



# Pathogenesis, Symptomatology, and Transmission of SARS-CoV-2 through Analysis of Viral Genomics and Structure

Halie M. Rando,<sup>a,b,c</sup> Adam L. MacLean,<sup>d</sup> Alexandra J. Lee,<sup>a</sup> Ronan Lordan,<sup>e</sup> Sandipan Ray,<sup>f</sup> Vikas Bansal,<sup>g</sup> Ashwin N. Skelly,<sup>h,i</sup> Elizabeth Sell,<sup>h</sup> John J. Dziak,<sup>j</sup> Lamonica Shinholster,<sup>k</sup> Lucy D'Agostino McGowan,<sup>l</sup> Marouen Ben Guebila,<sup>m</sup> Nils Wellhausen,<sup>a</sup> Sergey Knyazev,<sup>n</sup> Simina M. Boca,<sup>o</sup> Stephen Capone,<sup>p</sup> Yanjun Qi,<sup>q</sup> YoSon Park,<sup>a</sup> David Mai,<sup>r</sup> Yuchen Sun,<sup>q</sup> Joel D. Boerckel,<sup>r,s</sup> Christian Brueffer,<sup>t</sup> James Brian Byrd,<sup>u</sup> Jeremy P. Kamil,<sup>v</sup> Jinhui Wang,<sup>h</sup> Ryan Velazquez,<sup>w</sup> Gregory L. Szeto,<sup>x</sup> John P. Barton,<sup>y</sup> Rishi Raj Goel,<sup>i</sup> Serghei Mangul,<sup>z</sup> Tiago Lubiana,<sup>aa</sup> COVID-19 Review Consortium Anthony Gitter,<sup>bb,cc</sup> Casey S. Greene<sup>a,b,c,dd</sup>

<sup>a</sup>Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, Pennsylvania, USA

<sup>b</sup>Department of Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, Colorado, USA

<sup>c</sup>Center for Health AI, University of Colorado School of Medicine, Aurora, Colorado, USA

<sup>d</sup>Department of Quantitative and Computational Biology, University of Southern California, Los Angeles, California, USA

<sup>e</sup>Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

<sup>f</sup>Department of Biotechnology, Indian Institute of Technology Hyderabad, Sangareddy, Telangana, India

<sup>g</sup>Biomedical Data Science and Machine Learning Group, German Center for Neurodegenerative Diseases, Tübingen, Germany

<sup>h</sup>Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

<sup>i</sup>Institute for Immunology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

<sup>j</sup>Edna Bennett Pierce Prevention Research Center, The Pennsylvania State University, University Park, Pennsylvania, USA

<sup>k</sup>Mercer University, Macon, Georgia, USA

<sup>l</sup>Department of Mathematics and Statistics, Wake Forest University, Winston-Salem, North Carolina, USA

<sup>m</sup>Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts, USA

<sup>n</sup>Georgia State University, Atlanta, Georgia, USA

<sup>o</sup>Innovation Center for Biomedical Informatics, Georgetown University Medical Center, Washington, DC, USA

<sup>p</sup>St. George's University School of Medicine, St. George's, Grenada

<sup>q</sup>Department of Computer Science, University of Virginia, Charlottesville, Virginia, USA

<sup>r</sup>Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania, USA

<sup>s</sup>Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

<sup>t</sup>Department of Clinical Sciences, Lund University, Lund, Sweden

<sup>u</sup>University of Michigan School of Medicine, Ann Arbor, Michigan, USA

<sup>v</sup>Department of Microbiology and Immunology, Louisiana State University Health Sciences Center Shreveport, Shreveport, Louisiana, USA

<sup>w</sup>Azimuth1, McLean, Virginia, USA

<sup>x</sup>Allen Institute for Immunology, Seattle, Washington, USA

<sup>y</sup>Department of Physics and Astronomy, University of California-Riverside, Riverside, California, USA

<sup>z</sup>Department of Clinical Pharmacy, School of Pharmacy, University of Southern California, Los Angeles, California, USA

<sup>aa</sup>Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

<sup>bb</sup>Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison, Madison, Wisconsin, USA

<sup>cc</sup>Morgridge Institute for Research, Madison, Wisconsin, USA

<sup>dd</sup>Childhood Cancer Data Lab, Alex's Lemonade Stand Foundation, Philadelphia, Pennsylvania, USA

**ABSTRACT** The novel coronavirus SARS-CoV-2, which emerged in late 2019, has since spread around the world and infected hundreds of millions of people with coronavirus disease 2019 (COVID-19). While this viral species was unknown prior to January 2020, its similarity to other coronaviruses that infect humans has allowed for rapid insight into the mechanisms that it uses to infect human hosts, as well as the ways in which the human immune system can respond. Here, we contextualize SARS-CoV-2 among other

**Citation** Rando HM, MacLean AL, Lee AJ, Lordan R, Ray S, Bansal V, Skelly AN, Sell E, Dziak JJ, Shinholster L, D'Agostino McGowan L, Ben Guebila M, Wellhausen N, Knyazev S, Boca SM, Capone S, Qi Y, Park Y, Mai D, Sun Y, Boerckel JD, Brueffer C, Byrd JB, Kamil JP, Wang J, Velazquez R, Szeto GL, Barton JP, Goel RR, Mangul S, Lubiana T, COVID-19 Review Consortium, Gitter A, Greene CS. 2021. Pathogenesis, symptomatology, and transmission of SARS-CoV-2 through analysis of viral genomics and structure. *mSystems* 6:e00095-21. <https://doi.org/10.1128/mSystems.00095-21>.

**Editor** Jack A. Gilbert, University of California San Diego

**Copyright** © 2021 Rando et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Casey S. Greene, [casey.s.greene@cuanschutz.edu](mailto:casey.s.greene@cuanschutz.edu).

This represents one section of a larger evolving review on SARS-CoV-2 and COVID-19, which is regularly updated and available at <https://greenelab.github.io/covid19-review/>.

This is a review paper that is authored by scientists for an audience of scientists to discuss research that is in progress. If you are interested in guidelines on testing, therapies, or other issues related to your health, you should not use this document. Instead, you should collect information from your local health department, the CDC's guidance, or your own government.

**Accepted** 27 September 2021

**Published** 26 October 2021

[This article was published on 26 October 2021 but required additional changes, now reflected in the Note Added after Publication on p. 22. The changes to the article were made on 18 January 2022.]

coronaviruses and identify what is known and what can be inferred about its behavior once inside a human host. Because the genomic content of coronaviruses, which specifies the virus's structure, is highly conserved, early genomic analysis provided a significant head start in predicting viral pathogenesis and in understanding potential differences among variants. The pathogenesis of the virus offers insights into symptomatology, transmission, and individual susceptibility. Additionally, prior research into interactions between the human immune system and coronaviruses has identified how these viruses can evade the immune system's protective mechanisms. We also explore systems-level research into the regulatory and proteomic effects of SARS-CoV-2 infection and the immune response. Understanding the structure and behavior of the virus serves to contextualize the many facets of the COVID-19 pandemic and can influence efforts to control the virus and treat the disease.

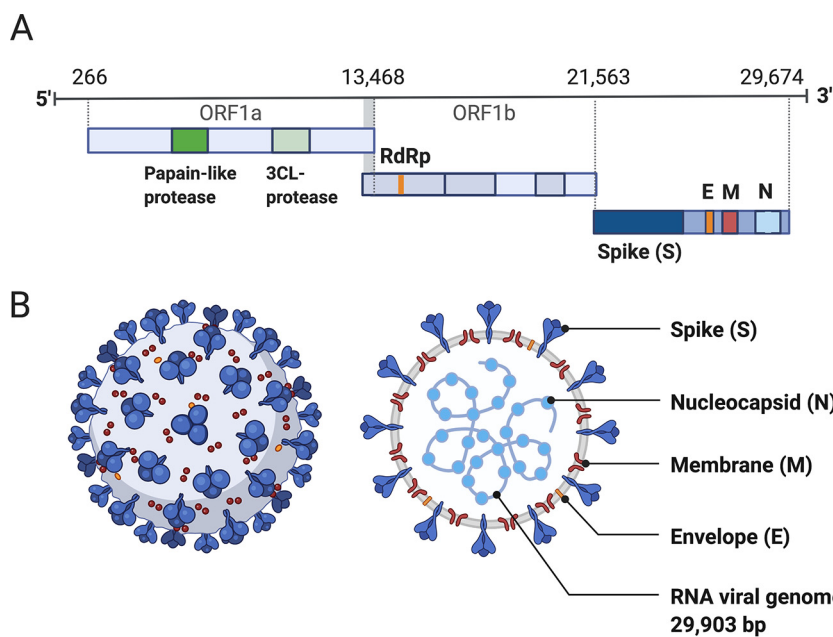
**IMPORTANCE** COVID-19 involves a number of organ systems and can present with a wide range of symptoms. From how the virus infects cells to how it spreads between people, the available research suggests that these patterns are very similar to those seen in the closely related viruses SARS-CoV-1 and possibly Middle East respiratory syndrome-related CoV (MERS-CoV). Understanding the pathogenesis of the SARS-CoV-2 virus also contextualizes how the different biological systems affected by COVID-19 connect. Exploring the structure, phylogeny, and pathogenesis of the virus therefore helps to guide interpretation of the broader impacts of the virus on the human body and on human populations. For this reason, an in-depth exploration of viral mechanisms is critical to a robust understanding of SARS-CoV-2 and, potentially, future emergent human CoVs (HCoVs).

**KEYWORDS** COVID-19, genomics, review, viral pathogenesis

The current coronavirus disease 2019 (COVID-19) pandemic, caused by the *Severe acute respiratory syndrome-related coronavirus 2* (SARS-CoV-2) virus, represents an acute global health crisis. Symptoms of the disease can range from mild to severe or fatal (1) and can affect a variety of organs and systems (2). Outcomes of infection can include acute respiratory distress syndrome (ARDS) and acute lung injury, as well as damage to other organ systems (2, 3). Understanding the progression of the disease, including these diverse symptoms, depends on understanding how the virus interacts with the host. Additionally, the fundamental biology of the virus can provide insights into how it is transmitted among people, which can, in turn, inform efforts to control its spread. As a result, a thorough understanding of the pathogenesis of SARS-CoV-2 is a critical foundation on which to build an understanding of COVID-19 and the pandemic as a whole.

The rapid identification and release of the genomic sequence of the virus in January 2020 (4) provided early insight into the virus in a comparative genomic context. The viral genomic sequence clusters with known coronaviruses (order *Nidovirales*, family *Coronaviridae*, subfamily *Orthocoronavirinae*). Phylogenetic analysis of the coronaviruses reveals four major subclades, each corresponding to a genus: the alpha, beta, gamma, and delta coronaviruses. Among them, alpha and beta coronaviruses infect mammalian species, gamma coronaviruses infect avian species, and delta coronaviruses infect both mammalian and avian species (5). The novel virus now known as SARS-CoV-2 was identified as a beta coronavirus belonging to the B lineage based on phylogenetic analysis of a PCR amplicon fragment from five patients along with the full genomic sequence (6). This lineage also includes the *Severe acute respiratory syndrome-related coronavirus* (SARS-CoV-1) that caused the 2002–2003 outbreak of severe acute respiratory syndrome (SARS) in humans (6). (Note that these subclades are not to be confused with variants of concern [VOC] within SARS-CoV-2 labeled with Greek letters; i.e., the Delta variant of SARS-CoV-2 is still a beta coronavirus).

Because viral structure and mechanisms of pathogenicity are highly conserved within the order, this phylogenetic analysis provided a basis for forming hypotheses about how the virus interacts with hosts, including which tissues, organs, and systems would be most susceptible to SARS-CoV-2 infection. Coronaviruses that infect humans (HCoVs) are not common, but prior research into other HCoVs such as SARS-CoV-1 and *Middle East respiratory syndrome-related*



**FIG 1** Structure of SARS-CoV-2 capsid and genome. (A) The genomic structure of coronaviruses is highly conserved and includes three main regions. Open reading frames (ORFs) 1a and 1b contain two polyproteins that encode the nonstructural proteins (nsp). The nsp include enzymes such as RNA-dependent RNA polymerase (RdRp). The last third of the genome encodes structural proteins, including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. Accessory genes can also be interspersed throughout the genome (13). (B) The physical structure of the coronavirus virion, including the components determined by the conserved structural proteins S, E, M, and N. This figure was adapted from “Human Coronavirus Structure,” by BioRender.com (2020), retrieved from <https://app.biorender.com/biorender-templates>.

coronavirus (MERS-CoV), as well as other viruses infecting humans such as a variety of influenza virus species, established a strong foundation that accelerated the pace of SARS-CoV-2 research.

Coronaviruses are large viruses that can be identified by their distinctive “crown-like” shape (Fig. 1). Their spherical virions are made from lipid envelopes ranging from 100 to 160 nm in which peplomers (protruding structures) of two to three spike (S) glycoproteins are anchored, creating the crown (7, 8). These spikes, which are critical both to viral pathogenesis and to the response by the host immune system, have been visualized using cryo-electron microscopy (9). Because they induce the human immune response, they are also the target of many proposed therapeutic agents (10, 11). Viral pathogenesis is typically broken down into three major components: entry, replication, and spread (12). However, in order to draw a more complete picture of pathogenesis, it is also necessary to examine how infection manifests clinically, identify systems-level interactions between the virus and the human body, and consider the possible effects of variation or evolutionary change on pathogenesis and virulence. Thus, clinical medicine and traditional biology are both important pieces of the puzzle of SARS-CoV-2 presentation and pathogenesis.

## CORONAVIRUS STRUCTURE AND PATHOGENESIS

**Structure of coronaviruses.** Genome structure is highly conserved among coronaviruses, meaning that the relationship between the SARS-CoV-2 genome and its pathogenesis can be inferred from prior research in related viral species. The genomes of viruses in the *Nidovirales* order share several fundamental characteristics. They are nonsegmented, which means the viral genome is a single continuous strand of RNA, and are enveloped, which means that the genome and capsid are encased by a lipid bilayer. Coronaviruses have large positive-sense RNA (ssRNA+) genomes ranging from 27 to 32 kb in length (13, 14). The SARS-CoV-2 genome lies in the middle of this range at 29,903 bp (14). Genome organization is highly conserved within the order (13). There are three major genomic regions: one containing the replicase gene, one containing the genes encoding structural proteins, and interspersed

accessory genes (13) (Fig. 1). The replicase gene comprises about two-thirds of the genome and consists of two open reading frames that are translated with ribosomal frameshifting (13). This polypeptide is then translated into 16 nonstructural proteins (nsp), except in gamma coronaviruses where nsp1 is absent, which form the replication machinery used to synthesize viral RNA (15). The remaining third of the genome encodes structural proteins, including the spike (S), membrane, envelope, and nucleocapsid proteins. Additional accessory genes are sometimes present between these two regions, depending on the species or strain. Much attention has been focused on the S protein, which is a critical structure involved in cell entry.

**Pathogenic mechanisms of coronaviruses.** While it is possible that SARS-CoV-1 and SARS-CoV-2, like most viruses, enter cells through endocytosis, a process conserved among coronaviruses enables them to target cells for entry through fusion with the plasma membrane (16, 17). Cell entry proceeds in three steps: binding, cleavage, and fusion. First, the viral spike protein binds to a host cell via a recognized receptor or entry point. Coronaviruses can bind to a range of host receptors (18, 19), with binding conserved only at the genus level (5). Viruses in the beta coronavirus genus, to which SARS-CoV-2 belongs, are known to bind to the CEACAM1 protein, 5-*N*-acetyl-9-*O*-acetyl neuraminic acid, and to angiotensin-converting enzyme 2 (ACE2) (18). This recognition is driven by domains in the S1 subunit (20). SARS-CoV-2 has a high affinity for human ACE2, which is expressed in the vascular epithelium, other epithelial cells, and cardiovascular and renal tissues (21, 22), as well as many others (23). The binding process is guided by the molecular structure of the spike protein, which is structured in three segments: an ectodomain, a transmembrane anchor, and an intracellular tail (24). The ectodomain forms the crown-like structures on the viral membrane and contains two subdomains known as the S1 and S2 subunits (25). The S1 (N-terminal) domain forms the head of the crown and contains the receptor binding motif, and the S2 (C-terminal) domain forms the stalk that supports the head (25). The S1 subunit guides the binding of the virus to the host cell, and the S2 subunit guides the fusion process (24).

After the binding of the S1 subunit to an entry point, the spike protein of coronaviruses is often cleaved at the S1/S2 boundary into the S1 and S2 subunits by a host protease (20, 26, 27). This proteolytic priming is important because it prepares the S protein for fusion (26, 27). The two subunits remain bound by van der Waals forces, with the S1 subunit stabilizing the S2 subunit throughout the membrane fusion process (20). Cleavage at a second site within S2 (S2') activates S for fusion by inducing conformational changes (20). Similar to SARS-CoV-1, SARS-CoV-2 exhibits redundancy in which host proteases can cleave the S protein (28). Both transmembrane protease serine protease-2 (TMPRSS-2) and cathepsins B/L have been shown to mediate SARS-CoV-2 S protein proteolytic priming, and small-molecule inhibition of these enzymes fully inhibited viral entry *in vitro* (28, 29). Other proteases known to cleave the S1/S2 boundary in coronaviruses include TMPRSS-4, trypsin, furin, cathepsins, and human airway trypsin-like protease (HAT) (29).

Unlike in SARS-CoV-1, a second cleavage site featuring a furin-like binding motif is also present near the S1/S2 boundary in SARS-CoV-2 (30). This site is found in HCoV-229E belonging to the A and C lineages of beta coronavirus, including MERS-CoV, but not in the other known members of the B lineage of beta coronavirus that contains SARS-CoV-1 and SARS-CoV-2 (30). It is associated with increased virulence in other viral species (30) and may facilitate membrane fusion of SARS-CoV-2 in the absence of other proteases that prime the S1/S2 site (31). However, given that proteases such as HAT are likely to be present in targets like the human airway, the extent to which this site has had a real-world effect on the spread of SARS-CoV-2 was initially unclear (31). Subsequent research has supported this site as an important contributor to pathogenesis: *in vitro* analyses have reported that it bolsters pathogenicity specifically in cell lines derived from human airway cells (Calu3 cell line) (32–34) and that furin inhibitors reduced pathogenic effects in VeroE6 cells (35).

Electron microscopy suggests that in some coronaviruses, including SARS-CoV-1 and MERS-CoV, a six-helix bundle separates the two subunits in the postfusion conformation, and the unusual length of this bundle facilitates membrane fusion through the release of additional energy (5). The viral membrane can then fuse with the endosomal membrane to release the viral genome into the host cytoplasm. Once the virus enters

a host cell, the replicase gene is translated and assembled into the viral replicase complex. This complex then synthesizes the double-stranded RNA (dsRNA) genome from the genomic ssRNA(+). The dsRNA genome is transcribed and replicated to create viral mRNAs and new ssRNA(+) genomes (13, 36). From there, the virus can spread into other cells. In SARS-CoV-2, the insertion of the furin-like binding site near the S1/S2 boundary is also thought to increase cell-cell adhesion, making it possible for the viral genome to spread directly from cell to cell rather than needing to propagate the virion itself (37). In this way, the genome of SARS-CoV-2 provides insight into the pathogenic behavior of the virus.

Evidence also suggests that SARS-CoV-2 may take advantage of the specific structure of endothelial cells to enter the circulatory system. Endothelial cells are specialized epithelial cells (38) that form a barrier between the bloodstream and surrounding tissues. The endothelium facilitates nutrient, oxygen, and cellular exchange between the blood and vascularized tissues (39). The luminal (interior) surface of the endothelium is lined with glycocalyx, a network of both membrane-bound and soluble proteins and carbohydrates, primarily proteoglycans and glycoproteins (40, 41). The glycocalyx varies in thickness from 0.5  $\mu\text{m}$  in the capillaries to 4.5  $\mu\text{m}$  in the carotid arteries and forms a meshwork that localizes both endothelium- and plasma-derived signals to the inner vessel wall (40). Heparan sulfate is the dominant proteoglycan in the glycocalyx, representing 50 to 90% of glycocalyx proteoglycan content (42). The SARS-CoV-2 spike protein can bind directly to heparan sulfate, which serves in part as a scaffolding molecule to facilitate ACE2 binding and entry into endothelial cells (41). A heparan sulfate binding site has also been identified near the ACE2 binding site on the viral receptor binding domain (RBD), and modeling has suggested that heparan sulfate binding yields an open conformation that facilitates binding to ACE2 on the cell surface (41). Degrading or removing heparan sulfate was associated with decreased binding (41). Heparan sulfate may also interact with the S1/S2 proteolytic cleavage site and other binding sites to promote binding affinity (43). Notably, treatment with soluble heparan sulfate or even heparin (a commonly used anticoagulant and vasodilator that is similar in structure to heparan sulfate [44]) potentially blocked spike protein binding and viral infection (41). This finding is particularly interesting because degradation of heparan sulfate in the glycocalyx has previously been identified as an important contributor to ARDS and sepsis (45), two common and severe outcomes of COVID-19, and suggests that heparan sulfate could be a target for pharmaceutical inhibition of cell entry by SARS-CoV-2 (46–50). Together, this evidence suggests that heparan sulfate can serve as an important adhesion molecule for SARS-CoV-2 cell entry. It may represent a therapeutic target but has not been pursued as much as other candidate targets (10).

**Immune evasion strategies.** Research in other HCoV provides some indication of how SARS-CoV-2 infection can proceed despite human immune defenses. Infecting the epithelium can help viruses such as SARS-CoV-1 bypass the physical barriers, such as mucus, that comprise the immune system's first line of defense (51). Once the virus infiltrates host cells, it is adept at evading detection. CD163<sup>+</sup> and CD68<sup>+</sup> macrophage cells are especially crucial for the establishment of SARS-CoV-1 in the body (51). These cells most likely serve as viral reservoirs that help shield SARS-CoV-1 from the innate immune response. According to a study on the viral dissemination of SARS-CoV-1 in Chinese macaques, viral RNA could be detected in some monocytes throughout the process of differentiation into dendritic cells (51). This lack of active viral replication allows SARS-CoV-1 to escape the innate immune response because reduced levels of detectable viral RNA allow the virus to avoid both natural killer cells and Toll-like receptors (51). Even during replication, SARS-CoV-1 is able to mask its dsRNA genome from detection by the immune system. Although dsRNA is a pathogen-associated molecular pattern that would typically initiate a response from the innate immune system (52), *in vitro* analysis of nidoviruses including SARS-CoV-1 suggests that these viruses can induce the development of double-membrane vesicles that protect the dsRNA signature from being detected by the host immune system (53). This protective envelope can therefore insulate these coronaviruses from the innate immune system's detection mechanism (54).



HCoVs are also known to interfere with the host immune response, rather than just evade it. For example, the virulence of SARS-CoV-2 is increased by nsp1, which can suppress host gene expression by stalling mRNA translation and inducing endonucleolytic cleavage and mRNA degradation (55). SARS-CoV-1 also evades the immune response by interfering with type I interferon (IFN) induction signaling, which is a mechanism that leads to cellular resistance to viral infections. SARS-CoV-1 employs methods such as ubiquitination and degradation of RNA sensor adaptor molecules MAVS and TRAF3/6 (56). Also, MERS-CoV downregulates antigen presentation via major histocompatibility complex (MHC) class I and MHC class II, which leads to a reduction in T cell activation (56). These evasion mechanisms, in turn, may facilitate systemic infection. Coronaviruses such as SARS-CoV-1 are also able to evade the humoral immune response through other mechanisms, such as inhibiting certain cytokine pathways or downregulating antigen presentation by the cells (53).

**Host cell susceptibility.** ACE2 and TMPRSS-2 have been identified as the primary entry portal and as a critical protease, respectively, in facilitating the entry of SARS-CoV-1 and SARS-CoV-2 into a target cell (9, 28, 57–59). This finding has led to a hypothesized role for the expression of these molecules in determining which cells, tissues, and organs are most susceptible to SARS-CoV-2 infection. ACE2 is expressed in numerous organs, such as the heart, kidney, and intestine, but it is most prominently expressed in alveolar epithelial cells; this pattern of expression is expected to contribute to the virus' association with lung pathology (21, 60, 61) as well as that of SARS (62). A retrospective observational study reported indirect evidence that certain antineoplastic therapies, such as the chemotherapy drug gemcitabine, may reduce risk of SARS-CoV-2 infection in patients with cancer, possibly via decreased ACE2 expression (63). Additionally, the addition of the furin site insertion at the S1/S2 boundary means that SARS-CoV-2 does not require TMPRSS-2 when furin, a ubiquitously expressed endoprotease (64), is present, enabling cell-cell fusion independent of TMPRSS-2 availability (65).

Clinical investigations of COVID-19 patients have detected SARS-CoV-2 transcripts in bronchoalveolar lavage fluid (BALF) (93% of specimens), sputum (72%), nasal swabs (63%), fibrobronchoscopy brush biopsy specimens (46%), pharyngeal swabs (32%), feces (29%), and blood (1%) (66). Two studies reported that SARS-CoV-2 could not be detected in urine specimens (66, 67); however, a third study identified four urine samples (out of 58) that were positive for SARS-CoV-2 nucleic acids (68). Although respiratory failure remains the leading cause of death for COVID-19 patients (69), SARS-CoV-2 infection can damage many other organ systems including the heart (70), kidneys (71, 72), liver (73), and gastrointestinal tract (74, 75). As it becomes clear that SARS-CoV-2 infection can damage multiple organs, the scientific community is pursuing multiple avenues of investigation in order to build a consensus about how the virus affects the human body.

