SARS-CoV-2 and COVID-19: An Evolving Review of Diagnostics and Therapeutics

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Abstract

Since late 2019, Coronavirus disease 2019 (COVID-19) has spread around the world, resulting in the declaration of a pandemic by the World Health Organization (WHO). This infectious disease is caused by the newly identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Research on the virus SARS-CoV-2 and the disease it causes is emerging rapidly through global scientific efforts. The development of approaches for the diagnosis and treatment of the disease will be critical to mitigating the impact of the virus. Scientific discussion of new and existing technologies and methods under investigation must be contextualized alongside a solid fundamental understanding of the virus and the disease it causes.

This manuscript represents a collaborative effort to organize and consolidate the rapidly emerging scientific literature related to SARS-CoV-2 and COVID-19. We present information about the virus in the context of what is known about related viruses, describe the pathogenesis of COVID-19, and synthesize studies emerging about the diagnosis and treatment of COVID-19 alongside literature about related illnesses. A broad scientific effort to understand this pandemic and related viruses and diseases will be fundamental to efforts to predict possible interventions. This text is an evolving and collaborative document that seeks to incorporate the ever-expanding body of information related to SARS-CoV-2 and COVID-19.

Where to Contribute

Introduce Yourself (GitHub Issue) https://github.com/greenelab/covid19-review/issues/17

Community Chat (Gitter Room) https://gitter.im/covid19-review/community

More Info (GitHub Readme) https://github.com/greenelab/covid19-review#sars-cov-2-and-covid-19-an-evolving-review-of-diagnostics-and-therapeutics

1 Introduction

1.1 General Background

On January 21, 2020, the World Health Organization (WHO) released its first report concerning what is now known as the Coronavirus Disease 2019 (COVID-19) [1]. This infectious disease came to international attention on December 31, 2019 following an announcement by national officials in China about 44 cases of a respiratory infection of unknown cause. The first known cases were located in Wuhan City within the Hubei province of China, but the disease spread rapidly beyond Wuhan within China and subsequently around the world. At the time of the first situation report [1], 282 confirmed cases had been identified, primarily in China, but also 1-2 exported cases had been identified in several neighboring countries (Thailand, Japan, and the Republic of Korea). One week later, 4593 confirmed cases had been identified, spanning not only Asia, but also Australia, North America, and Europe [2]. On March 11, 2020, WHO formally classified the situation as a pandemic [3]. On April 4, 2020, the WHO reported that the global number of confirmed cases had surpassed one million [4].

At this time, over 130,000 deaths had been reported due to COVID-19 worldwide (April 15, 2020).

[Note: Maybe add a graph here, update as new reports come out.] [So this is where manubot is particularly useful. it could pull from a public database / data table.]

In this review, we seek to consolidate information about the virus in the context of related viruses and to synthesize what is known about the diagnosis and treatment of COVID-19 and related diseases. This is a real-time, collaborative effort that welcomes submissions from scientists worldwide.

1.2 About Coronaviruses

1.2.1 Classification:

Coronaviruses (CoVs; order *Nidovirales*, family *Coronaviridae*, subfamily *Orthocoronavirinae*) are enveloped viruses with some of the largest viral RNA genomes and helical capsids with the solar corona-like appearance [5]. All viruses in the *Nidovirales* order are characterized by: 1) enveloped, non-segmented, positive-sense RNA genomes with a highly conserved genomic organization; 2) many non-structural genes by ribosomal shifting; 3) several unusual enzymatic activities encoded within the replicase-transcriptase polyprotein; and 4) 3’ nested sub-genomic mRNAs [6]. Coronaviruses are classified into four genera: alpha, beta, delta and gamma coronaviruses. Among them, alpha and beta coronaviruses infect mammalian species, gamma coronaviruses infect avian species and delta coronaviruses infect both mammalian and avian species [7]. The viruses were initially subdivided into these genera based on antigenic relationships of the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins but are now divided by phylogenetic clustering.

Most coronaviruses are considered zoonotic viruses with little to no transmission observed in humans. A major group of coronaviruses include human coronaviruses (HCoVs) strains associated with multiple respiratory diseases of varying severity, ranging from common cold to severe pneumonia, with severe symptoms mostly observed in immunocompromised individuals [8]. Approximately one-third of common cold infections in humans is attributable to four out of six previously known human coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1) that are globally circulating in the human population [9,10]. In the past two decades, however, highly pathogenic human coronaviruses have been identified, including the severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) and the Middle East respiratory syndrome coronavirus (MERS-

CoV) although both infections were confined to specific geographic regions [9,11]. The current pandemic of COVID-19, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), represents an acute and rapidly spreading global health crisis with severe or fatal symptoms such as acute respiratory distress, acute lung injury and other pulmonary complications. The transmission and mortality rate estimations of COVID-19 remain to be determined.

[this section may be better as a figure panel + legend] Phylogenetic analysis of a PCR amplicon fragment from five patients along with the total virus genome of 29.8 kilobases indicates that the virus is a novel betacoronavirus belonging to the B lineage, also known as sarbecovirus. The sarbecovirus lineage also includes the human SARS coronavirus [12].

1.2.2 Virion, morphology, structure:

Coronavirus virions are spherical with diameters ranging between 100 to 160nm. The virion has a lipid envelope with spike (S) glycoproteins on the surface creating a distinctive “crown” shape, for which the family of viruses was named [5].

1.2.3 Genome structure and replication:

Coronavirus genomes are single-stranded non-segmented positive RNA (ssRNA+) that are 27 to 32kb in length. The SARS-CoV-2 genome lies in the middle of this range at 29,903 bp in length [13]. Coronavirus genomes are comprised of a replicase gene that is 2/3 of the genome and the remaining 1/3 that encodes structural proteins including: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. There are additional accessory genes that are present depending on the strain. The non-structural polypeptide is translated into 16 non-structural proteins (nsp1-16, except Gammacoronaviruses that do not contain nsp1), which form replication machinery to synthesize viral RNA [14].

Replication initiates with the binding of the virus to a host cell via the S protein. The viral membrane fuses with the endosomal membrane to release the viral genome into the host cytoplasm. The replicase gene is translated and assembled into the viral replicase complex, which can synthesize dsRNA genome from the genomic ssRNA(+). The dsRNA genome is then transcribed and replicated to create viral mRNAs and new ssRNA(+) genomes [6,15].

1.2.4 Transmission

In general, respiratory viruses like coronavirus can have multiple routes of person-to-person transmission including droplet transmission (i.e. inhalation for cough, sneeze), aerosol transmission (i.e. virus suspended in air), and contact transmission (i.e. contact with oral, nasal, and eye mucous membranes). While there does not appear to be experimental evidence to conclude which mode of transmission different coronaviruses use [16], it has been suggested that the coronavirus person-to-person transmission is droplet-based using simulations [17] and trace studies [18].

1.3 SARS-CoV-2: Origin, Phylogenetics, and Evolution

1.3.1 Origin of SARS-CoV-2

The evolutionary origins of the SARS-CoV-2 virus are not yet fully understood. Genomic analyses and comparisons to other known coronaviruses suggest that SARS-CoV-2 is unlikely to have originated from a laboratory – either purposely engineered and released, or escaped – and instead evolved naturally in an animal host [19].

Among known coronaviruses, SARS-CoV-19 has the closest overall sequence similarity to RaTG13 (~96%) found in a *Rhinolophus affinis* bat [20], while the receptor binding domain (RBD) is highly similar to that of viruses found in pangolins [21]. This suggests that SARS-CoV-19 may have originated in viral reservoirs of similar hosts, however current evidence cannot discriminate an origin of the virus before or after zoonotic transfer to humans [19].

1.4 COVID-19: Mechanisms and Presentation

1.4.1 Mechanisms of Coronavirus-driven Disease in Humans

Coronaviruses are known to cause respiratory illnesses in humans through the following possible mechanisms...[Summarize relevant mechanisms for cell entry & address evidence for/against ACE2 being important, etc.]

1.4.2 Presentation of COVID-19

Retrospective samples of COVID-19 patients described the clinical presentations of patients infected with SARS-CoV-2 which included lower respiratory tract infection with fever, dry cough, and dyspnea [22]. [22] noted that upper respiratory tract symptoms were less common, which suggests that the virus targets cells located in the lower respiratory tract. The symptoms of infection by SARS-CoV-2 can vary greatly, making it difficult for public health agencies to provide clear recommendations for citizens regarding what symptoms indicate infection and should prompt isolation. [22] found that a higher probability of mortality was associated with older age and higher Sequential Organ Failure Assessment scores, as well as high levels of d-dimer. Mortality might be associated with other biomarkers measured in blood samples including lactate dehydrogenase and cardiac troponin I, although these analyses may not have been appropriately corrected for multiple testing. They also found that survivors continued to shed the virus for a median of 20 days and a maximum of at least 37 days.

1.5 Approaches to Understanding COVID-19

Scientific characterization of the SARS-CoV-2 virus and of the COVID-19 disease it causes is critical to controlling the current pandemic. Several broad areas of research interact with each other, offering different pieces of information critical to understanding the virus and disease. A comprehensive understanding of the epidemic must unify basic scientific and medical research with public health and biotechnology.

1.5.1 Science & Medicine

Understanding how the virus functions and interacts with the host is foundational to understanding pathogenesis and disease progression and to identifying available and novel approaches to treatment. Therefore, the fields of virology, immunology, and molecular biology are fundamental to characterizing SARS-CoV-2 and COVID-19. These topics can be approached using a range of techniques, including characterization of the host response from the cellular to systems level. Contextualizing SARS-CoV-2 in relation to other viruses that infect humans and other animals can further serve to elucidate the reaction of the human host to viral exposure. This information, when combined with an understanding of the biology of pharmaceutical and medical interventions, can guide new approaches to treatment.

1.5.2 Public Health

One necessary component of determining how to manage the outbreak is to understand epidemiological factors related to the transmission of the SARS-CoV-2 virus. These can include characteristics such as when infected individuals are contagious, how the virus is transmitted between individuals, the range of symptoms associated with infection and/or contagiousness in different individuals, and how rapidly the virus propogates between individuals, etc. The development of diagnostic tools is critical to this goal. Accurate diagnoses on a large scale is necessary to collect the data needed to develop epidemiological models. Other areas of public health that address resource availability, inequity, human behavior, and other components that influence people's exposure to pathogens and ability to manage illness will also be critical to mounting a global response to the pandemic. Currently, this manuscript focuses primarily on contextualizing epidemiological characteristics such as reproduction number and dynamics of transmission that intersect with the fundamental biology of the virus or the development of therapeutic and diagnostic technologies.

1.5.3 Biotechnology

1.5.3.1 Diagnostics

Two major concerns within diagnosis include the detection of current infections in individuals with and without symptoms, and the detection of past exposure without an active infection. In the latter category, identifying whether individuals can develop or have developed sustained immunity is also a major consideration. The development of high-throughput, affordable methods for detecting active infections and sustained immunity will be critical to understanding and controlling the disease.

- What are approaches that allow us to detect current infection or past exposure for other viruses?
- What is sustained immunity and what are the indicators?

1.5.3.2 Therapeutics

The identification of interventions that can mitigate the effect of the virus on exposed and infected individuals is a significant research priority. Some possible approaches include the identification of existing pharmaceuticals that reduce the severity of infection, either by reducing the virus' virulence (e.g., antivirals) or managing the most severe symptoms of infection. Due to the long timeline for the development of novel pharmaceuticals, in most cases, research surrounding possible pharmaceutical interventions focuses on the identification and investigation of existing compounds whose mechanisms may be relevant to COVID-19. Other foci of current research include the identification of antibodies produced by survivors of COVID-19 and the development of vaccines. Understanding the mechanisms describing host-virus interactions between humans and SARS-CoV-2 are thus critical to identifying candidate therapeutics.

1.6 Summary

In this review, we seek to consolidate information about efforts to develop strategies for diagnosis and therapeutics as new information is released by the scientific community. We include information from both traditional peer-reviewed scientific literature and from preprints, which typically have not undergone peer review but have been critically evaluated by the scientists involved in this effort. The goal of this manuscript is to present preliminary findings within the broader context of COVID-19 research and to identify the broad interpretations of new research, as well as limitations to interpretability.

2 Pathogenesis

2.1 Mechanism of Host Infection by SARS-CoV-2

This section would also be great for the introduction of zoonotic diseases which has been shown to be the origin of SARS-CoV2.

2.1.1 Primary Transmission and Viral Entry

Like that of SARS-CoV, SARS-CoV-2 entry into host cells is mediated by the interaction between the viral spike glycoprotein (S) and human angiotensin-converting enzyme 2 (ACE2) in humans and other animals [23,24,25,26,27,28,29,30]. The ACE2 receptor is expressed in numerous organs, such as the heart, kidney, and intestine, but most prominently in alveolar epithelial cells, which is expected to contribute to the virus' association with lung pathology [31,32]. The S protein is a highly glycosylated trimer that requires two proteolytic cleavage events, leading to substantial conformational changes, to achieve viral fusion with the host cell membrane [24,33]. Each protomer is composed of an S1 and an S2 subunit, which mediate receptor binding and viral fusion, respectively. The priming proteolytic events occur sequentially, first at the S1/S2 junction and then at the S2' site, ultimately resulting in the shedding of the S1 subunit and transitioning of the S2 subunit to a more stable, fusion-conducive conformation.

Similar to SARS-CoV, SARS-CoV-2 exhibits redundancy in which host proteases it can use to cleave the S protein [28]. Specifically, both transmembrane protease serine protease-2 (TMPRSS2) 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* [28,34]. Interestingly, SARS-CoV-2 S protein also contains a RRAR furin recognition site at the S1/S2 junction [24,33], setting it apart from bat coronavirus RaTG13, with which it shares 96% genome sequence identity, and SARS-CoV [20]. Such furin cleavage sites are commonly found in highly virulent influenza viruses, and as such may contribute to the heightened pathogenicity of SARS-CoV-2 [35,36]. Differences in S protein sequence between SARS-CoV and SARS-CoV-2 may also partially account for the increased transmissibility seen in the current COVID-19 pandemic. Recent studies have reported conflicting binding constants for the S protein-hACE2 interaction, though they agree that SARS-CoV-2 S protein binds with equal if not greater affinity than does SARS-CoV S protein [24,27,33]. 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 the human ACE2 (hACE2) receptor, and the crystal structure of the C-terminal domain of SARS-CoV-2 S protein in complex with human ACE2 reveals stronger interaction and higher affinity for receptor binding than SARS-CoV [doi:10.1016/j.cell.2020.03.045]. Among the 14 key binding residues identified in the SARS-CoV S protein, 8 are conserved in SARS-CoV-2 and the remaining 6 are semi-conservatively substituted, potentially explaining variation in binding affinity [24,27]. Recent crystal structures have shown that the receptor-binding domain (RBD) of 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 [24,33]. Because the RBD plays such a critical role in viral entry, blocking its interaction with ACE2 represents a promising therapeutic approach. Nevertheless, despite the high structural homology between SARS-CoV-2 RBD and that from SARS-CoV, monoclonal antibodies targeting SARS-CoV-RBD failed to bind SARS-CoV-2-RBD [33]. Promisingly though, sera from convalescent SARS patients inhibited SARS-CoV-2 viral entry in vitro, albeit with lower efficiency than it inhibited SARS-CoV [28].

2.1.2 Viral Replication and Spreading

- Basic introduction into replication cycle
- What are the routes of transmission

2.1.2.1 Mechanism of viral replication within cells

2.1.2.2 Mechanism of viral spreading to neighbor cells

2.1.2.3 Factors enhancing viral spreading

Viral progression may be enhanced by active upregulation of ACE2 on cell surfaces following or during a response to infection. In several preliminary assays and an analysis of previous microarray data, Wang et. al. reported that ACE2 is significantly upregulated following infection of other coronaviruses, including SARS-CoV and MERS-CoV, as well as viruses such as rhinovirus and influenza virus [31]. Additionally, direct stimulation with inflammatory cytokines such as type I interferons resulted in upregulation of ACE2, with treated groups showing 4-fold higher ACE2 expression as compared to control groups at 18 hours post-treatment [31]. Though whether SARS-CoV-2 infection facilitates positive regulation of its own transmission between host cells is still unclear, the host immune response itself likely plays a key role in mediating infection-associated pathologies. One severe example includes reports of cytokine storm-like responses in patients with particularly severe infections, in which the overproduction of inflammatory cytokines leads to systemic inflammation and potentially multi-organ failure, and may very well accelerate the spread of virus in the host [31,37].

2.1.2.4 Person-to-person transmission

2.1.3 Reproduction Number and Dynamics of Transmission

Accurate estimates of the reproduction number of a virus are crucial to understanding the dynamics of infection and to predict the effects of different interventions. The basic reproduction number, R_0, is the expected number of new infections caused by one infected person, assuming no time dependence and a wholly susceptible population [38]. The effective reproduction number, R_t, describes how the reproduction number may change over time, and is used to quantify deviations in R from R_0, for example as some fraction of the population becomes infected, or as interventions are put into place. R_0 and R_t can be estimated directly from epidemiological data or inferred using mathematical modeling. Modeling approaches are typically based upon a classic epidemiological model structure: the susceptible-infected-recovered (SIR) model and its extensions [39].

R_0 for COVID-19 is estimated to lie in the range R_0=1.4-6.5 [40,41,42]; estimates vary considerably depending on the data and the methods used. Data-derived estimates (i.e. those that do not incorporate SIR-type models into their analysis) typically predict lower values of R_0. For data-derived estimates, in one study of international cases, the predicted value is R_0=1.7 [43], in China (both Hubei province and nationwide), the value is predicted to lie in the range R_0=2.0-3.6 [40,44,45], and on a cruise ship where an outbreak occurred, predicted R_0=2.28 [46]. SIR model-derived estimates of R_0 range from 2.0 - 6.5 in China [47,48,49,50] to R_0=4.8 in France [51]. Using the same model as for the French population, this study estimated R_0=2.6 in South Korea [51], which is consistent with other studies [52]. From a meta-analysis of studies estimating R_0, [41] predict the median as R_0=2.79.

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 1.6-2.6 in Jan 2020, before travel restrictions [53]. R_t decreased from 2.35 one week before travel restrictions were imposed (Jan 23, 2020), to 1.05 one week after. Using their model, the authors also estimate the probability of new outbreaks occurring: the probability of a single individual exporting virus causing a large outbreak is 17-25% assuming MERS-like or SARS-like transmission, and the probability of a large outbreak occurring after ≥4 infections exist at a new location is greater than 50%. An independent study came to similar conclusions: in a two-week period before Jan 23 finding R_t=2.38, and decreasing to R_t = 1.34 (using data from Jan 24 to Feb 3) or R_t=0.98 (using data from Jan 24 to Feb 8) [42]. In South Korea, R_t was inferred for Feb-Mar 2020 in two cities: Daegu (the center of the outbreak), and Seoul [52]. Metro data was 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). This highlights that social distancing measures appeared to work to contain

the infection in Daegu, but that in Seoul, R_t remains above 1, thus secondary outbreaks are possible. It also shows the importance of region-specific analysis: the large decline in case load nationwide is mainly due to the Daegu region, and could hide 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 first week to 2.1 by the fourth week after the first cases were reported [54]. In Europe, analysis of 11 countries inferred the dynamics of R_t over a time range from the beginning of the outbreak until March 28, 2020, by which point most countries had implemented major interventions (such as school closures, public gathering bans, and stay-at-home orders) [55]. 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 (note that this is in part because 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.

