ritonavir/lopinavir	WHO Solidarity, DisCoVeRy (NCT04315948), NCT04276688, NCT04291729, and more.
Ritonavir/lopinavir	WHO Solidarity, DisCoVeRy, NCT04321174, NCT04307693, and more.

Slide Courtesy of Cole Maguire

COVID19 Challenges Beyond Flattering the Curve: What will the “new normal” look like?

- ?recurrent interval testing
- ?digital tracing
- ?intermittent quarantine
- continued social distancing

Google

“joint effort to enable the use of Bluetooth technology to help governments and health agencies reduce the spread of the virus, with user privacy and security central to the design.”

THE NEW YORKER

HOW MY SOVIET MOM PREPARED ME FOR THE CORONAVIRUS PANDEMIC

By Jenny Kroik April 22, 2020

"Cheer up! Pushkin spent months in quarantine during the cholera epidemic."

"We used to stand in a long line for hours to get toilet paper!"

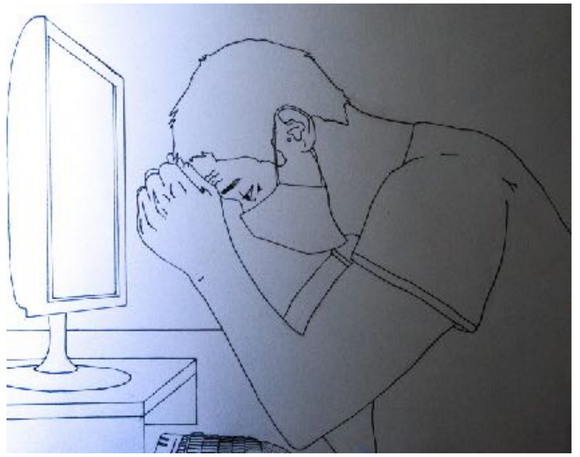
"Is that how you wash vegetables? You have to use soap! And soak your grapes in baking soda for thirty minutes."

Melamed Lab

Before COVID-19

After COVID-19

COVID19 BLOG
http://sites.utexas.edu/melamed-lab/blog/



SAM BAZZI
Bluelight Blues
March 2020
Ink on paper