## CLINICAL PRESENTATION OF COVID-19

SARS-CoV-2 pathogenesis is closely linked with the clinical presentation of the COVID-19 disease. Reports have described diverse symptom profiles associated with COVID-19, with a great deal of variability both within and between institutions and regions. Definitions for nonsevere, severe, and critical COVID-19, along with treatment recommendations, are available from the World Health Organization living guidelines (76). A large study from Wuhan, China, conducted early in the pandemic identified fever and cough as the two most common symptoms that patients reported at hospital admission (77), while a retrospective study in China described the clinical presentations of patients infected with SARS-CoV-2 as including lower respiratory tract infection with fever, dry cough, and dyspnea (shortness of breath) (78). This study (78) noted that upper respiratory tract symptoms were less common, suggesting that the virus preferentially targets cells located in the lower respiratory tract. However, data from the New York City region (79, 80) showed variable rates of fever as a presenting symptom, suggesting that symptoms may not be consistent across individuals. For example, even within New York City, one study (79) identified low oxygen saturation (<90% without the use of supplemental oxygen or ventilation support) in 20.4% of patients upon presentation, with fever being present in 30.7%, while another study (80)

reported cough (79.4%), fever (77.1%), and dyspnea (56.5%) as the most common presenting symptoms; both of these studies considered only hospitalized patients. A later study reported radiographic findings such as ground-glass opacity and bilateral patchy shadowing in the lungs of many hospitalized patients, with most COVID-19 patients having lymphocytopenia, or low levels of lymphocytes (a type of white blood cell) (77). Patients may also experience loss of smell, myalgias (muscle aches), fatigue, or headache. Gastrointestinal symptoms can also present (81), and the CDC includes nausea and vomiting, as well as congestion and runny nose, on its list of symptoms consistent with COVID-19 (1). An analysis of an app-based survey of 500,000 individuals in the United States found that among those tested for SARS-CoV-2, a loss of taste or smell, fever, and a cough were significant predictors of a positive test result (82). It is important to note that in this study, the predictive value of symptoms may be underestimated if they are not specific to COVID-19. This underestimation could occur because the outcome measured was a positive, as opposed to a negative, COVID-19 test result, meaning an association would be more easily identified for symptoms that were primarily or exclusively found with COVID-19. At the time the surveys were conducted, due to limits in U.S. testing infrastructure, respondents typically needed to have some symptoms known to be specific to COVID-19 in order to qualify for testing. Widespread testing of asymptomatic individuals may therefore provide additional insight into the range of symptoms associated with COVID-19.

Consistent with the wide range of symptoms observed and the pathogenic mechanisms described above, COVID-19 can affect a variety of systems within the body in addition to causing respiratory problems (83). For example, COVID-19 can lead to acute kidney injury, especially in patients with severe respiratory symptoms or certain preexisting conditions (84). Some patients are at risk for collapsing glomerulopathy (85).

COVID-19 can also cause neurological complications (86–88), potentially including stroke, seizures, or meningitis (89, 90). One study on autopsy samples suggested that SARS-CoV-2 may be able to enter the central nervous system via the neural-mucosal interface (91). However, a study of 41 autopsied brains (92) found no evidence that the virus can actually infect the central nervous system. Although there was viral RNA in some brain samples, it was found in only very small amounts, and no viral protein was found. The RNA may have been in the blood vessels or blood components and not in the brain tissue itself. Instead, the neuropathological effects of COVID-19 are more likely to be caused indirectly by hypoxia, coagulopathy, or inflammatory processes rather than by infection in the brain (92). COVID-19 has been associated with an increased incidence of large vessel stroke, particularly in patients under the age of 40 (93), and other thrombotic events including pulmonary embolism and deep vein thrombosis (94). The mechanism behind these complications has been suggested to be related to coagulopathy, with reports indicating the presence of antiphospholipid antibodies (95) and elevated levels of D-dimer and fibrinogen degradation products in deceased patients (96). Other viral infections have been associated with coagulation defects and changes to the coagulation cascade; notably, SARS was also found to lead to disseminated intravascular coagulation and was associated with both pulmonary embolism and deep vein thrombosis (97). The mechanism behind these insults has been suggested to be related to inflammation-induced increases in the von Willebrand factor clotting protein, leading to a procoagulative state (97). Abnormal clotting (thromboinflammation or coagulopathy) has been increasingly discussed recently as a possible key mechanism in many cases of severe COVID-19 and may be associated with the high D-dimer levels often observed in severe cases (98–100). This excessive clotting in lung capillaries has been suggested to be related to a dysregulated activation of the complement system, part of the innate immune system (101, 102).

Finally, concerns have been raised about long-term sequelae of COVID-19. Some COVID-19 patients have reported that various somatic symptoms (such as shortness of breath, fatigue, and chest pain) and psychological symptoms (depression, anxiety, or mild cognitive impairment) can last for months after infection (103). Such long-term effects occur in both adults (104) and children (105). Sustained symptoms affecting a

variety of biological systems have been reported across many studies (e.g., references 103, 106, and 107). The phenomenon of “long COVID” is not fully understood, although various possible explanations have been proposed, including damage caused by immune response to infection as well as by the infection itself, in addition to negative consequences of the experience of lengthy illness and hospitalization. However, a lack of consistency among definitions used in different studies makes it difficult to develop precise definitions or identify specific symptoms associated with long-term effects of COVID-19 (108, 109). Patient and family support groups for “long haulers” have been formed online, and patient-driven efforts to collect data about postacute COVID-19 provide valuable sources of information (e.g., reference 106). The specific relationship between viral pathogenesis and these reported sequelae remains to be uncovered, however.

**Pediatric presentation.** The presentation of COVID-19 infection can vary greatly among pediatric patients and, in some cases, manifests in distinct ways from COVID-19 in adults. Evidence suggests that children and adolescents tend to have mostly asymptomatic infections and that those who are symptomatic typically exhibit mild illness (110–113). One review examined symptoms reported in 17 studies of children infected with COVID-19 during the early months of the COVID-19 epidemic in China and one study from Singapore (114). In the more than a thousand cases described, the most common reports were for mild symptoms such as fever, dry cough, fatigue, nasal congestion, and/or runny nose, while three children were reported to be asymptomatic. Severe lower respiratory infection was described in only one of the pediatric cases reviewed. Gastrointestinal symptoms such as vomiting or diarrhea were occasionally reported. Radiologic findings were not always reported in the case studies reviewed, but when they were mentioned, they included bronchial thickening, ground-glass opacities, and/or inflammatory lesions (114). Neurological symptoms have also been reported (115).

These analyses indicate that most pediatric cases of COVID-19 are not severe. Indeed, it is estimated that less than 1% of pediatric cases result in critical illness (112, 116), although reporting suggests that pediatric hospitalizations may be greater with the emergence of the Delta variant of concern (VOC) (117–119). Serious complications and, in relatively rare cases, deaths have occurred (120). Of particular interest, children have occasionally experienced a serious inflammatory syndrome, multisystem inflammatory syndrome in children (MIS-C), following COVID-19 infection (121). This syndrome is similar in some respects to Kawasaki disease, including Kawasaki disease shock syndrome (122–124), and is thought to be a distinct clinical manifestation of SARS-CoV-2 due to its distinct cytokine profile and the presence of burr cells in peripheral blood smears (125, 126). MIS-C has been associated with heart failure in some cases (127). A small number of case studies have identified presentations similar to MIS-C in adults associated with SARS-CoV-2 (128–131). However, not all cases of severe COVID-19 in children are characterizable as MIS-C. A recent study (132) described demographic and clinical variables associated with MIS-C in comparison with non-MIS-C severe acute COVID-19 in young people in the United States. Efforts to characterize long-term sequelae of SARS-CoV-2 infection in children face the same challenges as in adults, but long-term effects remain a concern in pediatric patients (105, 133, 134), although some early studies have suggested that they may be less of a concern than in adults (135–137). Research is ongoing into the differences between the pediatric and adult immune responses to SARS-CoV-2, and future research may shed light on the factors that lead to MIS-C; it is also unknown whether the relative advantages of children against severe COVID-19 will remain in the face of current and future variants (138).

**Cytokine release syndrome.** The inflammatory response was identified early on as a potential driver of COVID-19 outcomes due to existing research in SARS and emerging research in COVID-19. While too low of an inflammatory response is a concern because it will fail to eliminate the immune threat (139), excessive proinflammatory cytokine activity can cascade (140) and cause cell damage, among other problems (141). A dysregulated immune response can cause significant damage to the host (142–144), including pathogenesis associated with sepsis. Sepsis, which can lead to multiorgan failure and death (145, 146), is traditionally associated with bacterial infections. However, sepsis associated with viral



infections may be underidentified (147), and sepsis has emerged as a major concern associated with SARS-CoV-2 infection (148). Hyperactivity of the proinflammatory response due to lung infection is commonly associated with acute lung injury and more rarely with the more severe manifestation, ARDS, which can arise from pneumonia, SARS, and COVID-19 (140, 145). Damage to the capillary endothelium can cause leaks that disrupt the balance between proinflammatory cytokines and their regulators (149), and heightened inflammation in the lungs can also serve as a source for systemic inflammation, or sepsis, and potentially multiorgan failure (145). The shift from local to systemic inflammation is a phenomenon often referred to broadly as a cytokine storm (145) or, more precisely, as cytokine release syndrome (150).

Cytokine dysregulation is therefore a significant concern in the context of COVID-19. In addition to the known role of cytokines in ARDS and lung infection more broadly, immunohistological analysis at autopsy of deceased SARS patients revealed that ACE2-expressing cells that were infected by SARS-CoV-1 showed elevated expression of the cytokines interleukin-6 (IL-6), IL-1 $\beta$ , and tumor necrosis factor alpha (TNF- $\alpha$ ) (151). Similarly, the introduction of the S protein from SARS-CoV-1 to mouse macrophages was found to increase production of IL-6 and TNF- $\alpha$  (152). For SARS-CoV-2 infection leading to COVID-19, early reports described a cytokine storm syndrome-like response in patients with particularly severe infections (60, 153, 154). Sepsis has been identified as a major contributor to COVID-19-related death. Among patients hospitalized with COVID-19 in Wuhan, China, 112 out of 191 (59%) developed sepsis, including all 54 of the nonsurvivors (78).

While IL-6 is sometimes used as a biomarker for cytokine storm activity in sepsis (145), the relationship between cytokine profiles and the risks associated with sepsis may be more complex. One study of patients with and at risk for ARDS, specifically those who were intubated for medical ventilation, found that shortly after the onset of ARDS, anti-inflammatory cytokine concentration in BALF increased relative to the concentration of proinflammatory cytokines (149). The results suggest that an increase in proinflammatory cytokines such as IL-6 may signal the onset of ARDS, but recovery depends on an increased anti-inflammatory response (149). However, patients with severe ARDS were excluded from this study. Another analysis of over 1,400 pneumonia patients in the United States reported that IL-6, tumor necrosis factor (TNF), and IL-10 were elevated at intake in patients who developed severe sepsis and/or ultimately died (155). However, unlike the study analyzing pro- and anti-inflammatory cytokines in ARDS patients (149), this study reported that unbalanced pro/anti-inflammatory cytokine profiles were rare. This discrepancy could be related to the fact that the sepsis study measured only three cytokines. Although IL-6 has traditionally been considered proinflammatory, its pleiotropic effects via both classical and *trans* signaling allow it to play an integral role in both the inflammatory and anti-inflammatory responses (156), leading it to be associated with both healthy and pathological responses to viral threat (157). While the cytokine levels observed in COVID-19 patients fall outside the normal range, they are not as high as typically found in patients with ARDS (158). Regardless of variation in the anti-inflammatory response, prior work has therefore made it clear that pulmonary infection and injury are associated with systemic inflammation and with sepsis. Inflammation has received significant interest in regard to both the pathology of COVID-19 as well as potential avenues for treatment, as the relationship between the cytokine storm and the pathophysiology of COVID-19 has led to the suggestion that a number of immunomodulatory pharmaceutical interventions could hold therapeutic value for the treatment of COVID-19 (10, 159).

### INSIGHTS FROM SYSTEMS BIOLOGY

Systems biology provides a cross-disciplinary analytical paradigm through which the host response to an infection can be analyzed. This field integrates the “omics” fields (genomics, transcriptomics, proteomics, metabolomics, etc.) using bioinformatics and other computational approaches. Over the last decade, systems biology approaches have been

used widely to study the pathogenesis of diverse types of life-threatening acute and chronic infectious diseases (160). Omics-based studies have also provided meaningful information regarding host immune responses and surrogate protein markers in several viral, bacterial, and protozoan infections (161). Though the complex pathogenesis and clinical manifestations of SARS-CoV-2 infection are not yet fully understood, omics technologies offer the opportunity for discovery-driven analysis of biological changes associated with SARS-CoV-2 infection.

**Transcriptomics.** Through transcriptomic analysis, the effect of a viral infection on gene expression can be assessed. Transcriptomic analyses, whether *in vivo* or *in situ*, can potentially reveal insights into viral pathogenesis by elucidating the host response to the virus. For example, infection by some viruses, including by the coronaviruses SARS-CoV-2, SARS-CoV-1, and MERS-CoV, is associated with the upregulation of ACE2 in human embryonic kidney cells and human airway epithelial cells (60). This finding suggests that SARS-CoV-2 facilitates the positive regulation of its own transmission between host cells (60). The host immune response also likely plays a key role in mediating infection-associated pathologies. Therefore, transcriptomics is one critical tool for characterizing the host response in order to gain insight into viral pathogenesis. For this reason, the application of omics technologies to the process of characterizing the host response is expected to provide novel insights into how hosts respond to SARS-CoV-2 infection and how these changes might influence COVID-19 outcomes.

Several studies have examined the cellular response to SARS-CoV-2 *in vitro* in comparison to other viruses. One study (162) compared the transcriptional responses of three human cell lines to SARS-CoV-2 and to other respiratory viruses, including MERS-CoV, SARS-CoV-1, *Human parainfluenza virus 3*, *Respiratory syncytial virus*, and *Influenza A virus*. The transcriptional response differed between the SARS-CoV-1-infected cells and the cells infected by other viruses, with changes in differential expression specific to each infection type. Where SARS-CoV-2 was able to replicate efficiently, differential expression analysis revealed that the transcriptional response was significantly different from the response to all of the other viruses tested. A unique proinflammatory cytokine signature associated with SARS-CoV-2 was present in cells exposed to both high and low doses of the virus, with the cytokines IL-6 and IL1RA uniquely elevated in response to SARS-CoV-2 relative to other viruses. However, one cell line showed significant IFN-I or IFN-III expression when exposed to high, but not low, doses of SARS-CoV-2, suggesting that IFN induction is dependent on the extent of exposure. These results suggest that SARS-CoV-2 induces a limited antiviral state with low IFN-I or IFN-III expression and a moderate IFN-stimulated gene response, in contrast to other viruses. Other respiratory viruses have been found to encode antagonists to the IFN response (163, 164), including SARS-CoV-1 (165) and MERS-CoV (166).

The analysis of SARS-CoV-2 suggested that this transcriptional state was specific to cells expressing ACE2, as it was not observed in cells lacking expression of this protein except with ACE2 supplementation and at a very high (10-fold increase) level of SARS-CoV-2 exposure (162). In another study, direct stimulation with inflammatory cytokines such as type I interferons (e.g., IFN- $\beta$ ) was also associated with the upregulation of ACE2 in human bronchial epithelial cells, with treated groups showing 4-fold-higher ACE2 expression than control groups at 18 h posttreatment (167). This hypothesis was further supported by studies showing that several nsp in SARS-CoV-2 suppress interferon activity (168) and that the SARS-CoV-2 *ORF3b* gene suppresses IFNB1 promoter activity (IFN-I induction) more efficiently than the SARS-CoV-1 *ORF3b* gene (169). Taken together, these findings suggest that a unique cytokine profile is associated with the response to the SARS-CoV-2 virus and that this response differs depending on the magnitude of exposure.

Susceptibility and IFN induction may also vary by cell type. Using poly(A) bulk transcriptome sequencing (RNA-seq) to analyze dynamic transcriptional responses to SARS-CoV-2 and SARS-CoV-1 revealed negligible susceptibility of cells from the H1299 line (<0.08 viral read percentage of total reads) compared to those from the Caco-2 and Calu-3 lines (>10% of viral reads) (170). This finding suggests that the risk of infection varies among cell types and that cell type could influence which hosts are more or less susceptible. Based on visual

inspection of microscopy images alongside transcriptional profiling, the authors also showed distinct responses among the host cell lines evaluated (170). In contrast to Caco-2, Calu-3 cells infected with SARS-CoV-2 showed signs of impaired growth and cell death at 24 h post-infection, as well as moderate IFN induction with a strong upregulation of IFN-stimulated genes. Interestingly, the results were similar to those reported in Calu-3 cells exposed to much higher levels of SARS-CoV-2 (162), as described above. This finding suggests that IFN induction in Calu-3 cells is not dependent on the level of exposure, in contrast to A549-ACE2 cells. The discrepancy could be explained by the observations that Calu-3 cells are highly susceptible to SARS-CoV-2 and show rapid viral replication (29), whereas A549 cells are incompatible with SARS-CoV-2 infection (171). This discrepancy raises the concern that *in vitro* models may vary in their similarity to the human response, underscoring the importance of follow-up studies in additional models.

As a result, transcriptional analysis of patient tissue is an important application of omics technology to understanding COVID-19. Several studies have collected blood samples from COVID-19 patients and analyzed them using RNA-seq (172–177). Analyzing gene expression in the blood is valuable to understanding host-pathogen interactions because of the potential to identify alterations associated with the immune response and to gain insights into inflammation, among other potential insights (172). One study compared gene expression in 39 COVID-19 inpatients admitted with community-acquired pneumonia to that of control donors using whole-blood-cell transcriptomes (172). They also evaluated the effect of mild versus severe disease. A greater number of differentially expressed genes were found in severe patients compared to controls than in mild patients compared to controls. They also identified that the transcriptional profiles clustered into five groups and that the groups could not be explained by disease severity. Most severe cases fell into two clusters associated with increased inflammation and granulocyte and neutrophil activation. The presence of these clusters suggests the possibility that personalized medicine could be useful in the treatment of COVID-19 (172). Longitudinal analysis of granulocytes from patients with mild versus severe COVID-19 revealed that granulocyte activation-associated factors differentiated the disease states, with greater numbers of differentially expressed genes early in the disease course (172). This study therefore revealed distinct patterns associated with COVID-19 and identified genes and pathways associated with each cluster.

Many other studies have also identified transcriptomic signatures associated with the immune response and inflammation. Other studies have profiled the transcriptome of BALF (174) and the nasopharynx (178). One study used single-cell transcriptomics techniques to investigate cell types including brain and choroid plexus cells compared to healthy controls and controls with influenza; among other signals of neuroinflammation, this study reported cortical T cells only in COVID-19 patients (179). Transcriptomic analysis can thus provide insight into the pathogenesis of SARS-CoV-2 and may also be useful in identifying candidate therapeutics (172).

**Proteomics.** Proteomics analysis offers an opportunity to characterize the response to a pathogen at a level above transcriptomics. Especially early on, this primarily involved evaluating the effect of the virus on cell lines. One early proteomics study investigated changes associated with *in vitro* SARS-CoV-2 infection using Caco-2 cells (180). This study reported that SARS-CoV-2 induced alterations in multiple vital physiological pathways, including translation, splicing, carbon metabolism, and nucleic acid metabolism in the host cells. Another area of interest is whether SARS-CoV-2 is likely to induce changes similar to those by other HCoVs. For example, because of the high level of sequence homology between SARS-CoV-2 and SARS-CoV-1, it has been hypothesized that sera from convalescent SARS-CoV-1 patients might show some efficacy in cross-neutralizing SARS-CoV-2-driven entry (28). However, despite the high level of sequence homology, certain protein structures might be immunologically distinct, which would be likely to prohibit effective cross-neutralization across different SARS species (181). Consequently, proteomic analyses of SARS-CoV-1 might also provide some essential information regarding the new pathogen (182, 183).

Proteomics research has been able to get ahead of the timeline for development of omics-level big data sets specific to SARS-CoV-2 by adopting a comparative bioinformatics

approach. Data hubs such as UniProt (184), NCBI Genome Database (185), The Immune Epitope Database and Analysis Resource (186), and The Virus Pathogen Resource (187) contain a wealth of data from studies in other viruses and even HCoVs. Such databases facilitate the systems-level reconstruction of protein-protein interaction networks, providing opportunities to generate hypotheses about the mechanism of action of SARS-CoV-2 and identify potential drug targets. In an initial study (188), 26 of the 29 SARS-CoV-2 proteins were cloned and expressed in HEK293T kidney cells, allowing for the identification of 332 high-confidence human proteins interacting with them. Notably, this study suggested that SARS-CoV-2 interacts with innate immunity pathways. Ranking pathogens by the similarity between their interactomes and that of SARS-CoV-2 suggested *West Nile virus*, *Mycobacterium tuberculosis*, and *Human papillomavirus* infections as the top three hits. The fact that the host-pathogen interactome of the bacterium *Mycobacterium tuberculosis* was found to be similar to that of SARS-CoV-2 suggests that changes related to lung pathology might comprise a significant contributor to these expression profiles. Additionally, it was suggested that the envelope protein, E, could disrupt host bromodomain-containing proteins, i.e., BRD2 and BRD4, which bind to histones, and the spike protein could likely intervene in viral fusion by modulating the GOLGA7-ZDHHC5 acyl-transferase complex to increase palmitoylation, which is a posttranslational modification that affects how proteins interact with membranes (189).

An example of an application of this *in silico* approach comes from another study (190), which used patient-derived peripheral blood mononuclear cells to identify 251 host proteins targeted by SARS-CoV-2. This study also reported that more than 200 host proteins were disrupted following infection. In particular, a network analysis showed that nsp9 and nsp10 interacted with NF- $\kappa$ B-repressing factor, which encodes a transcriptional repressor that mediates repression of genes responsive to nuclear factor kappa-light-chain-enhancer of activated B cells. These genes are important to pro-, and potentially also anti-, inflammatory signaling (191). This finding could explain the exacerbation of the immune response that shapes the pathology and the high cytokine levels characteristic of COVID-19, possibly due to the chemotaxis of neutrophils mediated by IL-8 and IL-6. Finally, it was suggested (192) that the E protein of both SARS-CoV-1 and SARS-CoV-2 has a conserved Bcl-2 homology 3-like motif, which could inhibit anti-apoptosis proteins, e.g., BCL2, and trigger the apoptosis of T cells. Several compounds are known to disrupt the host-pathogen protein interactome, largely through the inhibition of host proteins. Therefore, this research identifies candidate targets for intervention and suggests that drugs modulating protein-level interactions between virus and host could be relevant to treating COVID-19.

As with other approaches, analyzing the patterns found in infected versus healthy human subjects is also important. COVID-19 infection has been associated with quantitative changes in transcripts, proteins, metabolites, and lipids in patient blood samples (193). One longitudinal study (194) compared COVID-19 patients to symptomatic controls who were PCR negative for SARS-CoV-2. The longitudinal nature of this study allowed it to account for differences in the scale of inter- versus intraindividual changes. At the time of first sampling, common functions of proteins upregulated in COVID-19 patients relative to controls were related to immune system mediation, coagulation, lipid homeostasis, and protease inhibition. They compared these data to the patient-specific time points associated with the highest levels of SARS-CoV-2 antibodies and found that the actin-binding protein gelsolin, which is involved in recovery from disease, showed the steepest decline between those two time points. Immunoglobulins comprised the only proteins that were significantly different between the COVID-19 and control patients at both of these time points. The most significantly downregulated proteins between these time points were related to inflammation, while the most significantly upregulated proteins were immunoglobulins. Proteins related to coagulation also increased between the two time points. The selection of a symptomatic control cohort rather than healthy comparisons also suggests that the results are more likely to highlight the response to SARS-CoV-2 and COVID-19 specifically, rather than to disease more broadly. This study also compared the disease course in patients who ultimately survived to the course in those who died and found that ITIH4, a protein associated

with the inflammatory response to trauma, may be a biomarker useful to identify patients at risk of death. Thus, these results indicate the value of studying patients in a longitudinal manner over the disease course. By revealing which genes are perturbed during SARS-CoV-2 infection, proteomics-based analyses can thus provide novel insights into host-virus interaction and serve to generate new avenues of investigation for therapeutics.

### VIRAL VIRULENCE

Like that of SARS-CoV-1, the entry of SARS-CoV-2 into host cells is mediated by interactions between the viral spike glycoprotein, S, and human ACE2 (hACE2) (20, 28, 195–200). Differences in how the S proteins of the two viruses interact with hACE2 could partially account for the increased transmissibility of SARS-CoV-2. Studies have reported conflicting binding constants for the S-hACE2 interaction, though they have agreed that the SARS-CoV-2 S protein binds with equal affinity as, if not greater affinity than, the SARS-CoV-1 S protein does (9, 20, 198). The C-terminal domain of the SARS-CoV-2 S protein in particular was identified as the key region of the virus that interacts with hACE2, and the crystal structure of the C-terminal domain of the SARS-CoV-2 S protein in complex with hACE2 reveals stronger interaction and a higher affinity for receptor binding than that of SARS-CoV-1 (199). Among the 14 key binding residues identified in the SARS-CoV-1 S protein, eight are conserved in SARS-CoV-2, and the remaining six are semi-conservatively substituted, potentially explaining variation in binding affinity (20, 198). Studies of crystal structure have shown that the RBD of the SARS-CoV-2 S protein, like that of other coronaviruses, undergoes stochastic hinge-like movement that flips it from a “closed” conformation, in which key binding residues are hidden at the interface between protomers, to an “open” one (9, 20). Spike proteins cleaved at the furin-like binding site are substantially more likely to take an open conformation (66%) than those that are uncleaved (17%) (201). Because the RBD plays such a critical role in viral entry, blocking its interaction with ACE2 could represent a promising therapeutic approach. Nevertheless, despite the high structural homology between the SARS-CoV-2 RBD and that of SARS-CoV-1, monoclonal antibodies targeting SARS-CoV-1 RBD failed to bind to SARS-CoV-2-RBD (9). However, in early research, sera from convalescent SARS patients were found to inhibit SARS-CoV-2 viral entry *in vitro*, albeit with lower efficiency than it inhibited SARS-CoV-1 (28).