2.2 Immune Response to SARS-CoV-2

- Cellular responses to SARS-CoV-2 infection
- What is causing neutropenia and lymphopenia observed in COVID-19 patients
- Antibody production against SARS-CoV-2 by patient who recovered vs patient who did not recover
- Cytokines and other soluble factors contribution to immune response

2.3 Systems-level approaches for understanding SARS-CoV-2 pathogenesis

Systems biology provides a cross-disciplinary analytical platform integrating the different omics (genomics, transcriptomics, proteomics, metabolomics, and other omics approaches), bioinformatics, and computational strategies. These cutting-edge research approaches have enormous potential to study the complexity of biological systems and human diseases [56]. 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 [57]. Omics-based studies also provided meaningful information regarding host immune responses and surrogate protein markers in several viral, bacterial and protozoan infections [58].

The complex pathogenesis and clinical manifestations of SARS-CoV-2 infection are not understood adequately yet. A significant breakthrough in SARS-CoV-2 research was achieved through the successful full-length genome sequencing of the pathogen [13,20,59]. Multiple research groups have drafted the genome sequence of SARS-CoV-2 based on sequencing of clinical samples collected from bronchoalveolar lavage fluid (BALF) [20,59] or from BALF, throat swabs, or isolates of the virus cultured from BALF [13]. Importantly, SARS-CoV-2 has significant sequence homology with SARS-CoV (about 79%) and also to some extent with MERS-CoV (about 50%) [13]. However, a higher level of similarity (about 90%) has been observed between SARS-CoV-2 and bat-derived SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21), indicating a possible origin in bats [13,20].

The genome sequence of the pathogen subsequently allowed its phylogenetic characterization and prediction of its protein expression profile, which is crucial for understanding the pathogenesis and virulence of this novel viral infection. Availability of the genome sequence of SARS-CoV-2 enhances the potential for subsequent proteome-level studies to provide further mechanistic insights into the virus' complex pathogenesis. Of note, the cryo-electron microscopy structure of the SARS-CoV-2 spike (S) glycoprotein, which plays an important role in the early steps of viral infection, was reported very recently [33]. Even though no comprehensive proteomic analysis of the pathogen or of patients suffering from its infection has yet been reported, one forthcoming study has demonstrated SARS-CoV-2 infected host cell proteomics using human Caco–2 cells as an infection model [60]. The authors

observed SARS-CoV-2 induced alterations in multiple vital physiological pathways, including translation, splicing, carbon metabolism and nucleic acid metabolism in the host cells.

There is a high level of sequence homology between SARS-CoV-2 and SARS-CoV, and sera from convalescent SARS-CoV patients might show some efficacy to cross-neutralize SARS-CoV-2-S-driven entry [28]. However, despite the high level of sequence homology, certain protein structures might be immunologically distinct, prohibiting effective cross-neutralization across different SARS strains [61]. Consequently, earlier proteome-level studies on SARS-CoV can also provide some essential information regarding the new pathogen [62,63]. Considering the paucity of omics-level big data sets for SARS-CoV-2 up until now, existing data hubs that contain information for other coronaviruses such as UniProt, NCBI Genome Database, The Immune Epitope Database and Analysis Resource (IEDB), and The Virus Pathogen Resource (ViPR) will serve as useful resources for computational and bioinformatics research on SARS-CoV-2. Using such databases, the systems level reconstruction of the PPI (Protein-Protein Interaction) enabled the generation of hypotheses on the mechanism of action of SARS-CoV-2 and suggested drug targets.

2.3.1 Protein-protein interaction networks

In a first study [64], 26 of the 29 SARS-CoV-2 proteins were cloned and expressed in HEK293T kidney cells and 332 high confidence human proteins that interact with them were consequently identified. Notably, this study suggested the interaction of SARS-CoV-2 with innate immunity pathways. The ranking of pathogens with respect to their interactome similarity with SARS-CoV-2 suggested *West Nile Virus*, *Mycobacterium tuberculosis*, and *Human papillomavirus* as the top three hits. However, given the lung symptoms associated with COVID-19, *Mycobacterium tuberculosis* host-pathogen interactome could provide new insights to the mechanism SARS-CoV-2 infection. In addition, it was suggested that the envelope protein E could disrupt the host bromodomain-containing proteins, i.e., BRD2 and BRD4, binding to histone. The Spike protein S could likely intervene in the virus fusion through modulating the GOLGA7-ZDHHC5 acyl-transferase complex to increase palmitoylation.

Another study [65], used patient-derived peripheral blood mononuclear cells (PBMCs) to identify 251 host proteins targeted by SARS-CoV-2 and the disruption of more than 200 host proteins following the infection. The network analysis showed in particular that non-structural proteins 9 and 10 (nsp9 and nsp10) interacted with NKRF that usually represses NFKB. This finding could explain the exacerbation of the immune response that shape the pathology and the high cytokine levels possibly due to the chemotaxis of neutrophils mediated by IL-8 and IL-6. Finally, it was suggested [66] that protein E of both SARS-CoV and SARS-CoV-2 has a conserved Bcl-2 Homology 3 (BH3)-like motif, that could inhibit anti-apoptosis proteins BCL2 and trigger the apoptosis of T cells. Several known compounds were suggested to disrupt the host-pathogen protein interactome were suggested mostly through the inhibition of host proteins.

3 Diagnostics

Identifying individuals who have contracted COVID-19 is crucial to slowing down the global pandemic. Given the high transmissibility of SARS-CoV-2, the development of reliable assays to detect SARS-CoV-2 infection even in asymptomatic carriers is vitally important. For instance, the deployment of wide-scale diagnostic testing followed by the isolation of infected people has been a key factor in South Korea's successful strategy for controlling the spread of the virus. Following the first release of the genetic sequence of the virus by Chinese officials on January 10 2020, the first test was released about 13 days later [67]. A range of diagnostic approaches from a methodological standpoint are being or could possibly be developed. The assays available to date use the following approaches to identify the active virus in patient samples.

3.1 Molecular Tests

Molecular tests are used to identify distinct genomic subsequences of a viral molecule in a sample. This first requires identifying biospecimens that are likely to contain the virus in infected individuals and then acquiring these samples from the patient(s) to be tested. Common sources for a sample used in a molecular test include nasopharyngeal cavity samples, including throat wash and saliva [68], or stool samples [69]. Given a sample from a patient, molecular tests involve a number of steps to analyze a sample and produce results. These steps include sample pre-processing, followed by library preparation, and then sequencing itself [70]. When testing for RNA viruses like SARS-CoV-2, pre-processing is needed in order to create DNA, which can then be replicated during PCR, from the initial RNA sample. The DNA can then be manipulated and copied to produce quantities sufficient for the test, called amplification. Library preparation is the process of preparing the sample for sequencing, typically by fragmenting the sequences and adding adapters [70]. In some cases, library preparation can involve other modifications of the sample, such as adding "barcoding" to identify a particular sample in the sequence data, which is useful for pooling samples from multiple sources. Sequential pattern matching is then used to identify unique subsequences of the virus that identify it in specific. If sufficient subsequences are found, the test is considered positive.

3.1.1 RT-PCR

Real-Time Polymerase Chain Reaction (RT-PCR) tests measure the rate of amplification during PCR compared to a standard to detect the presence of a target. When the target is RNA, such as in the case of RNA viruses, the RNA must first be translated to DNA during pre-processing. There are different reagents used for library preparation that are specific to identifying one or more target sections with PCR [71]. The Drosten Lab, from Germany, was the first lab to establish and validate a diagnostic test to detect SARS-CoV-2. This test uses RT-PCR with reverse transcription [67] to detect several regions of the viral genome: the orf1b of the RNA dependent RNA polymerase (RdRP), the Envelope protein gene (E), and the Nucleocapsid protein gene (N). In collaboration with several other labs in Europe and in China, the researchers confirmed the specificity of this test with respect to other coronaviruses against specimens from 297 patients infected with a broad range of respiratory agents. Specifically this tests utilizes two probes against RdRP of which one is specific to SARS-CoV-2 [67]. Importantly, this assay did not give any false positive results.

3.1.2 qRT-PCR

Chinese researchers developed a quantitative real-time reverse transcription PCR (qRT-PCR) test to identify two gene regions of the viral genome, *ORF1b* and *N* [72]. Specifically, this assay was tested on samples coming from two COVID-19 patients, including a panel of positive and negative controls consisting of RNA extracted from several cultured viruses. The assay uses the *N* gene to screen patients, while the *ORF1b* gene region is used to confirm the infection [72]. In this case the test was designed to detect sequences conserved across sarbecoviruses, or viruses within the same subgenus as SARS-CoV-2. Considering that no other sarbecoviruses are currently known to infect humans, a positive test indicates that the patient is infected with SARS-CoV-2. However, this test is not able to discriminate the genetics of viruses within the sarbecovirus clade.

3.1.3 Pooled and Automated PCR Testing

Due to limited supplies and the need for more tests, several labs have found ways to pool or otherwise strategically design tests to increase throughput. The first such result came from Yelin et al. [73], who found they could pool up to 32 samples in a single qPCR run. This was followed by larger-scale pooling with slightly different methods [74]. Although these approaches are also PCR based,

they allow for more rapid scaling and higher efficiency for testing than the initial PCR-based methods developed.

3.1.3.1 CRISPR-based detection

Two American companies, Mammoth Biosciences and Sherlock Biosciences, adapted their CRISPR-based detection technology [75] for COVID-19 diagnostics to increase testing throughput and accessibility [76]. Their methodology involves purification of RNA extracted from patient specimens, amplification of extracted RNAs by loop-mediated amplification, a rapid, isothermal nucleic acid amplification technique, and application of their CRISPR-Cas12-based technology. In the assay designed by Mammoth Biosciences, guide RNAs were designed to recognize portions of sequences corresponding to the SARS-CoV-2 genome, specifically the N, E and RdRP regions. In the presence of SARS-CoV-2 genetic material, sequence recognition by the guide RNAs results in double-stranded DNA cleavage by Cas12, as well as cleavage of a single-stranded DNA molecular beacon. The cleavage of this molecular beacon acts as a colorimetric reporter that is subsequently read out in a lateral flow assay and indicates the positive presence of SARS-CoV-2 genetic material and therefore SARS-CoV-2 infection [76]. This assay has been reported to have a sensitivity as high as detection of 70-300 copies of the target RNA/µl, requires simple, accessible equipment, and can output an easily-interpretable result in approximately 30 minutes [77]. Initial testing with patient samples (n = 23) demonstrated a positive predictive value of 100% and a negative predictive value of 91.7%, which is highly competitive with the CDC's current testing standard using qRT-PCR. This test seeks to develop a practical solution for rapid, low-barrier testing in areas that are at greater risk of infection, such as airports and local community hospitals.

3.1.4 Limitation of Molecular Tests

Tests that identify SARS-CoV-2 using nucleic-acid-based technologies will identify only individuals with current infections and are not appropriate for identifying individuals who have recovered from a previous infection. Within this category, different types of tests have different limitations. For example, PCR-based test can be highly sensitive, but in high-throughput settings they can show several problems:

1. False-negative responses, which can present a significant problem to large-scale testing. To reduce occurrence of false negatives, correct execution of the analysis is crucial [78].
2. Uncertainty surrounding the SARS-CoV-2 viral shedding kinetics, which could affect the result of a test depending on when it was taken [78].
3. Type of specimen, as it is not clear which clinical samples are best to detect the virus [78].
4. Expensive machinery, which might be present in major hospitals and/or diagnostic centers but is often not available to smaller facilities [77].
5. Timing of the test, which might take up to 4 days to give results [77].
6. The availability of supplies for testing, including swabs and testing media, has been limited [79].
7. Because the guide RNA can recognize other interspersed sequences on the patient's genome, false positives and a loss of specificity can occur.

Similarly, in tests that use CRISPR, false positives can occur due to the specificity of the technique, as the guide RNA can recognize other interspersed sequences on the patient's genome. As noted above, false negatives are a significant concern for several reason. Importantly, clinical reports indicate that it is imperative to exercise caution when interpreting the results of molecular tests for SARS-CoV-2 because negative results do not necessarily mean a patient is virus-free [80].

3.2 Serological Tests

Although diagnostic tests based on the detection of the genetic material can be quite sensitive, they cannot provide information about the extent of the disease over time. Most importantly, they would not work on a patient who has fully recovered from the virus at the time of sample collection. In this context, immunity tests are significantly more informative. Additionally, they can help scientists to understand why the disease has a different course among patients, as well as what strategy might work to manage the spread of the infection. Furthermore, serological tests hold significant interest at present because they can provide information relevant to advancing economic recovery and allowing reopenings. For instance, people that have developed antibodies can plausibly return to work prior to the others, based on (still-unproven) protective immunity [81], and if extensive enough, herd immunity which will prevent further diffusion of the virus.

3.2.1 Current Approaches

Several countries are now focused on implementing antibody tests, and in the United States, the FDA recently approved a serological test by Cellex for use under emergency conditions [82]. Specifically, the Cellex qSARS-CoV-2 IgG/IgM Rapid Test is a chromatographic immunoassay designed to qualitatively detect IgM and IgG antibodies against SARS-CoV-2 in the plasma of patients (blood sample) suspected to have developed the infection [82]. Such tests allow for the progress of the viral disease to be understood, as IgM are the first antibodies produced by the body and indicate that the infection is active. Once the body has responded to the infection, IgG are produced and gradually replace IgM, indicating that the body has developed immunogenic memory [83]. The test cassette contains a pad of SARS-CoV-2 antigens and a nitrocellulose strip with lines for each of IgG and IgM, as well as a control (goat IgG) [82]. In a specimen that contains antibodies against the SARS-CoV-2 antigen, the antibodies will bind to the strip and be captured by the IgM and/or IgG line(s), resulting in a change of color [82]. With this particular assay results can be read within 15-20 minutes [82]. Other research groups, such as the Krammer lab of the Icahn School of Medicine at Mount Sinai proposed an ELISA test that detects IgG and IgM that react against the receptor binding domain (RBD) of the spike proteins (S) of the virus [84]. The authors are now working to get the assay into clinical use [85].

3.2.2 Limitations of Sierological Tests

Importantly, false-positives can occur due to the cross-reactivity with other antibodies according to the clinical condition of the patient [82]. Therefore, this test should be used in combination with RNA detection tests [82]. Due to the long incubation times and delayed immune responses of infected patients, serological tests are insufficiently sensitive for a diagnosis in the early stages of an infection. The limitations due to timing make serological tests far less useful for enabling test-and-trace strategies.

3.3 Possible Alternatives to Current Practices for Identifying Active Cases

Clinical symptoms are too similar to other types of pneumonia to be sufficient as a sole diagnostics criterion. In addition, as noted above, identifying asymptomatic cases is critical. Even among mildly symptomatic patients, a predictive model based on clinical symptoms had a sensitivity of only 56% and a specificity of 91% [86]. More problematic is that clinical symptom-based tests are only able to identify already symptomatic cases, not presymptomatic or asymptomatic cases. They may still be important for clinical practice, and for reducing tests needed for patients deemed unlikely to have COVID-19.

X-ray diagnostics have been reported to have high sensitivity but low specificity in some studies [87]. Other studies have shown that specificity varies between radiologists [88], though the sensitivity reported here was lower than that published in the previous paper. However, preliminary machine-learning results have shown far higher sensitivity and specificity from analyzing chest X-rays than was

possible with clinical examination [89]. X-ray tests with machine learning can potentially detect asymptomatic or presymptomatic infections that show lung manifestations. This approach would still not recognize entirely asymptomatic cases. Given the above, the widespread use of X-ray tests on otherwise healthy adults is likely inadvisable.

3.4 Challenges to Diagnostic Approaches

3.4.1 Limitations to Implementation of Large-Scale Testing

More information to follow.

3.4.2 Strategies and Considerations for Determining Whom to Test

Currently, Coronavirus tests are limited to people that are in danger of serious illness [90]. Specifically, the individuals at risk include:

- people with severe symptoms
- people showing mild symptoms that have been in contact with a person who has developed the infection
- people with underlying health conditions

However, this method of testing administration does not detect a high proportion of infections and does not allow for test-and-trace methods to be used. Individuals who are asymptomatic (i.e. potential spreaders) and individuals who are able to recover at home are therefore often unaware of their status. For instance, a recent study from the Imperial College estimates that in Italy the true number of infections is around 5.9 million against the 70,000 detected as of March 28th [55].

4 Therapeutics

Given the rapid predicted spread of the disease, the development of therapeutics will be critical to mitigating its effect on health and the mortality rate. Typically, therapeutics can take a few forms. First, the treatment and reduction of symptoms can result in the reduction of the severity and risk associated with an active infection. Second, the development of antiviral drugs can drive a reduced recovery time for patients by inhibiting the development of the virus once an individual is infected. Finally, vaccines present a strategy for bolstering the immune response of the populus broadly to the virus, resulting in a lower rate of infection. All three of these strategies have been valuable elements of responses to other viruses, including coronaviruses, and are being investigated by researchers at present. Additionally, there have been suggestions within the scientific community that nutraceutical or dietary supplement interventions may prime an individual's immune system to prevent or lessen the impact of RNA virus infections [91,92]. In the following sections, we critically appraise the literature surrounding the repurposing of existing treatments and development of novel therapeutics for the prevention, mitigation, and treatment of coronavirus infections.

4.1 Treatment of Symptoms

The clinical picture of SARS-CoV-2 infection differs dramatically between individuals. Some are asymptomatic, and many experience mild COVID-19 symptoms. Mild symptoms commonly include fever and respiratory symptoms such as cough and sore throat, and, less commonly, gastrointestinal symptoms such as loss of appetite and vomiting [93]; some patients experience a combination of respiratory and gastrointestinal symptoms. The most severe cases of COVID-19 include severe complications such as pneumonia and Acute Respiratory Distress Syndrome (ARDS), which can lead to

respiratory failure and death [94]. Vaccines are one avenue to mitigate harm from viral pathogens, but in the case of a rapidly growing pandemic the longer timeframe of vaccine development and distribution means that there can be a key for treatments that palliate symptoms to avoid the most severe outcomes from infection.

4.1.1 Tocilizumab

A recent study carried out on a sample of 191 adult COVID-19 in-patients at two Wuhan hospitals found that blood samples taken at admission contained significantly higher concentrations of interleukin-6 (IL-6) in patients who ultimately deceased compared to those who survived; average concentrations of IL-6 remained higher in the deceased group than the surviving group throughout hospitalization [22]. This suggests that these individuals may be experiencing a "cytokine storm", which refers to an excessive inflammatory response. IL-6 plays a key role in this response [95]. IL-6 is a pro-inflammatory cytokine belonging to the family of interleukins, which are immune system regulators that are primarily responsible for immune cell differentiation. Specifically, IL-6 promotes the differentiation of activated B cells into immunoglobulin-producing plasma cells [96] and acts as a growth factor for hybridoma and myeloma cells [97,98]. In addition, IL-6 also induces the differentiation of naïve CD4+ T cells into effector T-cell subsets [99]. In this way interleukins regulate both the pro- and anti-inflammatory responses. In this context, the observation of elevated IL-6 in patients who died may reflect an over-production of proinflammatory interleukins.

In a healthy situation the lung respiratory epithelium together with alveolar macrophages limits the activation of the immune system, ensuring homeostasis. The introduction of the S-protein from SARS-CoV to mouse macrophages was found to increase production of IL-6 and TNF-α [100], and deceased SARS-CoV patients were found to have intermediate levels of IL-6, IL-1β, and TNF-α expressed in a number of ACE2-expressing cell types sampled from the lung and bronchial tissues during autopsy [101]. However, other reports found the severe respiratory condition ARDS to be associated with elevated concentrations of IL-6 in BALF, but that concentrations of Tumor Necrosis Factor α (TNF-α) and IL-1β decreased with the onset of ARDS [102]. These cytokines enhance the pro-inflammatory reaction by increase acute-phase signaling, trafficking of immune cells to the site of primary infection, epithelial cell activation, and secondary cytokine production. The acute phase response to infection results in the heavily damage of the endothelium of blood vessels, which disrupts the balance between pro and anti-inflammatory response [102]. Thus, the holes generated allow not just for the passage of neutrophils, macrophages and lymphocytes to the site of the infection but also the accumulation of liquids into the lungs, which is the ultimate cause of the death as per Acute Distress Respiratory Syndrome (ADRS) or Severe Acute Respiratory Syndrome (SARS) [103], also caused by the new coronavirus. Recently Chinese and Italian doctors have found that the the Tocilizumab (actemra, by Roche), a drug commonly used in the rheumatoid arthritis, may palliate the most severe symptoms associated with COVID-19.

4.1.1.1 Anticipated Mechanism

Human IL-6 is a glycoprotein of 26 kDa and it consists of 184 amino acids containing 2 potential N-glycosylation sites and four cysteine residues. IL-6 binds to its receptor either in the insoluble (IL-6R) and soluble (sIL-6R) form. The receptor specificity determines the type of signaling. Specifically, the binding of IL-6 to the cell membrane receptor IL-6R gives rise to the "classical transduction of the signaling", while to binding to sIL-6R generate the so called "trans-signaling" [104,105]. IL-6 signaling occurs through 3 independent pathways: the Janus-activated kinase (JAK)-STAT3 pathway, the Ras/Mitogen-Activated Protein Kinases (MAPK) pathway and the Phosphoinositol-3 Kinase (PI3K)/Akt pathway [106]. The ultimate result of the IL-6 cascade is to direct transcriptional activity of various promoters of pro-inflammatory cytokines, such as IL-1 and TFN, including IL-6 own regulation through the activity of NF-κB [106]. Particularly, IL-6 synthesis is tightly regulated both transcriptionally and post-transcriptionally . In this context, it has been shown that viral proteins can enhance transcription

of the IL-6 gene, via strengthening the DNA-binding activity between several transciptional factors and IL-6 gene-cis-regulatory elements [107]. Tocilizumab is a humanised monoclonal antibody that binds both to the insoluble and soluble receptor of IL-6, de facto inhibiting the IL-6 immune cascade.

4.1.1.2 Current Evidence

Also, The AIFA (the Italian Drug Agency) approved the start of a new trial on March 19 recruiting patients at the initial stage of the infection [108]. Together with these independent trials, Roche, also in collaboration with the FDA, will start a randomised, double-blind, placebo-controlled phase III trial early April. The trial will enroll 330 patients globally, which will be followed for 60 days upon use of the drug via injection to analyze its efficiency/safety (Biopharma-reporter.com). However, previous studies on RA showed that in patients treated with TCZ the rate of incident infections in clinical practice patients was higher than the one observed during clinical trial [109]. Also, RA patients with chronic hepatitis B (HB) infection showed high risk of HB virus reactivation upon TCZ administration in combination with other RA drugs [110]. These last findings highlight the need to search for a balance between impairing a harmful immune response, such as the one generated by the cytokine storm, and preventing the worsening of the clinical picture of the patients by potential new viral infections. This aspect is probably crucial to be investigated further in the trials that are about to start.

Perhaps, the TCZ treatment would best suit patients with severely compromised lungs due to the COVID-19 infection and are therefore at greater risk of death, in order to stop the uncontrolled immune response before it's too late. <!-##### Summary

Summarize the state of the symptom management approach. –>

4.2 Small Molecule Drugs for Targeting SARS-CoV-2

The replication cycle of a virus within an epithelial host cell includes 6 basic steps which can be shortly summarised as follow: i) attachment of the virus to the host cell; ii) penetration by endocytosis; iii) uncoating, classically defined as the release of viral contents into the host cell; iv) biosynthesis, during which the viral genetic material enters the nucleus where it gets replicated; v) assembly, where viral proteins are translated and new viral particles are assembled; vi) release, when the new viruses are released into the extracellular environment [111]. Antiviral drugs do not kill the virus, rather they inhibit its amplification by impairing one of these steps. Nowadays, many of these drugs act during the biosynthesis step in order to inhibit the virus' genetic material replication. Importantly, SARS-CoV-2 is an RNA virus. Differently from a DNA virus, which can use the host enzymes to propagate itself, RNA viruses depends on their own polymerase, the RNA-dependent RNA polymerase (RdRP), in order to be replicated [112,113].

4.2.1 Nucleotide Analogs

Removing this header for now, if we add additional nucleotide analogs, we can put it back Why one might use nucleotide analogs. #### Favipiravir

Favipiravir (T-705) was discovered by Toyama Chemical Co., Ltd. [114]. The drug was found to effective at blocking viral amplification in several Influenza subtypes as well as other RNA viruses (Flaviviridae, Picornaviridae) based on reduction in plaque formations [115] and viral replication in MDCK cells [116]. Furthermore, inoculation of mice with favipiravir was shown to increase survivability. In 2014, the drug was approved in Japan for the treatment of patients infected with influenza that was resistant to conventional treatments, like neuraminidase inhibitors [117].

4.2.1.1 Anticipated Mechanism

Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide) acts as a purine and purine nucleoside analogue that inhibits viral RNA polymerase in a dose dependent manner across a range of RNA viruses, including Influenza virus [118,119,120,121,122]. Nucleotide/side are the natural building blocks for RNA synthesis. Because of this, modifications to these nucleotides/sides can disrupt key processes including replication [123]. Biochemical experiments showed that favipiravir was recognized as a purine nucleoside analogue and incorporated into the viral RNA template. A single incorporation does not influence RNA transcription; however, multiple events of incorporation lead to the arrest of RNA synthesis [124]. Evidence for T-705 inhibiting viral RNA polymerase are based on time-of-drug addition studies that found that viral loads were reduced with the addition of Favipiravir in early times post-infection [118,121,122].

4.2.1.2 Current Evidence

The effectiveness of favipiravir for treating patients with COVID-19 is currently under investigation. An open-label, nonrandomized, before-after controlled study was recently conducted [125]. The study included 80 COVID-19 patients (35 treated with favipiravir, 45 control) from the isolation ward of the National Clinical Research Center for Infectious Diseases (The Third People's Hospital of Shenzhen), Shenzhen, China. The patients in the control group were treated with other antivirals, such as lopinavir and ritonavir. Treatment was applied on days 2-14; treatment stopped either when viral clearance was confirmed or at day 14. The efficacy of the treatment was measured by 1) the time until viral clearance using Kaplan-Meier survival curves, and 2) the improvement rate of chest computed tomography (CT) scans on day 14 after treatment. The study found that favipiravir increased the speed of recovery (measured as viral clearance from the patient by RT-PCR) to 4 days compared to 11 days using other antivirals such as lopinavir and ritonavir. Additionally, the lung CT scans of patients treated with favipiravir showed significantly higher improvement rates (91%) on day 14 compared to control patients (62%). However, there were adverse side effects in 4 (11%) favipiravir-treated patients and 25 (56%) control patients. The adverse side effects included: diarrhea, vomiting, nausea, rash, and liver and kidney injury. Overall, despite the study reporting clinical improvement in favipiravir-treated patients, due to some issues with study design, it cannot be determined whether treatment with Favipiravir had an effect or whether these patients would have recovered regardless of any treatment. For example, although there were significant differences between the two treatment groups, follow-up analysis is necessary due to the small sample size. The selection of patients did not take into consideration important factors such as previous clinical conditions or sex, and there was no age categorization. The study was not randomized or blinded, and the baseline control group was another antiviral instead of a placebo. Therefore, randomized controlled trials are still required.

4.2.1.3 Summary

Summarize the state of the antiviral approach.

4.2.2 Remdesivir

Remdesivir (GS-5734) was developed by Gilead Sciences to treat Ebola Virus Disease. It does not have any FDA-approved use. However, on May 1, 2020, the FDA issued an Emergency Use Authorization (EUA) for remdesivir for the treatment of hospitalized COVID-19 patients. The EUA was based on information from two clinical trials, NCT04280705 and NCT04292899 [126,127,128].

4.2.2.1 Anticipated Mechanism

Remdesivir is metabolized to GS-441524, an adenosine analog that inhibits a broad range of polymerases and then evades exonuclease repair causing chain termination [129,130,131]. Although it was developed against Ebola, it also inhibits the MERS-CoV and SARS-CoV polymerase and inhibits

coronavirus replication in cell culture assays with submicromolar IC50s [132]. It also inhibits SARS-CoV-2, showing synergy with chloroquine *in vitro* [131].

4.2.2.2 Current Evidence

In addition to the previous work showing remdesivir to be an effective treatment for viral pathogens such as SARS-CoV and MERS-CoV in cultured cells and animal models, a recent study found that administration of remdesivir to non-human primate models resulted in 100% protection against infection by the Ebola virus. Although a clinical trial in the Democratic Republic of Congo found some evidence of effectiveness against ebola, two antibody preparations were found to be more effective, and remdesivir was not pursued [133]. Remdesivir has also been reported to inhibit SARS-CoV-2 infection in a human cell line sensitive to the virus [131].

The effectiveness of remdesivir for treating patients with COVID-19 is currently under investigation. Remdesivir has been used on some COVID-19 patients under compassionate use guidelines [134,135]. All were in late stages of COVID-19 infection, and these reports are inconclusive about the drug's efficacy. Gilead Sciences, the maker of remdesivir, led a recent publication that reported outcomes for compassionate use of the drug in 61 patients hospitalized with confirmed COVID-19. Here, 200mg of remdesivir was administered intravenously on day 1, followed by a further 100mg/day for 9 days [137]. There were significant issues with the study design or lack thereof. There was no randomized control group. The inclusion criteria were variable: some patients only required low doses of oxygen, others required ventilation. The study included many sites, potentially with variable inclusion criteria and treatment protocols. Patients analyzed had mixed demographics. There was a short follow-up period of investigation. Some patients worsened, some patients died, and eight were excluded from the analysis mainly due to missing post-baseline information, thus their health is unaccounted for. Therefore, even though the study reported clinical improvement in 68% of the 53 patients ultimately evaluated, due to the significant issues with study design, it cannot be determined whether treatment with remdesivir had an effect or whether these patients would have recovered regardless of treatment. As a result, the study does not alter our understanding of the efficacy of remdesivir in this setting, and randomized controlled trials are still required. Remdesivir recently entered controlled clinical trials, and as of March 2020, there are six clinical trials underway to treat COVID-19 patients at both early and late stages of infection and in combinations with other drugs [126,127,131,138,139,140,141].

4.2.2.3 Summary

Remdesivir is a major drug candidate since it attacks the virus with high potency and known mechanism. Moreover, one of the most successful therapies for viral diseases is to target the viral replication machinery, which are typically virally encoded polymerases. Small molecule drugs targeting viral polymerases are the backbones of treatments for other viral diseases including HIV and Herpes. Note that the HIV and Herpes polymerases are a reverse transcriptase and a DNA polymerase respectively, whereas SARS-CoV-2 encodes an RNA dependent RNA polymerase, so most of the commonly used polymerase inhibitors are not likely to be active against SARS-CoV-2. In clinical use, polymerase inhibitors show short term benefits for HIV patients but for long term benefits they must be part of combination regimens. They are typically combined with protease inhibitors, integrase inhibitors and even other polymerase inhibitors.

4.2.3 Protease Inhibitors

Several studies showed that viral proteases play an important role in the life cycle of (corona)viruses by modulating the cleavage of viral polyprotein precursors [142]. Several FDA-approved drugs target proteases like like Lopinavir and Ritonavir in HIV infection, and Simeprevir in hepatitis C virus infection. In particular, serine protease inhibitors were suggested for the treatment of SARS and MERS

viruses [143]. Recently, a study [28] suggested that camostat mesylate, an FDA-approved protease inhibitor (PI) could block the the entry of SARS-CoV-2 into lung cells in vitro. However, to test the efficacy of PIs in patients, randomized clinical trials have to be conducted on patients and healthy volunteers.

4.2.3.1 N3

N3 is an inhibitor of Mpro, a 33.8-kDa SARS-CoV-2 protease that is involved in viral replication and transcription.

4.2.3.1.1 Anticipated Mechanism

N3 inhibits Mpro through binding to its substrate pocket.

4.2.3.2 Current Evidence

N3 was first designed computationally [144] to bind in the substrate binding pocket of the Mpro protease of SARS-like coronaviruses [145]. Consequently, the structure N3-bound SARS-CoV-2 Mpro has been solved [146], which confirmed the computational prediction. Finally, N3 reduced the viral load in samples taken from patients.

4.2.3.3 Summary

N3 is a computationally designed molecule that inhibits the viral transcription through inhibiting Mpro. Although N3 is a strong inhibitor of SARS-CoV-2 in vitro, its safety and efficacy have to be tested in healthy volunteers and patients.

4.2.3.4 Ebselen

Ebselen identified as Mpro protease inhibitor. It is currently investigated as an anti-oxidant drug [147].

4.2.3.4.1 Anticipated Mechanism

Ebselen inhibits Mpro through binding to its substrate pocket.

4.2.3.4.2 Current Evidence

After the design and confirmation of N3 as a highly potent Michael acceptor inhibitor and the identification of Mpro structure [146,148], 10000 compounds were screened for their in vitro anti-Mpro activity. The six leads that were identified were Ebselen, Disulfiram, Tideglusib, Carmofour, PX-12. When the compounds were further assayed on patient viral samples, Ebselen had the strongest potency in reducing the viral load. However, the authors cautioned that these compounds are likely promiscuous binders, which would diminish their therapeutic potential.

4.2.3.4.3 Summary

Ebselen is both a strong Mpro inhibitor and strong inhibitor of viral replication in vitro. The reduction of the viral load after exposure to Ebselen was even larger than N3. Ebselen is a very promising compound since its safety has been demonstrated in other indications. However, Ebselen is likely a false positive since it is a promiscuous compound that can have many targets [149]. Therefore, compounds with higher specificity are required to effectively translate to clinical trials.

4.2.4 Molecules Targeting the Viral Envelope

Why it may be useful

4.3 Drugs Targeting Host Proteins

Brief background on the therapeutic.

4.3.1 Viral Entry Receptors

Entry of SARS-CoV-2 into the cell depends on the ACE2 receptor and the enzyme encoded by *TMPRSS2* [28]. In principle, drugs that reduce the expression of these proteins or sterically hinder viral interactions with them might reduce viral entry into cells.

4.3.2 Current Evidence

A list of current studies and their results, using carefully the information requested in the therapeutic paper tickets.

4.3.3 Summary

Summarize the state of the antiviral approach.

4.4 Broad-Spectrum Pharmaceuticals

- Add some background on broad-spectrum pharmaceuticals

4.4.1 Hydroxychloroquine and Chloroquine

CQ and HCQ are lysosomotropic agents, meaning they are weak bases that can pass through the plasma membrane. Both drugs increase cellular pH by accumulating in their protonated form inside lysosomes [150,151]. This shift in pH inhibits the breakdown of proteins and peptides by the lysosomes during the process of proteolysis [151]. A number of mechanisms have been proposed through which these drugs could influence the immune response to pathogen challenge. For example, CQ/HCQ can interfere with digestion of antigens within the lysosome and inhibit CD4 T-cell stimulation while promoting the stimulation of CD8 T-cells [151]. CQ/HCQ can also decrease the production of certain key cytokines involved in the immune response including IL-6 and inhibit the stimulation of toll-like receptors (TLR) and TLR signaling [151]. The drugs also have anti-inflammatory and photoprotective effects and may also affect rates of cell death, blood clotting, glucose tolerance, and cholesterol levels [151].

Interest in CQ and HCQ for treating COVID-19 was catalyzed by a mechanism observed in *in vitro* studies of both SARS-CoV and SARS-CoV-2. In one study, CQ inhibited viral entry of SARS-CoV into Vero E6 cells, a cell line derived from the epithelial cells of an African green monkey kidney, through the elevation of endosomal pH and the terminal glycosylation of angiotensin-converting enzyme 2 (ACE2), which is the cellular entry receptor [152]. Increased pH within the cell, as discussed above, inhibits proteolysis, and terminal glycosylation of ACE2 is thought to interfere with virus-receptor binding. An *in vitro* study of SARS-CoV-2 infection of Vero cells, a line from which the Vero E6 clone has been separated since 1968, found both HCQ and CQ to be effective in inhibiting viral replication, with HCQ being more potent [153]. Additionally, an early case study of three COVID-19 patients reported the presence of antiphospholipid antibodies in all three patients [154]. Antiphospholipid antibodies are central to the diagnosis of the antiphospholipid syndrome, and HCQ has a history of use in treating

Antiphospholipid Syndrome [155,156,157]. Together, these studies triggered initial enthusiasm about the therapeutic potential for HCQ and CQ against COVID-19. However, as results from clinical studies have emerged, concerns about the efficacy and risks of treating COVID-19 with HCQ and CQ has led to the removal of these drugs from standard of care practices in several countries [158,159]. This section will discuss the current information available administration of CQ and HCQ for the treatment of COVID-19.

4.4.1.1 Current Evidence

Gautret et al conducted a non-randomized, non-blinded, non-placebo clinical trial on 42 hospitalized patients comparing HCQ to standard care [160]. This trial found patients who received HCQ showed higher rates of virological clearance by nasopharyngeal swab on Days 3-6 when compared to standard care. This study also treated six patients with both HCQ + azithromycin and found this combination therapy to be more effective than HCQ alone. This study showed design and analysis weaknesses that severely limit interpretability of results. These weaknesses include: lack of randomization, lack of blinding, lack of placebo, lack of Intention-To-Treat analysis, lack of correction for sequential multiple comparisons, trial arms entirely confounded by hospital, false negatives in outcome measurements, lack of trial pre-registration, and small sample size. Two of these weaknesses are due to inappropriate data analysis and can therefore be corrected post-hoc by recalculating p-values (lack of Intention-To-Treat analysis and multiple comparisons.) However, all other weaknesses are fundamental design flaws and can not be corrected for. Thus, conclusions cannot be generalized outside of the study. Additionally, the International Society of Antimicrobial Chemotherapy, the scientific organization that publishes *International Journal of Antimicrobial Agents* where the article appeared, has announced that the article does not meet its expected standard for publications [161], although it has not been officially retracted. Because of the preliminary data presented in this study, the use of HCQ in COVID-19 treatment has subsequently been explored by other researchers.