Comparative genomic analysis reveals that several regions of the coronavirus genome are likely critical to virulence. The S1 domain of the spike protein, which contains the receptor binding motif, evolves more rapidly than the S2 domain (18, 19). However, even within the S1 domain, some regions are more conserved than others, with the receptors in S1’s N-terminal domain (S1-NTD) evolving more rapidly than those in its C-terminal domain (S1-CTD) (19). Both S1-NTD and S1-CTD are involved in receptor binding and can function as RBDs to bind proteins and sugars (18), but RBDs in the S1-NTD typically bind to sugars, while those in the S1-CTD recognize protein receptors (5). Viral receptors show higher affinity with protein receptors than sugar receptors (5), which suggests that positive selection on or relaxed conservation of the S1-NTD might reduce the risk of a deleterious mutation that would prevent binding. The SARS-CoV-2 S protein also contains an RRAR furin recognition site at the S1/S2 junction (9, 20), setting it apart from both bat coronavirus RaTG13, with which it shares 96% genome sequence identity, and SARS-CoV-1 (202). Such furin cleavage sites are commonly found in highly virulent influenza viruses (203, 204). The furin recognition site at the S1/S2 junction is likely to increase pathogenicity via destabilization of the spike protein during fusion to ACE2 and the facilitation of cell-cell adhesion (9, 20, 37, 201, 203, 204). These factors may influence the virulence of SARS-CoV-2 relative to other beta coronaviruses. Additionally, a major concern has been the emergence of SARS-CoV-2 variants with increased virulence. The extent to which evolution within SARS-CoV-2 may affect pathogenesis is reviewed below.

### MOLECULAR SIGNATURES, TRANSMISSION, AND VARIANTS OF CONCERN

Genetic variation in SARS-CoV-2 has been used to elucidate patterns over time and space. Many mutations are neutral in their effect and can be used to trace transmission



patterns. Such signatures within SARS-CoV-2 have provided insights during outbreak investigations (205–207). Similar mutations observed in several patients may indicate that the patients belong to the same transmission group. The tracking of SARS-CoV-2 mutations is recognized as an essential tool for controlling future outbreaks and tracing the path of the spread of SARS-CoV-2. In the first months of the pandemic in early 2020, early genomic surveillance efforts in Guangdong, China, revealed that local transmission rates were low and that most cases arising in the province were imported (208). Since then, efforts have varied widely among countries: for example, the United Kingdom has coordinated a national database of viral genomes (209), but efforts to collect this type of data in the United States have been more limited (210). Studies have applied phylogenetic analyses of viral genomes to determine the source of local COVID-19 outbreaks in Connecticut (USA) (211), the New York City area (USA) (212), and Iceland (213). There has been an ongoing effort to collect SARS-CoV-2 genomes throughout the COVID-19 outbreak, and as of summer 2021, millions of genome sequences have been collected from patients. The sequencing data can be found at GISAID (214), NCBI (215), and the COVID-19 data portal (216).

Ongoing evolution can be observed in genomic data collected through molecular surveillance efforts. In some cases, mutations can produce functional changes that can impact pathogenesis. One early example is the spike protein mutation D614G, which appeared in March 2020 and became dominant worldwide by the end of May 2020 (217, 218). This variant was associated with increased infectivity and increased viral load but not with more severe disease outcomes (217, 219). This increased virulence is likely achieved by altering the conformation of the S1 domain to facilitate binding to ACE2 (219). Similarly, the N439K mutation within the RBD of the spike protein is likely associated with increased transmissibility and enhanced binding affinity for hACE2, although it is also not thought to affect disease outcomes (220). In contrast, a mutation in ORF8 that was identified in Singapore in the early months of 2020 was associated with cases of COVID-19 that were less likely to require treatment with supplemental oxygen (221), and a deletion surrounding the furin site insertion at the S1/S2 boundary has been identified only rarely in clinical settings (222), suggesting that these mutations may disadvantage viral pathogenesis in human hosts. Thus, mutations have been associated with both virological and clinical differences in pathogenesis.

Several VOCs have also been identified and designated through molecular surveillance efforts (223). The Alpha variant (lineage B.1.1.7) was first observed in the United Kingdom in October 2020 before it quickly spread around the world (224). Other variants meriting further investigation have also been identified, including the Beta variant (B.1.351 lineage) first identified in South Africa and the Gamma variant (P.1 lineage) initially associated with outbreaks in Brazil. These lineages share independently acquired mutations that may affect pathogenicity (225–229). For example, they are all associated with a greater binding affinity for hACE2 than that of the wild-type variant (227, 230, 231), but they were not found to have more efficient cell entry than the wild-type virus (232). A fourth VOC, the Delta variant (B.1.617.2 and AY.1, AY.2, and AY.3 lineages), was identified in India in late 2020 (233). Some of the mutations associated with this lineage may alter fusogenicity and enhance furin cleavage, among other effects associated with increased pathogenicity (234). The changes in these VOC demonstrate how ongoing evolution in SARS-CoV-2 can drive changes in how the virus interacts with host cells.

## QUANTIFYING VIRAL PRESENCE

Assessing whether a virus is present in a sample is a more complex task than it initially seems. Many diagnostic tests rely on real-time PCR (RT-PCR) to test for the presence versus absence of a virus (235). They may report the cycle threshold ( $C_T$ ) indicating the number of doubling cycles required for the target (in this case, SARS-CoV-2) to become detectable. A lower  $C_T$  therefore corresponds to a higher viral load. The  $C_T$  that corresponds to a positive can vary widely but is often around 35. This information is sufficient to answer many questions, since an amplicon must be present in order to be duplicated in RT-PCR. For example,

if a patient is presenting with COVID-19 symptoms, a positive RT-PCR test can confirm the diagnosis.

However, RT-PCR analysis alone cannot provide the information needed to determine whether a virus is present at sufficient levels to be infectious (236). Some studies have therefore taken the additional step of cultivating samples *in vitro* in order to observe whether cells become infected with SARS-CoV-2. One study collected upper respiratory tract samples from COVID-19 patients, analyzed them with RT-PCR to determine the cycle threshold, and then attempted to cultivate the SARS-CoV-2 virus in VeroE6 cells (236). This study found that out of 246 samples, fewer than half (103 samples) produced a positive culture. Moreover, at a  $C_T$  of 35, only 5 out of 60 samples grew *in vitro*. Therefore, the RT-PCR-confirmed presence of SARS-CoV-2 in a sample does not necessarily indicate that the virus is present at a high-enough concentration to grow and/or spread.

## MECHANISMS OF TRANSMISSION

When a human host is infected with a virus and is contagious, person-to-person viral transmission can occur through several possible mechanisms. When a contagious individual sneezes, coughs, or exhales, they produce respiratory droplets that can contain a large number of viral particles (237). Viral particles can enter the body of a new host when they then come in contact with the oral, nasal, eye, or other mucus membranes (237). The primary terms typically used to discuss the transmission of viruses via respiratory droplets are droplet, aerosol, and contact transmission (238). The distinction between droplet and aerosol transmission is typically anchored on whether a particle containing the virus is larger or smaller than  $5\ \mu\text{m}$  (239, 240). Droplet transmission typically refers to contact with large droplets that fall quickly to the ground at close range, such as breathing in droplets produced by a sneeze (237, 239). Aerosol transmission typically refers to much smaller particles (less than  $5\ \mu\text{m}$ ) produced by sneezing, coughing, or exhaling (237, 238) that can remain suspended over a longer period of time and potentially be moved by air currents (237). It is also possible that viral particles deposited on surfaces via large respiratory droplets could later be aerosolized (237). The transmission of viral particles that have settled on a surface is typically referred to as contact or fomite transmission (237, 241). Any respiratory droplets that settle on a surface could contribute to fomite transmission (237). Droplet and contact transmission are both well-accepted modes of transmission for many viruses associated with common human illnesses, including influenza virus and rhinovirus (237).

The extent to which aerosol transmission contributes to the spread of respiratory viruses is more widely debated. In influenza A, for example, viral particles can be detected in aerosols produced by infected individuals, but it is not clear to what extent these particles drive the spread of influenza A virus infection (237, 238, 242–244). Regardless of its role in the spread of influenza A, however, aerosol transmission likely played a role in outbreaks such as the 1918 Spanish influenza (H1N1) and 2009 “swine flu” (pH1N1) (244). All three of these mechanisms have been identified as possible contributors to the transmission of HCoVs (237), including the highly pathogenic coronaviruses SARS-CoV-1 and MERS-CoV (245, 246). Transmission of SARS-CoV-1 is thought to proceed primarily through droplet transmission, but aerosol transmission is also considered possible (237, 247, 248), and fomite transmission may have also played an important role in some outbreaks (249). Similarly, the primary mechanism of MERS transmission is thought to be droplets because interindividual transmission appears to be associated with close interpersonal contact (e.g., household or health care settings), but aerosolized particles of the MERS virus have been reported to persist much more robustly than influenza A virus under a range of environmental conditions (250, 251). However, few of these analyses have sought to grow positive samples in culture and thus to confirm their potential to infect new hosts.

Contact, droplet, and aerosol transmission are therefore all worth evaluating when considering possible modes of transmission for a respiratory virus like SARS-CoV-2. The stability of the SARS-CoV-2 virus both in aerosols and on a variety of surfaces was found to be similar to that of SARS-CoV-1 (252). Droplet-based and contact transmission were initially

put forward as the greatest concern for the spread of SARS-CoV-2 (253), with droplet transmission considered the dominant mechanism driving the spread of the virus (254) because the risk of fomite transmission under real-world conditions is likely to be substantially lower than the conditions used for experimental analyses (255). The COVID-19 pandemic has, however, exposed significant discrepancies in how terms pertaining to airborne viral particles are interpreted in different contexts (239). The 5- $\mu$ m distinction between “droplets” and “aerosols” is typical in the biological literature but is likely an artifact of historical science rather than a meaningful boundary in biology or physics (240). Additionally, various ambient conditions such as airflow can influence how particles of different sizes fall or spread (239). Despite initial skepticism about airborne transmission of SARS-CoV-2 through small particles (240), evidence now suggests that small particles can contribute to SARS-CoV-2 transmission (252, 256–258). For example, one early study detected SARS-CoV-2 viral particles in air samples taken from hospitals treating COVID-19 patients, although the infectivity of these samples was not assessed (259). Subsequently, other studies have been successful in growing SARS-CoV-2 in culture with samples taken from the air (260, 261) while others have not (262, 263) (see reference 264 for a systematic review of available findings as of July 2020). The fact that viable SARS-CoV-2 may exist in aerosolized particles calls into question whether some axioms of COVID-19 prevention, such as 2-m social distancing, are sufficient (240, 260, 265).

**Symptoms and viral spread.** Other aspects of pathogenesis are also important to understanding how the virus spreads, especially the relationship between symptoms, viral shedding, and contagiousness. Symptoms associated with reported cases of COVID-19 range from mild to severe (1), but some individuals who contract COVID-19 remain asymptomatic throughout the duration of the illness (266). The incubation period, or the time period between exposure and the onset of symptoms, has been estimated at 5 to 8 days, with means of 4.91 (95% confidence interval [CI], 4.35 to 5.69) and 7.54 (95% CI, 6.76 to 8.56) reported in two different Asian cities and a median of 5 (interquartile range [IQR], 1 to 6) reported in a small number of patients in a Beijing hospital (267, 268).

However, the exact relationship between contagiousness and viral shedding remains unclear. Estimates suggest that viral shedding can, in some cases, begin as early as 12.3 days (95% CI, 5.9 to 17.0) before the onset of symptoms, although this was found to be very rare, with fewer than 0.1% of transmission events occurring 7 or more days before symptom onset (269). Transmissibility appeared to peak around the onset of symptoms (95% CI, -0.9 to 0.9 days), and only 44% (95% CI, 30 to 57%) of transmission events were estimated to occur from presymptomatic contacts (269). A peak in viral load corresponding to the onset of symptoms was also confirmed by another study (236). As these trends became apparent, concerns arose due to the potential for individuals who did not yet show symptoms to transmit the virus (270). Recovered individuals may also be able to transmit the virus after their symptoms cease. A study of the communicable period based on 24 individuals who tested positive for SARS-CoV-2 prior to or without developing symptoms estimated that individuals may be contagious for 1 to 21 days, but the authors note that this estimate may be low (266). In an early study, viral nucleic acids were reported to remain at observable levels in the respiratory specimens of recovering hospitalized COVID-19 patients for a median of 20 days and with a maximum observed duration through 37 days, when data collection for the study ceased (78).

As more estimates of the duration of viral shedding were released, they converged around approximately 3 weeks from first positive PCR test and/or onset of symptoms (which, if present, are usually identified within 3 days of the initial PCR test). For example, in some studies, viral shedding was reported for up to 28 days following symptom onset (271) and for 1 to 24 days from first positive PCR test, with a median of 12 days (67). On the other hand, almost 70% of patients were reported to still have symptoms at the time that viral shedding ceased, although all symptoms reduced in prevalence between onset and cessation of viral shedding (272). The median time that elapsed between the onset of symptoms and cessation of viral RNA shedding was 23 days, and that between first positive PCR test and cessation of viral shedding was 17 days (272). The fact that this study reported symptom onset to predate the first positive PCR test

by an average of 3 days, however, suggests that there may be some methodological differences between it and related studies. Furthermore, an analysis of residents of a nursing home with a known SARS-CoV-2 case measured similar viral loads in residents who were asymptomatic regardless of whether they later developed symptoms, and the load in the asymptomatic residents was comparable to that of residents who displayed either typical or atypical symptoms (273). Taken together, these results suggest that the presence or absence of symptoms is not a reliable predictor of viral shedding or of SARS-CoV-2 status (e.g., reference 274). However, it should be noted that viral shedding is not necessarily a robust indicator of contagiousness. The risk of spreading the infection was low after 10 days from the onset of symptoms, as viral load in sputum was found to be unlikely to pose a significant risk based on efforts to culture samples *in vitro* (271). The relationship between symptoms, detectable levels of the virus, and risk of viral spread is therefore complex.

The extent to which asymptomatic or presymptomatic individuals are able to transmit SARS-CoV-2 has been a question of high scientific and community interest. Early reports (February and March 2020) described transmission from presymptomatic SARS-CoV-2-positive individuals to close family contacts (275, 276). One of these reports (276) also included a description of an individual who tested positive for SARS-CoV-2 but never developed symptoms. Later analyses also sought to estimate the proportion of infections that could be traced back to a presymptomatic or asymptomatic individual (e.g., reference 277). Estimates of the proportion of individuals with asymptomatic infections have varied widely. The proportion of asymptomatic individuals on board the *Diamond Princess* cruise ship, which was the site of an early COVID-19 outbreak, was estimated at 17.9% (278). In contrast, a model using the prevalence of antibodies among residents of Wuhan, China, estimated a much higher rate of asymptomatic cases, at approximately 7 in 8, or 87.5% (279). An analysis of the populations of care homes in London found that, among the residents (median age 85 years), the rate of asymptomatic infection was 43.8%, and among the caretakers (median age 47 years), the rate was 49.1% (280). The duration of viral shedding may also be longer in individuals with asymptomatic cases of COVID-19 than in those who do show symptoms (281). As a result, the potential for individuals who do not know they have COVID-19 to spread the virus raises significant concerns. In Singapore and Tianjin, two cities studied to estimate incubation period, an estimated 40 to 50% and 60 to 80% of cases, respectively, were considered to be caused by contact with asymptomatic individuals (267). An analysis of viral spread in the Italian town of Vo', which was the site of an early COVID-19 outbreak, revealed that 42.5% of cases were asymptomatic and that the rates were similar across age groups (282). The argument was thus made that the town's lockdown was imperative for controlling the spread of COVID-19 because it isolated asymptomatic individuals. While more models are likely to emerge to better explore the effect of asymptomatic individuals on SARS-CoV-2 transmission, these results suggest that strategies for identifying and containing asymptomatic but contagious individuals are important for managing community spread.

**Estimating the fatality rate.** Estimating the occurrence of asymptomatic and mild COVID-19 cases is important to identifying the mortality rate associated with COVID-19. The mortality rate of greatest interest would be the total number of fatalities as a fraction of the total number of people infected. One commonly reported metric is the case fatality rate (CFR), which compares the number of COVID-19-related deaths to the number of confirmed or suspected cases. However, in locations without universal testing protocols, it is impossible to identify all infected individuals because so many asymptomatic or mild cases go undetected. Therefore, a more informative metric is the infection fatality rate (IFR), which compares the known deaths to the estimated number of cases. It thus requires the same numerator as CFR but divides by an approximation of the total number of cases rather than only the observed/suspected cases. IFR varies regionally, with some locations observed to have IFRs as low as 0.17% while others are as high as 1.7% (283). Estimates of CFR at the national and continental level and IFR at the continent level are maintained by the Centre for Evidence-Based Medicine (284). Several meta-analyses have also sought to estimate IFR at the global scale. These estimates have varied; one peer-reviewed study aggregated data from 24 other studies and estimated IFR at 0.68% (95% CI, 0.53% to 0.82%), but a preprint

that aggregated data from 139 countries calculated a global IFR of 1.04% (95% CI, 0.77% to 1.38%) when false negatives were considered in the model (283, 285). A similar prevalence estimate was identified through a repeated cross-sectional serosurvey conducted in New York City that estimated the IFR as 0.97% (286). Examination of serosurvey-based estimates of IFR identified convergence on a global IFR estimate of 0.60% (95% CI, 0.42% to 0.77%) (283). All of these studies note that IFR varies widely by location, and it is also expected to vary with demographic and health-related variables such as age, sex, prevalence of comorbidities, and access to health care and testing (287). Estimates of infection rates are becoming more feasible as more data become available for modeling and will be bolstered as serological testing becomes more common and more widely available. However, this research may be complicated due to the emergence of variants over time, as well as the varying availability and acceptance of vaccines in different communities and locations.

## DYNAMICS OF TRANSMISSION

Disease spread dynamics can be estimated using  $R_0$ , the basic reproduction number, and  $R_t$ , the effective reproduction number. Accurate estimates of both are crucial to understanding the dynamics of infection and to predicting the effects of different interventions.  $R_0$  is the average number of new (secondary) infections caused by one infected person, assuming a wholly susceptible population (288), and is one of the most important epidemiological parameters (289). A simple mechanistic model used to describe infectious disease dynamics is a susceptible-infected-recovered compartmental model (290, 291). In this model, individuals move through three states: susceptible, infected, and recovered; two parameters,  $\gamma$  and  $\beta$ , specify the rate at which the infectious recover and the infection transmission rate, respectively, and  $R_0$  is estimated as the ratio of  $\beta$  and  $\gamma$  (289, 292). A pathogen can invade a susceptible population only if  $R_0$  is  $>1$  (289, 293). The spread of an infectious disease at a particular time  $t$  can be quantified by  $R_t$ , the effective reproduction number, which assumes that part of the population has already recovered (and thus gained immunity to reinfection) or that mitigating interventions have been put into place. For example, if only a fraction  $S_t$  of the population is still susceptible,  $R_t = S_t \times R_0$ . When  $R_t$  is greater than 1, an epidemic grows (i.e., the proportion of the population that is infectious increases); when  $R_t$  is less than 1, the proportion of the population that is infectious decreases.  $R_0$  and  $R_t$  can be estimated directly from epidemiological data or inferred using susceptible-infected-recovered-type models. To capture the dynamics of SARS-CoV-2 accurately, the addition of a fourth compartment, i.e., a susceptible-exposed-infectious-recovered model, may be appropriate because such models account for the relative lengths of incubation and infectious periods (294).

Original estimates of  $R_0$  for COVID-19 lie in the range  $R_0 = 1.4$  to 6.5 (295–297). Variation in  $R_0$  is expected between different populations, and the estimated values of  $R_0$  discussed below are for specific populations in specific environments. The different estimates of  $R_0$  should not necessarily be interpreted as a range of estimates of the same underlying parameter. In one study of international cases, the predicted value was  $R_0 = 1.7$  (298). In China (both Hubei province and nationwide), the value was predicted to lie in the range  $R_0 = 2.0$  to 3.6 (295, 299, 300). Another estimate based on a cruise ship where an outbreak occurred predicted  $R_0 = 2.28$  (301). Susceptible-exposed-infectious-recovered model-derived estimates of  $R_0$  range from 2.0 to 6.5 in China (302–305) to  $R_0 = 4.8$  in France (306). Using the same model as for the French population, a study estimated  $R_0 = 2.6$  in South Korea (306), which is consistent with other studies (307). From a meta-analysis of studies estimating  $R_0$  (296), the median  $R_0$  was estimated to be 2.79 (IQR 1.16) based on 12 studies published between 1 January and 7 February 2020.

Inference of the effective reproduction number can provide insight into how populations respond to an infection and the effectiveness of interventions. In China,  $R_t$  was predicted to lie in the range of 1.6 to 2.6 in January 2020, before travel restrictions (308).  $R_t$  decreased from 2.35 1 week before travel restrictions were imposed (23 January 2020), to 1.05 1 week after. Using their model, the authors also estimated the probability of new outbreaks occurring. Assuming individual-level variation in transmission comparable to that of MERS or SARS, the probability of a single individual exporting the virus and causing a large outbreak is 17 to



25%, and assuming variation like that of SARS and transmission patterns like those observed for COVID-19 in Wuhan, the probability of a large outbreak occurring after  $\geq 4$  infections exist at a new location is greater than 50%. An independent study came to similar conclusions, finding  $R_t = 2.38$  in the 2-week period before January 23 with a decrease to  $R_t = 1.34$  (using data from January 24 to February 3) or  $R_t = 0.98$  (using data from January 24 to February 8) (297). In South Korea,  $R_t$  was inferred for February through March 2020 in two cities, Daegu (the center of the outbreak) and Seoul (307). Metro data were also analyzed to estimate the effects of social distancing measures.  $R_t$  decreased in Daegu from around 3 to  $< 1$  over the period that social distancing measures were introduced. In Seoul,  $R_t$  decreased slightly but remained close to 1 (and larger than  $R_t$  in Daegu). These findings indicate that social distancing measures appeared to be effective in containing the infection in Daegu, but in Seoul,  $R_t$  remained above 1, meaning secondary outbreaks remained possible. The study also shows the importance of region-specific analysis: the large decline in caseload nationwide was mainly due to the Daegu region and could mask persistence of the epidemic in other regions, such as Seoul and Gyeonggi-do. In Iran, estimates of  $R_t$  declined from 4.86 in the 1st week to 2.1 by the 4th week after the first cases were reported (309). In Europe, analysis of 11 countries inferred the dynamics of  $R_t$  over a time range from the beginning of the outbreak until 28 March 2020, by which point most countries had implemented major interventions (such as stay-at-home orders, public gathering bans, and school closures) (310). Across all countries, the mean  $R_t$  before interventions began was estimated as 3.87;  $R_t$  varied considerably, from below 3 in Norway to above 4.5 in Spain. After interventions,  $R_t$  decreased by an average of 64% across all countries, with mean  $R_t = 1.43$ . The lowest predicted value was 0.97 for Norway, and the highest was 2.64 for Sweden, which could be related to the fact that Sweden did not implement social distancing measures on the same scale as other countries. The study concludes that while large changes in  $R_t$  are observed, it is too early to tell whether the interventions put into place are sufficient to decrease  $R_t$  below 1.

Evolution within SARS-CoV-2 has also driven changes in the estimated reproduction number for different populations at different times. As of June 2021, the reproduction number had increased globally relative to 2020, and increased transmissibility over the wild-type variant was observed for the Alpha, Beta, Gamma, and Delta VOC (311). In the United States, between December 2020 and January 2021, B.1.1.7 (Alpha) was estimated to have an increased transmission of 35% to 45% relative to common SARS-CoV-2 variants at the time, with B.1.1.7 being the dominant SARS-CoV-2 variant in some places at some time points (312). This lineage was estimated to have increased transmissibility of 43% to 90% in the United Kingdom (313). An estimate of the reproduction number of B.1.1.7 in the United Kingdom from September to December 2020 yielded 1.59 overall and between 1.56 and 1.95 in different regions of the country (229). The Delta variant is particularly transmissible, and it has been estimated to be twice as transmissible as the wild-type variant of SARS-CoV-2 (311). A review of the literature describing the Delta variant identified a mean estimated  $R_0$  of 5.08 (314). Such differences can affect fitness and therefore influence the relative contributions of different lineages to a given viral gene pool over time (315). Therefore, the evolution of the virus can result in shifts in the reproduction rate.