A randomized, non-placebo trial of 62 COVID-19 patients at the Renmin Hospital of Wuhan University studied whether HCQ decreased time to fever break or time to cough relief when compared to standard care [162]. This trial found HCQ decreased both average time to fever break and average time to cough relief, defined as mild or no cough. However, this study also had flaws in trial design and analysis that prevent generalization of the results. These weaknesses include: lack of placebo, lack of correction for multiple primary outcomes, inappropriate choice of outcomes, lack of sufficient detail to understand analysis, drastic disparities between pre-registration and published protocol, and small sample size. The choice of outcomes may be inappropriate as both fevers and cough may break periodically without resolution of illness. Additionally, for these outcomes, the authors report 23/62 patients did not have fever and 25/62 patients did not have cough at the start of the study - the authors fail to describe how these patients were included in a study assessing time to fever break and time to cough relief. It is important to note here that the authors claim “neither the research performers nor the patients were aware of the treatment assignments.” This seems impossible in a non-placebo trial - at the very least, providers would know whether they were administering a medication or not, and this knowledge could lead to systematic differences in how care is given. Correction for multiple primary outcomes can be adjusted post-hoc by recalculating p-values, but all other issues are design and statistical weaknesses that cannot be corrected for. Additionally, the observation of drastic disparities between pre-registration and published protocol may be suggestive of p-hacking. Conclusions cannot de generalized outside of the study, but the results support further investigation.

A randomized trial from the Shanghai Public Health Clinical Center of 30 COVID-19 patients studied whether HCQ increased rates of virological clearance by respiratory pharyngeal swab on Day 7 post-treatment compared to standard care [163]. This trial was published in Chinese with an abstract also in English. Only the English abstract was read and interpreted. The trial found HCQ showed comparable outcomes to standard care with regard to virological clearance rate, time to virological

clearance, and time to body temperature normalization. A known weakness is small sample size. This problem suggests the study is underpowered to detect potentially useful differences and precludes interpretation of results. Additionally, because only the abstract could be read, other design and analysis issues could be present. Conclusions should be interpreted very cautiously. However, these preliminary negative results do reiterate the need for further study.

A case study treated 11 consecutive patients with HCQ + azithromycin using the same dosing regime reported by Gautret et al. [164]. One patient died, two were transferred to the ICU, and one developed a prolonged QT interval leading to discontinuation of HCQ + azithromycin. As in the Gautret et al study, the outcome measurement was virological clearance at Day 6 post-treatment by nasopharyngeal swabs. Of the ten living patients on Day 6, eight of the patients remained positive for SARS-CoV-2 RNA. Interpretation of conclusions are severely limited by lack of comparison group and small sample size. However, these results stand in contrast to claims by Gautret et al that all six patients treated with HCQ + azithromycin tested negative for SARS-CoV-2 RNA by Day 6 post-treatment. This case study illustrates the need for better and further investigation.

A Letter to the Editor was published in BioScience Trends claiming numerous clinical trials showed HCQ is superior to control treatment in inhibiting the exacerbation of COVID-19 pneumonia [165]. This Letter has been cited by numerous primary literature, review articles, and media alike [166,167]. Yet, this Letter's claims of clinical trials supporting HCQ use refer only to 15 pre-registration identifiers from the Chinese Clinical Trial Registry. When these identifiers are followed back to the registry, most trials claim they are not yet recruiting patients or are currently recruiting patients. For all of these 15 identifiers, no data uploads or links to publications could be located on the pre-registrations. At the very least, an inability to locate primary data means such claims cannot be verified. Additionally, citation of unavailable sources cast doubt on policies and research based on the assumption that HCQ is effective in treating COVID-19. A randomized, open-label, non-placebo trial of 150 COVID-19 patients was conducted in parallel in 16 government-designated COVID-19 centers in China to assess the safety and efficacy of HCQ [168]. The trial examined the efficacy of HCQ in conjunction with standard of care (SOC) compared to SOC alone in 150 infected patients assigned randomly to the two respective groups (75 per group). Most of the SARS-CoV-2 cases in this cohort were mild to moderate (98%), and the average age of the patients was 46 years. The primary endpoint of the study was the negative conversion rate of SARS-CoV-2 in 28 days, and the investigators found no difference in this parameter between the groups. The secondary endpoints were an amelioration of the symptoms of the disease such as axillary temperature ≤36.6°C, SpO2 >94% on room air, and disappearance of symptoms like shortness of breath, cough, and sore throat. The median time to symptom alleviation was similar across different conditions (19 days in HCQ+SOC vs. 21 days in SOC). However, the investigators reported an interesting finding revealed in *post hoc* analysis: controlling for the administration of antivirals yielded an effect of HCQ on the alleviation of symptoms, with a reported hazard ratio of 8.83. However, the 95% confidence interval of 1.09 to 71.3 suggests that this analysis may be underpowered and should be interpreted cautiously, as only 28 patients total (14 in each group) met this criterion. Additionally, there was a non-significant trend towards a greater number of lymphocytes in the SOC+HCQ group compared to the SOC alone group (mean of 0.062 versus 0.008; p=0.57). Given the improvement in CRP levels and the possible improvement in lymphocyte count, the authors hypothesized that the addition of HCQ to the current SOC could decrease the inflammatory response and subsequently prevent multiorgan failure and death. Additionally, one of the key results of this trial was that the 30% of the patients receiving SOC+HCQ reported adverse outcomes as opposed to 8.8% of patients receiving only SOC. The most common adverse outcome in the SOC+HCQ group was diarrhea (10% vs. 0% in the SOC group, p=0.004).

The study had a few limitations. One of the limitations of the study was that the cohort mostly consisted of patients with mild to moderate symptoms, and the average age of analyzed patients was 46. This average may not be representative for COVID-19 patients because older age groups are known to be at higher risk. Although the authors claimed that HCQ could have beneficial effects in

hindering disease progression, multiorgan failure, and death in COVID-19, this finding cannot be extrapolated to older patients or severe cases based on the evidence they present. Indeed, a larger sample needs to be tested to validate whether lymphocyte counts do increase in patients treated with SOC+HCQ; although the result in the present analysis appeared promising, it lacked statistical significance. Additionally, in this study, SOC included the use of antivirals (Lopinavir-Ritonavir, Arbidol, Oseltamivir, Virazole, Entecavir, Ganciclovir, and Interferon alfa), which appeared to introduce confounding effects. Thus, to better assess the effects of HCQ, an alternate study design needs to be outlined where SOC does not involve the use of anti-virals. In this trial, the samples used to test for presence of the SARS-CoV-2 virus were collected from the upper respiratory tract, and the authors indicated that the use of upper respiratory samples may have introduced false negatives (e.g., [169]); thus, the identification of biomarkers that can be collected non-invasively would be valuable to studies such as this one. Another limitation of the study that the authors acknowledge was that the HCQ treatment began, on average, at a 16-day delay from the symptom onset. A quicker treatment regimen with HCQ needs to be investigated to elucidate how the drug affects early disease management. Overall, the study provides promising data, although all of the findings still need to be validated in independent population cohorts. Their safety analysis indicated that the adverse effects of administering HCQ to mild and moderate COVID-19 cases were manageable. Further investigation is necessary to confirm whether the drug indeed ameliorates symptoms and reduces inflammatory response. ##### Summary

In vitro evidence shows HCQ may be an effective therapeutic against SARS-CoV-2 and COVID-19. Multiple clinical studies have already been carried out to assess this possibility. All current studies are low-quality and have small sample sizes. Thus, interpretation is severely limited and must be done cautiously. Additionally, disagreements between studies demonstrate there is controversy on the effectiveness of HCQ, as well as HCQ + azithromycin combination therapy. This uncertainty is important to recognize clinically because HCQ-based treatments can lead to dangerous side effects like prolonged QT interval [170]. HCQ use for COVID-19 also leads to shortages for anti-malarial or anti-rheumatic use, where it has been definitively proven to be effective. Further investigation of HCQ in large, rigorous, multi-center clinical trials is necessary.

4.4.2 Nutraceuticals

Considering the current pandemic, scientists and the medical community are scrambling to repurpose or discover novel host-directed therapies for which nutraceuticals hold some promise. Nutraceuticals are classified as supplements with health benefits beyond their basic nutritional value designed for the prophylaxis and treatment of disease [171,172]. Nutraceuticals purported to boost the immune response, reduce immunopathology, exhibit antiviral activities or prevent acute respiratory distress syndrome (ARDS) are being considered for their potential therapeutic value [92]. A host of potential candidates have been highlighted in the literature that target various aspects of the COVID-19 viral pathology, while others are thought to prime the host immune system. These candidates include vitamins and minerals along with extracts and omega-3 polyunsaturated fatty acids (n-3 PUFA) [173]. Considerable evidence *in vitro* and *in vivo* suggests that nutraceuticals containing phycocyanobilin, N-acetylcysteine, glucosamine, selenium or phase 2 inductive nutraceuticals (e.g. ferulic acid, lipoic acid, or sulforaphane) can prevent or modulate RNA virus infections via amplification of the signaling activity of mitochondrial antiviral-signaling protein (MAVS) and activation of toll-like receptor 7 (TLR7) [91]. While promising, further animal and human studies are required to assess the therapeutic potential of these various nutraceuticals against COVID-19.

4.4.2.1 n-3 PUFA

Another potential nutraceutical that has exhibited beneficial effects against various viral infections is n-3 PUFA [173], such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA

intake can come from a diet high in fish intake or through dietary supplementation with fish oils or purified oils [174].

4.4.2.1.1 Potential Mechanisms

N-3 PUFA nutraceuticals can mediate inflammation and they have the capacity to modulate the adaptive immune response [172,174,175]. Another potential mechanism by which n-3 PUFA could exert beneficial effects against viral infections is by acting as precursor molecules for the biosynthesis of endogenous specialised proresolving mediators (SPM) like protectins and resolvins that actively resolve inflammation and infection [176].

4.4.2.1.2 Current Evidence

SPM have exhibited beneficial effects against a variety of lung infections including RNA viruses [177]. Indeed, protectin D1 has been shown to increase survival from H1N1 viral infection in mice by affecting the viral replication machinery [178]. Moreover, not all studies are in agreement that n-3 PUFA are effective against infections [179]. Indeed, the effectiveness of n-3 PUFA against infections is dependent on the dosage, timing, and the specific pathogens responsible [180].

4.4.2.1.3 Summary

However, the overall lack of human studies in this area means there is limited evidence as to whether these nutraceuticals could affect COVID-19 infection.

4.4.2.2 Zinc Supplements

There is evidence that nutrient supplements may exhibit some benefit against RNA viral infections. Zinc is a trace metal obtained from dietary sources or supplementation that is important for the maintenance of immune cells involved in adaptive and innate immunity [181]. Zinc supplements can be administered orally as a tablet or as a lozenge and they are available in many forms, such as zinc picolinate, zinc acetate, and zinc citrate. Zinc is also available from dietary sources including meat, seafood, nuts, seeds, legumes, and dairy.

4.4.2.2.1 Potential Mechanisms

The role of zinc in immune function has been extensively reviewed [181]. Zinc is an important signaling molecule and zinc levels can alter host defense systems. In inflammatory situations such as an infection, zinc can regulate leukocyte immune responses and it can activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), thus altering cytokine production [182,183]. In particular, zinc supplementation can increase natural killer cell levels, which are important cells for host defense against viral infections [181,184].

4.4.2.2.2 Current Evidence

Adequate zinc intake has been associated with reduced incidence of infection [185] and antiviral immunity [186]. Similarly, a randomized, double-blind, placebo-controlled trial that administered zinc supplementation to elderly subjects over the course of a year found zinc deficiency to be associated with increased susceptibility to infection and that zinc deficiency could be prevented through supplementation [185]. Clinical trial data supports the utility of zinc to diminish the duration and severity of symptoms associated with common colds when it is provided within 24 hours of the onset of symptoms [187,188]. In coronaviruses specifically, in vitro evidence demonstrates that the combination of zinc (Zn^{2+}) and zinc ionophores (pyrithione) can interrupt the replication mechanisms of SARS-CoV-GFP (a flourescently tagged SARS-CoV) and a variety of other RNA viruses [189,190].

4.4.2.2.3 Summary

While overall there is encouraging data for zinc supplementation against the common cold and viral infections, there is currently limited evidence to suggest zinc supplementation has any beneficial effects against the current novel COVID-19, thus further research is imperative. However, it would be advisable to maintain a healthy diet to ensure an adequate zinc status to prevent the likelihood of an infection.

4.4.2.3 Vitamin C

Vitamins B, C, D, and E have also been suggested as potential nutrient supplement interventions for COVID-19 [173,191]. In particular vitamin C has been proposed as a potential prophylactic and therapeutic agent against COVID-19. Vitamin C can be obtained via dietary sources such as fruit and vegetable or via supplementation.

4.4.2.3.1 Potential Mechanisms

Vitamin C plays a significant role in promoting immune function due to its effects on various immune cells. Vitamin C affects inflammation by modulating cytokine production, decreasing histamine levels, enhancing the differentiation and proliferation of T- and B-lymphocytes, increasing antibody levels, and protecting against the negative effects of reactive oxygen species amongst other effects [192,193,194]. During viral infections vitamin C is utilised as evinced by lower concentrations in leukocytes and lower concentrations of urinary vitamin C. Post-infection, these levels return to baseline ranges [195,196,197,198,199].

4.4.2.3.2 Current Evidence

A recent meta-analysis found consistent support for regular vitamin C supplementation reducing the duration of the common cold, but that supplementation with vitamin C (> 200 mg) failed to reduce the incidence of colds [200]. Individual studies have found Vitamin C to reduce the susceptibility of patients to lower respiratory tract infections such as pneumonia [201]. Another meta-analysis has demonstrated in twelve trials that vitamin C supplementation reduced the length of stay of patients in intensive care units (ICUs) by 7.8% (95% CI: 4.2% to 11.2%; p = 0.00003). Furthermore, high doses (1-3 g/day) significantly reduced the length of an ICU stay by 8.6% in six trials (p = 0.003). Vitamin C also shortened the duration of mechanical ventilation by 18.2% in three trials in which patients required intervention for over 24 hours (95% CI 7.7% to 27%; p = 0.001) [202]. Despite these findings, the CITRUS ALI study failed to show a benefit of a 96-hour infusion of vitamin C to treat ARDS, which is a severe complication of COVID-19 infection [203]. Nevertheless, a randomized placebo-controlled trial [204] has begun in Wuhan, China to investigate the intravenous infusion of vitamin C to treat pneumonia in 140 severe COVID-19 infected patients. As summarized by Carr [205] the trial will not be completed until September 2020. Another trial in Italy [206] intends to deliver a 10 g infusion of vitamin C to 500 severe COVID-19 patients with pneumonia to assess in-hospital mortality over a 72 hr period as the primary outcome. The trial is currently recruiting and is due to run until March 2021. We will not know how effective vitamin C is as a therapeutic for quite some time due to the length of both trials. When completed, the trials will provide crucial evidence on the efficacy of vitamin C as a therapeutic for COVID-19 infection.

4.4.2.3.3 Summary

Some evidence suggests that vitamin C supplementation can shorten the duration of a cold, reduce an individual's susceptibility to infections, and shorten a patient's stay in an ICU when administered at high doses. We don't yet understand if these findings apply to COVID-19. There are ongoing trials in China and Italy that will inform our understanding of the therapeutic value of vitamin C supplementation for COVID-19.

4.4.2.4 Vitamin D

In terms of other dietary supplements, vitamin D can modulate the adaptive and innate immune system and has been associated with various aspects of health. Vitamin D can be sourced through diet or supplementation, but it is mainly biosynthesized by the body on exposure to sunlight.

4.4.2.4.1 Potential Mechanisms

Vitamin D deficiency is associated with an increased susceptibility to infection [207]. In particular, vitamin D deficient patients are at risk of developing acute respiratory infections [208] and ARDS [208]. 1,25-dihydroxyvitamin D3 is the active form of vitamin D that is involved in adaptive and innate responses, whereby the vitamin D receptor is expressed in various immune cells and vitamin D is an immunomodulator of antigen presenting cells, dendritic cells, macrophages, monocytes, and T- and B-lymphocytes [207,209]. Due to its potential immunomodulating properties, vitamin D supplementation may be advantageous to maintain a healthy immune system.

4.4.2.4.2 Current Evidence

A recent preprint postulated that an individual's vitamin D status may significantly affect one's risk of developing COVID-19. This hypothesis was derived from the fact that the current pandemic occurred in winter in Wuhan China when 25-hydroxyvitamin D concentrations are at their lowest due to lack of sunlight, whereas in the Southern Hemisphere, where it was nearing the end of the summer, the number of cases was low at that time coinciding with higher 25-hydroxyvitamin D concentrations [210]. The authors suggest that people at risk of developing COVID-19 should increase their vitamin D3 intake to reach 25-hydroxyvitamin D plasma concentrations above 40–60 ng/ml. The authors also suggest supplementation of vitamin D to treat infected patients and to prevent infection in hospital staff. While vitamin D is relatively inexpensive and safe to consume, caution is warranted when interpreting this review as it has yet to be determined whether vitamin D levels effect COVID-19 specifically. Likewise, it is assumed that COVID-19 may be seasonal, but there are multiple other factors at play that can affect vitamin D levels that need to be considered. These factors include an individual's nutritional status, their age, their occupation, skin pigmentation, potential comorbidities, and the variation of exposure to sunlight due to latitude amongst others [211].

4.4.2.4.3 Summary

Supplementation of vitamin D and maintaining a healthy diet for optimum vitamin D status warrants further investigation. This is particularly important considering 'stay in place' guidance has been implemented in many densely populated cities around the world. This measure is likely to limit people's exposure to sunlight and thus reduce endogenous synthesis of vitamin D, potentially weakening the immune system and increasing the risk of COVID-19 infection.

4.4.2.5 Nutraceutical Conclusions

Despite all the potential benefits of nutraceutical and dietary supplement interventions presented, currently there is a paucity of clinical evidence to support their use for the prevention or mitigation of COVID-19 infections. Nevertheless, optimal nutritional status will undoubtedly prime an individual's immune system to protect against the effects of acute respiratory viral infections by supporting normal maintenance of the immune system [212]. Overall, supplementation of vitamin C, vitamin D, and zinc may be an effective method of ensuring their adequate intake to maintain optimal immune function, which may also convey beneficial effects against viral infections due to their immunomodulatory effects. However, many supplements and nutraceuticals designed for various ailments are available in the United States and beyond that are not strictly regulated [213]. Indeed, there can be safety and efficacy concerns associated with many of these products. Often, the vulnerable members of society can be exploited in this regard and unfortunately the COVID-19

pandemic is no different. The Food and Drug Administration (FDA) has issued warnings to several companies for advertising falsified claims in relation to the preventative and therapeutic capabilities of their products against COVID-19 [214].
In light of these serious occurrences, it is pertinent to clarify that the nutraceuticals discussed in this review have been selected because of their possible relevance to the biological mechanisms that can beneficially affect viral and respiratory infections. Therefore, further intensive investigation is required to establish the effects of these nutraceuticals, if any, against COVID-19.

4.5 Biological Drugs for COVID-19

4.5.1 Neutralizing Antibodies

Monoclonal antibodies (mAbs) have revolutionized the way we treat human diseases. As a result, they have become some of the best-selling drugs in the pharmaceutical market in recent years [215]. There are currently 79 FDA approved mAbs on the market including antibodies for viral infections (e.g. Ibalizumab for HIV and Palivizumab for RSV) [215,216]. Although vaccines remain the most important way to prevent viral infections, their development process is long and they fail to provide immediate prophylactic protection or treat ongoing infections [217]. For that reason, neutralizing antibodies have emerged to address these shortcomings. Virus-specific neutralizing antibodies commonly target viral surface glycoproteins or host structures, thereby inhibiting viral entry through receptor binding interference [218,219]. This section discusses current efforts in developing neutralizing antibodies against SARS-CoV-2 and how expertise gained from previous approaches for MERS-CoV and SARS-CoV may benefit antibody development.

4.5.1.1 Spike (S) Neutralizing Antibody

During the first SARS epidemic in 2002, nAbs were found in SARS-CoV infected patients [220,221]. Several studies following up on these findings identified various S glycoprotein epitopes as the major targets of neutralizing antibodies against SARS-CoV [222]. The passive transfer of immune serum containing nAbs from SARS-CoV-infected mice resulted in protection of naïve mice from viral lower respiratory tract infection upon intranasal challenge [223]. Similarly, a meta-analysis suggested that administration of plasma from recovered SARS-CoV patients reduced mortality upon SARS-CoV infection [224].

Similar results have been observed for MERS-CoV infections, which emerged as the second coronavirus-related epidemic. Neutralizing antibodies have been identified against various epitopes of the RBD of the S glycoprotein [225; doi:10.1128/JVI.00912-14].

4.5.1.1.1 Anticipated Mechanisms

Coronaviruses use trimeric spike (S) glycoproteins on their surface to bind to host cell receptors, such as ACE2, allowing for cell entry [24,28]. Each S glycoprotein protomer is comprised of an S1 domain, also called the receptor binding domain (RBD), and an S2 domain. The S1 domain binds to host cell receptors while the S2 domain facilitates the fusion between the viral envelope and host cell membranes [222]. Although targeting of the host cell receptor ACE2 shows efficacy in inhibiting SARS-CoV-2 infection [226], given the physiological relevance of ACE2 [227], it would be favorable to target virus-specific structures rather than host receptors. This forms the rationale of developing neutralizing antibodies against the S glycoprotein, disrupting its interaction with ACE2 and other receptors and thereby inhibiting viral entry.

4.5.1.1.2 Current Evidence

The first human neutralizing antibody against SARS-CoV-2 targeting the trimeric spike (S) glycoproteins has been developed using hybridoma technology, [228], where antibody-producing B-cells developed by mice can be inserted into myeloma cells to produce a hybrid cell line (the hybridoma) that is grown in culture. The 47D11 clone was able to cross-neutralize SARS-CoV and SARS-CoV2 by a mechanism that is different from receptor binding interference. The exact mechanism of how this clone neutralizes SARS-CoV-2 and inhibits infection in vitro remains unknown, but a potential mechanism might be antibody induced destabilization of the membrane prefusion structure [228,229]. The ability of this antibody to prevent infection at a feasible dose needs to be validated in vivo, especially since in vitro neutralization effects have been shown to not be reflective of in vivo efficacy [230]. Only a week later, a different group successfully isolated multiple nAbs targeting the RBD of the S glycoprotein from blood samples taken from COVID-19 patients in China [61]. Interestingly, the patient isolated antibodies did not cross-react with RBDs from SARS-CoV and MERS-CoV, although cross-reactivity to the trimeric spike proteins of SARS-CoV and MERS-CoV was observed. This suggests that the RBDs between the three coronavirus species are immunologically distinct and that the isolated nAbs targeting the RBD of SARS-CoV-2 are species specific. While this specificity is desirable, it also raises the question of whether these antibodies are more susceptible to viral escape mechanisms. Viral escape is a common resistance mechanism to nAbs therapy due to selective pressure from neutralizing antibodies [231,232]. For HIV, broadly neutralizing antibodies (bnAbs) targeting the CD4 binding site (CD4bs) show greater neutralization breadth than monoclonal antibodies, which target only specific HIV strains [233]. For MERS-CoV, a combination of multiple neutralizing antibodies targeting different antigenic sites prevented neutralization escape [234]. It was found that the different antibody isolates did not target the same epitopes, suggesting that using them in combination might produce a synergistic effect that prevents viral escape [61]. It was also demonstrated that binding affinity of the antibodies does not reflect their capability to compete with ACE2 binding. Furthermore, no conclusions about correlations between the severity of disease and the ability to produce neutralizing antibodies can be drawn at this point. Rather, higher neutralizing antibody titers were more frequently found in patients with severe disease. Correspondingly, higher levels of anti-spike IgG were observed in patients that deceased from infection compared to patient that recovered [235].

4.5.1.1.3 Summary

Results from the SARS-CoV and MERS-CoV epidemics can provide valuable lessons for the design of neutralizing antibodies for the current outbreak. The findings for SARS-CoV and MERS can aid in identifying which structures constitute suitable targets for nAbs, despite the fact that the RBD appears to be distinct between the three coronavirus species. These studies also suggest that a combination of nAbs targeting distinct antigens might be necessary to provide protection [234]. The biggest challenge remains identifying antibodies that not only bind to their target, but also prove to be beneficial for disease management. On that note, a recently published study indicates that anti-spike antibodies could make the disease worse rather than eliminating the virus [235]. These findings underscores our current lack of understanding the full immune response to SARS-CoV-2.

4.5.2 Interferons

Interferons (IFNs) are a family of cytokines crucial to activate the first (innate) immune system response against viral infections. Interferons are classified into three categories based on their receptor specificity: type I, II and III [95]. Specifically, IFNs I (IFN-α and β) and II (IFN-γ) induce the expression of antiviral proteins which bring the viral RNA to degradation [236]. Among these IFNs, IFN- β was already found to strongly inhibit the replication of other corona viruses, such as SARS-CoV, in cell culture, while IFN-α and γ were shown to be less effective in this context [236]. There are evidences that patients with higher susceptibility to develop Acute respiratory distress syndrome (ARDS) show indeed deficiency of IFN- β. For instance, upon other Corona viruses infection IFN- β

expression and synthesis is impaired, so that the virus can in fact escape the innate immune response [237].

On March 18 2020 Synairgen plc has received approval to start a phase II trial for SNG001, an IFN-β-1a formulation to be delivered to lungs via inhalation. SNG001 was already shown to be effective reducing viral load in swine flu in vivo model, as well as it has been shown to be effective in the protection from other Corona virus infection in vitro (Synairgen plc, press release).

4.5.2.1 Anticipated Mechanism

Why it may be useful

4.5.2.2 Current Evidence

A list of current studies and their results, using carefully the information requested in the therapeutic paper tickets.

4.5.2.3 Summary

Summarize the state of interferons.

4.6 Vaccines

4.6.1 Strategies for and challenges to vaccine development

Today, the first step in producing a vaccine often is characterizing the target. The genetic sequence of SARS-CoV-2 was published on January 11, 2020, which aided the global effort to develop a vaccine to prevent COVID-19. The Coalition for Epidemic Preparedness Innovations (CEPI) is coordinating global health agencies and pharmaceutical companies to develop vaccines against SARS-CoV-2. As of April 8, 2020, there were 115 vaccine candidates to prevent COVID-19, of which 78 were active. Of the 78 active vaccine programs, 73 were in the preclinical or exploratory stage [238].

Historically, an H1N1 influenza vaccine was developed relatively efficiently, mainly because influenza-vaccine technology had already been developed and regulatory agencies had already decided that vaccines produced using egg- and cell-based platforms could be licensed under the regulations used for a strain change. Critiques of the experience producing and distributing the H1N1 vaccine have stressed the need for alternative development-and-manufacturing platforms that can be readily adapted to new pathogens. Although a monovalent H1N1 vaccine was not available before the pandemic peaked in the United States and Europe, it was available soon afterward as a stand-alone vaccine that was eventually incorporated into the commercially available seasonal influenza vaccines [239]. If H1N1 vaccine development provides any indication, considering developing and manufacturing platforms for promising COVID-19 vaccine trials early could hasten the emergence of an effective prophylactic vaccine against SARS-CoV-2.

Unlike many global vaccine development programs previously, such as with H1N1, the vaccine development landscape for COVID-19 includes vaccines produced by a wide array of technologies. Experience in the field of oncology is encouraging COVID-19 vaccine developers to use next-generation approaches to vaccine development, which have led to the great diversity of vaccine development programs [240]. Diverse technology platforms include DNA, RNA, virus-like particle, recombinant protein, both replicating and non-replicating viral vectors, live attenuated virus, and inactivated virus approaches. Given the wide range of vaccines under development, it is possible that

some vaccine products may eventually be shown to be more effective in certain subpopulations, such as children, pregnant women, immunocompromised patients, the elderly, etc.

4.6.2 DNA Vaccines

This vaccination method involves the direct introduction of a plasmid containing a DNA sequence encoding the antigen(s) against which an immune response is sought into appropriate tissues [241].

4.6.2.1 Anticipated Mechanism

This approach may offer several advantages over traditional vaccination approaches, such as the stimulation of both B- as well as T-cell responses and the absence of any infectious agent.

4.6.2.2 Current Evidence

Currently, a Phase I safety and immunogenicity clinical trial of INO-4800, a prophylactic vaccine against SARS-CoV-2, is underway [242]. The vaccine developer Inovio Pharmaceuticals Technology is overseeing administration of INO-4800 by intradermal injection followed by electroporation with the CELLECTRA® device to healthy volunteers. Electroporation is the application of brief electric pulses to tissues in order to permeabilize cell membranes in a transient and reversible manner. It has been shown that electroporation can enhance vaccine efficacy by up to 100-fold, as measured by increases in antigen-specific antibody titers [243]. The safety of the CELLECTRA® device has been studied for over seven years, and these studies support the further development of electroporation as a safe vaccine delivery method [244]. The temporary formation of pores through electroporation facilitates the successful transportation of macromolecules into cells, allowing cells to robustly take up INO-4800 for the production of an antibody response.

Approved by the U.S. Food and Drug Administration (FDA) on April 6, 2020, the Phase I study is enrolling up to 40 healthy adult volunteers in Philadelphia, PA at the Perelman School of Medicine and at the Center for Pharmaceutical Research in Kansas City, MO. The trial has two experimental arms corresponding to the two locations. Participants in Experimental Group 1 will receive one intradermal injection of 1.0 milligram (mg) of INO-4800 followed by electroporation using the CELLECTRA® 2000 device twice, administered at Day 0 and Week 4. Participants in Experimental Group 2 will receive two intradermal injections of 1.0 mg (total 2.0 mg per dosing visit) of INO-4800 followed by electroporation using the CELLECTRA® 2000 device, administered at Day 0 and Week 4. Safety data and the initial immune responses of participants from the trial are expected by the end of the summer of 2020.

4.6.2.3 Summary

The development of a DNA vaccine against SARS-CoV-2 by Inovio could be an important step forward in the world's search for a COVID-19 vaccine. Although exciting, the cost of vaccine manufacturing and electroporation may make scaling the use of this technology for prophylactic use for the general public difficult.

4.6.3 RNA Vaccines

RNA vaccines are nucleic-acid based modalities that code for viral antigens against which the human body elicits a humoral and cellular immune response. The mRNA technology is transcribed *in vitro* and delivered to cells via lipid nanoparticles (LNP). They are recognized by ribosomes *in vivo* and then translated and modified into functional proteins [245]. The resulting intracellular viral proteins are displayed on surface MHC proteins, provoking a strong CD8+ T cell response as well as a CD4+ T cell and B cell-associated antibody responses [245].

Naturally, mRNA is not very stable and can degrade quickly in the extracellular environment or the cytoplasm. The LNP covering protects the mRNA from enzymatic degradation outside of the cell [246]. Codon optimization to prevent secondary structure formation and modifications of the poly-A tail as well as the 5' untranslated region to promote ribosomal complex binding can increase mRNA expression in cells. Furthermore, purifying out dsRNA and immature RNA with FPLC (fast performance liquid chromatography) and HPLC (high performance liquid chromatography) technology will improve translation of the mRNA in the cell [245,247].

mRNA vaccines confer many advantages over traditional viral vectored vaccines and DNA vaccines. In comparison to live attenuated viruses, mRNA vaccines are non-infectious and can be synthetically produced in an egg-free, cell-free environment, and thereby reducing the risk of a detrimental immune response in the host [248]. Unlike DNA vaccines, mRNA technologies are naturally degradable and non-integrating, and they do not need to cross the nuclear membrane in addition to the plasma membrane for their effects to be seen [245].
Furthermore, mRNA vaccines are easily, affordably, and rapidly scalable.

Although mRNA vaccines have been developed for therapeutic and prophylactic purposes, none have been licensed or commercialized thus far. Nevertheless, they have show promise in animal models and preliminary clinical trials for several indications, including rabies, coronavirus, influenza, rabies, and cytomegalovirus [249]. Preclinical data from Pardi et al. identified effective antibody generation against full-length FPLC-purified influenza hemagglutinin stalk-encoding mRNA in mice, rabbits, and ferrets [250]. Similar immunological responses for mRNA vaccines were observed in humans in Phase I and II clinical trials operated by the pharmaceutical-development companies Curevac and Moderna for rabies, flu, and zika [247].

4.6.3.1 Anticipated Mechanism

Positively charged bilayer LNPs carrying the mRNA attract negatively charged cell membranes, endocytose into the cytoplasm [246], and facilitate endosomal escape. LNPs can be coated with modalities recognized and engulfed by specific cell types. LNPs 150nm or less effectively enter into lymphatic vessels.

There are three types of RNA vaccines: non-replicating, *in vivo* self-replicating, and *in vitro* dendritic cell non-replicating [251].
Non-replicating mRNA vaccines consist of a simple open reading frame (ORF) for the viral antigen flanked by the 5' UTR and 3' poly-A tail. *In vivo* self-replicating vaccines encode a modified viral genome derived from single-stranded, positive sense RNA alphaviruses [245,247]. The RNA genome encodes the viral antigen along with proteins of the genome replication machinery, including an RNA polymerase. Structural proteins required for viral assembly are not included in the engineered genome [245]. Self-replicating vaccines produce more viral antigens over a longer period of time, thereby evoking a more robust immune response[251].
Finally, *in vitro* dendritic cell non-replicating RNA vaccines limit transfection to dendritic cells. Dendritic cells are potent antigen presenting immune cells that easily take up mRNA and present fragments of the translated peptide on their MHC proteins, which can then interact with T cell receptors.
Ultimately, primed T follicular helper cells can stimulate germinal center B cells that also present the viral antigen to produce antibodies against the virus [252]. These cells are isolated from the patient, grown and transfected *ex vivo*, and reintroduced to the patient [253].

4.6.3.2 Current Evidence

mRNA-1273 is the first COVID-19 vaccine to enter a phase I clinical in the United States.
ModernaTX, Inc. is currently spearheading an investigation on the immunogenicity and reactogenicity of mRNA-1273, a conventional lipid nanoparticle encapsulated RNA encoding a full-length prefusion

stabilized spike (S) protein for SARS-CoV-2 [254]. Forty-five participants will be enrolled in the study and given an intramuscular injection of mRNA-1273 in their deltoid muscle on Day 1 and Day 29, and then followed for the next twelve months. Healthy males and non-pregnant females aged 18-55 years are being recruited and will be divided in three dosage groups receiving either 25 micrograms, 100 mcg, or 250 mcg of the vaccine.

The study started on March 3rd, 2020, and is expected to complete by June 1st, 2021. Emory Vaccine Center and Kaiser Permanent Washington Health Research Institute are currently recruiting participants with NIH Clinical Center expecting to recruit soon. Reports on patient safety and reactogenicity will be recorded soon. IgG ELISA assays on patient serology samples will study the immunogenicity of the vaccine [254].

4.6.3.3 Summary

mRNA vaccines are promising tools in the prevention and control of pandemics. mRNA-1273 is the only RNA vaccine for SARS-CoV-2 currently being tested in clinical trials and results are expected soon.

4.6.4 Viral Particle Vaccines

Brief background on the therapeutic.

4.6.5 Oligonucleotide Therapies

Add background and other information below

4.7 Underexplored Therapeutics

The majority of current clinical trials and lines of investigation have focused on repurposing existing therapies to counter SARS-CoV-2 and treat its symptoms. This is necessary given the urgency of the situation as well as the extensive time required for developing and testing new therapies. However, in the long-term, new drugs specific for treatment of COVID-19 may also enter development. There is thus value in investigating two lines of inquiry for treatment of COVID-19: 1) new therapeutics specific for treatment of COVID-19 or its symptoms, and 2) repurposing of existing therapeutics for treatment of COVID-19 or its symptoms. Here we consider further avenues that scientific investigators may explore in the development of therapies for COVID-19.

Given the great focus in investigating hydroxychloroquine (HCQ) as a potential antiviral treatment for SARS-CoV-2, it may be of interest to researchers to explore related alternatives. For example, hydroxyferroquine derivatives of HCQ have been described as a class of bioorganometallic compounds that exert antiviral effects with some selectivity for SARS-CoV [255]. Future work could explore whether these compounds exert antiviral effects against SARS-CoV-2 and whether they are safe for use in animals and humans.

The tocilizumab trial described in an above section [22] studies the possibility of using an anti-inflammatory agent typically used for the treatment of autoimmune disease to counter the effects of the “cytokine storm” induced by the virus. Another anti-IL-6 antibody, sarilumab, is also being investigated [256,257]. Typically, immunosuppressive drugs such as these are contraindicated in the setting of infection [258]. However, COVID-19 results in hyperinflammation that appears to contribute to mortality via lung damage, suggesting that immunosuppression may be a helpful approach to treatment [37]. The decision of whether and/or when to counter hyperinflammation with immunosuppression in the setting of COVID-19 remains in debate as the risks of inhibiting antiviral immunity continue to be weighed against the beneficial anti-inflammatory effects [259]. If the need to

curtail the “cytokine storm” inflammatory response to the virus transcends the risks of immunosuppression, exploration of more anti-inflammatory agents may be warranted; these agents are considered here. While tocilizumab targets IL-6, several other inflammatory markers could be potential targets, including TNF-alpha. Inhibition of TNF-alpha by an inhibitor such as Etanercept has been previously suggested for treatment of SARS-CoV [260] and may be relevant for SARS-CoV-2 as well. Baricitinib and other small molecule inhibitors of the JAK kinase pathway also curtail the inflammatory response and have been suggested as potential options for SARS-CoV-2 infections [261]. Baricitinib in particular may be able to reduce the ability of SARS-CoV-2 to infect lung cells [262]. Clinical trials studying baricitinib in COVID-19 have already begun in the US and in Italy [263,264]. Identification and targeting of further inflammatory markers that are relevant in SARS-CoV-2 infection may be of value for curtailing the inflammatory response and lung damage. Lastly, it is also worth noting the high costs of tocilizumab therapy and other biologics: at doses used for rheumatoid arthritis patients, the cost for tocilizumab ranges from $179.20 to $896 per dose for the IV form and $355 for the pre-filled syringe [265]. Cyclosporine may be a more cost-effective and readily-available alternative than biologics [266], if it proves effective against the cytokine storm induced by SARS-CoV-2.