More generally, population-level epidemic dynamics can be both observed and modeled (292). Data and empirically determined biological mechanisms inform models, while models can be used to try to understand data and systems of interest or to make predictions about possible future dynamics, such as the estimation of capacity needs (316) or the comparison of predicted outcomes among prevention and control strategies (317, 318). Many current efforts to model  $R_t$  have also led to tools that assist the visualization of estimates in real time or over recent intervals (319, 320). These are valuable resources, yet it is also important to note that the estimates arise from models containing many assumptions and are dependent on the quality of the data they use, which varies widely by region.

## CONCLUSIONS

The novel coronavirus SARS-CoV-2 is the third HCoV to emerge in the 21st century, and research into previous HCoVs has provided a strong foundation for characterizing

the pathogenesis and transmission of SARS-CoV-2. Critical insights into how the virus interacts with human cells have been gained from previous research into HCoVs and other viral infections. With the emergence of three devastating HCoVs over the past 20 years, emergent viruses are likely to represent an ongoing threat. Contextualizing SARS-CoV-2 alongside other viruses not only serves to provide insights that can be immediately useful for combating this virus itself but may also prove valuable in the face of future viral threats.

Host-pathogen interactions provide a basis not only for understanding COVID-19 but also for developing a response. As with other HCoVs, the immune response to SARS-CoV-2 is likely driven by detection of its spike protein, which allows it to enter cells through ACE2. Epithelial cells have also emerged as the major cellular target of the virus, contextualizing the respiratory and gastrointestinal symptoms that are frequently observed in COVID-19. Many of the mechanisms that facilitate the pathogenesis of SARS-CoV-2 are currently under consideration as possible targets for the treatment or prevention of COVID-19 (10, 11). Research in other viruses also provides a foundation for understanding the transmission of SARS-CoV-2 among people and can therefore inform efforts to control the virus's spread. Airborne forms of transmission (droplet and aerosol transmission) have emerged as the primary modes by which the virus spreads to new hosts. Asymptomatic transmission was also a concern in the SARS outbreak of 2002 to 2003 and, as in the current pandemic, presented challenges for estimating rates of infection (321). These insights are important for developing a public health response, such as the CDC's shift in its recommendations surrounding masking (322).

Even with the background obtained from research in SARS and MERS, COVID-19 has revealed itself to be a complex and difficult-to-characterize disease that has many possible presentations that vary with age. Variability in presentation, including cases with no respiratory symptoms or with no symptoms altogether, was also reported during the SARS epidemic at the beginning of the 21st century (321). The variability of both which symptoms present and their severity has presented challenges for public health agencies seeking to provide clear recommendations regarding which symptoms indicate SARS-CoV-2 infection and should prompt isolation. Asymptomatic cases add complexity to efforts to estimate statistics both such as  $R_0$  and  $R_t$ , which are critical to understanding the transmission of the virus, and IFR, which is an important component of understanding its impact on a given population. The development of diagnostic technologies over the course of the pandemic has facilitated more accurate identification, including of asymptomatic cases (235). As more cases have been diagnosed, the health conditions and patient characteristics associated with more severe infection have also become more clear, although there are likely to be significant sociocultural elements that also influence these outcomes (323). While many efforts have focused on adults, and especially older adults because of the susceptibility of this demographic, additional research is needed to understand the presentation of COVID-19 and MIS-C in pediatric patients. As more information is uncovered about the pathogenesis of HCoV and SARS-CoV-2 specifically, the diverse symptomatology of COVID-19 has and likely will continue to conform with the ever-broadening understanding of how SARS-CoV-2 functions within a human host.

While the SARS-CoV-2 virus is very similar to other HCoVs in several ways, including in its genomic structure and the structure of the virus itself, there are also some differences that may account for differences in the COVID-19 pandemic compared to the SARS and MERS epidemics of the past 2 decades. The  $R_0$  of SARS-CoV-2 has been estimated to be similar to that of SARS-CoV-1 but much higher than that of MERS-CoV (324), although a higher  $R_0$  has been estimated for some VOC. While the structures of the viruses are very similar, evolution among these species may account for differences in their transmissibility and virulence. For example, the acquisition of a furin cleavage site at the S1/S2 boundary within the SARS-CoV-2 S protein may be associated with increased virulence. Additionally, concerns have been raised about the accumulation of mutations within the SARS-CoV-2 species itself, and whether these could influence virulence (325). These novel variants may be resistant to vaccines and antibody

treatments such as bamlanivimab that were designed based on the wild-type spike protein (10, 326). As a consequence of reliance on targeting the SARS-CoV-2 spike protein for many therapeutic and prophylactic strategies, increased surveillance is required to rapidly identify and prevent the spread of novel SARS-CoV-2 variants with alterations to the spike protein. The coming of age of genomic technologies has made these types of analyses feasible, and genomics research characterizing changes in SARS-CoV-2 along with temporal and spatial movement is likely to provide additional insights into whether within-species evolution influences the effect of the virus on the human host. Additionally, the rapid development of sequencing technologies over the past decade has made it possible to rapidly characterize the host response to the virus. For example, proteomics analysis of patient-derived cells revealed candidate genes whose regulation is altered by SARS-CoV-2 infection, suggesting possible approaches for pharmaceutical invention and providing insight into which systems are likely to be disrupted in COVID-19 (190). As more patient data become available, the biotechnological advances of the 2000s are expected to allow for more rapid identification of potential drug targets than was feasible during the SARS, or even MERS, pandemic.

Thus, the COVID-19 crisis continues to evolve, but the insights acquired over the past 20 years of HCoV research have provided a solid foundation for understanding the SARS-CoV-2 virus and the disease it causes. As the scientific community continues to respond to COVID-19 and to elucidate more of the relationships between pathogenesis, transmission, host regulatory responses, and symptomatology, this understanding will no doubt continue to evolve and to reveal additional connections among virology, pathogenesis, and health. This review represents a collaboration between scientists from diverse backgrounds to contextualize this virus at the union of many different biological disciplines (327). At present, understanding the SARS-CoV-2 virus and its pathogenesis is critical to a holistic understanding of the COVID-19 pandemic. In the future, interdisciplinary work on SARS-CoV-2 and COVID-19 may guide a response to a new viral threat.

### ACKNOWLEDGMENTS

We thank Yael Evelyn Marshall, who contributed writing (original draft) as well as reviewing and editing of pieces of the text but who did not formally approve the manuscript, as well as Ronnie Russell, who contributed text to and helped develop the structure of the manuscript early in the writing process. We are grateful to the following contributors for reviewing pieces of the text: Nadia Danilova, James Eberwine, and Ipsita Krishnan.

L.D.M. received consulting fees from Acelyt and Sanofi in the past 5 years. S.M.B. is currently an employee at AstraZeneca, Gaithersburg, MD, USA, and may own stock or stock options. Y.P. is affiliated with Pfizer Worldwide Research; the author has no financial interests to declare and contributed as an author prior to joining Pfizer, and the work was not part of a Pfizer collaboration nor was it funded by Pfizer. C.B. is an employee and shareholder of SAGA Diagnostics AB. J.B.B. was funded by FastGrants to conduct a COVID-19-related clinical trial. A.G. filed a patent application with the Wisconsin Alumni Research Foundation related to classifying activated T cells. The other authors declare no conflicts of interest.

Author contributions: Halie M. Rando: Project Administration, Writing - Original Draft, Writing - Review & Editing; Adam L. MacLean: Writing - Original Draft, Writing - Review & Editing; Alexandra J. Lee: Writing - Original Draft, Writing - Review & Editing; Ronan Lordan: Writing - Review & Editing; Sandipan Ray: Writing - Original Draft, Writing - Review & Editing; Vikas Bansal: Writing - Original Draft, Writing - Review & Editing; Ashwin N. Skelly: Writing - Original Draft, Writing - Review & Editing; Elizabeth Sell: Writing - Original Draft, Writing - Review & Editing; John J. Dziak: Writing - Original Draft, Writing - Review & Editing; Lamonica Shinholster: Writing - Original Draft; Lucy D'Agostino McGowan: Writing - Original Draft, Writing - Review & Editing; Marouen Ben Guebila: Writing - Original Draft, Writing - Review & Editing; Nils Wellhausen: Visualization, Writing - Original Draft, Writing - Review & Editing; Sergey Knyazev: Writing - Original Draft, Writing - Review & Editing; Simina M. Boca: Writing - Review & Editing; Stephen Capone: Writing - Original Draft, Writing - Review & Editing; Yanjun Qi: Visualization; YoSon Park: Writing - Original Draft, Writing - Review & Editing; Yuchen Sun:

Visualization; David Mai: Writing - Original Draft, Writing - Review & Editing; Joel D. Boerckel: Writing - Review & Editing; Christian Brueffer: Writing - Original Draft, Writing - Review & Editing; James Brian Byrd: Writing - Original Draft, Writing - Review & Editing; Jeremy P. Kamil: Writing - Review & Editing; Jinhui Wang: Writing - Review & Editing; Ryan Velazquez: Writing - Review & Editing; Gregory L Szeto: Writing - Review & Editing; John P. Barton: Writing - Original Draft, Writing - Review & Editing; Rishi Raj Goel: Writing - Original Draft, Writing - Review & Editing; Serghei Mangul: Writing - Review & Editing; Tiago Lubiana: Writing - Review & Editing; COVID-19 Review Consortium: Project Administration; Anthony Gitter: Methodology, Project Administration, Software, Writing - Review & Editing; Casey S. Greene: Conceptualization, Software, Writing - Review & Editing.

The COVID-19 Review Consortium includes Vikas Bansal, John P. Barton, Simina M. Boca, Joel D. Boerckel, Christian Brueffer, James Brian Byrd, Stephen Capone, Shikta Das, Anna Ada Dattoli, John J. Dziak, Jeffrey M. Field, Soumita Ghosh, Anthony Gitter, Rishi Raj Goel, Casey S. Greene, Marouen Ben Guebila, Daniel S. Himmelstein, Fengling Hu, Nafisa M. Jadavji, Jeremy P. Kamil, Sergey Knyazev, Likhitha Kolla, Alexandra J. Lee, Ronan Lordan, Tiago Lubiana, Temitayo Lukan, Adam L. MacLean, David Mai, Serghei Mangul, David Manheim, Lucy D'Agostino McGowan, Amruta Naik, YoSon Park, Dimitri Perrin, Yanjun Qi, Diane N. Rafizadeh, Bharath Ramsundar, Halie M. Rando, Sandipan Ray, Michael P. Robson, Vincent Rubinetti, Elizabeth Sell, Lamonica Shinholster, Ashwin N. Skelly, Yuchen Sun, Yusha Sun, Gregory L. Szeto, Ryan Velazquez, Jinhui Wang, and Nils Wellhausen.

Anthony Gitter was funded by the John W. and Jeanne M. Rowe Center for Research in Virology.

#### Note Added after Publication

After original publication of this paper, changes were required and have been made in this version of the article. ORCIDs were added for several authors. In addition, original author affiliation p was deleted on page 1, and author conflicts of interest were updated in Acknowledgments. Finally, attribution has been added to the Fig. 1 legend.

#### REFERENCES

- Centers for Disease Control and Prevention. 2021. COVID-19. Symptoms of COVID-19. Centers for Disease Control and Prevention, Atlanta, GA. <https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html>.
- Cucinotta D, Vanelli M. 2020. WHO declares COVID-19 a pandemic. *Acta Biomed* 91:157–160. <https://doi.org/10.23750/abm.v91i1.9397>.
- Li L, Huang Q, Wang DC, Ingbar DH, Wang X. 2020. Acute lung injury in patients with COVID-19 infection. *Clin Transl Med* 10:20–27. <https://doi.org/10.1002/ctm2.16>.
- Tan W, Zhao X, Ma X, Wang W, Niu P, Xu W, Gao GF, Wu G. 2020. A novel coronavirus genome identified in a cluster of pneumonia cases - Wuhan, China 2019-2020. *China CDC Wkly* 2:61–62. <https://doi.org/10.46234/ccdcw2020.017>.
- Li F. 2016. Structure, function, and evolution of coronavirus spike proteins. *Annu Rev Virol* 3:237–261. <https://doi.org/10.1146/annurev-virology-110615-042301>.
- Chan JF-W, Yuan S, Kok K-H, To KK-W, Chu H, Yang J, Xing F, Liu J, Yip CC-Y, Poon RW-S, Tsoi H-W, Lo SK-F, Chan K-H, Poon VK-M, Chan W-M, Ip JD, Cai J-P, Cheng VC-C, Chen H, Hui CK-M, Yuen K-Y. 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 395:514–523. [https://doi.org/10.1016/S0140-6736\(20\)30154-9](https://doi.org/10.1016/S0140-6736(20)30154-9).
- Fields BN, Knipe DM, Howley PM (ed). 2007. *Fields virology*. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, PA.
- Broer R, Boson B, Spaan W, Cosset F-L, Corver J. 2006. Important role for the transmembrane domain of severe acute respiratory syndrome coronavirus spike protein during entry. *J Virol* 80:1302–1310. <https://doi.org/10.1128/JVI.80.3.1302-1310.2006>.
- Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, Graham BS, McLellan JS. 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367:1260–1263. <https://doi.org/10.1126/science.abb2507>.
- Rando HM, Wellhausen N, Ghosh S, Lee AJ, Dattoli AA, Hu F, Byrd JB, Rafizadeh DN, Lordan R, Qi Y, Sun Y, Brueffer C, Field JM, Ben Guebila M, Jadavji NM, Skelly AN, Ramsundar B, Wang J, Goel RR, Park Y, COVID-19 Review Consortium, Boca SM, Gitter A, Greene CS. 2021. Identification and development of therapeutics for COVID-19. *arXiv* <https://arxiv.org/abs/2103.02723>.
- Lordan R, Rando HM, COVID-19 Review Consortium, Greene CS. 2021. Dietary supplements and nutraceuticals under investigation for COVID-19 prevention and treatment. *mSystems* 6:e00122-21. <https://doi.org/10.1128/mSystems.00122-21>.
- Baron S (ed). 1996. *Medical microbiology*. University of Texas Medical Branch at Galveston, Galveston, TX.
- Fehr AR, Perlman S. 2015. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol* 1282:1–23. [https://doi.org/10.1007/978-1-4939-2438-7\\_1](https://doi.org/10.1007/978-1-4939-2438-7_1).
- Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395:565–574. [https://doi.org/10.1016/S0140-6736\(20\)30251-8](https://doi.org/10.1016/S0140-6736(20)30251-8).
- Chen Y, Liu Q, Guo D. 2020. Emerging coronaviruses: genome structure, replication, and pathogenesis. *J Med Virol* 92:418–423. <https://doi.org/10.1002/jmv.25681>.
- Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, Jiang C. 2008. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res* 18:290–301. <https://doi.org/10.1038/cr.2008.15>.
- Mercer J, Schelhaas M, Helenius A. 2010. Virus entry by endocytosis. *Annu Rev Biochem* 79:803–833. <https://doi.org/10.1146/annurev-biochem-060208-104626>.



18. Belouzard S, Millet JK, Licitra BN, Whittaker GR. 2012. Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses* 4: 1011–1033. <https://doi.org/10.3390/v4061011>.
19. Jaimes JA, André NM, Chappie JS, Millet JK, Whittaker GR. 2020. Phylogenetic analysis and structural modeling of SARS-CoV-2 spike protein reveals an evolutionary distinct and proteolytically sensitive activation loop. *J Mol Biol* 432: 3309–3325. <https://doi.org/10.1016/j.jmb.2020.04.009>.
20. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell* 181:281–292.e6. <https://doi.org/10.1016/j.cell.2020.02.058>.
21. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. 2020. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med* 46:586–590. <https://doi.org/10.1007/s00134-020-05985-9>.
22. Jia HP, Look DC, Hickey M, Shi L, Pewe L, Netland J, Farzan M, Wohlford-Lenane C, Perlman S, McCray PB, Jr. 2006. Infection of human airway epithelia by SARS coronavirus is associated with ACE2 expression and localization. *Adv Exp Med Biol* 581:479–484. [https://doi.org/10.1007/978-0-387-33012-9\\_85](https://doi.org/10.1007/978-0-387-33012-9_85).
23. Hikmet F, Méar L, Edvinsson Å, Micic P, Uhlén M, Lindskog C. 2020. The protein expression profile of ACE2 in human tissues. *Mol Syst Biol* 16: e9610. <https://doi.org/10.15252/msb.20209610>.
24. Li F. 2015. Receptor recognition mechanisms of coronaviruses: a decade of structural studies. *J Virol* 89:1954–1964. <https://doi.org/10.1128/JVI.02615-14>.
25. Du L, He Y, Zhou Y, Liu S, Zhang B-J, Jiang S. 2009. The spike protein of SARS-CoV-a target for vaccine and therapeutic development. *Nat Rev Microbiol* 7:226–236. <https://doi.org/10.1038/nrmicro2090>.
26. de Haan CAM, Rottier PJM. 2005. Molecular interactions in the assembly of coronaviruses. *Adv Virus Res* 64:165–230. [https://doi.org/10.1016/S0065-3527\(05\)64006-7](https://doi.org/10.1016/S0065-3527(05)64006-7).
27. Tang T, Bidon M, Jaimes JA, Whittaker GR, Daniel S. 2020. Coronavirus membrane fusion mechanism offers a potential target for antiviral development. *Antiviral Res* 178:104792. <https://doi.org/10.1016/j.antiviral.2020.104792>.
28. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A, Müller MA, Drosten C, Pöhlmann S. 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181:271–280.e8. <https://doi.org/10.1016/j.cell.2020.02.052>.
29. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* 11:1620. <https://doi.org/10.1038/s41467-020-15562-9>.
30. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. 2020. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res* 176:104742. <https://doi.org/10.1016/j.antiviral.2020.104742>.
31. Xia S, Lan Q, Su S, Wang X, Xu W, Liu Z, Zhu Y, Wang Q, Lu L, Jiang S. 2020. The role of furin cleavage site in SARS-CoV-2 spike protein-mediated membrane fusion in the presence or absence of trypsin. *Signal Transduct Target Ther* 5:92. <https://doi.org/10.1038/s41392-020-0184-0>.
32. Johnson BA, Xie X, Kalveram B, Lokugamage KG, Muruato A, Zou J, Zhang X, Juelich T, Smith JK, Zhang L, Bopp N, Schindewolf C, Vu M, Vanderheiden A, Swetnam D, Plante JA, Aguilar P, Plante KS, Lee B, Weaver SC, Suthar MS, Routh AL, Ren P, Ku Z, An Z, Debbink K, Shi PY, Freiberg AN, Menachery VD. 2020. Furin cleavage site is key to SARS-CoV-2 pathogenesis. *bioRxiv* <https://doi.org/10.1101/2020.08.26.268854>.
33. Johnson BA, Xie X, Bailey AL, Kalveram B, Lokugamage KG, Muruato A, Zou J, Zhang X, Juelich T, Smith JK, Zhang L, Bopp N, Schindewolf C, Vu M, Vanderheiden A, Winkler ES, Swetnam D, Plante JA, Aguilar P, Plante KS, Popov V, Lee B, Weaver SC, Suthar MS, Routh AL, Ren P, Ku Z, An Z, Debbink K, Diamond MS, Shi P-Y, Freiberg AN, Menachery VD. 2021. Loss of furin cleavage site attenuates SARS-CoV-2 pathogenesis. *Nature* 591: 293–299. <https://doi.org/10.1038/s41586-021-03237-4>.
34. Peacock TP, Goldhill DH, Zhou J, Baillon L, Frise R, Swann OC, Kugathasan R, Penn R, Brown JC, Sanchez-David RY, Braga L, Kavanagh-Williamson M, Hassard JA, Staller E, Hanley B, Osborn M, Giacca M, Davidson AD, Matthews DA, Barclay WS. 2021. The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. *Nat Microbiol* 6:899–909. <https://doi.org/10.1038/s41564-021-00908-w>.
35. Cheng Y-W, Chao T-L, Li C-L, Chiu M-F, Kao H-C, Wang S-H, Pang Y-H, Lin C-H, Tsai Y-M, Lee W-H, Tao M-H, Ho T-C, Wu P-Y, Jang L-T, Chen P-J, Chang S-Y, Yeh S-H. 2020. Furin inhibitors block SARS-CoV-2 spike protein cleavage to suppress virus production and cytopathic effects. *Cell Rep* 33:108254. <https://doi.org/10.1016/j.celrep.2020.108254>.
36. Swiss Institute of Bioinformatics. 2020. ViralZone. Coronaviridae. <https://viralzone.expasy.org/30>.
37. Jaimes JA, Millet JK, Whittaker GR. 2020. Proteolytic cleavage of the SARS-CoV-2 spike protein and the role of the novel S1/S2 site. *iScience* 23:101212. <https://doi.org/10.1016/j.isci.2020.101212>.
38. Dyer L, Patterson C. 2010. Development of the endothelium: an emphasis on heterogeneity. *Semin Thromb Hemost* 36:227–235. <https://doi.org/10.1055/s-0030-1253446>.
39. Thorgeirsson G, Robertson AL. 1978. The vascular endothelium-pathobiologic significance. *Am J Pathol* 93:803–848.
40. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. 2007. The endothelial glycocalyx: composition, functions, and visualization. *Pflügers Arch* 454:345–359. <https://doi.org/10.1007/s00424-007-0212-8>.
41. Clausen TM, Sandoval DR, Spliid CB, Pihl J, Perrett HR, Painter CD, Narayanan A, Majowicz SA, Kwong EM, McVicar RN, Thacker BE, Glass CA, Yang Z, Torres JL, Golden GJ, Bartels PL, Porell RN, Garretson AF, Laubach L, Feldman J, Yin X, Pu Y, Hauser BM, Caradonna TM, Kellman BP, Martino C, Gordts PLSM, Chanda SK, Schmidt AG, Godula K, Leibel SL, Jose J, Corbett KD, Ward AB, Carlin AF, Esko JD. 2020. SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. *Cell* 183:1043–1057.e15. <https://doi.org/10.1016/j.cell.2020.09.033>.
42. Ihrcke NS, Wrenshall LE, Lindman BJ, Platt JL. 1993. Role of heparan sulfate in immune system-blood vessel interactions. *Immunol Today* 14: 500–505. [https://doi.org/10.1016/0167-5699\(93\)90265-M](https://doi.org/10.1016/0167-5699(93)90265-M).
43. Liu L, Chopra P, Li X, Bouwman KM, Tompkins S, Wolfert MA, de Vries RP, Boons G-J. 2021. Heparan sulfate proteoglycans as attachment factor for SARS-CoV-2. *bioRxiv* <https://doi.org/10.1101/2020.05.10.087288>.
44. Shriver Z, Capila I, Venkataraman G, Sasisekharan R. 2012. Heparin and heparan sulfate: analyzing structure and microheterogeneity. *Handb Exp Pharmacol* (207):159–176. [https://doi.org/10.1007/978-3-642-23056-1\\_8](https://doi.org/10.1007/978-3-642-23056-1_8).
45. LaRivière WB, Schmidt EP. 2018. The pulmonary endothelial glycocalyx in ARDS: a critical role for heparan sulfate. *Curr Top Membranes* 82: 33–52. <https://doi.org/10.1016/bs.ctm.2018.08.005>.
46. Zhang Q, Chen CZ, Swaroop M, Xu M, Wang L, Lee J, Wang AQ, Pradhan M, Hagen N, Chen L, Shen M, Luo Z, Xu X, Xu Y, Huang W, Zheng W, Ye Y. 2020. Heparan sulfate assists SARS-CoV-2 in cell entry and can be targeted by approved drugs in vitro. *Cell Discov* 6:80. <https://doi.org/10.1038/s41421-020-00222-5>.
47. Buijssers B, Yanginlar C, Maciej-Hulme ML, de Mast Q, van der Vlag J. 2020. Beneficial non-anticoagulant mechanisms underlying heparin treatment of COVID-19 patients. *EBioMedicine* 59:102969. <https://doi.org/10.1016/j.ebiom.2020.102969>.
48. Salah HM, Naser JA, Calcaterra G, Bassareo PP, Mehta JL. 2020. The effect of anticoagulation use on mortality in COVID-19 infection. *Am J Cardiol* 134:155–157. <https://doi.org/10.1016/j.amjcard.2020.08.005>.
49. Salaris C, Scarpa M, Elli M, Bertolini A, Guglielmetti S, Pregliasco F, Blandizzi C, Brun P, Castagliuolo I. 2021. Protective effects of lactoferrin against SARS-CoV-2 infection in vitro. *Nutrients* 13:328. <https://doi.org/10.3390/nu13020328>.
50. Wadowski PP, Jilma B, Kopp CW, Ertl S, Gremmel T, Koppstein R. 2021. Glycocalyx as possible limiting factor in COVID-19. *Front Immunol* 12:607306. <https://doi.org/10.3389/fimmu.2021.607306>.
51. Liu L, Wei Q, Nishiura K, Peng J, Wang H, Midkiff C, Alvarez X, Qin C, Lackner A, Chen Z. 2016. Spatiotemporal interplay of severe acute respiratory syndrome coronavirus and respiratory mucosal cells drives viral dissemination in rhesus macaques. *Mucosal Immunol* 9:1089–1101. <https://doi.org/10.1038/mi.2015.127>.
52. Poynter SJ, DeWitte-Orr SJ. 2018. Understanding viral dsRNA-mediated innate immune responses at the cellular level using a rainbow trout model. *Front Immunol* 9:829. <https://doi.org/10.3389/fimmu.2018.00829>.
53. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJ, van der Meulen J, Koerten HK, Mommaas A. 2006. Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. *J Virol* 80:5927–5940. <https://doi.org/10.1128/JVI.02501-05>.
54. Li X, Geng M, Peng Y, Meng L, Lu S. 2020. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal* 10:102–108. <https://doi.org/10.1016/j.jpba.2020.03.001>.
55. Thoms M, Buschauer R, Ameisemeier M, Koepke L, Denk T, Hirschenberger M, Kratzat H, Hayn M, Mackens-Kiani T, Cheng J, Straub H, Stürzel CM, Fröhlich T, Berninghausen O, Becker T, Kirchhoff F, Sparrer KMJ, Beckmann R. 2020. Structural basis for translational shutdown and immune evasion by