Another approach is the development of antivirals, which could be broad-spectrum, specific to coronaviruses, or targeted to SARS-CoV-2. Given the increasingly apparent role of the cytokine storm in disease pathogenesis, it is possible that antivirals could be less effective in more severe cases of COVID-19, but this is not yet known; regardless, it is likely that early-stage patients could benefit from antiviral therapy. The potential for remdesivir as an antiviral has already been described in an above section. Development of new antivirals is complicated by the fact that none have yet been approved for human coronaviruses. Intriguing new options are emerging, however. Beta-D-N4-hydroxycytidine (NHC) is an orally bioavailable ribonucleotide analog showing broad-spectrum activity against RNA viruses, which may inhibit SARS-CoV-2 replication [267]. Various other antivirals are in development. Development of antivirals will be further facilitated as research reveals more information about the interaction of SARS-CoV-2 with the host cell and host cell genome, mechanisms of viral replication, mechanisms of viral assembly, and mechanisms of viral release to other cells; this can allow researchers to target specific stages and structures of the viral life cycle.

Antibodies against viruses, also known as antiviral monoclonal antibodies, could be an alternative as well and are described in detail in an above section. The goal of antiviral antibodies is to neutralize viruses through either cell-killing activity or blocking of viral replication [268]. They may also engage the host immune response, encouraging the immune system to hone in on the virus. Given the cytokine storm that results from immune system activation in response to the virus, which has been implicated in worsening of the disease, a neutralizing antibody (nAb) may be preferable. Upcoming work may explore the specificity of nAbs for their target, mechanisms by which the nAbs impede the virus, and improvements to antibody structure that may enhance the ability of the antibody to block viral activity.

In the longer term, as more information becomes available about the structures of SARS-CoV-2 components, small molecule inhibitors of those components may become candidates for drug discovery. For example, crystal structures of the SARS-CoV-2 main protease have recently been resolved [269,270]. Efforts have already been in place to perform screens for small molecule inhibitors of the main protease, yielding potential hits [269]. Much work remains to be done to determine further crystal structures of other viral components, understand the relative utility of targeting different viral components, perform additional small molecule inhibitor screens, and determine the safety and efficacy of the potential inhibitors. While still nascent, work in this area is promising.

5 Methods

5.1 Article Selection and Evaluation

The authors solicited relevant articles to be submitted via GitHub for review. Articles were classified as *diagnostic*, *therapeutic*, or *other*. Following a framework often used for assessing medical literature, the review consisted of examining methods used in each relevant article, assignment (whether the study was observational or randomized), assessment, results, interpretation, and how well the study extrapolates [271].

5.1.1 Diagnostic Papers

5.1.1.1 Methods

Reviewers began by describing the study question(s) being investigated by the article. They then described the study population, the sample size, the prevalence of the disease in the study population, countries / regions considered in case of human subjects, demographics of participants, the setting, and any remaining inclusion / exclusion criteria considered. They then described the reference test or "gold standard," if one was utilized.

5.1.1.2 Assignment

Reviewers described how new and reference tests were assigned, including additional relevant details about the study design. For example, reviewers were asked whether the diagnostic test resulted in rigorous assignments of case status or was biased towards sicker or healthier individuals.

5.1.1.3 Assessment

Reviewers described how the test was performed. For example, for both standard and reference tests, reviewers described technical details of assays used, when measurements were taken and by whom. Subsequently, they described how individuals were classified as positive or negative cases and whether results were precise and reproducible with repeated tests. Reviewers described whether there were any missing data, whether some participants underwent only one test, or whether there were individuals with inconclusive results.

5.1.1.4 Results

Reviewers reported the estimated sensitivity, specificity, positive predictive value (PPV), and negative predicted value (NPV), as well as confidence bounds around these measures, if provided.

5.1.1.5 Interpretation

Reviewers reported how well the test ruled in or ruled out disease based on the population, if there were identified side effects, and patient adherence.

5.1.1.6 Extrapolation

Reviewers described how well this test will extrapolate outside the measured population.

5.1.2 Therapeutic Papers

5.1.2.1 Methods

Reviewers began by describing the study question(s) being investigated by the article. They then described the study population, the sample size, the prevalence of the disease in the study population, countries / regions considered in case of human subjects, demographics of participants, the setting, and any remaining inclusion / exclusion criteria considered.

5.1.2.2 Assignment

Reviewers described how the treatment is assigned, whether it was an interventional or observational study, whether randomization took place, etc.

5.1.2.3 Assessment

5.1.2.3.1 Outcome Assessment

Reviewers described the outcome that was assessed and evaluated whether it was appropriate given the underlying study question. They described whether there were any missing data such as whether there were individuals lost to follow up. They then describe whether there were any potential sources of bias such as lack of blinding in a randomized controlled trial.

5.1.2.3.2 Statistical Methods Assessment

Reviewers described which statistical methods were used for inference and whether applied methods were appropriate for the study. They then described whether adjustments were made for possible confounders.

5.1.2.4 Results

Reviewers described the estimated association between the treatment and outcome. They described measures of confidence or statistical significance, if provided.

5.1.2.5 Interpretation

Reviewers described whether a causal claim could be made. They described whether any side effects or interactions with other drugs were identified, as well as any subgroup findings.

5.1.2.6 Extrapolation

Reviewers describe how the study may extrapolate to a different species or population.

5.2 Collaborative Writing

Crowd-sourced writing with Manubot [272].

6 Additional Items

6.1 Competing Interests

Author	Competing Interests	Last Reviewed
Halie M. Rando	None	2020-03-22
Casey S. Greene	None	2020-03-22
Michael P. Robson	None	2020-03-23
Simina M. Boca	None	2020-03-23
Nils Wellhausen	None	2020-03-22
Ronan Lordan	None	2020-03-25
Christian Brueffer	None	2020-03-25
Sadipan Ray	None	2020-03-25
Lucy D'Agostino McGowan	None	2020-03-26
Anthony Gitter	None	2020-03-26
Ronnie M. Russell	None	2020-04-07
Anna Ada Dattoli	None	2020-03-26
Ryan Velazquez	None	2020-04-04
John P. Barton	None	2020-04-06
Jeffrey M. Field	None	2020-03-30
Bharath Ramsundar	None	2020-04-06
Adam L. MacLean	None	2020-04-06
Alexandra J. Lee	None	2020-04-07
Immunology Institute of the Icahn School of Medicine	None	2020-04-07
Fengling Hu	None	2020-04-08
Nafisa M. Jadavji	None	2020-04-09
Elizabeth Sell	None	2020-04-10
Jinhui Wang	None	2020-04-13
Diane N. Rafizadeh	None	2020-04-14
Ashwin N. Skelly	None	2020-04-16
Marouen Ben Guebila	None	2020-04-17
Likhitha Kolla	None	2020-04-23
David Manheim	None	2020-04-28
Soumita Ghosh	None	2020-04-28
Matthias Fax	None	2020-04-30
James Brian Byrd	Funded by FastGrants to conduct a COVID-19-related clinical trial	2020-04-23

6.2 Author Contributions

Author	Contributions
Halie M. Rando	Project Administration, Writing - Original Draft, Writing - Review & Editing, Methodology
Casey S. Greene	Conceptualization, Software
Michael P. Robson	Software
Simina M. Boca	Methodology
Nils Wellhausen	Writing - Original Draft, Writing - Review & Editing, Project Administration
Ronan Lordan	Writing - Original Draft, Writing - Review & Editing
Christian Brueffer	Writing - Original Draft, Writing - Review & Editing, Project Administration
Sadipan Ray	Writing - Original Draft
Lucy D'Agostino McGowan	Methodology, Writing - Original Draft
Anthony Gitter	Methodology, Software, Project Administration
Ronnie M. Russell	Writing - Original Draft, Writing - Review & Editing
Anna Ada Dattoli	Writing - Original Draft
Ryan Velazquez	Methodology, Software
John P. Barton	Writing - Original Draft, Writing - Review & Editing
Jeffrey M. Field	Writing - Original Draft
Bharath Ramsundar	Investigation, Writing - Review & Editing
Adam L. MacLean	Writing - Original Draft
Alexandra J. Lee	Writing - Original Draft
Immunology Institute of the Icahn School of Medicine	Data Curation
Fengling Hu	Writing - Original Draft, Writing - Review & Editing
Nafisa M. Jadavji	Writing - Original Draft, Writing - Review & Editing
Elizabeth Sell	Writing - Original Draft, Writing - Review & Editing
Jinhui Wang	Writing - Revising & Editing
Diane N. Rafizadeh	Writing - Original Draft, Writing - Review & Editing
Ashwin N. Skelly	Writing - Original Draft, Writing - Review & Editing
Marouen Ben Guebila	Writing - Original Draft
Likhitha Kolla	Writing - Original Draft
David Manheim	Writing - Original Draft, Investigation
Soumita Ghosh	Writing - Original Draft
Matthias Fax	Writing - Review & Editing
James Brian Byrd	Writing - Original Draft, Writing - Review & Editing

7 References

1. Cramer
(2020-01-27) https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019-ncov.pdf

2. Ikejezie, Mr. Juniorcaius (WDC)
(2020-01-28) https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200128-sitrep-8-ncov-cleared.pdf

3. Ikejezie, Mr. Juniorcaius (WDC)
(2020-03-11) https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200311-sitrep-51-covid-19.pdf

4. Ikejezie, Mr. Juniorcaius (WDC)
(2020-04-05) https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200404-sitrep-75-covid-19.pdf?sfvrsn

5. **Fields virology**
Bernard N. Fields, David M. Knipe, Peter M. Howley (editors)
Wolters Kluwer Health/Lippincott Williams & Wilkins (2007)
ISBN: 9780781760607

6. **Coronaviruses: An Overview of Their Replication and Pathogenesis**
Anthony R. Fehr, Stanley Perlman
Methods in Molecular Biology (2015) https://doi.org/ggpc6n
DOI: 10.1007/978-1-4939-2438-7_1 · PMID: 25720466 · PMCID: PMC4369385

7. **Structure, Function, and Evolution of Coronavirus Spike Proteins**
Fang Li
Annual Review of Virology (2016-09-29) https://doi.org/ggr7gv
DOI: 10.1146/annurev-virology-110615-042301 · PMID: 27578435 · PMCID: PMC5457962

8. **Coronavirus 229E-Related Pneumonia in Immunocompromised Patients**
F. Pene, A. Merlat, A. Vabret, F. Rozenberg, A. Buzyn, F. Dreyfus, A. Cariou, F. Freymuth, P. Lebon
Clinical Infectious Diseases (2003-10-01) https://doi.org/dcjk64
DOI: 10.1086/377612 · PMID: 13130404 · PMCID: PMC7107892

9. **Origin and evolution of pathogenic coronaviruses**
Jie Cui, Fang Li, Zheng-Li Shi
Nature Reviews Microbiology (2018-12-10) https://doi.org/ggh4vb
DOI: 10.1038/s41579-018-0118-9 · PMID: 30531947 · PMCID: PMC7097006

10. **Human Coronaviruses: A Review of Virus–Host Interactions**
Yvonne Lim, Yan Ng, James Tam, Ding Liu
Diseases (2016-07-25) https://doi.org/ggjs23
DOI: 10.3390/diseases4030026 · PMID: 28933406 · PMCID: PMC5456285

11. **SARS and MERS: recent insights into emerging coronaviruses**
Emmie de Wit, Neeltje van Doremalen, Darryl Falzarano, Vincent J. Munster
Nature Reviews Microbiology (2016-06-27) https://doi.org/f8v5cv
DOI: 10.1038/nrmicro.2016.81 · PMID: 27344959 · PMCID: PMC7097822

12. **A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster**
Jasper Fuk-Woo Chan, Shuofeng Yuan, Kin-Hang Kok, Kelvin Kai-Wang To, Hin Chu, Jin Yang, Fanfan Xing, Jieling Liu, Cyril Chik-Yan Yip, Rosana Wing-Shan Poon, … Kwok-Yung Yuen
The Lancet (2020-02) https://doi.org/ggjs7j
DOI: 10.1016/s0140-6736(20)30154-9 · PMID: 31986261 · PMCID: PMC7159286

13. **Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding**
Roujian Lu, Xiang Zhao, Juan Li, Peihua Niu, Bo Yang, Honglong Wu, Wenling Wang, Hao Song, Baoying Huang, Na Zhu, … Wenjie Tan
The Lancet (2020-02) https://doi.org/ggjr43
DOI: 10.1016/s0140-6736(20)30251-8 · PMID: 32007145 · PMCID: PMC7159086

14. **Emerging coronaviruses: Genome structure, replication, and pathogenesis**
Yu Chen, Qianyun Liu, Deyin Guo
Journal of Medical Virology (2020-02-07) https://doi.org/ggjvwj
DOI: 10.1002/jmv.25681 · PMID: 31967327 · PMCID: PMC7167049

15. **Coronaviridae ~ ViralZone page** https://viralzone.expasy.org/30?outline

16. **Transmission routes of 2019-nCoV and controls in dental practice**
Xian Peng, Xin Xu, Yuqing Li, Lei Cheng, Xuedong Zhou, Biao Ren
International Journal of Oral Science (2020-03-03) https://doi.org/ggnf47
DOI: 10.1038/s41368-020-0075-9 · PMID: 32127517 · PMCID: PMC7054527

17. **Evidence of Airborne Transmission of the Severe Acute Respiratory Syndrome Virus**
Ignatius T. S. Yu, Yuguo Li, Tze Wai Wong, Wilson Tam, Andy T. Chan, Joseph H. W. Lee, Dennis Y. C. Leung, Tommy Ho
New England Journal of Medicine (2004-04-22) https://doi.org/bz7skr
DOI: 10.1056/nejmoa032867 · PMID: 15102999

18. **Airflow as a Possible Transmission Route of Middle East Respiratory Syndrome at an Initial Outbreak Hospital in Korea**
Minki Sung, Seongmin Jo, Sang-Eun Lee, Moran Ki, Bo Choi, JinKwan Hong
International Journal of Environmental Research and Public Health (2018-12-06) https://doi.org/ggqsnk
DOI: 10.3390/ijerph15122757 · PMID: 30563206 · PMCID: PMC6313554

19. **The proximal origin of SARS-CoV-2**
Kristian G. Andersen, Andrew Rambaut, W. Ian Lipkin, Edward C. Holmes, Robert F. Garry
Nature Medicine (2020-03-17) https://doi.org/ggn4dn
DOI: 10.1038/s41591-020-0820-9 · PMID: 32284615 · PMCID: PMC7095063

20. **A pneumonia outbreak associated with a new coronavirus of probable bat origin**
Peng Zhou, Xing-Lou Yang, Xian-Guang Wang, Ben Hu, Lei Zhang, Wei Zhang, Hao-Rui Si, Yan Zhu, Bei Li, Chao-Lin Huang, … Zheng-Li Shi

Nature (2020-02-03) https://doi.org/ggj5cg
DOI: 10.1038/s41586-020-2012-7 · PMID: 32015507 · PMCID: PMC7095418

21. **Pangolin homology associated with 2019-nCoV**
Tao Zhang, Qunfu Wu, Zhigang Zhang
bioRxiv (2020-02-20) https://doi.org/ggpvpt
DOI: 10.1101/2020.02.19.950253

22. **Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study**
Fei Zhou, Ting Yu, Ronghui Du, Guohui Fan, Ying Liu, Zhibo Liu, Jie Xiang, Yeming Wang, Bin Song, Xiaoying Gu, … Bin Cao
The Lancet (2020-03) https://doi.org/ggnxb3
DOI: 10.1016/s0140-6736(20)30566-3

23. **Structure of SARS Coronavirus Spike Receptor-Binding Domain Complexed with Receptor**
F. Li
Science (2005-09-16) https://doi.org/fww324
DOI: 10.1126/science.1116480 · PMID: 16166518

24. **Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein**
Alexandra C. Walls, Young-Jun Park, M. Alejandra Tortorici, Abigail Wall, Andrew T. McGuire, David Veesler
Cell (2020-03) https://doi.org/dpvh
DOI: 10.1016/j.cell.2020.02.058 · PMID: 32155444 · PMCID: PMC7102599

25. **Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2**
Renhong Yan, Yuanyuan Zhang, Yaning Li, Lu Xia, Yingying Guo, Qiang Zhou
Science (2020-03-27) https://doi.org/ggpxc8
DOI: 10.1126/science.abb2762 · PMID: 32132184 · PMCID: PMC7164635

26. **Structural basis of receptor recognition by SARS-CoV-2**
Jian Shang, Gang Ye, Ke Shi, Yushun Wan, Chuming Luo, Hideki Aihara, Qibin Geng, Ashley Auerbach, Fang Li
Nature (2020-03-30) https://doi.org/ggqspv
DOI: 10.1038/s41586-020-2179-y · PMID: 32225175

27. **Crystal structure of the 2019-nCoV spike receptor-binding domain bound with the ACE2 receptor**
Jun Lan, Jiwan Ge, Jinfang Yu, Sisi Shan, Huan Zhou, Shilong Fan, Qi Zhang, Xuanling Shi, Qisheng Wang, Linqi Zhang, Xinquan Wang
bioRxiv (2020-02-20) https://doi.org/ggqzp5
DOI: 10.1101/2020.02.19.956235

28. **SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor**
Markus Hoffmann, Hannah Kleine-Weber, Simon Schroeder, Nadine Krüger, Tanja Herrler, Sandra Erichsen, Tobias S. Schiergens, Georg Herrler, Nai-Huei Wu, Andreas Nitsche, … Stefan Pöhlmann
Cell (2020-03) https://doi.org/ggnq74
DOI: 10.1016/j.cell.2020.02.052 · PMID: 32142651 · PMCID: PMC7102627

29. **Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2**
Qihui Wang, Yanfang Zhang, Lili Wu, Sheng Niu, Chunli Song, Zengyuan Zhang, Guangwen Lu,

Chengpeng Qiao, Yu Hu, Kwok-Yung Yuen, … Jianxun Qi
Cell (2020-04) https://doi.org/ggr2cz
DOI: 10.1016/j.cell.2020.03.045 · PMID: 32275855 · PMCID: PMC7144619

30. **Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS**
Yushun Wan, Jian Shang, Rachel Graham, Ralph S. Baric, Fang Li
Journal of Virology (2020-01-29) https://doi.org/ggjvwn
DOI: 10.1128/jvi.00127-20 · PMID: 31996437 · PMCID: PMC7081895

31. **Increasing Host Cellular Receptor—Angiotensin-Converting Enzyme 2 (ACE2) Expression by Coronavirus may Facilitate 2019-nCoV Infection**
Pei-Hui Wang, Yun Cheng
bioRxiv (2020-02-27) https://doi.org/ggscwd
DOI: 10.1101/2020.02.24.963348

32. **Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target**
Haibo Zhang, Josef M. Penninger, Yimin Li, Nanshan Zhong, Arthur S. Slutsky
Intensive Care Medicine (2020-03-03) https://doi.org/ggpx6p
DOI: 10.1007/s00134-020-05985-9 · PMID: 32125455 · PMCID: PMC7079879

33. **Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation**
Daniel Wrapp, Nianshuang Wang, Kizzmekia S. Corbett, Jory A. Goldsmith, Ching-Lin Hsieh, Olubukola Abiona, Barney S. Graham, Jason S. McLellan
Science (2020-02-19) https://doi.org/ggmtk2
DOI: 10.1126/science.abb2507 · PMID: 32075877 · PMCID: PMC7164637

34. **Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV**
Xiuyuan Ou, Yan Liu, Xiaobo Lei, Pei Li, Dan Mi, Lili Ren, Li Guo, Ruixuan Guo, Ting Chen, Jiaxin Hu, … Zhaohui Qian
Nature Communications (2020-03-27) https://doi.org/ggqsrf
DOI: 10.1038/s41467-020-15562-9 · PMID: 32221306 · PMCID: PMC7100515

35. **Structure of the Hemagglutinin Precursor Cleavage Site, a Determinant of Influenza Pathogenicity and the Origin of the Labile Conformation**
Jue Chen, Kon Ho Lee, David A Steinhauer, David J Stevens, John J Skehel, Don C Wiley
Cell (1998-10) https://doi.org/bvgh5b
DOI: 10.1016/s0092-8674(00)81771-7

36. **Role of Hemagglutinin Cleavage for the Pathogenicity of Influenza Virus**
David A. Steinhauer
Virology (1999-05) https://doi.org/fw3jz4
DOI: 10.1006/viro.1999.9716 · PMID: 10329563

37. **COVID-19: consider cytokine storm syndromes and immunosuppression**
Puja Mehta, Daniel F McAuley, Michael Brown, Emilie Sanchez, Rachel S Tattersall, Jessica J Manson
The Lancet (2020-03) https://doi.org/ggnzmc
DOI: 10.1016/s0140-6736(20)30628-0

38. **The concept of R_0 in epidemic theory**
J. A. P. Heesterbeek, K. Dietz

Statistica Neerlandica (1996-03) https://doi.org/d29ch4
DOI: 10.1111/j.1467-9574.1996.tb01482.x

39. **Population Dynamics of Immune Responses to Persistent Viruses**
M. A. Nowak, C. R. M. Bangham
Science (1996-04-05) https://doi.org/fqgprd
DOI: 10.1126/science.272.5258.74 · PMID: 8600540

40. **Nowcasting and forecasting the potential domestic and international spread of the 2019-nCoV outbreak originating in Wuhan, China: a modelling study**
Joseph T Wu, Kathy Leung, Gabriel M Leung
The Lancet (2020-01) https://doi.org/ggjvr7
DOI: 10.1016/s0140-6736(20)30260-9 · PMID: 32014114 · PMCID: PMC7159271

41. **The reproductive number of COVID-19 is higher compared to SARS coronavirus**
Ying Liu, Albert A Gayle, Annelies Wilder-Smith, Joacim Rocklöv
Journal of Travel Medicine (2020-02-13) https://doi.org/ggnntv
DOI: 10.1093/jtm/taaa021 · PMID: 32052846 · PMCID: PMC7074654

42. **Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV2)**
Ruiyun Li, Sen Pei, Bin Chen, Yimeng Song, Tao Zhang, Wan Yang, Jeffrey Shaman
Science (2020-03-16) https://doi.org/ggn6c2
DOI: 10.1126/science.abb3221 · PMID: 32179701 · PMCID: PMC7164387

43. **Epidemiological parameters of coronavirus disease 2019: a pooled analysis of publicly reported individual data of 1155 cases from seven countries**
Shujuan Ma, Jiayue Zhang, Minyan Zeng, Qingping Yun, Wei Guo, Yixiang Zheng, Shi Zhao, Maggie H Wang, Zuyao Yang
medRxiv (2020-03-24) https://doi.org/ggqhzz
DOI: 10.1101/2020.03.21.20040329

44. **Early Transmissibility Assessment of a Novel Coronavirus in Wuhan, China**
Maimuna Majumder, Kenneth D. Mandl
SSRN Electronic Journal (2020) https://doi.org/ggqhz3
DOI: 10.2139/ssrn.3524675

45. **Time-varying transmission dynamics of Novel Coronavirus Pneumonia in China**
Tao Liu, Jianxiong Hu, Jianpeng Xiao, Guanhao He, Min Kang, Zuhua Rong, Lifeng Lin, Haojie Zhong, Qiong Huang, Aiping Deng, … Wenjun Ma
bioRxiv (2020-02-13) https://doi.org/dkx9
DOI: 10.1101/2020.01.25.919787

46. **Estimation of the reproductive number of novel coronavirus (COVID-19) and the probable outbreak size on the Diamond Princess cruise ship: A data-driven analysis**
Sheng Zhang, MengYuan Diao, Wenbo Yu, Lei Pei, Zhaofen Lin, Dechang Chen
International Journal of Infectious Diseases (2020-04) https://doi.org/ggpx56
DOI: 10.1016/j.ijid.2020.02.033 · PMID: 32097725 · PMCID: PMC7110591

47. **Estimation of the Transmission Risk of the 2019-nCoV and Its Implication for Public Health Interventions**
Tang, Wang, Li, Bragazzi, Tang, Xiao, Wu

Journal of Clinical Medicine (2020-02-07) https://doi.org/ggmkf4
DOI: 10.3390/jcm9020462 · PMID: 32046137 · PMCID: PMC7074281

48. **Estimating the effective reproduction number of the 2019-nCoV in China**
Zhidong Cao, Qingpeng Zhang, Xin Lu, Dirk Pfeiffer, Zhongwei Jia, Hongbing Song, Daniel Dajun Zeng
medRxiv (2020-01) https://www.medrxiv.org/content/10.1101/2020.01.27.20018952v1
DOI: 10.1101/2020.01.27.20018952

49. **Modelling the epidemic trend of the 2019 novel coronavirus outbreak in China**
Mingwang Shen, Zhihang Peng, Yanni Xiao, Lei Zhang
bioRxiv (2020-01-25) https://doi.org/ggqhzw
DOI: 10.1101/2020.01.23.916726

50. **Novel coronavirus 2019-nCoV: early estimation of epidemiological parameters and epidemic predictions**
Jonathan M Read, Jessica RE Bridgen, Derek AT Cummings, Antonia Ho, Chris P Jewell
medRxiv (2020-01-28) https://doi.org/dkzb
DOI: 10.1101/2020.01.23.20018549

51. **Using early data to estimate the actual infection fatality ratio from COVID-19 in France**
Lionel Roques, Etienne Klein, Julien Papaix, Antoine Sar, Samuel Soubeyrand
medRxiv (2020-05-07) https://doi.org/ggqhz2
DOI: 10.1101/2020.03.22.20040915

52. **Potential roles of social distancing in mitigating the spread of coronavirus disease 2019 (COVID-19) in South Korea**
Sang Woo Park, Kaiyuan Sun, Cécile Viboud, Bryan T Grenfell, Jonathan Dushoff
GitHub (2020) https://github.com/parksw3/Korea-analysis/blob/master/v1/korea.pdf

53. **Early dynamics of transmission and control of COVID-19: a mathematical modelling study**
Adam J Kucharski, Timothy W Russell, Charlie Diamond, Yang Liu, John Edmunds, Sebastian Funk, Rosalind M Eggo, Fiona Sun, Mark Jit, James D Munday, … Stefan Flasche
The Lancet Infectious Diseases (2020-03) https://doi.org/ggptcf
DOI: 10.1016/s1473-3099(20)30144-4 · PMID: 32171059 · PMCID: PMC7158569

54. **Estimating the reproduction number of COVID-19 in Iran using epidemic modeling**
Ebrahim Sahafizadeh, Samaneh Sartoli
medRxiv (2020-04-23) https://doi.org/ggqhzx
DOI: 10.1101/2020.03.20.20038422

55. **Report 13: Estimating the number of infections and the impact of non-pharmaceutical interventions on COVID-19 in 11 European countries**
S Flaxman, S Mishra, A Gandy, H Unwin, H Coupland, T Mellan, H Zhu, T Berah, J Eaton, P Perez Guzman, … S Bhatt
Imperial College London (2020-03-30) https://doi.org/ggrbmf
DOI: 10.25561/77731

56. **Systems Approaches to Biology and Disease Enable Translational Systems Medicine**
Leroy Hood, Qiang Tian
Genomics, Proteomics & Bioinformatics (2012-08) https://doi.org/f4f599
DOI: 10.1016/j.gpb.2012.08.004 · PMID: 23084773 · PMCID: PMC3844613

57. **A systems approach to infectious disease**
Manon Eckhardt, Judd F. Hultquist, Robyn M. Kaake, Ruth Hüttenhain, Nevan J. Krogan
Nature Reviews Genetics (2020-02-14) https://doi.org/ggnv63
DOI: 10.1038/s41576-020-0212-5 · PMID: 32060427

58. **Differential expression of serum/plasma proteins in various infectious diseases: Specific or nonspecific signatures**
Sandipan Ray, Sandip K. Patel, Vipin Kumar, Jagruti Damahe, Sanjeeva Srivastava
PROTEOMICS - Clinical Applications (2014-02) https://doi.org/f2px3h
DOI: 10.1002/prca.201300074 · PMID: 24293340 · PMCID: PMC7168033

59. **A new coronavirus associated with human respiratory disease in China**
Fan Wu, Su Zhao, Bin Yu, Yan-Mei Chen, Wen Wang, Zhi-Gang Song, Yi Hu, Zhao-Wu Tao, Jun-Hua Tian, Yuan-Yuan Pei, … Yong-Zhen Zhang
Nature (2020-02-03) https://doi.org/dk2w
DOI: 10.1038/s41586-020-2008-3 · PMID: 32015508 · PMCID: PMC7094943

60. **SARS-CoV-2 infected host cell proteomics reveal potential therapy targets**
Denisa Bojkova, Kevin Klann, Benjamin Koch, Marek Widera, David Krause, Sandra Ciesek, Jindrich Cinatl, Christian Münch
(2020-03-11) https://doi.org/ggn4ds
DOI: 10.21203/rs.3.rs-17218/v1

61. **Potent human neutralizing antibodies elicited by SARS-CoV-2 infection**
Bin Ju, Qi Zhang, Xiangyang Ge, Ruoke Wang, Jiazhen Yu, Sisi Shan, Bing Zhou, Shuo Song, Xian Tang, Jinfang Yu, … Linqi Zhang
bioRxiv (2020-03-26) https://doi.org/ggp7t4
DOI: 10.1101/2020.03.21.990770

62. **Plasma proteome of severe acute respiratory syndrome analyzed by two-dimensional gel electrophoresis and mass spectrometry**
J.-H. Chen, Y.-W. Chang, C.-W. Yao, T.-S. Chiueh, S.-C. Huang, K.-Y. Chien, A. Chen, F.-Y. Chang, C.-H. Wong, Y.-J. Chen
Proceedings of the National Academy of Sciences (2004-11-30) https://doi.org/dtv8sx
DOI: 10.1073/pnas.0407992101 · PMID: 15572443 · PMCID: PMC535397

63. **Analysis of multimerization of the SARS coronavirus nucleocapsid protein**
Runtao He, Frederick Dobie, Melissa Ballantine, Andrew Leeson, Yan Li, Nathalie Bastien, Todd Cutts, Anton Andonov, Jingxin Cao, Timothy F. Booth, … Xuguang Li
Biochemical and Biophysical Research Communications (2004-04) https://doi.org/dbfwr9
DOI: 10.1016/j.bbrc.2004.02.074 · PMID: 15020242 · PMCID: PMC7111152

64. **A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing**
David E. Gordon, Gwendolyn M. Jang, Mehdi Bouhaddou, Jiewei Xu, Kirsten Obernier, Matthew J. O'Meara, Jeffrey Z. Guo, Danielle L. Swaney, Tia A. Tummino, Ruth Hüttenhain, … Nevan J. Krogan
bioRxiv (2020-03-22) https://doi.org/ggpptg
DOI: 10.1101/2020.03.22.002386

65. **Virus-host interactome and proteomic survey of PMBCs from COVID-19 patients reveal potential virulence factors influencing SARS-CoV-2 pathogenesis**
Jingjiao Li, Mingquan Guo, Xiaoxu Tian, Chengrong Liu, Xin Wang, Xing Yang, Ping Wu, Zixuan Xiao, Yafei Qu, Yue Yin, … Qiming Liang

bioRxiv (2020-04-02) https://doi.org/ggrgbv
DOI: 10.1101/2020.03.31.019216

66. **The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) envelope (E) protein harbors a conserved BH3-like sequence**
Vincent Navratil, Loïc Lionnard, Sonia Longhi, J. Marie Hardwick, Christophe Combet, Abdel Aouacheria
bioRxiv (2020-04-21) https://doi.org/ggrp43
DOI: 10.1101/2020.04.09.033522

67. **Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR**
Victor M Corman, Olfert Landt, Marco Kaiser, Richard Molenkamp, Adam Meijer, Daniel KW Chu, Tobias Bleicker, Sebastian Brünink, Julia Schneider, Marie Luisa Schmidt, … Christian Drosten
Eurosurveillance (2020-01-23) https://doi.org/ggjs7g
DOI: 10.2807/1560-7917.es.2020.25.3.2000045 · PMID: 31992387 · PMCID: PMC6988269

68. **Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study**
Kelvin Kai-Wang To, Owen Tak-Yin Tsang, Wai-Shing Leung, Anthony Raymond Tam, Tak-Chiu Wu, David Christopher Lung, Cyril Chik-Yan Yip, Jian-Piao Cai, Jacky Man-Chun Chan, Thomas Shiu-Hong Chik, … Kwok-Yung Yuen
The Lancet Infectious Diseases (2020-03) https://doi.org/ggp4qx
DOI: 10.1016/s1473-3099(20)30196-1 · PMID: 32213337 · PMCID: PMC7158907

69. **Fecal specimen diagnosis 2019 novel coronavirus–infected pneumonia**
JingCheng Zhang, SaiBin Wang, YaDong Xue
Journal of Medical Virology (2020-03-12) https://doi.org/ggpx6d
DOI: 10.1002/jmv.25742 · PMID: 32124995

70. **Library preparation for next generation sequencing: A review of automation strategies**
J. F. Hess, T. A. Kohl, M. Kotrová, K. Rönsch, T. Paprotka, V. Mohr, T. Hutzenlaub, M. Brüggemann, R. Zengerle, S. Niemann, N. Paust
Biotechnology Advances (2020-03) https://doi.org/ggth2v
DOI: 10.1016/j.biotechadv.2020.107537 · PMID: 32199980

71. **Diagnosing COVID-19: The Disease and Tools for Detection**
Buddhisha Udugama, Pranav Kadhiresan, Hannah N. Kozlowski, Ayden Malekjahani, Matthew Osborne, Vanessa Y. C. Li, Hongmin Chen, Samira Mubareka, Jonathan B. Gubbay, Warren C. W. Chan
ACS Nano (2020-03-30) https://doi.org/ggq8ds
DOI: 10.1021/acsnano.0c02624 · PMID: 32223179 · PMCID: PMC7144809

72. **Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia**
Daniel KW Chu, Yang Pan, Samuel MS Cheng, Kenrie PY Hui, Pavithra Krishnan, Yingzhi Liu, Daisy YM Ng, Carrie KC Wan, Peng Yang, Quanyi Wang, … Leo LM Poon
Clinical Chemistry (2020-01-31) https://doi.org/ggnbpp
DOI: 10.1093/clinchem/hvaa029 · PMID: 32031583 · PMCID: PMC7108203

73. **Evaluation of COVID-19 RT-qPCR test in multi-sample pools**
Idan Yelin, Noga Aharony, Einat Shaer-Tamar, Amir Argoetti, Esther Messer, Dina Berenbaum, Einat Shafran, Areen Kuzli, Nagam Gandali, Tamar Hashimshony, … Roy Kishony
medRxiv (2020-03-27) https://doi.org/ggrn74
DOI: 10.1101/2020.03.26.20039438

74. **Analytical Validation of a COVID-19 qRT-PCR Detection Assay Using a 384-well Format and Three Extraction Methods**
Andrew C. Nelson, Benjamin Auch, Matthew Schomaker, Daryl M. Gohl, Patrick Grady, Darrell Johnson, Robyn Kincaid, Kylene E. Karnuth, Jerry Daniel, Jessica K. Fiege, … Sophia Yohe
bioRxiv (2020-04-05) https://doi.org/ggs45d
DOI: 10.1101/2020.04.02.022186

75. **Development and Applications of CRISPR-Cas9 for Genome Engineering**
Patrick D. Hsu, Eric S. Lander, Feng Zhang
Cell (2014-06) https://doi.org/f6d3wg
DOI: 10.1016/j.cell.2014.05.010 · PMID: 24906146 · PMCID: PMC4343198

76. **Rapid Detection of 2019 Novel Coronavirus SARS-CoV-2 Using a CRISPR-based DETECTR Lateral Flow Assay**
James P Broughton, Xianding Deng, Guixia Yu, Clare L Fasching, Jasmeet Singh, Jessica Streithorst, Andrea Granados, Alicia Sotomayor-Gonzalez, Kelsey Zorn, Allan Gopez, … Charles Y Chiu
medRxiv (2020-03-27) https://doi.org/ggrmwt
DOI: 10.1101/2020.03.06.20032334

77. **The standard coronavirus test, if available, works well—but can new diagnostics help in this pandemic?**
Robert Service
Science (2020-03-22) https://doi.org/ggq9wm
DOI: 10.1126/science.abb8400

78. **Coronavirus and the race to distribute reliable diagnostics**
Cormac Sheridan
Nature Biotechnology (2020-02-19) https://doi.org/ggm4nt
DOI: 10.1038/d41587-020-00002-2 · PMID: 32265548

79. **The Laboratory Diagnosis of COVID-19 Infection: Current Issues and Challenges**
Yi-Wei Tang, Jonathan E. Schmitz, David H. Persing, Charles W. Stratton
Journal of Clinical Microbiology (2020-04-03) https://doi.org/ggq7h8
DOI: 10.1128/jcm.00512-20 · PMID: 32245835

80. **Negative Nasopharyngeal and Oropharyngeal Swab Does Not Rule Out COVID-19**
Poramed Winichakoon, Romanee Chaiwarith, Chalerm Liwsrisakun, Parichat Salee, Aree Goonna, Atikun Limsukon, Quanhathai Kaewpoowat
Journal of Clinical Microbiology (2020-02-26) https://doi.org/ggpw9m
DOI: 10.1128/jcm.00297-20 · PMID: 32102856 · PMCID: PMC7180262

81. **A systematic review of antibody mediated immunity to coronaviruses: antibody kinetics, correlates of protection, and association of antibody responses with severity of disease**
Angkana T. Huang, Bernardo Garcia-Carreras, Matt D. T. Hitchings, Bingyi Yang, Leah Katzelnick, Susan M Rattigan, Brooke Borgert, Carlos Moreno, Benjamin D. Solomon, Isabel Rodriguez-Barraquer, … Derek A. T. Cummings
medRxiv (2020-04-17) https://doi.org/ggsfmz
DOI: 10.1101/2020.04.14.20065771

82.
James
(2020-04-07) https://www.fda.gov/media/136625/download

83. **Detection of antibodies against SARS-CoV-2 in patients with COVID-19**
Zhe Du, Fengxue Zhu, Fuzheng Guo, Bo Yang, Tianbing Wang
Journal of Medical Virology (2020-04-10) https://doi.org/ggq7m2
DOI: 10.1002/jmv.25820 · PMID: 32243608