- the Nsp1 protein of SARS-CoV-2. *Science* 369:1249–1255. <https://doi.org/10.1126/science.abc8665>.
56. Prompetchchara E, Ketloy C, Palaga T. 2020. Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. *Asian Pac J Allergy Immunol* 38:1–9. <https://doi.org/10.12932/ap-200220-0772>.
  57. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 426:450–454. <https://doi.org/10.1038/nature02145>.
  58. Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F. 2010. Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. *J Virol* 84:12658–12664. <https://doi.org/10.1128/JVI.01542-10>.
  59. Glowacka I, Bertram S, Muller MA, Allen P, Soilleux E, Pfefferle S, Steffen I, Tsegaye TS, He Y, Gnirss K, Niemeyer D, Schneider H, Drosten C, Pohlmann S. 2011. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *J Virol* 85:4122–4134. <https://doi.org/10.1128/JVI.02232-10>.
  60. Zhuang M-W, Cheng Y, Zhang J, Jiang X-M, Wang L, Deng J, Wang P-H. 2020. Increasing host cellular receptor-angiotensin-converting enzyme 2 expression by coronavirus may facilitate 2019-nCoV (or SARS-CoV-2) infection. *J Med Virol* 92:2693–2701. <https://doi.org/10.1002/jmv.26139>.
  61. Li Y, Zhou W, Yang L, You R. 2020. Physiological and pathological regulation of ACE2, the SARS-CoV-2 receptor. *Pharmacol Res* 157:104833. <https://doi.org/10.1016/j.phrs.2020.104833>.
  62. Hamming I, Timens W, Bultuis MLC, Lely AT, Navis GJ, van Goor H. 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 203:631–637. <https://doi.org/10.1002/path.1570>.
  63. Foote MB, White JR, Jee J, Argilés G, Wan JC, Rousseau B, Pessin MS, Diaz LA. 2021. Association of antineoplastic therapy with decreased SARS-CoV-2 infection rates in patients with cancer. *JAMA Oncol* <https://doi.org/10.1001/jamaoncol.2021.3585>.
  64. Thomas G. 2002. Furin at the cutting edge: from protein traffic to embryogenesis and disease. *Nat Rev Mol Cell Biol* 3:753–766. <https://doi.org/10.1038/nrm934>.
  65. Hoffmann M, Kleine-Weber H, Pöhlmann S. 2020. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol Cell* 78:779–784.e5. <https://doi.org/10.1016/j.molcel.2020.04.022>.
  66. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. 2020. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* 323:1843–1844. <https://doi.org/10.1001/jama.2020.3786>.
  67. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, Ng O-T, Marimuthu K, Ang LW, Mak TM, Lau SK, Anderson DE, Chan KS, Tan TY, Ng TY, Cui L, Said Z, Kurupatham L, Chen MI-C, Chan M, Vasoo S, Wang L-F, Tan BH, Lin RTP, Lee VJM, Leo Y-S, Lye DC, Singapore 2019 Novel Coronavirus Outbreak Research Team. 2020. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA* 323:1488–1494. <https://doi.org/10.1001/jama.2020.3204>.
  68. Ling Y, Xu S-B, Lin Y-X, Tian D, Zhu Z-Q, Dai F-H, Wu F, Song Z-G, Huang W, Chen J, Hu B-J, Wang S, Mao E-Q, Zhu L, Zhang W-H, Lu H-Z. 2020. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. *Chin Med J* 133:1039–1043. <https://doi.org/10.1097/CM9.0000000000000774>.
  69. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, Frank S, Turek D, Willi N, Pargger H, Bassetti S, Leuppi JD, Cathomas G, Tolnay M, Mertz KD, Tzankov A. 2020. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. *Histopathology* 77:198–209. <https://doi.org/10.1111/his.14134>.
  70. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, Gong W, Liu X, Liang J, Zhao Q, Huang H, Yang B, Huang C. 2020. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan. *JAMA Cardiol* 5:802–810. <https://doi.org/10.1001/jamacardio.2020.0950>.
  71. Wang S, Zhou X, Zhang T, Wang Z. 2020. The need for urogenital tract monitoring in COVID-19. *Nat Rev Urol* 17:314–315. <https://doi.org/10.1038/s41585-020-0319-7>.
  72. Fanelli V, Fiorentino M, Cantaluppi V, Gesualdo L, Stallone G, Ronco C, Castellano G. 2020. Acute kidney injury in SARS-CoV-2 infected patients. *Crit Care* 24:155. <https://doi.org/10.1186/s13054-020-02872-z>.
  73. Zhang C, Shi L, Wang F-S. 2020. Liver injury in COVID-19: management and challenges. *Lancet Gastroenterol Hepatol* 5:428–430. [https://doi.org/10.1016/S2468-1253\(20\)30057-1](https://doi.org/10.1016/S2468-1253(20)30057-1).
  74. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H. 2020. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology* 158:1831–1833.e3. <https://doi.org/10.1053/j.gastro.2020.02.055>.
  75. Gao QY, Chen YX, Fang JY. 2020. 2019 novel coronavirus infection and gastrointestinal tract. *J Dig Dis* 21:125–126. <https://doi.org/10.1111/1751-2980.12851>.
  76. Rochwerf B, Agarwal A, Siemieniuk RA, Agoritsas T, Lamontagne F, Askie L, Lytvyn L, Leo Y-S, Macdonald H, Zeng L, Amin W, Burhan E, Bausch FJ, Calfee CS, Cecconi M, Chanda D, Du B, Geduld H, Gee P, Harley N, Hashimi M, Hunt B, Kabra SK, Kanda S, Kawano-Dourado L, Kim Y-J, Kissoon N, Kwizera A, Mahaka I, Manai H, Mino G, Nsutebu E, Preller J, Pshenichnaya N, Qadir N, Sabzwari S, Sarin R, Shankar-Hari M, Sharland M, Shen Y, Ranganathan SS, Souza JP, Stegeman M, Sutter AD, Ugarte S, Venkatapuram S, Dat VQ, Vuysika D, Wijewickrama A, Maguire B, Zeraatkar D, Bartoszko JJ, Ge L, Brignardello-Petersen R, Owen A, Guyatt G, Diaz J, Jacobs M, Vandvik PO. 2020. A living WHO guideline on drugs for covid-19. *BMJ* 370:m3379. <https://doi.org/10.1136/bmj.m3379>.
  77. Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, Liu L, Shan H, Lei C-L, Hui DSC, Du B, Li L-J, Zeng G, Yuen K-Y, Chen R-C, Tang C-L, Wang T, Chen P-Y, Xiang J, Li S-Y, Wang J-L, Liang Z-J, Peng Y-X, Wei L, Liu Y, Hu Y-H, Peng P, Wang J-M, Liu J-Y, Chen Z, Li G, Zheng Z-J, Qiu S-Q, Luo J, Yu C-J, Zhu S-Y, Zhong N-S. 2020. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 382:1708–1720. <https://doi.org/10.1056/NEJMoa2002032>.
  78. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B. 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395:1054–1062. [https://doi.org/10.1016/S0140-6736\(20\)30566-3](https://doi.org/10.1016/S0140-6736(20)30566-3).
  79. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. 2020. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA* 323:2052–2059. <https://doi.org/10.1001/jama.2020.6775>.
  80. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, Satlin MJ, Campion TR, Nahid M, Ringel JB, Hoffman KL, Alshak MN, Li HA, Wehmeyer GT, Rajan M, Reshetnyak E, Hupert N, Horn EM, Martinez FJ, Gulick RM, Safford MM. 2020. Clinical characteristics of covid-19 in New York City. *N Engl J Med* 82:2372–2374. <https://doi.org/10.1056/NEJMc2010419>.
  81. Burke RM, Killerby ME, Newton S, Ashworth CE, Berns AL, Brennan S, Bressler JM, Bye E, Crawford R, Morano LH, Lewis NM, Markus TM, Read JS, Rissman T, Taylor J, Tate JE, Midgley CM, Case Investigation Form Working Group. 2020. Symptom profiles of a convenience sample of patients with COVID-19 - United States, January-April 2020. *MMWR Morb Mortal Wkly Rep* 69:904–908. <https://doi.org/10.15585/mmwr.mm6928a2>.
  82. Allen WE, Altae-Tran H, Briggs J, Jin X, McGee G, Shi A, Raghavan R, Kamariza M, Nova N, Pereta A, Danford C, Kamel A, Gothe P, Milam E, Aurambault J, Primke T, Li W, Inkenbrandt J, Huynh T, Chen E, Lee C, Croatto M, Bentley H, Lu W, Murray R, Travassos M, Coull BA, Openshaw J, Greene CS, Shalem O, King G, Probasco R, Cheng DR, Silbermann B, Zhang F, Lin X. 2020. Population-scale longitudinal mapping of COVID-19 symptoms, behaviour and testing. *Nat Hum Behav* 4:972–982. <https://doi.org/10.1038/s41562-020-00944-2>.
  83. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, Bikdeli B, Ahluwalia N, Ausiello JC, Wan EY, Freedberg DE, Kirtane AJ, Parikh SA, Maurer MS, Nordvig AS, Accili D, Bathon JM, Mohan S, Bauer KA, Leon MB, Krumholz HM, Uriel N, Mehra MR, Elkind MSV, Stone GW, Schwartz A, Ho DD, Bilezikian JP, Landry DW. 2020. Extrapulmonary manifestations of COVID-19. *Nat Med* 26:1017–1032. <https://doi.org/10.1038/s41591-020-0968-3>.
  84. Hirsch JS, Ng JH, Ross DW, Sharma P, Shah HH, Barnett RL, Hazzan AD, Fishbane S, Jhaveri KD, Northwell COVID-19 Research Consortium, Northwell Nephrology COVID-19 Research Consortium. 2020. Acute kidney injury in patients hospitalized with COVID. *Kidney Int* 98:209–218. <https://doi.org/10.1016/j.kint.2020.05.006>.
  85. Velez JCQ, Caza T, Larsen CP. 2020. COVAN is the new HIVAN: the re-emergence of collapsing glomerulopathy with COVID-19. *Nat Rev Nephrol* 16:565–567. <https://doi.org/10.1038/s41581-020-0332-3>.

86. Wu Y, Xu X, Chen Z, Duan J, Hashimoto K, Yang L, Liu C, Yang C. 2020. Nervous system involvement after infection with COVID-19 and other coronaviruses. *Brain Behav Immun* 87:18–22. <https://doi.org/10.1016/j.bbi.2020.03.031>.
87. Ellul MA, Benjamin L, Singh B, Lant S, Michael BD, Easton A, Kneen R, Defres S, Sejvar J, Solomon T. 2020. Neurological associations of COVID-19. *Lancet Neurol* 19:767–783. [https://doi.org/10.1016/S1474-4422\(20\)30221-0](https://doi.org/10.1016/S1474-4422(20)30221-0).
88. Boldrini M, Canoll PD, Klein RS. 2021. How COVID-19 affects the brain. *JAMA Psychiatry* 78:682–683. <https://doi.org/10.1001/jamapsychiatry.2021.0500>.
89. Finsterer J, Stollberger C. 2020. Update on the neurology of COVID-19. *J Med Virol* 92:2316–2318. <https://doi.org/10.1002/jmv.26000>.
90. Koralnik IJ, Tyler KL. 2020. COVID-19: a global threat to the nervous system. *Ann Neurol* 88:1–11. <https://doi.org/10.1002/ana.25807>.
91. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, Laue M, Schneider J, Brünink S, Greuel S, Lehmann M, Hassan O, Aschman T, Schumann E, Chua RL, Conrad C, Eils R, Stenzel W, Windgassen M, Rößler L, Goebel H-H, Gelderblom HR, Martin H, Nitsche A, Schulz-Schaeffer WJ, Hakroush S, Winkler MS, Tampe B, Scheibe F, Körtvélyessy P, Reinhold D, Siegmund B, Kühl AA, Elezkurtaj S, Horst D, Oesterhelweg L, Tsokos M, Ingold-Heppner B, Stadelmann C, Drosten C, Corman VM, Radrbruch H, Heppner FL. 2021. Olfactory transnucosol SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat Neurosci* 24:168–175. <https://doi.org/10.1038/s41593-020-00758-5>.
92. Thakur KT, Miller EH, Glendinning MD, Al-Dalahmah O, Banu MA, Boehme AK, Boubour AL, Bruce SS, Chong AM, Claassen J, Faust PL, Hargus G, Hickman RA, Jambawalikar S, Khandji AG, Kim CY, Klein RS, Lignelli-Dipple A, Lin C-C, Liu Y, Miller ML, Moonis G, Nordvig AS, Overdeest JB, Prust ML, Przedborski S, Roth WH, Soung A, Tanji K, Teich AF, Agalliu D, Uhlemann A-C, Goldman JE, Canoll P. 2021. COVID-19 neuropathology at Columbia University Irving Medical Center/New York Presbyterian Hospital. *Brain* <https://doi.org/10.1093/brain/awab148>.
93. Oxley TJ, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh I, De Leacy RA, Shigematsu T, Ladner TR, Yaeger KA, Skliut M, Weinberger J, Dangayach NS, Bederson JB, Tuhim S, Fifi JT. 2020. Large-vessel stroke as a presenting feature of covid-19 in the young. *N Engl J Med* 382:e60. <https://doi.org/10.1056/NEJMc2009787>.
94. Klok FA, Kruip MJHA, van der Meer NJM, Arbous MS, Gommers D, Kant KM, Kaptein FHJ, van Paassen J, Stals MAM, Huisman MV, Endeman H. 2020. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res* 191:145–147. <https://doi.org/10.1016/j.thromres.2020.04.013>.
95. Zhang Y, Xiao M, Zhang S, Xia P, Cao W, Jiang W, Chen H, Ding X, Zhao H, Zhang H, Wang C, Zhao J, Sun X, Tian R, Wu W, Wu D, Ma J, Chen Y, Zhang D, Xie J, Yan X, Zhou X, Liu Z, Wang J, Du B, Qin Y, Gao P, Qin X, Xu Y, Zhang W, Li T, Zhang F, Zhao Y, Li Y, Zhang S. 2020. Coagulopathy and antiphospholipid antibodies in patients with covid-19. *N Engl J Med* 382:e38. <https://doi.org/10.1056/NEJMc2007575>.
96. Tang N, Li D, Wang X, Sun Z. 2020. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 18:844–847. <https://doi.org/10.1111/jth.14768>.
97. Goeijenbier M, van Wissen M, van de Weg C, Jong E, Gerdes VEA, Meijers JCM, Brandjes DPM, van Gorp ECM. 2012. Review: viral infections and mechanisms of thrombosis and bleeding. *J Med Virol* 84:1680–1696. <https://doi.org/10.1002/jmv.23354>.
98. McGonagle D, O'Donnell JS, Sharif K, Emery P, Bridgwood C. 2020. Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. *Lancet Rheumatol* 2:e437–e445. [https://doi.org/10.1016/S2665-9913\(20\)30121-1](https://doi.org/10.1016/S2665-9913(20)30121-1).
99. Guglielmetti G, Quaglia M, Sainaghi PP, Castello LM, Vaschetto R, Pirisi M, Corte FD, Avanzi GC, Stratta P, Cantaluppi V. 2020. “War to the knife” against thromboinflammation to protect endothelial function of COVID-19 patients. *Crit Care* 24:365. <https://doi.org/10.1186/s13054-020-03060-9>.
100. Becker RC. 2020. COVID-19 update: covid-19-associated coagulopathy. *J Thromb Thrombolysis* 50:54–67. <https://doi.org/10.1007/s11239-020-02134-3>.
101. Java A, Apicelli AJ, Liszewski M, Coler-Reilly A, Atkinson JP, Kim AH, Kulkarni HS. 2020. The complement system in COVID-19: friend and foe? *JCI Insight* 5:e140711. <https://doi.org/10.1172/jci.insight.140711>.
102. Song W-C, FitzGerald GA. 2020. COVID-19, microangiopathy, hemostatic activation, and complement. *J Clin Invest* 130:3950–3953. <https://doi.org/10.1172/JCI140183>.
103. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, Cook JR, Nordvig AS, Shalev D, Sehrawat TS, Ahluwalia N, Bikdeli B, Dietz D, Der-Nigoghossian C, Liyanage-Don N, Rosner GF, Bernstein EJ, Mohan S, Beckley AA, Seres DS, Choueiri TK, Uriel N, Ausiello JC, Acclli D, Freedberg DE, Baldwin M, Schwartz A, Brodie D, Garcia CK, Elkind MSV, Connors JM, Bilezikian JP, Landry DW, Wan EY. 2021. Post-acute COVID-19 syndrome. *Nat Med* 27:601–615. <https://doi.org/10.1038/s41591-021-01283-z>.
104. Horwitz LI, Garry K, Prete AM, Sharma S, Mendoza F, Kahan T, Karpel H, Duan E, Hochman KA, Weerahandi H. 2021. Six-month outcomes in patients hospitalized with severe COVID-19. *J Gen Intern Med* <https://doi.org/10.1007/s11606-021-07032-9>.
105. Buonsenso D, Munblit D, De Rose C, Sinatti D, Ricchiuto A, Carfi A, Valentini P. 2021. Preliminary evidence on long COVID in children. *Acta Paediatr* 110:2208–2211. <https://doi.org/10.1111/apa.15870>.
106. Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ, Re'em Y, Redfield S, Austin JP, Akrami A. 2021. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. *EclinicalMedicine* 38:101019. <https://doi.org/10.1016/j.eclinm.2021.101019>.
107. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, Kang L, Guo L, Liu M, Zhou X, Luo J, Huang Z, Tu S, Zhao Y, Chen L, Xu D, Li Y, Li C, Peng L, Li Y, Xie W, Cui D, Shang L, Fan G, Xu J, Wang G, Wang Y, Zhong J, Wang C, Wang J, Zhang D, Cao B. 2021. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet* 397:220–232. [https://doi.org/10.1016/S0140-6736\(20\)32656-8](https://doi.org/10.1016/S0140-6736(20)32656-8).
108. Rando HM, Bennett TD, Byrd JB, Bramante C, Callahan TJ, Chute CG, Davis HE, Deer R, Gagnier J, Korashy FM, Liu F, McMurry JA, Moffitt RA, Pfaff ER, Reese JT, Relevo R, Robinson PN, Saltz JH, Solomonides A, Sule A, Topaloglu U, Haendel MA. 2021. Challenges in defining long COVID: striking differences across literature, electronic health records, and patient-reported information. *medRxiv* <https://doi.org/10.1101/2021.03.20.21253896>.
109. Deer RR, Rock MA, Vasilevsky N, Carmody L, Rando H, Anzalone AJ, Callahan TJ, Bramante CT, Chute CG, Greene CS, Gagnier J, Chu H, Korashy FM, Liang C, Liu F, Madlock-Brown CR, Mazzotti DR, McNair DS, Parker AM, Coleman BD, Davis HE, Perry MA, Reese JT, Saltz J, Solomonides AE, Sule AA, Stein GS, Köhler S, Monteith TS, Madhira V, Kimble WD, Kavuluru R, Hillegass WB, Chan LE, Byrd JB, Boudreau EA, Liu H, McMurry JA, Pfaff E, Matentzoglou N, Relevo R, Moffitt RA, Schuff RA, Solway J, Spratt H, Bergquist T, Bennett TD, Basson MD, Topaloglu U, Wang L, Haendel MA, Robinson PN. 2021. Characterizing long COVID: deep phenotype of a complex condition. *medRxiv* <https://doi.org/10.1101/2021.06.23.21259416>.
110. Fontanet A, Grant R, Tondeur L, Madec Y, Grzelak L, Cailleau I, Ungeheuer M-N, Renaudat C, Fernandes Pellerin S, Kuhmel L, Staropoli I, Anna F, Charneau P, Demeret C, Bruel T, Schwartz O, Hoen B. 2020. SARS-CoV-2 infection in primary schools in northern France: a retrospective cohort study in an area of high transmission. *medRxiv* <https://doi.org/10.1101/2020.06.25.20140178>.
111. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, Zhang W, Wang Y, Bao S, Li Y, Wu C, Liu H, Liu D, Shao J, Peng X, Yang Y, Liu Z, Xiang Y, Zhang F, Silva RM, Pinkerton KE, Shen K, Xiao H, Xu S, Wong GWK. 2020. SARS-CoV-2 infection in children. *N Engl J Med* 382:1663–1665. <https://doi.org/10.1056/NEJMc2005073>.
112. Ludvigsson JF. 2020. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. *Acta Paediatr* 109:1088–1095. <https://doi.org/10.1111/apa.15270>.
113. Lordan R, FitzGerald GA, Grosser T. 2020. Reopening schools during COVID-19. *Science* 369:1146. <https://doi.org/10.1126/science.abe5765>.
114. Castagnoli R, Votto M, Licari A, Brambilla I, Bruno R, Perlini S, Rovida F, Baldanti F, Marseglia GL. 2020. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents. *JAMA Pediatr* 174:882–889. <https://doi.org/10.1001/jamapediatrics.2020.1467>.
115. Abdel-Mannan O, Eyre M, Löbel U, Bamford A, Eltze C, Hameed B, Hemingway C, Hacoen Y. 2020. Neurologic and radiographic findings associated with COVID-19 infection in children. *JAMA Neurol* 77:1440–1445. <https://doi.org/10.1001/jamaneurol.2020.2687>.
116. Parri N, Lenge M, Buonsenso D, Coronavirus Infection in Pediatric Emergency Departments (CONFIDENCE) Research Group. 2020. Children with covid-19 in pediatric emergency departments in Italy. *N Engl J Med* 383:187–190. <https://doi.org/10.1056/NEJMc2007617>.
117. Centers for Disease Control and Prevention. 2020. COVID data tracker. Centers for Disease Control and Prevention, Atlanta, GA. <https://covid.cdc.gov/covid-data-tracker>.
118. Mahase E. 2021. Delta variant: what is happening with transmission, hospital admissions, and restrictions? *BMJ* 373:n1513. <https://doi.org/10.1136/bmj.n1513>.
119. Anthes E. 2021. The delta variant is sending more children to the hospital. Are they sicker, too? *The New York Times*, New York, NY. <https://www.nytimes.com/2021/08/09/health/coronavirus-children-delta.html>.
120. Hoang A, Chorath K, Moreira A, Evans M, Burmeister-Morton F, Burmeister F, Naqvi R, Petershack M, Moreira A. 2020. COVID-19 in 7780 pediatric patients:

- a systematic review. *EClinicalMedicine* 24:100433. <https://doi.org/10.1016/j.eclinm.2020.100433>.
121. Koskela U, Helve O, Sarviki E, Helminen M, Nieminen T, Peltola V, Renko M, Saxén H, Pasma H, Pokka T, Honkila M, Tapiainen T. 2021. Multi-inflammatory syndrome and Kawasaki disease in children during the COVID-19 pandemic: a nationwide register-based study and time series analysis. *Acta Paediatr* <https://doi.org/10.1111/apa.16051>.
  122. Chiotos K, Bassiri H, Behrens EM, Blatz AM, Chang J, Diorio C, Fitzgerald JC, Topjian A, Odom John AR. 2020. Multisystem inflammatory syndrome in children during the coronavirus 2019 pandemic: a case series. *J Pediatr Infect Dis Soc* 9:393–398. <https://doi.org/10.1093/jpids/piaa069>.
  123. Whittaker E, Bamford A, Kenny J, Kaforou M, Jones CE, Shah P, Ramnarayan P, Fraise A, Miller O, Davies P, Kucera F, Brierley J, McDougall M, Carter M, Tremoulet A, Shimizu C, Herberg J, Burns JC, Lyall H, Levin M, PIMS-TS Study Group and EUCLIDS and PERFORM Consortia. 2020. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA* 324:259–269. <https://doi.org/10.1001/jama.2020.10369>.
  124. Greene AG, Saleh M, Roseman E, Sinert R. 2020. Toxic shock-like syndrome and COVID-19: multisystem inflammatory syndrome in children (MIS-C). *Am J Emerg Med* 38:2492.e5–2492.e6. <https://doi.org/10.1016/j.ajem.2020.05.117>.
  125. Diorio C, Henrickson SE, Vella LA, McNERney KO, Chase J, Burudpakdee C, Lee JH, Jasen C, Balamuth F, Barrett DM, Banwell BL, Bernt KM, Blatz AM, Chiotos K, Fisher BT, Fitzgerald JC, Gerber JS, Gollomp K, Gray C, Grupp SA, Harris RM, Kilbaugh TJ, John ARO, Lambert M, Lieblich EJ, Paessler ME, Petrosa W, Phillips C, Reilly AF, Romberg ND, Seif A, Sesok-Pizzini DA, Sullivan KE, Vardaro J, Behrens EM, Teachey DT, Bassiri H. 2020. Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2. *J Clin Invest* 130:5967–5975. <https://doi.org/10.1172/JCI140970>.
  126. Rosat Consiglio C, Cotugno N, Sardh F, Pou C, Amodio D, Rodriguez L, Tan Z, Zicari S, Ruggiero A, Pascucci GR, Santilli V, Campbell T, Bryceson Y, Eriksson D, Wang J, Marchesi A, Lakshminanth T, Campana A, Villani A, Rossi P, CACTUS Study Team, Landegren N, Palma P, Brodin P. 2020. The immunology of multisystem inflammatory syndrome in children with COVID-19. *Cell* 183:968–981.e7. <https://doi.org/10.1016/j.cell.2020.09.016>.
  127. Belhadjer Z, Méot M, Bajolle F, Khraiche D, Legendre A, Abakka S, Auriau J, Grimaud M, Oualha M, Beghetti M, Wacker J, Ovaert C, Hascoet S, Selegny M, Malekzadeh-Milani S, Maltret A, Bosser G, Giroux N, Bonnemains L, Bordet J, Di Filippo S, Mauran P, Falcon-Eicher S, Thambo J-B, Lefort B, Moceri P, Houyel L, Renolleau S, Bonnet D. 2020. Acute heart failure in multisystem inflammatory syndrome in children in the context of global SARS-CoV-2 pandemic. *Circulation* 142:429–436. <https://doi.org/10.1161/CIRCULATIONAHA.120.048360>.
  128. Shaigany S, Gnirke M, Guttmann A, Chong H, Meehan S, Raabe V, Louie E, Solitar B, Femia A. 2020. An adult with Kawasaki-like multisystem inflammatory syndrome associated with COVID-19. *Lancet* 396:e8–e10. [https://doi.org/10.1016/S0140-6736\(20\)31526-9](https://doi.org/10.1016/S0140-6736(20)31526-9).
  129. Nune A, Iyengar KP, Goddard C, Ahmed AE. 2021. Multisystem inflammatory syndrome in an adult following the SARS-CoV-2 vaccine (MIS-V). *BMJ Case Rep* 14:e243888. <https://doi.org/10.1136/bcr-2021-243888>.
  130. Sokolovsky S, Soni P, Hoffman T, Kahn P, Scheers-Masters J. 2021. COVID-19 associated Kawasaki-like multisystem inflammatory disease in an adult. *Am J Emerg Med* 39:253.e1–253.e2. <https://doi.org/10.1016/j.ajem.2020.06.053>.
  131. Boudhabhay I, Rabant M, Roumenina LT, Couprie L-M, Poillierat V, Marchal A, Frémeaux-Bacchi V, El Karoui K, Monchi M, Pourcine F. 2021. Case report: adult post-COVID-19 multisystem inflammatory syndrome and thrombotic microangiopathy. *Front Immunol* 12:680567. <https://doi.org/10.3389/fimmu.2021.680567>.
  132. Feldstein LR, Tenforde MW, Friedman KG, Newhams M, Rose EB, Dapul H, Soma VL, Maddux AB, Mourani PM, Bowens C, Maamari M, Hall MW, Riggs BJ, Giuliano JS, Singh AR, Li S, Kong M, Schuster JE, McLaughlin GE, Schwartz SP, Walker TC, Loftis LL, Hobbs CV, Halasa NB, Doymaz S, Babbitt CJ, Hume JR, Gertz SJ, Irby K, Clouser KN, Cvijanovich NZ, Bradford TT, Smith LS, Heidemann SM, Zackai SP, Wellnitz K, Nofziger RA, Horwitz SM, Carroll RW, Rowan CM, Tarquinio KM, Mack EH, Fitzgerald JC, Coates BM, Jackson AM, Young CC, Son MBF, Patel MM, Newburger JW, Randolph AG, Overcoming COVID-19 Investigators. 2021. Characteristics and outcomes of US children and adolescents with multisystem inflammatory syndrome in children (MIS-C) compared with severe acute COVID-19. *JAMA* 325:1074–1087. <https://doi.org/10.1001/jama.2021.2091>.
  133. Buonsenso D, Munblit D, De Rose C, Sinatti D, Ricchiuto A, Carfi A, Valentini P. 2021. Preliminary evidence on long COVID in children. *medRxiv* <https://doi.org/10.1101/2021.01.23.21250375>.
  134. Brackel CLH, Lap CR, Buddingh EP, van Houten MA, van der Sande LJT, Langereis EJ, Bannier MAGE, Pijnenburg MWH, Hashimoto S, Terheggen-Lagro SWJ. 2021. Pediatric long-COVID: an overlooked phenomenon? *Pediatr Pulmonol* 56:2495–2502. <https://doi.org/10.1002/ppul.25521>.
  135. Say D, Crawford N, McNab S, Wurzel D, Steer A, Tosif S. 2021. Post-acute COVID-19 outcomes in children with mild and asymptomatic disease. *Lancet Child Adolesc Health* 5:e22–e23. [https://doi.org/10.1016/S2352-4642\(21\)00124-3](https://doi.org/10.1016/S2352-4642(21)00124-3).
  136. Radtke T, Ulyte A, Puhan MA, Kriemler S. 2021. Long-term symptoms after SARS-CoV-2 infection in school children: population-based cohort with 6-months follow-up. *medRxiv* <https://doi.org/10.1101/2021.05.16.21257255>.
  137. Radtke T, Ulyte A, Puhan MA, Kriemler S. 2021. Long-term symptoms after SARS-CoV-2 infection in children and adolescents. *JAMA* 326:869–871. <https://doi.org/10.1001/jama.2021.11880>.
  138. Mallapaty S. 2021. Kids and COVID: why young immune systems are still on top. *Nature* 597:166–168. <https://doi.org/10.1038/d41586-021-02423-8>.
  139. Cicchese JM, Evans S, Hult C, Joslyn LR, Wessler T, Millar JA, Marino S, Cilfone NA, Mattila JT, Linderman JJ, Kirschner DE. 2018. Dynamic balance of pro- and anti-inflammatory signals controls disease and limits pathology. *Immunol Rev* 285:147–167. <https://doi.org/10.1111/immr.12671>.
  140. Elenkov IJ, Iezzoni DG, Daly A, Harris AG, Chrousos GP. 2005. Cytokine dysregulation, inflammation and well-being. *Neuroimmunomodulation* 12:255–269. <https://doi.org/10.1159/000087104>.
  141. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. 2017. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 9:7204–7218. <https://doi.org/10.18632/oncotarget.23208>.
  142. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. 2002. *Molecular biology of the cell*. Garland Science, New York, NY.
  143. Widmaier EP, Raff H, Strang KT. 2008. *Vander's human physiology: the mechanisms of body function*. McGraw-Hill Higher Education, New York, NY.
  144. McKechnie JL, Blish CA. 2020. The innate immune system: fighting on the front lines or fanning the flames of COVID-19? *Cell Host Microbe* 27:863–869. <https://doi.org/10.1016/j.chom.2020.05.009>.
  145. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. 2012. Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* 76:16–32. <https://doi.org/10.1128/MMBR.05015-11>.
  146. Hall MJ, Williams SN, DeFrances CJ, Golosinskiy A. 2011. Inpatient care for septicemia or sepsis: a challenge for patients and hospitals. *NCHS Data Brief* (62):1–8.
  147. Gu X, Zhou F, Wang Y, Fan G, Cao B. 2020. Respiratory viral sepsis: epidemiology, pathophysiology, diagnosis and treatment. *Eur Respir Rev* 29:200038. <https://doi.org/10.1183/16000617.0038-2020>.
  148. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, Su X, Cao B. 2020. SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet* 395:1517–1520. [https://doi.org/10.1016/S0140-6736\(20\)30920-X](https://doi.org/10.1016/S0140-6736(20)30920-X).
  149. Park WY, Goodman RB, Steinberg KP, Ruzinski JT, Radella F, Park DR, Pugin J, Skerrett SJ, Hudson LD, Martin TR. 2001. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 164(10 Pt 1):1896–1903. <https://doi.org/10.1164/ajrccm.164.10.2104013>.
  150. Shimabukuro-Vornhagen A, Gödel P, Subklewe M, Stemmler HJ, Schlößer HA, Schlaak M, Kochanek M, Böll B, von Bergwelt-Baildon MS. 2018. Cytokine release syndrome. *J Immunother Cancer* 6:56. <https://doi.org/10.1186/s40425-018-0343-9>.
  151. He L, Ding Y, Zhang Q, Che X, He Y, Shen H, Wang H, Li Z, Zhao L, Geng J, Deng Y, Yang L, Li J, Cai J, Qiu L, Wen K, Xu X, Jiang S. 2006. Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2<sup>+</sup> cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. *J Pathol* 210:288–297. <https://doi.org/10.1002/path.2067>.
  152. Wang W, Ye L, Ye L, Li B, Gao B, Zeng Y, Kong L, Fang X, Zheng H, Wu Z, She Y. 2007. Up-regulation of IL-6 and TNF- $\alpha$  induced by SARS-coronavirus spike protein in murine macrophages via NF- $\kappa$ B pathway. *Virus Res* 128:1–8. <https://doi.org/10.1016/j.virusres.2007.02.007>.
  153. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. 2020. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet* 395:1033–1034. [https://doi.org/10.1016/S0140-6736\(20\)30628-0](https://doi.org/10.1016/S0140-6736(20)30628-0).
  154. Mangalmurti N, Hunter CA. 2020. Cytokine storms: understanding COVID-19. *Immunity* 53:19–25. <https://doi.org/10.1016/j.immuni.2020.06.017>.



155. Kellum JA. 2007. Understanding the inflammatory cytokine response in pneumonia and sepsis. *Arch Intern Med* 167:1655. <https://doi.org/10.1001/archinte.167.15.1655>.
156. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* 813:878–888. <https://doi.org/10.1016/j.bbamcr.2011.01.034>.
157. Velazquez-Salinas L, Verdugo-Rodriguez A, Rodriguez LL, Borca MV. 2019. The role of interleukin 6 during viral infections. *Front Microbiol* 10:1057. <https://doi.org/10.3389/fmicb.2019.01057>.
158. Sinha P, Matthay MA, Calfee CS. 2020. Is a “cytokine storm” relevant to COVID-19? *JAMA Intern Med* 180:1152–1154. <https://doi.org/10.1001/jamainternmed.2020.3313>.
159. Liu B, Li M, Zhou Z, Guan X, Xiang Y. 2020. Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID)-induced cytokine release syndrome (CRS)? *J Autoimmun* 111:102452. <https://doi.org/10.1016/j.jaut.2020.102452>.
160. Eckhardt M, Hultquist JF, Kaake RM, Hüttenhain R, Krogan NJ. 2020. A systems approach to infectious disease. *Nat Rev Genet* 21:339–354. <https://doi.org/10.1038/s41576-020-0212-5>.
161. Ray S, Patel SK, Kumar V, Damahe J, Srivastava S. 2014. Differential expression of serum/plasma proteins in various infectious diseases: Specific or nonspecific signatures. *Proteomics Clin Appl* 8:53–72. <https://doi.org/10.1002/prca.201300074>.
162. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, Uhl S, Hoagland D, Møller R, Jordan TX, Oishi K, Panis M, Sachs D, Wang TT, Schwartz RE, Lim JK, Albrecht RA, tenOever BR. 2020. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* 181:1036–1045.e9. <https://doi.org/10.1016/j.cell.2020.04.026>.
163. Versteeg GA, García-Sastre A. 2010. Viral tricks to grid-lock the type I interferon system. *Curr Opin Microbiol* 13:508–516. <https://doi.org/10.1016/j.mib.2010.05.009>.
164. Stewart CE, Randall RE, Adamson CS. 2014. Inhibitors of the interferon response enhance virus replication in vitro. *PLoS One* 9:e112014. <https://doi.org/10.1371/journal.pone.0112014>.
165. Kopecky-Bromberg SA, Martínez-Sobrido L, Frieman M, Baric RA, Palese P. 2007. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. *J Virol* 81:548–557. <https://doi.org/10.1128/JVI.01782-06>.
166. Niemeyer D, Zillinger T, Muth D, Zielecki F, Horvath G, Suliman T, Barchet W, Weber F, Drosten C, Müller MA. 2013. Middle East respiratory syndrome coronavirus accessory protein 4a is a type I interferon antagonist. *J Virol* 87:12489–12495. <https://doi.org/10.1128/JVI.01845-13>.
167. Wang P-H, Cheng Y. 2020. Increasing host cellular receptor—angiotensin-converting enzyme 2 (ACE2) expression by coronavirus may facilitate 2019-nCoV infection. *bioRxiv* <https://doi.org/10.1101/2020.02.24.963348>.
168. Yuen C-K, Lam J-Y, Wong W-M, Mak L-F, Wang X, Chu H, Cai J-P, Jin D-Y, To KK-W, Chan JF-W, Yuen K-Y, Kok K-H. 2020. SARS-CoV-2 nsp13, nsp14, nsp15 and orf6 function as potent interferon antagonists. *Emerg Microbes Infect* 9:1418–1428. <https://doi.org/10.1080/22221751.2020.1780953>.
169. Konno Y, Kimura I, Uriu K, Fukushi M, Irie T, Koyanagi Y, Sauter D, Gifford RJ, USFQ-COVID19 Consortium, Nakagawa S, Sato K. 2020. SARS-CoV-2 ORF3b is a potent interferon antagonist whose activity is increased by a naturally occurring elongation variant. *Cell Rep* 32:108185. <https://doi.org/10.1016/j.celrep.2020.108185>.
170. Wyler E, Mösbauer K, Franke V, Diag A, Gottula LT, Arsie R, Klironomos F, Koppstein D, Ayoub S, Buccitelli C, Richter A, Legnini I, Ivanov A, Mari T, Del Guidice S, Papias JP, Müller MA, Niemeyer D, Selbach M, Akalin A, Rajewsky N, Drosten C, Landthaler M. 2020. Bulk and single-cell gene expression profiling of SARS-CoV-2 infected human cell lines identifies molecular targets for therapeutic intervention. *bioRxiv* <https://doi.org/10.1101/2020.05.05.079194>.
171. Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J, Queen K, Tao Y, Paden CR, Zhang J, Li Y, Uehara A, Wang H, Goldsmith C, Bullock HA, Wang L, Whitaker B, Lynch B, Gautam R, Schindewolf C, Lokugamage KG, Scharton D, Plante JA, Mirchandani D, Widén SG, Narayanan K, Makino S, Ksiazek TG, Plante KS, Weaver SC, Lindstrom S, Tong S, Menachery VD, Thornburg NJ. 2020. Isolation and characterization of SARS-CoV-2 from the first US COVID-19 patient. *bioRxiv* <https://doi.org/10.1101/2020.03.02.972935>.
172. Aschenbrenner AC, Mouktaroudi M, Krämer B, Oestreich M, Antonakos N, Nuesch-Germano M, Gkizeli K, Bonaguro L, Reusch N, Baßler K, Saridakis M, Knoll R, Pecht T, Kapellos TS, Doulou S, Kröger C, Herbert M, Holsten L, Horne A, Gemünd ID, Rovina N, Agrawal S, Dahm K, van Uelft M, Drews A, Lenkeit L, Bruse N, Gerretsen J, Gierlich J, Becker M, Händler K, Kraut M, Theis H, Mengiste S, De Domenico E, Schulte-Schrepping J, Seep L, Raabe J, Hoffmeister C, ToVinh M, Keitel V, Rieke G, Talevi V, Skowasch D, Aziz NA, Pickkers P, van de Veerdonk FL, Netea MG, Schultze JL, Kox M, Breteler MMB, Nattermann J, Koutsoukou A, Giamarellos-Bourboulis EJ, Ulas T, German COVID-19 Omics Initiative (DeCOI). 2021. Disease severity-specific neutrophil signatures in blood transcriptomes stratify COVID-19 patients. *Genome Med* 13:7. <https://doi.org/10.1186/s13073-020-00823-5>.
173. Bernardes JP, Mishra N, Tran F, Bahmer T, Best L, Blase JI, Bordoni D, Franzenburg J, Geisen U, Josephs-Spaulding J, Köhler P, Künstner A, Rosati E, Aschenbrenner AC, Bacher P, Baran N, Boysen T, Brandt B, Bruse N, Dörr J, Dräger A, Elke G, Ellinghaus D, Fischer J, Forster M, Franke A, Franzenburg S, Frey N, Friedrichs A, Fuß J, Glück A, Hamm J, Hinrichsen F, Hoepfner MP, Imm S, Junker R, Kaiser S, Kan YH, Knoll R, Lange C, Laue G, Lier C, Lindner M, Marinos G, Markewitz R, Nattermann J, Noth R, Pickkers P, Rabe KF, Renz A, Röcken C, Rupp J, Schaffarczyk A, Scheffold A, Schulte-Schrepping J, Schunk D, Skowasch D, Ulas T, Wandinger K-P, Wittig M, Zimmermann J, Busch H, Hoyer BF, Kaleta C, Heyckendorf J, Kox M, Rybniker J, Schreiber S, Schultze JL, Rosenstiel P, HCA Lung Biological Network, Deutsche COVID-19 Omics Initiative (DeCOI). 2020. Longitudinal multi-omics analyses identify responses of megakaryocytes, erythroid cells, and plasmablasts as hallmarks of severe COVID-19. *Immunity* 53:1296–1314.e9. <https://doi.org/10.1016/j.immuni.2020.11.017>.
174. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, Guo D, Hu W, Yang J, Tang Z, Wu H, Lin Y, Zhang M, Zhang Q, Shi M, Liu Y, Zhou Y, Lan K, Chen Y. 2020. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. *Emerg Microbes Infect* 9:761–770. <https://doi.org/10.1080/22221751.2020.1747363>.
175. Liu C, Martins AJ, Lau WW, Rachmaninoff N, Chen J, Imberti L, Mostaghimi D, Fink DL, Burbelo PD, Dobbs K, Delmonte OM, Bansal N, Failla L, Sottini A, Quiros-Roldan E, Han KL, Sellers BA, Cheung F, Sparks R, Chun T-W, Moir S, Lionakis MS, NIAID COVID Consortium, COVID Clinicians, Rossi C, Su HC, Kuhns DB, Cohen JI, Notarangelo LD, Tsang JS. 2021. Time-resolved systems immunology reveals a late juncture linked to fatal COVID-19. *Cell* 184:1836–1857.e22. <https://doi.org/10.1016/j.cell.2021.02.018>.
176. Prokop JW, Hartog NL, Chesla D, Faber W, Love CP, Karam R, Abualkheir N, Feldmann B, Teng L, McBride T, Leimanis ML, English BK, Holsworth A, Frisch A, Bauss J, Kalpage N, Derbedrossian A, Pinti RM, Hale N, Mills J, Eby A, VanSickle EA, Pageau SC, Shankar R, Chen B, Carcillo JA, Sanfilippo D, Olivero R, Bupp CP, Rajasekaran S. 2021. High-density blood transcriptomics reveals precision immune signatures of SARS-CoV-2 infection in hospitalized individuals. *Front Immunol* 12:694243. <https://doi.org/10.3389/fimmu.2021.694243>.
177. Arunachalam PS, Wimmers F, Mok CKP, Perera RA, Scott M, Hagan T, Sigal N, Feng Y, Bristow L, Tsang OT-Y, Wagh D, Collier J, Pellegrini KL, Kazmin D, Alaaeddine G, Leung WS, Chan JMC, Chik TSH, Choi CYC, Huerta C, McCullough MP, Lv H, Anderson E, Edupuganti S, Upadhyay AA, Bosinger SE, Maecker HT, Khatri P, Roupael N, Peiris M, Pulendran B. 2020. Systems biological assessment of immunity to mild versus severe COVID-19 infection in humans. *Science* 369:1210–1220. <https://doi.org/10.1126/science.abc6261>.
178. Cheemarla NR, Watkins TA, Mihaylova VT, Wang B, Zhao D, Wang G, Landry ML, Foxman EF. 2021. Dynamic innate immune response determines susceptibility to SARS-CoV-2 infection and early replication kinetics. *J Exp Med* 218:e20210583. <https://doi.org/10.1084/jem.20210583>.
179. Yang AC, Kern F, Losada PM, Agam MR, Maat CA, Schmartz GP, Fehlmann T, Stein JA, Schaum N, Lee DP, Calcuttawala K, Vest RT, Berndik D, Lu N, Hahn O, Gate D, Mc Nerney MW, Channappa D, Cobos I, Ludwig N, Schulz-Schaeffer WJ, Keller A, Wyss-Coray T. 2021. Dysregulation of brain and choroid plexus cell types in severe COVID-19. *Nature* 595:565–571. <https://doi.org/10.1038/s41586-021-03710-0>.
180. Bojkova D, Klann K, Koch B, Widera M, Krause D, Ciesek S, Cinatl J, Münch C. 2020. Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. *Nature* 583:469–472. <https://doi.org/10.1038/s41586-020-2332-7>.
181. Ju B, Zhang Q, Ge X, Wang R, Yu J, Shan S, Zhou B, Song S, Tang X, Yu J, Ge J, Lan J, Yuan J, Wang H, Zhao J, Zhang S, Wang Y, Shi X, Liu L, Wang X, Zhang Z, Zhang L. 2020. Potent human neutralizing antibodies elicited by SARS-CoV-2 infection. *bioRxiv* <https://doi.org/10.1101/2020.03.21.990770>.
182. Chen J-H, Chang Y-W, Yao C-W, Chiueh T-S, Huang S-C, Chien K-Y, Chen A, Chang F-Y, Wong C-H, Chen Y-J. 2004. Plasma proteome of severe acute respiratory syndrome analyzed by two-dimensional gel electrophoresis and

- mass spectrometry. *Proc Natl Acad Sci U S A* 101:17039–17044. <https://doi.org/10.1073/pnas.0407992101>.
183. He R, Dobie F, Ballantine M, Leeson A, Li Y, Bastien N, Cutts T, Andonov A, Cao J, Booth TF, Plummer FA, Tyler S, Baker L, Li X. 2004. Analysis of multimerization of the SARS coronavirus nucleocapsid protein. *Biochem Biophys Res Commun* 316:476–483. <https://doi.org/10.1016/j.bbrc.2004.02.074>.
  184. UniProt Consortium. 2019. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res* 47(D1):D506–D515. <https://doi.org/10.1093/nar/gky1049>.
  185. National Center for Biotechnology Information. 2021. Genome. <https://www.ncbi.nlm.nih.gov/genome/>.
  186. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, Wheeler DK, Sette A, Peters B. 2019. The Immune Epitope Database (IEDB): 2018 update. *Nucleic Acids Res* 47(D1):D339–D343. <https://doi.org/10.1093/nar/gky1006>.
  187. Pickett BE, Sadat EL, Zhang Y, Noronha JM, Squires RB, Hunt V, Liu M, Kumar S, Zaremba S, Gu Z, Zhou L, Larson CN, Dietrich J, Klem EB, Scheuermann RH. 2012. ViPR: an open bioinformatics database and analysis resource for virology research. *Nucleic Acids Res* 40(Database issue): D593–D598. <https://doi.org/10.1093/nar/gkr859>.
  188. Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, O'Meara MJ, Rezelj VV, Guo JZ, Swaney DL, Tummino TA, Hüttenhain R, Kaake RM, Richards AL, Tutuncuoglu B, Foussard H, Batra J, Haas K, Modak M, Kim M, Haas P, Polacco BJ, Braberg R, Fabius JM, Eckhardt M, Soucraey M, Bennett MJ, Cakir M, McGregor MJ, Li Q, Meyer B, Roesch F, Vallet T, Mac Kain A, Miorin L, Moreno E, Naing ZCZ, Zhou Y, Peng S, Shi Y, Zhang Z, Shen W, Kirby IT, Melnyk JE, Chorba JS, Lou K, Dai SA, Barrio-Hernandez I, Memon D, Hernandez-Armenta C, Lyu J, Mathy CJ, Perica T, Pilla KB, Ganesan SJ, Saltzberg DJ, Rakesh R, Liu X, Rosenthal SB, Calviello L, Venkataraman S, Liboy-Lugo J, Lin Y, Huang XP, Liu Y, Wankowicz SA, Bohn M, Safari M, Ugur FS, Koh C, Savar NS, Tran QD, Shengjuler D, Fletcher SJ, O'Neal MC, Cai Y, Chang JCJ, Broadhurst DJ, Klippsten S, Sharp PP, Wenzel NA, Kuzuoglu-Ozturk D, Wang HY, Trenker R, Young JM, Caverio DA, Hiatt J, Roth TL, Rathore U, Subramanian A, Noack J, Hubert M, Stroud RM, Frankel AD, Rosenberg OS, Verba KA, Agard DA, Ott M, Emerman M, Jura N, von Zastrow M, Verdini E, Ashworth A, Schwartz O, d'Enfert C, Mukherjee S, Jacobson M, Malik HS, Fujimori DG, Ideker T, Craik CS, Floor SN, Fraser JS, Gross JD, Salvi A, Roth BL, Ruggero D, Taunton J, Kortemme T, Beltrao P, Vignuzzi M, García-Sastre A, Shokat KM, Shoichet BK, Krogan NJ. 2020. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* 583:459–468. <https://doi.org/10.1038/s41586-020-2286-9>.
  189. Sobocińska J, Roszczenko-Jasińska P, Ciesielska A, Kwiatkowska K. 2017. Protein palmitoylation and its role in bacterial and viral infections. *Front Immunol* 8:2003. <https://doi.org/10.3389/fimmu.2017.02003>.
  190. Li J, Guo M, Tian X, Liu C, Wang X, Yang X, Wu P, Xiao Z, Qu Y, Yin Y, Fu J, Zhu Z, Liu Z, Peng C, Zhu T, Liang Q. 2020. Virus-host interactome and proteomic survey of PMBCs from COVID-19 patients reveal potential virulence factors influencing SARS-CoV-2 pathogenesis. *bioRxiv* <https://doi.org/10.1101/2020.03.31.019216>.
  191. Lawrence T. 2009. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol* 1:a001651. <https://doi.org/10.1101/cshperspect.a001651>.
  192. Navratil V, Lionnard L, Longhi S, Hardwick J, Combet C, Aouacheria A. 2020. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) envelope (E) protein harbors a conserved BH3-like sequence. *bioRxiv* (Withdrawn.). <https://doi.org/10.1101/2020.04.09.033522>.
  193. Overmyer KA, Shishkova E, Miller IJ, Balnis J, Bernstein MN, Peters-Clarke TM, Meyer JG, Quan Q, Muehlbauer LK, Trujillo EA, He Y, Chopra A, Chieng HC, Tiwari A, Judson MA, Paulson B, Brademan DR, Zhu Y, Serrano LR, Linke V, Drake LA, Adam AP, Schwartz BS, Singer HA, Swanson S, Mosher DF, Stewart R, Coon JJ, Jaitovich A. 2021. Large-scale multi-omic analysis of COVID-19 severity. *Cell Syst* 12:23–40.e7. <https://doi.org/10.1016/j.cels.2020.10.003>.
  194. Geyer PE, Arend FM, Doll S, Louisset M-L, Virreira Winter S, Müller-Reif JB, Torun FM, Weigand M, Eichhorn P, Bruegel M, Strauss MT, Holdt LM, Mann M, Tupser D. 2021. High-resolution serum proteome trajectories in COVID-19 reveal patient-specific seroconversion. *EMBO Mol Med* 13: e14167. <https://doi.org/10.15252/emmm.202114167>.
  195. Li F, Li W, Farzan M, Harrison SC. 2005. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 309: 1864–1868. <https://doi.org/10.1126/science.1116480>.
  196. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. 2020. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 367: 1444–1448. <https://doi.org/10.1126/science.abb2762>.
  197. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F. 2020. Structural basis of receptor recognition by SARS-CoV-2. *Nature* 581:221–224. <https://doi.org/10.1038/s41586-020-2179-y>.
  198. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L, Wang X. 2020. Crystal structure of the 2019-nCoV spike receptor-binding domain bound with the ACE2 receptor. *bioRxiv* <https://doi.org/10.1101/2020.02.19.956235>.
  199. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen K-Y, Wang Q, Zhou H, Yan J, Qi J. 2020. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell* 181:894–904.e9. <https://doi.org/10.1016/j.cell.2020.03.045>.
  200. Wan Y, Shang J, Graham R, Baric RS, Li F. 2020. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol* 94:e00127–20. <https://doi.org/10.1128/JVI.00127-20>.
  201. Wrobel AG, Benton DJ, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gamblin SJ. 2020. SARS-CoV-2 and bat RaTG13 spike glycoprotein structures inform on virus evolution and furin-cleavage effects. *Nat Struct Mol Biol* 27:763–767. <https://doi.org/10.1038/s41594-020-0468-7>.
  202. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, Si H-R, Zhu Y, Li B, Huang C-L, Chen H-D, Chen J, Luo Y, Guo H, Jiang R-D, Liu M-Q, Chen Y, Shen X-R, Wang X, Zheng X-S, Zhao K, Chen Q-J, Deng F, Liu L-L, Yan B, Zhan F-X, Wang Y-Y, Xiao G-F, Shi Z-L. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579: 270–273. <https://doi.org/10.1038/s41586-020-2012-7>.
  203. Chen J, Lee KH, Steinhauer DA, Stevens DJ, Skehel JJ, Wiley DC. 1998. Structure of the hemagglutinin precursor cleavage site, a determinant of influenza pathogenicity and the origin of the labile conformation. *Cell* 95:409–417. [https://doi.org/10.1016/S0092-8674\(00\)81771-7](https://doi.org/10.1016/S0092-8674(00)81771-7).
  204. Steinhauer DA. 1999. Role of hemagglutinin cleavage for the pathogenicity of influenza virus. *Virology* 258:1–20. <https://doi.org/10.1006/viro.1999.9716>.
  205. Pachetti M, Marini B, Benedetti F, Giudici F, Mauro E, Storici P, Masciovecchio C, Angeletti S, Ciccocci M, Gallo RC, Zella D, Ippodirino R. 2020. Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. *J Transl Med* 18:179. <https://doi.org/10.1186/s12967-020-02344-6>.
  206. Tang X, Wu C, Li X, Song Y, Yao X, Wu X, Duan Y, Zhang H, Wang Y, Qian Z, Cui J, Lu J. 2020. On the origin and continuing evolution of SARS-CoV-2. *Natl Sci Rev* 7:1012–1023. <https://doi.org/10.1093/nsr/nwaa036>.
  207. van Dorp L, Acman M, Richard D, Shaw LP, Ford CE, Ormond L, Owen CJ, Pang J, Tan CC, Boshier FA, Torres Ortiz A, Balloux F. 2020. Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. *Infect Genet Evol* 83:104351. <https://doi.org/10.1016/j.meegid.2020.104351>.
  208. Lu J, Du Plessis L, Liu Z, Hill V, Kang M, Lin H, Sun J, François S, Kraemer MU, Faria NR, McCrone JT, Peng J, Xiong Q, Yuan R, Zeng L, Zhou P, Liang C, Yi L, Liu J, Xiao J, Hu J, Liu T, Ma W, Li W, Su J, Zheng H, Peng B, Fang S, Su W, Li K, Sun R, Bai R, Tang X, Liang M, Quick J, Song T, Rambaut A, Loman N, Raghwanji J, Pybus OG, Ke C. 2020. Genomic epidemiology of SARS-CoV-2 in Guangdong Province, China. *Cell* 181: 997–1003.e9. <https://doi.org/10.1016/j.cell.2020.04.023>.
  209. COVID-19 Genomics UK (COG-UK). 2020. An integrated national scale SARS-CoV-2 genomic surveillance network. *Lancet Microbe* 1:e99–e100. [https://doi.org/10.1016/s2666-5247\(20\)30054-9](https://doi.org/10.1016/s2666-5247(20)30054-9).
  210. CDC. 2021. SPHERES: SARS-CoV-2 sequencing for public health emergency response, epidemiology, and surveillance. CDC, Atlanta, GA. <https://www.cdc.gov/coronavirus/2019-ncov/variants/spheres.html>.
  211. Fauver JR, Petrone ME, Hodcroft EB, Shioda K, Ehrlich HY, Watts AG, Vogels CB, Brito AF, Alpert T, Muyombwe A, Razeq J, Downing R, Cheemarla NR, Wyllie AL, Kalinich CC, Ott IM, Quick J, Loman NJ, Neugebauer KM, Greninger AL, Jerome KR, Roychoudhury P, Xie H, Shrestha L, Huang ML, Pitzer VE, Iwasaki A, Omer SB, Khan K, Bogoch II, Martinello RA, Foxman EF, Landry ML, Neher RA, Ko AI, Grubaugh ND. 2020. Coast-to-coast spread of SARS-CoV-2 during the early epidemic in the United States. *Cell* 181: 990–996.e5. <https://doi.org/10.1016/j.cell.2020.04.021>.
  212. Gonzalez-Reiche AS, Hernandez MM, Sullivan MJ, Ciferri B, Alshamary H, Obla A, Fabre S, Kleiner G, Polanco J, Khan Z, Alburquerque B, van de Guchte A, Dutta J, Francoeur N, Melo BS, Oussenko I, Deikus G, Soto J, Sridhar SH, Wang YC, Twyman K, Kasarskis A, Altman DR, Smith M, Sebra R, Aberg J, Krammer F, García-Sastre A, Luksza M, Patel G, Paniz-Mondolfi A, Gitman M, Sordillo EM, Simon V, van Bakel H. 2020. Introductions and early spread of SARS-CoV-2 in the New York City area. *Science* 369:297–301. <https://doi.org/10.1126/science.abc1917>.
  213. Gudbjartsson DF, Helgason A, Jonsson H, Magnusson OT, Melsted P, Norddahl GL, Saemundsdottir J, Sigurdsson A, Sulem P, Agustsdottir AB,



- Eiriksdottir B, Fridriksdottir R, Gardarsdottir EE, Georgsson G, Gretarsdottir OS, Gudmundsson KR, Gunnarsdottir TR, Gylfason A, Holm H, Jensson BO, Jonasdottir A, Jonsson F, Josefsdottir KS, Kristjansson T, Magnusdottir DN, Le Roux L, Sigmundsdottir G, Sveinbjornsson G, Sveinsdottir KE, Sveinsdottir M, Thorarensen EA, Thorbjornsson B, Löve A, Masson G, Jonsdottir I, Möller AD, Gudnason T, Kristinsson KG, Thorsteinsdottir U, Stefansson K. 2020. Spread of SARS-CoV-2 in the Icelandic population. *N Engl J Med* 382:2302–2315. <https://doi.org/10.1056/NEJMoa2006100>.
214. GISAID. 2021. GISAID initiative. <https://www.gisaid.org/>.
215. National Center for Biotechnology Information. 2021. SARS-CoV-2 resources. National Institutes of Health, Bethesda, MD. <https://www.ncbi.nlm.nih.gov/sars-cov-2/>.
216. COVID-19 Data Portal. 2021. Accelerating scientific research through data. <https://www.covid19dataportal.org/>.
217. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, Hengartner N, Giorgi EE, Bhattacharya T, Foley B, Hastie KM, Parker MD, Partridge DG, Evans CM, Freeman TM, de Silva TI, Sheffield COVID-19 Genomics Group, McDanal C, Perez LG, Tang H, Moon-Walker A, Whelan SP, LaBranche CC, Saphire EO, Montefiori DC. 2020. Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell* 182:812–827.e19. <https://doi.org/10.1016/j.cell.2020.06.043>.
218. Yurkovetskiy L, Wang X, Pascal KE, Tomkins-Tinch C, Nyalile TP, Wang Y, Baum A, Diehl WE, Dauphin A, Carbone C, Veinotte K, Egri SB, Schaffner SF, Lemieux JE, Munro JB, Rafique A, Barve A, Sabeti PC, Kyratsous CA, Dudkina NV, Shen K, Luban J. 2020. Structural and functional analysis of the D614G SARS-CoV-2 spike protein variant. *Cell* 183:739–751.e8. <https://doi.org/10.1016/j.cell.2020.09.032>.
219. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, Zhang X, Muruato AE, Zou J, Fontes-Garfias CR, Mirchandani D, Scharton D, Bilello JP, Ku Z, An Z, Kalveram B, Freiberg AN, Menachery VD, Xie X, Plante KS, Weaver SC, Shi P-Y. 2021. Spike mutation D614G alters SARS-CoV-2 fitness. *Nature* 592:116–121. <https://doi.org/10.1038/s41586-020-2895-3>.
220. Thomson EC, Rosen LE, Shepherd JG, Spreafico R, da Silva Filipe A, Wojcechowskyj JA, Davis C, Piccoli L, Pascall DJ, Dillen J, Lytras S, Czudnochowski N, Shah R, Meury M, Jesudason N, De Marco A, Li K, Bassi J, O'Toole A, Pinto D, Colquhoun RM, Culap K, Jackson B, Zatta F, Rambaut A, Jaconi S, Sreenu VB, Nix J, Zhang I, Jarrett RF, Glass WG, Beltramo M, Nomikou K, Pizzuto M, Tong L, Cameroni E, Croll TI, Johnson N, Di Iulio J, Wickenhagen A, Ceschi A, Harbison AM, Mair D, Ferrari P, Smollett K, Sallusto F, Carmichael S, Garzoni C, Nichols J, Galli M, Hughes J, Riva A, Ho A, Schiama M, Semple MG, Openshaw PJM, Fadda E, Baillie JK, Chodera JD, ISARIC4C Investigators, COVID-19 Genomics UK (COG-UK) Consortium, Rihh SJ, Lycett SJ, Virgin HW, Telenti A, Corti D, Robertson DL, Snell G. 2021. Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity. *Cell* 184:1171–1187.e20. <https://doi.org/10.1016/j.cell.2021.01.037>.
221. Young BE, Fong S-W, Chan Y-H, Mak T-M, Ang LW, Anderson DE, Lee C-Y, Amrun SN, Lee B, Goh YS, Su YCF, Wei WE, Kalimuddin S, Chai LYA, Pada S, Tan SY, Sun L, Parthasarathy P, Chen YYC, Barkham T, Lin RTP, Maurer-Stroh S, Leo Y-S, Wang L-F, Renia L, Lee VJ, Smith GJD, Lye DC, Ng LFP. 2020. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. *Lancet* 396: 603–611. [https://doi.org/10.1016/S0140-6736\(20\)31757-8](https://doi.org/10.1016/S0140-6736(20)31757-8).
222. Liu Z, Zheng H, Lin H, Li M, Yuan R, Peng J, Xiong Q, Sun J, Li B, Wu J, Yi L, Peng X, Zhang H, Zhang W, Hulswit RJG, Loman N, Rambaut A, Ke C, Bowden TA, Pybus OG, Liu J. 2020. Identification of common deletions in the spike protein of severe acute respiratory syndrome coronavirus 2. *J Virol* 94:e00790–20. <https://doi.org/10.1128/JVI.00790-20>.
223. Tao K, Tzou PL, Nouhin J, Gupta RK, de Oliveira T, Kosakovsky Pond SL, Fera D, Shafer RW. 2021. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nat Rev Genet* <https://doi.org/10.1038/s41576-021-00408-x>.
224. Grabowski F, Preibisch G, Giziński S, Kochańczyk M, Lipniacki T. 2021. SARS-CoV-2 variant of concern 202012/01 has about twofold replicative advantage and acquires concerning mutations. *Viruses* 13:392. <https://doi.org/10.3390/v13030392>.
225. Kemp SA, Meng B, Ferriera IATM, Dattir R, Harvey WT, Papa G, Lytras S, Collier DA, Mohamed A, Gallo G, Thakur N, The COVID-19 Genomics UK (COG-UK) Consortium, Carabelli AM, Kenyon JC, Lever AM, De Marco A, Saliba C, Culap K, Cameroni E, Piccoli L, Corti D, James LC, Bailey D, Robertson DL, Gupta RK. 2021. Recurrent emergence and transmission of a SARS-CoV-2 Spike deletion H69/V70. *bioRxiv* <https://doi.org/10.1101/2020.12.14.422555>.
226. cov-lineages.org. 2021. B.1.351 2021-10-07. [https://cov-lineages.org/global\\_report\\_B.1.351.html](https://cov-lineages.org/global_report_B.1.351.html).
227. Ramanathan M, Ferguson ID, Miao W, Khavari PA. 2021. SARS-CoV-2 B.1.1.7 and B.1.351 spike variants bind human ACE2 with increased affinity. *Lancet Infect Dis* 21:1070. [https://doi.org/10.1016/S1473-3099\(21\)00262-0](https://doi.org/10.1016/S1473-3099(21)00262-0).
228. Spratt AN, Kannan SR, Woods LT, Weisman GA, Quinn TP, Lorson CL, Sönerborg A, Byrareddy SN, Singh K. 2021. Evolution, correlation, structural impact and dynamics of emerging SARS-CoV-2 variants. *Comput Struct Biotechnol J* 19:3799–3809. <https://doi.org/10.1016/j.csbj.2021.06.037>.
229. Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, Hinsley WR, Laydon DJ, Dabrera G, O'Toole Á, Amato R, Ragonnet-Cronin M, Harrison I, Jackson B, Ariani CV, Boyd O, Loman NJ, McCrone JT, Gonçalves S, Jorgensen D, Myers R, Hill V, Jackson DK, Gaythorpe K, Groves N, Sillitoe J, Kwiatkowski DP, The COVID-19 Genomics UK (COG-UK) consortium, Flaxman S, Ratmann O, Bhatt S, Hopkins S, Gandy A, Rambaut A, Ferguson NM. 2021. Transmission of SARS-CoV-2 lineage B.1.1.7 in England: insights from linking epidemiological and genetic data. *medRxiv* <https://doi.org/10.1101/2020.12.30.20249034>.
230. Laffeb C, de Koning K, Kanaar R, Lebbink JH. 2021. Experimental evidence for enhanced receptor binding by rapidly spreading SARS-CoV-2 variants. *J Mol Biol* 433:167058. <https://doi.org/10.1016/j.jmb.2021.167058>.
231. Ali F, Kasry A, Amin M. 2021. The new SARS-CoV-2 strain shows a stronger binding affinity to ACE2 due to N501Y mutant. *Med Drug Discov* 10: 100086. <https://doi.org/10.1016/j.medidd.2021.100086>.
232. Hoffmann M, Arora P, Groß R, Seidel A, Hörnich BF, Hahn AS, Krüger N, Graichen L, Hofmann-Winkler H, Kempf A, Winkler MS, Schulz S, Jäck HM, Jahrsdörfer B, Schrezenmeier H, Müller M, Kleger A, Münch J, Pöhlmann S. 2021. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell* 184:2384–2393.e12. <https://doi.org/10.1016/j.cell.2021.03.036>.
233. World Health Organization. 2021. Tracking SARS-CoV-2 variants. World Health Organization, Geneva, Switzerland. <https://www.who.int/emergencies/emergency-health-kits/trauma-emergency-surgery-kit-who-tesk-2019/tracking-SARS-CoV-2-variants>.
234. Saito A, Irie T, Suzuki R, Maemura T, Nasser H, Uriu K, Kosugi Y, Shirakawa K, Sadamasu K, Kimura I, Ito J, Wu J, Iwatsuki-Horimoto K, Ito M, Yamayoshi S, Ozono S, Butlertanaka EP, Tanaka YL, Shimizu R, Shimizu K, Yoshimatsu K, Kawabata R, Sakaguchi T, Tokunaga K, Yoshida I, Asakura H, Nagashima M, Kazuma Y, Nomura R, Horisawa Y, Yoshimura K, Takaori-Kondo A, Imai M, The Genotype to Phenotype Japan (G2P-Japan) Consortium, Nakagawa S, Ikeda T, Fukuhara T, Kawaoka Y, Sao K. 2021. SARS-CoV-2 spike P681R mutation, a hallmark of the Delta variant, enhances viral fusogenicity and pathogenicity. *bioRxiv* <https://doi.org/10.1101/2021.06.17.448820>.
235. Rando HM, Greene CS, Robson MP, Boca SM, Wellhausen N, Lordan R, Brueffer C, Ray S, McGowan LD, Gitter A, Dattoli AA, Velazquez R, Barton JP, Field JM, Ramsundar B, MacLean AL, Lee AJ, Immunology Institute of the Icahn School of Medicine, Hu F, Jadavji NM, Sell E, Rubineti V, Wang J, Rafizadeh DN, Skelly AN, Ben Guebila M, Kolla L, Manheim D, Ghosh S, Byrd JB, Park Y, Bansal V, Capone S, Dziak JJ, Sun Y, Qi Y, Shinholster L, Lukan T, Knyazev S, Perrin D, Mangul S, Das S, Szeto GI, Lubiana T, Mai D, COVID-19 Review Consortium, Goel RR, Boerckel JD, Naik A, Sun Y. 2021. SARS-CoV-2 and COVID-19: an evolving review of diagnostics and therapeutics. 4. Diagnostics. <https://greenelab.github.io/covid19-review/v/32afa309f69f0466a91acec5d0df3151fe4d61b5/#diagnostics>.
236. Singanayagam A, Patel M, Charlett A, Lopez Bernal J, Saliba V, Ellis J, Ladhani S, Zambon M, Gopal R. 2020. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *Euro Surveill* 25:2001483. <https://doi.org/10.2807/1560-7917.ES.2020.25.32.2001483>.
237. La Rosa G, Fratini M, Della Libera S, Iaconelli M, Muscillo M. 2013. Viral infections acquired indoors through airborne, droplet or contact transmission. *Ann Ist Super Sanita* 49:124–132. [https://doi.org/10.4415/ANN\\_13\\_02\\_03](https://doi.org/10.4415/ANN_13_02_03).
238. Shiu EY, Leung NH, Cowling BJ. 2019. Controversy around airborne versus droplet transmission of respiratory viruses. *Curr Opin Infect Dis* 32: 372–379. <https://doi.org/10.1097/QCO.0000000000000563>.
239. Tang JW, Bahnfleth WP, Bluysen PM, Buonanno G, Jimenez JL, Kurnitski J, Li Y, Miller S, Sekhar C, Morawska L, Marr LC, Melikov AK, Nazaroff WW, Nielsen PV, Tellier R, Wargoocki P, Dancer SJ. 2021. Dismantling myths on the airborne transmission of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J Hosp Infect* 110:89–96. <https://doi.org/10.1016/j.jhin.2020.12.022>.
240. Randall K, Ewing ET, Marr L, Jimenez J, Bourouiba L. 2021. How did we get here: what are droplets and aerosols and how far do they go? *A*

- historical perspective on the transmission of respiratory infectious diseases. SSRN. [https://papers.ssrn.com/sol3/papers.cfm?abstract\\_id=3829873](https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3829873).
241. World Health Organization. 2020. Transmission of SARS-CoV-2: implications for infection prevention precautions. World Health Organization, Geneva, Switzerland. <https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions>.
  242. Lemieux C, Brankston G, Gitterman L, Hirji Z, Gardam M. 2007. Questioning aerosol transmission of influenza. *Emerg Infect Dis* 13:173–174. <https://doi.org/10.3201/eid1301.061202>.
  243. Smieszek T, Lazzari G, Salathé M. 2019. Assessing the dynamics and control of droplet- and aerosol-transmitted influenza using an indoor positioning system. *Sci Rep* 9:2185. <https://doi.org/10.1038/s41598-019-38825-y>.
  244. Richard M, Fouchier RA. 2016. Influenza A virus transmission via respiratory aerosols or droplets as it relates to pandemic potential. *FEMS Microbiol Rev* 40:68–85. <https://doi.org/10.1093/femsre/fuv039>.
  245. Weiss SR, Leibowitz JL. 2011. Coronavirus pathogenesis. *Adv Virus Res* 81:85–164. <https://doi.org/10.1016/B978-0-12-385885-6.00009-2>.
  246. de Wit E, van Doremalen N, Falzarano D, Munster VJ. 2016. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol* 14:523–534. <https://doi.org/10.1038/nrmicro.2016.81>.
  247. Li Y, Huang X, Yu ITS, Wong TW, Qian H. 2005. Role of air distribution in SARS transmission during the largest nosocomial outbreak in Hong Kong. *Indoor Air* 15:83–95. <https://doi.org/10.1111/j.1600-0668.2004.00317.x>.
  248. Booth TF, Kournikakis B, Bastien N, Ho J, Kobasa D, Stadnyk L, Li Y, Spence M, Paton S, Henry B, Mederski B, White D, Low DE, McGeer A, Simor A, Vearncombe M, Downey J, Jamieson FB, Tang P, Plummer F. 2005. Detection of airborne severe acute respiratory syndrome (SARS) coronavirus and environmental contamination in SARS outbreak units. *J Infect Dis* 191:1472–1477. <https://doi.org/10.1086/429634>.
  249. Xiao S, Li Y, Wong T-W, Hui DS. 2017. Role of fomites in SARS transmission during the largest hospital outbreak in Hong Kong. *PLoS One* 12:e0181558. <https://doi.org/10.1371/journal.pone.0181558>.
  250. van Doremalen N, Bushmaker T, Munster VJ. 2013. Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions. *Euro Surveill* 18:20590. <https://doi.org/10.2807/1560-7917.es2013.18.38.20590>.
  251. Mackay IM, Arden KE. 2015. MERS coronavirus: diagnostics, epidemiology and transmission. *Virology* 51:1222. <https://doi.org/10.1186/s12985-015-0439-5>.
  252. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ. 2020. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 382:1564–1567. <https://doi.org/10.1056/NEJMc2004973>.
  253. Peng X, Xu X, Li Y, Cheng L, Zhou X, Ren B. 2020. Transmission routes of 2019-nCoV and controls in dental practice. *Int J Oral Sci* 12:9. <https://doi.org/10.1038/s41368-020-0075-9>.
  254. Klompas M, Baker MA, Rhee C. 2020. Airborne transmission of SARS-CoV-2. *JAMA* 324:441–442. <https://doi.org/10.1001/jama.2020.12458>.
  255. Goldman E. 2020. Exaggerated risk of transmission of COVID-19 by fomites. *Lancet Infect Dis* 20:892–893. [https://doi.org/10.1016/S1473-3099\(20\)30561-2](https://doi.org/10.1016/S1473-3099(20)30561-2).
  256. Prather KA, Wang CC, Schooley RT. 2020. Reducing transmission of SARS-CoV-2. *Science* 368:1422–1424. <https://doi.org/10.1126/science.abc6197>.
  257. Morawska L, Milton DK. 2020. It is time to address airborne transmission of coronavirus disease 2019 (COVID). *Clin Infect Dis* 71:2311–2313. <https://doi.org/10.1093/cid/cia939>.
  258. Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. 2021. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. *Lancet* 397:1603–1605. [https://doi.org/10.1016/S0140-6736\(21\)00869-2](https://doi.org/10.1016/S0140-6736(21)00869-2).
  259. Liu Y, Ning Z, Chen Y, Guo M, Liu Y, Galí NK, Sun L, Duan Y, Cai J, Westerdahl D, Liu X, Xu K, Ho KF, Kan H, Fu Q, Lan K. 2020. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature* 582:557–560. <https://doi.org/10.1038/s41586-020-2271-3>.
  260. Lednicky JA, Lauzardo M, Fan Z, Jutla A, Tilly TB, Gangwar M, Usmani M, Shankar SN, Mohamed K, Eiguren-Fernandez A, Stephenson CJ, Alam MM, Elbadry MA, Loeb JC, Subramaniam K, Waltzek TB, Cherabuddi K, Morris JG, Jr, Wu C-Y. 2020. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. *Int J Infect Dis* 100:476–482. <https://doi.org/10.1016/j.ijid.2020.09.025>.
  261. Santarpia JL, Herrera VL, Rivera DN, Ratnesar-Shumate S, Reid S, Denton PW, Martens JW, Fang Y, Conoan N, Callahan MV, Lawler JV, Brett-Major DM, Lowe JJ. 2020. The infectious nature of patient-generated SARS-CoV-2 aerosol. medRxiv <https://doi.org/10.1101/2020.07.13.20041632>.
  262. Santarpia JL, Rivera DN, Herrera VL, Morwitzer M, Creager HM, Santarpia GW, Crown KK, Brett-Major DM, Schnaubelt ER, Broadhurst M, Lawler JV, Reid SP, Lowe JJ. 2020. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. *Sci Rep* 10:12732. <https://doi.org/10.1038/s41598-020-69286-3>.
  263. Zhou J, Otter JA, Price JR, Cimpeanu C, Garcia DM, Kinross J, Boshier PR, Mason S, Bolt F, Holmes AH, Barclay WS. 2021. Investigating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) surface and air contamination in an acute healthcare setting during the peak of the coronavirus disease 2019 (COVID) pandemic in London. *Clin Infect Dis* 73:e1870–e1877. <https://doi.org/10.1093/cid/ciaa905>.
  264. Comber L, Murchu EO, Drummond L, Carty PG, Walsh KA, De Gascun CF, Connolly MA, Smith SM, O'Neill M, Ryan M, Harrington P. 2021. Airborne transmission of SARS-CoV-2 via aerosols. *Rev Med Virol* 31:e2184. <https://doi.org/10.1002/rmv.2184>.
  265. Bourouiba L. 2020. Turbulent gas clouds and respiratory pathogen emissions: potential implications for reducing transmission of COVID-19. *JAMA* 323:1837–1838. <https://doi.org/10.1001/jama.2020.4756>.
  266. Hu Z, Song C, Xu C, Jin G, Chen Y, Xu X, Ma H, Chen W, Lin Y, Zheng Y, Zhang L, Hu Z, Yi Y, Shen H. 2020. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. *Sci China Life Sci* 63:706–711. <https://doi.org/10.1007/s11427-020-1661-4>.
  267. Tindale LC, Stockdale JE, Coombe M, Garlock ES, Lau WYV, Saraswat M, Zhang L, Chen D, Wallinga J, Colijn C. 2020. Evidence for transmission of COVID-19 prior to symptom onset. *Elife* 9:e57149. <https://doi.org/10.7554/eLife.57149>.
  268. Chang D, Mo G, Yuan X, Tao Y, Peng X, Wang F-S, Xie L, Sharma L, Dela Cruz CS, Qin E. 2020. Time kinetics of viral clearance and resolution of symptoms in novel coronavirus infection. *Am J Respir Crit Care Med* 201:1150–1152. <https://doi.org/10.1164/rccm.202003-0524LE>.
  269. He X, Lau EH, Wu P, Deng X, Wang J, Hao X, Lau YC, Wong JY, Guan Y, Tan X, Mo X, Chen Y, Liao B, Chen W, Hu F, Zhang Q, Zhong M, Wu Y, Zhao L, Zhang F, Cowling BJ, Li F, Leung GM. 2020. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med* 26:672–675. <https://doi.org/10.1038/s41591-020-0869-5>.
  270. CDC. 2020. COVID-19 and your health. Centers for Disease Control and Prevention, Atlanta, GA. <https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html>.
  271. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, Niemeyer D, Jones TC, Vollmar P, Rothe C, Hoelscher M, Bleicker T, Brünink S, Schneider J, Ehmann R, Zwirgmaier K, Drosten C, Wendtner C. 2020. Virological assessment of hospitalized patients with COVID-2019. *Nature* 581:465–469. <https://doi.org/10.1038/s41586-020-2196-x>.
  272. Corsini Campioli C, Cano Cevallos E, Assi M, Patel R, Binnicker MJ, O'Horo JC. 2020. Clinical predictors and timing of cessation of viral RNA shedding in patients with COVID-19. *J Clin Virol* 130:104577. <https://doi.org/10.1016/j.jcv.2020.104577>.
  273. Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, Taylor J, Spicer K, Bardossy AC, Oakley LP, Tanwar S, Dyal JW, Harney J, Chisty Z, Bell JM, Methner M, Paul P, Carlson CM, McLaughlin HP, Thornburg N, Tong S, Tamin A, Tao Y, Uehara A, Harcourt J, Clark S, Brostrom-Smith C, Page LC, Kay M, Lewis J, Montgomery P, Stone ND, Clark TA, Honein MA, Duchin JS, Jernigan JA, Public Health—Seattle and King County and CDC COVID-19 Investigation Team. 2020. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. *N Engl J Med* 382:2081–2090. <https://doi.org/10.1056/NEJMoa2008457>.
  274. Baggett TP, Keyes H, Sporn N, Gaeta JM. 2020. Prevalence of SARS-CoV-2 infection in residents of a large homeless shelter in Boston. *JAMA* 323:2191–2192. <https://doi.org/10.1001/jama.2020.6887>.
  275. Bai Y, Yao L, Wei T, Tian F, Jin D-Y, Chen L, Wang M. 2020. Presumed asymptomatic carrier transmission of COVID-19. *JAMA* 323:1406–1407. <https://doi.org/10.1001/jama.2020.2565>.
  276. Li P, Fu J-B, Li K-F, Liu J-N, Wang H-L, Liu L-J, Chen Y, Zhang Y-L, Liu S-L, Tang A, Tong Z-D, Yan J-B. 2020. Transmission of COVID-19 in the terminal stages of the incubation period: a familial cluster. *Int J Infect Dis* 96:452–453. <https://doi.org/10.1016/j.ijid.2020.03.027>.
  277. Zhang P, Tian F, Wan Y, Cai J, Qian Z, Wu R, Zhang Y, Zhang S, Li H, Li M, Trevathan E, Lin H. 2020. A cohort of SARS-CoV-2 infected asymptomatic and pre-symptomatic contacts from COVID-19 contact tracing in Hubei Province, China: short-term outcomes. SSRN <https://doi.org/10.2139/ssrn.3678556>.
  278. Mizumoto K, Kagaya K, Zarebski A, Chowell G. 2020. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Euro Surveill* 25:2000180. <https://doi.org/10.2807/1560-7917.ES.2020.25.10.2000180>.

279. Zhang K, Tong W, Wang X, Lau JY-N. 2020. Estimated prevalence and viral transmissibility in subjects with asymptomatic SARS-CoV-2 infections in Wuhan, China. *Precis Clin Med* 3:301–305. <https://doi.org/10.1093/pcmedi/pbaa032>.
280. Ladhani SN, Chow JY, Janarthanan R, Fok J, Crawley-Boevey E, Vusirikala A, Fernandez E, Perez MS, Tang S, Dun-Campbell K, Wynne-Evans E, Bell A, Patel B, Amin-Chowdhury Z, Aiano F, Paranthaman K, Ma T, Saavedra-Campos M, Myers R, Ellis J, Lackenby A, Gopal R, Patel M, Brown C, Chand M, Brown K, Ramsay ME, Hopkins S, Shetty N, Zambon M. 2020. Investigation of SARS-CoV-2 outbreaks in six care homes in London, April 2020. *EclinicalMedicine* 26:100533. <https://doi.org/10.1016/j.eclinm.2020.100533>.
281. Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, Hu J-L, Xu W, Zhang Y, Lv F-J, Su K, Zhang F, Gong J, Wu B, Liu XM, Li JJ, Qiu JF, Chen J, Huang A-L. 2020. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 26:1200–1204. <https://doi.org/10.1038/s41591-020-0965-6>.
282. Lavezzo E, Franchin E, Ciavarella C, Cuomo-Dannenburg G, Barzon L, Del Vecchio C, Rossi L, Manganelli R, Loregian A, Navarin N, Abate D, Sciro M, Merigliano S, De Canale E, Vanuzzo MC, Besutti V, Saluzzo F, Onelia F, Pacenti M, Parisi SG, Carretta G, Donato D, Flor L, Cocchio S, Masi G, Sperduti A, Cattarino L, Salvador R, Nicoletti M, Caldart F, Castelli G, Nieddu E, Labella B, Fava L, Drigo M, Gaythorpe KAM, Imperial College COVID-19 Response Team, Brazzale AR, Toppo S, Trevisan M, Baldo V, Donnelly CA, Ferguson NM, Dorigatti I, Crisanti A. 2020. Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'. *Nature* 584:425–429. <https://doi.org/10.1038/s41586-020-2488-1>.
283. Meyerowitz-Katz G, Merone L. 2020. A systematic review and meta-analysis of published research data on COVID-19 infection fatality rates. *Int J Infect Dis* 101:138–148. <https://doi.org/10.1016/j.ijid.2020.09.1464>.
284. Oke J, Heneghan C. 2020. Global Covid-19 case fatality rates. The Centre for Evidence-Based Medicine, Nuffield Department of Primary Care Health Sciences, Oxford, United Kingdom. <https://www.cebm.net/covid-19/global-covid-19-case-fatality-rates/>.
285. Grewelle R, Leo GD. 2020. Estimating the global infection fatality rate of COVID-19. *medRxiv* <https://doi.org/10.1101/2020.05.11.20098780>.
286. Stadlbauer D, Tan J, Jiang K, Hernandez MM, Fabre S, Amanat F, Teo C, Arunkumar GA, McMahon M, Capuano C, Twyman K, Jhang J, Nowak MD, Simon V, Sordillo EM, van BAKEL H, Krammer F. 2021. Repeated cross-sectional sero-monitoring of SARS-CoV-2 in New York City. *Nature* 590:146–150. <https://doi.org/10.1038/s41586-020-2912-6>.
287. Ritchie H, Roser M. 2020. What do we know about the risk of dying from COVID-19? Our World in Data. <https://ourworldindata.org/covid-mortality-risk>.
288. Heesterbeek JAP, Dietz K. 1996. The concept of  $R_0$  in epidemic theory. *Stat Neerl* 50:89–110. <https://doi.org/10.1111/j.1467-9574.1996.tb01482.x>.
289. Keeling MJ, Rohani P. 2008. Modeling infectious diseases in humans and animals. Princeton University Press, Princeton, NJ.
290. Kermack WO, McKendrick AG. 1927. A contribution to the mathematical theory of epidemics. *Proc R Soc Lond A* 115:700–721. <https://doi.org/10.1098/rspa.1927.0118>.
291. Anderson RM, May RM. 1979. Population biology of infectious diseases: part I. *Nature* 280:361–367. <https://doi.org/10.1038/280361a0>.
292. Cobe S. 2020. Modeling infectious disease dynamics. *Science* 368:713–714. <https://doi.org/10.1126/science.abb5659>.
293. May RM, McLean AR (ed). 2007. Theoretical ecology: principles and applications, 3rd ed. Oxford University Press, Oxford, United Kingdom.
294. Heng K, Althaus CL. 2020. The approximately universal shapes of epidemic curves in the Susceptible-Exposed-Infectious-Recovered (SEIR) model. *Sci Rep* 10:19365. <https://doi.org/10.1038/s41598-020-76563-8>.
295. Wu JT, Leung K, Leung GM. 2020. Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study. *Lancet* 395:689–697. [https://doi.org/10.1016/S0140-6736\(20\)30260-9](https://doi.org/10.1016/S0140-6736(20)30260-9).
296. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. 2020. The reproductive number of COVID-19 is higher compared to SARS coronavirus. *J Travel Med* 27:taaa021. <https://doi.org/10.1093/jtm/taaa021>.
297. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, Shaman J. 2020. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). *Science* 368:489–493. <https://doi.org/10.1126/science.abb3221>.
298. Ma S, Zhang J, Zeng M, Yun Q, Guo W, Zheng Y, Zhao S, Wang MH, Yang Z. 2020. Epidemiological parameters of coronavirus disease 2019: a pooled analysis of publicly reported individual data of 1155 cases from seven countries. *medRxiv* <https://doi.org/10.1101/2020.03.21.20040329>.
299. Majumder M, Mandl KD. 2020. Early transmissibility assessment of a novel coronavirus in Wuhan, China. SSRN <https://doi.org/10.2139/ssrn.3524675>.
300. Liu TT, Hu J, Xiao J, He G, Kang M, Rong Z, Lin L, Zhong H, Huang Q, Deng A, Zeng W, Tan X, Zeng S, Zhu Z, Li J, Gong D, Wan D, Chen S, Guo L, Li Y, Sun L, Liang W, Song T, He J, Ma W. 2020. Time-varying transmission dynamics of novel coronavirus pneumonia in China. *bioRxiv* <https://doi.org/10.1101/2020.01.25.919787>.
301. Zhang S, Diao M, Yu W, Pei L, Lin Z, Chen D. 2020. Estimation of the reproductive number of novel coronavirus (COVID) and the probable outbreak size on the Diamond Princess cruise ship: A data-driven analysis. *Int J Infect Dis* 93:201–204. <https://doi.org/10.1016/j.ijid.2020.02.033>.
302. Tang B, Wang X, Li Q, Bragazzi NL, Tang S, Xiao Y, Wu J. 2020. Estimation of the transmission risk of the 2019-nCoV and its implication for public health interventions. *J Clin Med* 9:462. <https://doi.org/10.3390/jcm9020462>.
303. Cao Z, Zhang Q, Lu X, Pfeiffer D, Jia Z, Song H, Zeng DD. 2020. Estimating the effective reproduction number of the 2019-nCoV in China. *medRxiv* <https://doi.org/10.1101/2020.01.27.20018952>.
304. Shen M, Peng Z, Xiao Y, Zhang L. 2020. Modelling the epidemic trend of the 2019 novel coronavirus outbreak in China. *bioRxiv* <https://doi.org/10.1101/2020.01.23.916726>.
305. Read JM, Bridgen JR, Cummings DA, Ho A, Jewell CP. 2020. Novel coronavirus 2019-nCoV: early estimation of epidemiological parameters and epidemic predictions. *medRxiv* <https://doi.org/10.1101/2020.01.23.20018549>.
306. Roques L, Klein E, Papaix J, Sar A, Soubeyrand S. 2020. Using early data to estimate the actual infection fatality ratio from COVID-19 in France. *medRxiv* <https://doi.org/10.1101/2020.03.22.20040915>.
307. Park SW, Sun K, Viboud C, Grenfell BT, Dushoff J. 2020. Potential role of social distancing in mitigating spread of coronavirus disease, South Korea. *Emerg Infect Dis* 26:2697–2700. <https://doi.org/10.3201/eid2611.201099>.
308. Kucharski AJ, Russell TW, Diamond C, Liu Y, Edmunds J, Funk S, Eggo RM, Sun F, Jit M, Munday JD, Davies N, Gimma A, van Zandvoort K, Gibbs H, Hellewell J, Jarvis CI, Clifford S, Quilty BJ, Bosse NI, Abbott S, Klepac P, Flasche S. 2020. Early dynamics of transmission and control of COVID-19: a mathematical modelling study. *Lancet Infect Dis* 20:553–558. [https://doi.org/10.1016/S1473-3099\(20\)30144-4](https://doi.org/10.1016/S1473-3099(20)30144-4).
309. Sahafzadeh E, Sartoli S. 2020. Estimating the reproduction number of COVID-19 in Iran using epidemic modeling. *medRxiv* <https://doi.org/10.1101/2020.03.20.20038422>.
310. Flaxman S, Mishra S, Gandy A, Unwin H, Coupland H, Mellan T, Zhu H, Berah T, Eaton J, Guzman PNP, Schmit N, Cilloni L, Ainslie KEC, Baguelin M, Blake I, Boonyasiri A, Boyd O, Cattarino L, Ciavarella C, Cooper L, Cucunubá Z, Cuomo-Dannenburg G, Dighe A, Djafaara B, Dorigatti I, van Elsland S, FitzJohn R, Fu H, Gaythorpe K, Geidelberg L, Grassly N, Green W, Hallett T, Hamlet A, Hinsley W, Jeffrey B, Jorgensen D, Knock E, Laydon D, Nedjati-Gilani G, Nouvellet P, Parag K, Siveroni I, Thompson H, Verity R, Volz E, Walters C, Wang H, Wang Y, Watson O, Winskill P, Xi X, Whittaker C, Walker PGT, Ghani A, Donnelly CA, Riley S, Okell LC, Vollmer MAC, Ferguson NM, Bhatt S. 2020. Report 13: estimating the number of infections and the impact of non-pharmaceutical interventions on COVID-19 in 11 European countries. Imperial College London, London, United Kingdom.
311. Campbell F, Archer B, Laurenson-Schafer H, Jinnai Y, Konings F, Batra N, Pavlin B, Vandemaële K, Van Kerckhove MD, Jombart T, Morgan O, le Polain de Waroux O. 2021. Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. *Euro Surveill* 26:2100509. <https://doi.org/10.2807/1560-7917.ES.2021.26.24.2100509>.
312. Washington NL, Gangavarapu K, Zeller M, Bolze A, Cirulli ET, Barrett KMS, Larsen BB, Anderson C, White S, Cassens T, Jacobs S, Levan G, Nguyen J, Ramirez JM, III, Rivera-Garcia C, Sandoval E, Wang X, Wong D, Spencer E, Robles-Sikisaka R, Kurzban E, Hughes LD, Deng X, Wang C, Servellita V, Valentine H, De Hoff P, Seaver P, Sathe S, Gietzen K, Sickler B, Antico J, Hoon K, Liu J, Harding A, Bakhtar O, Basler T, Austin B, Isaksson M, Febbo PG, Becker D, Laurent M, McDonald E, Yeo GW, Knight R, Laurent LC, de Feo E, Worobey M, Chiu C, Suchard MA, Lu JT, Lee W, Andersen KG. 2021. Genomic epidemiology identifies emergence and rapid transmission of SARS-CoV-2 B.1.1.7 in the United States. *medRxiv* <https://doi.org/10.1101/2021.02.06.21251159>.
313. Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, Pearson CA, Russell TW, Tully DC, Washburne AD, Wenseleers T, Gimma A, Waites W, Wong KLM, van Zandvoort K, Silverman JD, CMMID COVID-19 Working Group, COVID-19 Genomics UK (COG-UK) Consortium, Diaz-Ordaz K, Keogh R, Eggo RM, Funk S, Jit M, Atkins KE, Edmunds WJ. 2021. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science* 372:eabg3055. <https://doi.org/10.1126/science.abg3055>.



314. Liu Y, Rocklöv J. 2021. The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. *J Travel Med* <https://doi.org/10.1093/jtm/taab124>.
315. Ito K, Piantham C, Nishiura H. 2021. Predicted dominance of variant Delta of SARS-CoV-2 before Tokyo Olympic Games, Japan, July 2021. *Euro Surveill* 26: 2100570. <https://doi.org/10.2807/1560-7917.ES.2021.26.27.2100570>.
316. Moghadas SM, Shoukat A, Fitzpatrick MC, Wells CR, Sah P, Pandey A, Sachs JD, Wang Z, Meyers LA, Singer BH, Galvani AP. 2020. Projecting hospital utilization during the COVID-19 outbreaks in the United States. *Proc Natl Acad Sci U S A* 117:9122–9126. <https://doi.org/10.1073/pnas.2004064117>.
317. Prem K, Liu Y, Russell TW, Kucharski AJ, Eggo RM, Davies N, Jit M, Klepac P, Flasche S, Clifford S, Pearson CAB, Munday JD, Abbott S, Gibbs H, Rosello A, Quilty BJ, Jombart T, Sun F, Diamond C, Gimma A, van Zandvoort K, Funk S, Jarvis CI, Edmunds WJ, Bosse NI, Hellewell J. 2020. The effect of control strategies to reduce social mixing on outcomes of the COVID-19 epidemic in Wuhan, China: a modelling study. *Lancet Public Health* 5:e261–e270. [https://doi.org/10.1016/S2468-2667\(20\)30073-6](https://doi.org/10.1016/S2468-2667(20)30073-6).
318. Gatto M, Bertuzzo E, Mari L, Miccoli S, Carraro L, Casagrandi R, Rinaldo A. 2020. Spread and dynamics of the COVID-19 epidemic in Italy: effects of emergency containment measures. *Proc Natl Acad Sci U S A* 117: 10484–10491. <https://doi.org/10.1073/pnas.2004978117>.
319. Abbott S, Hellewell J, Thompson RN, Sherratt K, Gibbs HP, Bosse NI, Munday JD, Meakin S, Doughty EL, Chun JY, Chan Y-WD, Finger F, Campbell P, Endo A, Pearson CAB, Gimma A, Russell T, CMMID COVID modelling group, Flasche S, Kucharski AJ, Eggo RM, Funk S. 2020. Covid-19: temporal variation in transmission during the COVID-19 outbreak. <https://epiforecasts.io/covid/>.
320. System K, Vladeck T, Krieger M. 2020. Rt: effective reproduction number. <https://rt.live/>.
321. Anderson RM, Fraser C, Ghani AC, Donnelly CA, Riley S, Ferguson NM, Leung GM, Lam TH, Hedley AJ. 2004. Epidemiology, transmission dynamics and control of SARS: the 2002–2003 epidemic. *Philos Trans R Soc Lond B Biol Sci* 359:1091–1105. <https://doi.org/10.1098/rstb.2004.1490>.
322. Netburn D. 2021. A timeline of the CDC's advice on face masks. *Los Angeles Times*, Los Angeles, CA. <https://www.latimes.com/science/story/2021-07-27/timeline-cdc-mask-guidance-during-covid-19-pandemic>.
323. Rando HM, Greene CS, Robson MP, Boca SM, Wellhausen N, Lordan R, Brueffer C, Ray S, McGowan LD, Gitter A, Dattoli AA, Velazquez R, Barton JP, Field JM, Ramsundar B, MacLean AL, Lee AJ, Immunology Institute of the Icahn School of Medicine, Hu F, Jadavji NM, Sell E, Wang J, Rafizadeh DN, Skelly AN, Ben Guebila M, Kolla L, Manheim D, Ghosh S, Byrd JB, Park Y, Bansal V, Capone S, Dziak JJ, Sun Y, Qi Y, Shinholster L, Lukan T, Knyazev S, Perrin D, Mangul S, Das S, Szeto GL, Lubiana T, Mai D, COVID-19 Review Consortium, Goel RR. 2021. SARS-CoV-2 and COVID-19: an evolving review of diagnostics and therapeutics. 9. Social factors influencing COVID-19 exposure and outcomes. <https://greenelab.github.io/covid19-review/v/32afa309f69f0466a91acec5d0df3151fe4d61b5/#social-factors-influencing-covid-19-exposure-and-outcomes>.
324. Guarner J. 2020. Three emerging coronaviruses in two decades. *Am J Clin Pathol* 153:420–421. <https://doi.org/10.1093/ajcp/aqaa029>.
325. Rando HM, Greene CS, Robson MP, Boca SM, Wellhausen N, Lordan R, Brueffer C, Ray S, McGowan LD, Gitter A, Dattoli AA, Velazquez R, Barton JP, Field JM, Ramsundar B, MacLean AL, Lee AJ, Immunology Institute of the Icahn School of Medicine, Hu F, Jadavji NM, Sell E, Wang J, Rafizadeh DN, Skelly AN, Ben Guebila M, Kolla L, Manheim D, Ghosh S, Byrd JB, Park Y, Bansal V, Capone S, Dziak JJ, Sun Y, Qi Y, Shinholster L, Lukan T, Knyazev S, Perrin D, Mangul S, Das S, Szeto GL, Lubiana T, Mai D, COVID-19 Review Consortium, Goel RR, Boerckel JD. 2021. SARS-CoV-2 and COVID-19: an evolving review of diagnostics and therapeutics. 3. Evolutionary and genomic analysis of SARS-CoV-2. <https://greenelab.github.io/covid19-review/v/910dd7b7479f5336a1c911c57446829bef015dbe/#evolutionary-and-genomic-analysis-of-sars-cov-2>.
326. Rando HM, Greene CS, Robson MP, Boca SM, Wellhausen N, Lordan R, Brueffer C, Ray S, McGowan LD, Gitter A, Dattoli AA, Velazquez R, Barton JP, Field JM, Ramsundar B, MacLean AL, Lee AJ, Immunology Institute of the Icahn School of Medicine, Hu F, Jadavji NM, Sell E, Wang J, Rafizadeh DN, Skelly AN, Ben Guebila M, Kolla L, Manheim D, Ghosh S, Byrd JB, Park Y, Bansal V, Capone S, Dziak JJ, Sun Y, Qi Y, Shinholster L, Lukan T, Knyazev S, Perrin D, Mangul S, Das S, Szeto GL, Lubiana T, Mai D, COVID-19 Review Consortium, Goel RR. 2021. SARS-CoV-2 and COVID-19: an evolving review of diagnostics and therapeutics. 7. Vaccine development strategies for SARS-CoV-2. <https://greenelab.github.io/covid19-review/v/d9d90fd7e88ef547fb4cbed0ef73baef5fee7fb5/#vaccine-development-strategies-for-sars-cov-2>.
327. Rando HM, Boca SM, McGowan LD, Himmelstein DS, Robson MP, Rubineti V, Velazquez R, COVID-19 Review Consortium, Greene CS, Gitter A. 2021. An open-publishing response to the COVID-19 infodemic. *arXiv* <https://arxiv.org/abs/2109.08633>.