84. **A serological assay to detect SARS-CoV-2 seroconversion in humans**
Fatima Amanat, Daniel Stadlbauer, Shirin Strohmeier, Thi Nguyen, Veronika Chromikova, Meagan McMahon, Kaijun Jiang, Guha Asthagiri-Arunkumar, Denise Jurczyszak, Jose Polanco, … Thomas Moran
medRxiv (2020-04-16) https://doi.org/ggpn83
DOI: 10.1101/2020.03.17.20037713

85. **Coronavirus testing is ramping up. Here are the new tests and how they work.**
Stephanie Pappas-Live Science Contributor 31 March 2020
livescience.com https://www.livescience.com/coronavirus-tests-available.html

86. **Strong associations and moderate predictive value of early symptoms for SARS-CoV-2 test positivity among healthcare workers, the Netherlands, March 2020**
Alma Tostmann, John Bradley, Teun Bousema, Wing-Kee Yiek, Minke Holwerda, Chantal Bleeker-Rovers, Jaap ten Oever, Corianne Meijer, Janette Rahamat-Langendoen, Joost Hopman, … Heiman Wertheim
Eurosurveillance (2020-04-23) https://doi.org/ggthwx
DOI: 10.2807/1560-7917.es.2020.25.16.2000508 · PMID: 32347200 · PMCID: PMC7189649

87. **Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases**
Tao Ai, Zhenlu Yang, Hongyan Hou, Chenao Zhan, Chong Chen, Wenzhi Lv, Qian Tao, Ziyong Sun, Liming Xia
Radiology (2020-02-26) https://doi.org/ggmw6p
DOI: 10.1148/radiol.2020200642 · PMID: 32101510

88. **Performance of radiologists in differentiating COVID-19 from viral pneumonia on chest CT**
Harrison X. Bai, Ben Hsieh, Zeng Xiong, Kasey Halsey, Ji Whae Choi, Thi My Linh Tran, Ian Pan, Lin-Bo Shi, Dong-Cui Wang, Ji Mei, … Wei-Hua Liao
Radiology (2020-03-10) https://doi.org/ggnqw4
DOI: 10.1148/radiol.2020200823 · PMID: 32155105

89. **Covid-19: automatic detection from X-ray images utilizing transfer learning with convolutional neural networks**
Ioannis D. Apostolopoulos, Tzani A. Mpesiana
Physical and Engineering Sciences in Medicine (2020-04-03) https://doi.org/ggs448
DOI: 10.1007/s13246-020-00865-4 · PMCID: PMC7118364

90. **Coronavirus Disease 2019 (COVID-19)**
CDC
Centers for Disease Control and Prevention (2020-02-11) https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-criteria.html

91. **Nutraceuticals have potential for boosting the type 1 interferon response to RNA viruses including influenza and coronavirus**
Mark F. McCarty, James J. DiNicolantonio
Progress in Cardiovascular Diseases (2020-02) https://doi.org/ggpwx2
DOI: 10.1016/j.pcad.2020.02.007 · PMID: 32061635 · PMCID: PMC7130854

92. **Reducing mortality from 2019-nCoV: host-directed therapies should be an option**
Alimuddin Zumla, David S Hui, Esam I Azhar, Ziad A Memish, Markus Maeurer
The Lancet (2020-02) https://doi.org/ggkd3b
DOI: 10.1016/s0140-6736(20)30305-6 · PMID: 32035018 · PMCID: PMC7133595

93. **Digestive Symptoms in COVID-19 Patients With Mild Disease Severity**
Chaoqun Han, Caihan Duan, Shengyan Zhang, Brennan Spiegel, Huiying Shi, Weijun Wang, Lei Zhang, Rong Lin, Jun Liu, Zhen Ding, Xiaohua Hou
The American Journal of Gastroenterology (2020-04) https://doi.org/ggtzm2
DOI: 10.14309/ajg.0000000000000664 · PMID: 32301761 · PMCID: PMC7172493

94. **Coronavirus Disease 2019 (COVID-19)**
CDC
Centers for Disease Control and Prevention (2020-02-11) https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html

95. **Into the Eye of the Cytokine Storm**
J. R. Tisoncik, M. J. Korth, C. P. Simmons, J. Farrar, T. R. Martin, M. G. Katze
Microbiology and Molecular Biology Reviews (2012-03-05) https://doi.org/f4n9h2
DOI: 10.1128/mmbr.05015-11 · PMID: 22390970 · PMCID: PMC3294426

96. **Induction of cell cycle arrest and B cell terminal differentiation by CDK inhibitor p18(INK4c) and IL-6.**
L Morse, D Chen, D Franklin, Y Xiong, S Chen-Kiang
Immunity (1997-01) https://www.ncbi.nlm.nih.gov/pubmed/9052836
DOI: 10.1016/s1074-7613(00)80241-1 · PMID: 9052836

97. **The effects of hybridoma growth factor in conditioned media upon the growth, cloning, and antibody production of heterohybridoma cell lines.**
Y Zhu, B Jin, C Sun, C Huang, X Liu
Human antibodies and hybridomas (1993-01) https://www.ncbi.nlm.nih.gov/pubmed/8431556
PMID: 8431556

98. **Interleukin-6 is the central tumor growth factor in vitro and in vivo in multiple myeloma.**
B Klein, XG Zhang, M Jourdan, JM Boiron, M Portier, ZY Lu, J Wijdenes, J Brochier, R Bataille
European cytokine network https://www.ncbi.nlm.nih.gov/pubmed/2104241
PMID: 2104241

99. **The Biology and Medical Implications of Interleukin-6**
T. Tanaka, T. Kishimoto
Cancer Immunology Research (2014-04-03) https://doi.org/f56dzb
DOI: 10.1158/2326-6066.cir-14-0022 · PMID: 24764575

100. **Up-regulation of IL-6 and TNF-α induced by SARS-coronavirus spike protein in murine macrophages via NF-κB pathway**
Wei Wang, Linbai Ye, Li Ye, Baozong Li, Bo Gao, Yingchun Zeng, Lingbao Kong, Xiaonan Fang, Hong Zheng, Zhenghui Wu, Yinglong She
Virus Research (2007-09) https://doi.org/bm7m55
DOI: 10.1016/j.virusres.2007.02.007 · PMID: 17532082 · PMCID: PMC7114322

101. **Expression of elevated levels of pro-inflammatory cytokines in SARS-CoV-infected ACE2 $^{+}$ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS**
L He, Y Ding, Q Zhang, X Che, Y He, H Shen, H Wang, Z Li, L Zhao, J Geng, … S Jiang

The Journal of Pathology (2006-11) https://doi.org/bwb8ns
DOI: 10.1002/path.2067 · PMID: 17031779 · PMCID: PMC7167655

102. **Cytokine Balance in the Lungs of Patients with Acute Respiratory Distress Syndrome**
WILLIAM Y. PARK, RICHARD B. GOODMAN, KENNETH P. STEINBERG, JOHN T. RUZINSKI, FRANK RADELLA, DAVID R. PARK, JEROME PUGIN, SHAWN J. SKERRETT, LEONARD D. HUDSON, THOMAS R. MARTIN
American Journal of Respiratory and Critical Care Medicine (2001-11-15) https://doi.org/ggqfq7
DOI: 10.1164/ajrccm.164.10.2104013 · PMID: 11734443

103. **Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology**
Rudragouda Channappanavar, Stanley Perlman
Seminars in Immunopathology (2017-05-02) https://doi.org/ggqf2w
DOI: 10.1007/s00281-017-0629-x · PMID: 28466096 · PMCID: PMC7079893

104. **Plasticity and cross-talk of Interleukin 6-type cytokines**
Christoph Garbers, Heike M. Hermanns, Fred Schaper, Gerhard Müller-Newen, Joachim Grötzinger, Stefan Rose-John, Jürgen Scheller
Cytokine & Growth Factor Reviews (2012-06) https://doi.org/f3z743
DOI: 10.1016/j.cytogfr.2012.04.001 · PMID: 22595692

105. **Soluble receptors for cytokines and growth factors: generation and biological function**
S Rose-John, PC Heinrich
Biochemical Journal (1994-06-01) https://doi.org/ggqmgd
DOI: 10.1042/bj3000281 · PMID: 8002928 · PMCID: PMC1138158

106. **Hall of Fame among Pro-inflammatory Cytokines: Interleukin-6 Gene and Its Transcriptional Regulation Mechanisms**
Yang Luo, Song Guo Zheng
Frontiers in Immunology (2016-12-19) https://doi.org/ggqmgv
DOI: 10.3389/fimmu.2016.00604 · PMID: 28066415 · PMCID: PMC5165036

107. **Interleukin-6; pathogenesis and treatment of autoimmune inflammatory diseases**
Toshio Tanaka, Masashi Narazaki, Kazuya Masuda, Tadamitsu Kishimoto
Inflammation and Regeneration (2013) https://doi.org/ggqmgt
DOI: 10.2492/inflammregen.33.054

108. **COVID-19 – Italy launches an independent trial on tocilizumab**
COVID-19 – Italy launches an independent trial on tocilizumab | Univadis
https://www.univadis.co.uk/viewarticle/covid-19-italy-launches-an-independent-trial-on-tocilizumab-715741

109. **Risk of infections in rheumatoid arthritis patients treated with tocilizumab**
Veronika R. Lang, Matthias Englbrecht, Jürgen Rech, Hubert Nüsslein, Karin Manger, Florian Schuch, Hans-Peter Tony, Martin Fleck, Bernhard Manger, Georg Schett, Jochen Zwerina
Rheumatology (2012-05) https://doi.org/d3b3rh
DOI: 10.1093/rheumatology/ker223 · PMID: 21865281

110. **Short-course tocilizumab increases risk of hepatitis B virus reactivation in patients with rheumatoid arthritis: a prospective clinical observation**
Le-Feng Chen, Ying-Qian Mo, Jun Jing, Jian-Da Ma, Dong-Hui Zheng, Lie Dai

International Journal of Rheumatic Diseases (2017-07) https://doi.org/f9pbc5
DOI: 10.1111/1756-185x.13010 · PMID: 28160426

111. **Introduction to modern virology**
N. J. Dimmock, A. J. Easton, K. N. Leppard
Blackwell Pub (2007)
ISBN: 9781405136457

112. **Coronaviruses**
Methods in Molecular Biology
(2015) https://doi.org/ggqfqx
DOI: 10.1007/978-1-4939-2438-7 · PMID: 25870870

113. **The potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase**
Jrhau Lung, Yu-Shih Lin, Yao-Hsu Yang, Yu-Lun Chou, Li-Hsin Shu, Yu-Ching Cheng, Hung Te Liu, Ching-Yuan Wu
Journal of Medical Virology (2020-03-18) https://doi.org/ggp6fm
DOI: 10.1002/jmv.25761 · PMID: 32167173

114. **Favipiravir** https://www.drugbank.ca/drugs/DB12466

115. **In Vitro and In Vivo Activities of Anti-Influenza Virus Compound T-705**
Y. Furuta, K. Takahashi, Y. Fukuda, M. Kuno, T. Kamiyama, K. Kozaki, N. Nomura, H. Egawa, S. Minami, Y. Watanabe, ... K. Shiraki
Antimicrobial Agents and Chemotherapy (2002-04-01) https://doi.org/cndw7n
DOI: 10.1128/aac.46.4.977-981.2002 · PMID: 11897578 · PMCID: PMC127093

116. **Efficacy of Orally Administered T-705 on Lethal Avian Influenza A (H5N1) Virus Infections in Mice**
R. W. Sidwell, D. L. Barnard, C. W. Day, D. F. Smee, K. W. Bailey, M.-H. Wong, J. D. Morrey, Y. Furuta
Antimicrobial Agents and Chemotherapy (2006-12-28) https://doi.org/dm9xr2
DOI: 10.1128/aac.01051-06 · PMID: 17194832 · PMCID: PMC1803113

117. **Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase**
Yousuke FURUTA, Takashi KOMENO, Takaaki NAKAMURA
Proceedings of the Japan Academy, Series B (2017) https://doi.org/gbxcxw
DOI: 10.2183/pjab.93.027 · PMID: 28769016 · PMCID: PMC5713175

118. **Mechanism of Action of T-705 against Influenza Virus**
Y. Furuta, K. Takahashi, M. Kuno-Maekawa, H. Sangawa, S. Uehara, K. Kozaki, N. Nomura, H. Egawa, K. Shiraki
Antimicrobial Agents and Chemotherapy (2005-02-23) https://doi.org/dgbwdh
DOI: 10.1128/aac.49.3.981-986.2005 · PMID: 15728892 · PMCID: PMC549233

119. **Activity of T-705 in a Hamster Model of Yellow Fever Virus Infection in Comparison with That of a Chemically Related Compound, T-1106**
Justin G. Julander, Kristiina Shafer, Donald F. Smee, John D. Morrey, Yousuke Furuta
Antimicrobial Agents and Chemotherapy (2009-01) https://doi.org/brknds
DOI: 10.1128/aac.01074-08 · PMID: 18955536 · PMCID: PMC2612161

120. **In Vitro and In Vivo Activities of T-705 against Arenavirus and Bunyavirus Infections**
Brian B. Gowen, Min-Hui Wong, Kie-Hoon Jung, Andrew B. Sanders, Michelle Mendenhall, Kevin W. Bailey, Yousuke Furuta, Robert W. Sidwell

Antimicrobial Agents and Chemotherapy (2007-09) https://doi.org/d98c87
DOI: 10.1128/aac.00356-07 · PMID: 17606691 · PMCID: PMC2043187

121. **Favipiravir (T-705) inhibits in vitro norovirus replication**
J. Rocha-Pereira, D. Jochmans, K. Dallmeier, P. Leyssen, M. S. J. Nascimento, J. Neyts
Biochemical and Biophysical Research Communications (2012-08) https://doi.org/f369j7
DOI: 10.1016/j.bbrc.2012.07.034 · PMID: 22809499

122. **T-705 (Favipiravir) Inhibition of Arenavirus Replication in Cell Culture**
Michelle Mendenhall, Andrew Russell, Terry Juelich, Emily L. Messina, Donald F. Smee, Alexander N. Freiberg, Michael R. Holbrook, Yousuke Furuta, Juan-Carlos de la Torre, Jack H. Nunberg, Brian B. Gowen
Antimicrobial Agents and Chemotherapy (2011-02) https://doi.org/cppwsc
DOI: 10.1128/aac.01219-10 · PMID: 21115797 · PMCID: PMC3028760

123. **The evolution of nucleoside analogue antivirals: A review for chemists and non-chemists. Part 1: Early structural modifications to the nucleoside scaffold**
Katherine L. Seley-Radtke, Mary K. Yates
Antiviral Research (2018-06) https://doi.org/gdpn35
DOI: 10.1016/j.antiviral.2018.04.004 · PMID: 29649496 · PMCID: PMC6396324

124. **The Ambiguous Base-Pairing and High Substrate Efficiency of T-705 (Favipiravir) Ribofuranosyl 5'-Triphosphate towards Influenza A Virus Polymerase**
Zhinan Jin, Lucas K. Smith, Vivek K. Rajwanshi, Baek Kim, Jerome Deval
PLoS ONE (2013-07-10) https://doi.org/f5br92
DOI: 10.1371/journal.pone.0068347 · PMID: 23874596 · PMCID: PMC3707847

125. **TEMPORARY REMOVAL: Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study**
Qingxian Cai, Minghui Yang, Dongjing Liu, Jun Chen, Dan Shu, Junxia Xia, Xuejiao Liao, Yuanbo Gu, Qiue Cai, Yang Yang, … Lei Liu
Engineering (2020-03) https://doi.org/ggpprd
DOI: 10.1016/j.eng.2020.03.007 · PMID: 32346491 · PMCID: PMC7185795

126. **A Multicenter, Adaptive, Randomized Blinded Controlled Trial of the Safety and Efficacy of Investigational Therapeutics for the Treatment of COVID-19 in Hospitalized Adults**
National Institute of Allergy and Infectious Diseases (NIAID)
clinicaltrials.gov (2020-05-06) https://clinicaltrials.gov/ct2/show/NCT04280705

127. **A Phase 3 Randomized Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734™) in Participants With Severe COVID-19**
Gilead Sciences
clinicaltrials.gov (2020-05-05) https://clinicaltrials.gov/ct2/show/NCT04292899

128. **FDa logo for letterhead**
JBowers
(2020-05-01) https://www.fda.gov/media/137564/download

129. **The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus**
Calvin J Gordon, Egor P Tchesnokov, Joy Y. Feng, Danielle P Porter, Matthias Gotte
Journal of Biological Chemistry (2020-02-24) https://doi.org/ggqm6x
DOI: 10.1074/jbc.ac120.013056 · PMID: 32094225 · PMCID: PMC7152756

130. **Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease**
Maria L. Agostini, Erica L. Andres, Amy C. Sims, Rachel L. Graham, Timothy P. Sheahan, Xiaotao Lu, Everett Clinton Smith, James Brett Case, Joy Y. Feng, Robert Jordan, … Mark R. Denison
mBio (2018-03-06) https://doi.org/gc45v6
DOI: 10.1128/mbio.00221-18 · PMID: 29511076 · PMCID: PMC5844999

131. **Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro**
Manli Wang, Ruiyuan Cao, Leike Zhang, Xinglou Yang, Jia Liu, Mingyue Xu, Zhengli Shi, Zhihong Hu, Wu Zhong, Gengfu Xiao
Cell Research (2020-02-04) https://doi.org/ggkbsg
DOI: 10.1038/s41422-020-0282-0 · PMID: 32020029 · PMCID: PMC7054408

132. **Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses**
Timothy P. Sheahan, Amy C. Sims, Rachel L. Graham, Vineet D. Menachery, Lisa E. Gralinski, James B. Case, Sarah R. Leist, Krzysztof Pyrc, Joy Y. Feng, Iva Trantcheva, … Ralph S. Baric
Science Translational Medicine (2017-06-28) https://doi.org/gc3grb
DOI: 10.1126/scitranslmed.aal3653 · PMID: 28659436 · PMCID: PMC5567817

133. **A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics**
Sabue Mulangu, Lori E. Dodd, Richard T. Davey, Olivier Tshiani Mbaya, Michael Proschan, Daniel Mukadi, Mariano Lusakibanza Manzo, Didier Nzolo, Antoine Tshomba Oloma, Augustin Ibanda, … the PALM Writing Group
New England Journal of Medicine (2019-12-12) https://doi.org/ggqmx4
DOI: 10.1056/nejmoa1910993 · PMID: 31774950

134. **Did an experimental drug help a U.S. coronavirus patient?**
Jon Cohen
Science (2020-03-13) https://doi.org/ggqm62
DOI: 10.1126/science.abb7243

135. **First 12 patients with coronavirus disease 2019 (COVID-19) in the United States**
Stephanie A. Kujawski, Karen K Wong, Jennifer P. Collins, Lauren Epstein, Marie E. Killerby, Claire M. Midgley, Glen R. Abedi, N. Seema Ahmed, Olivia Almendares, Francisco N. Alvarez, … Jing Zhang
medRxiv (2020-03-12) https://doi.org/ggqm6z
DOI: 10.1101/2020.03.09.20032896

136. **First Case of 2019 Novel Coronavirus in the United States**
Michelle L. Holshue, Chas DeBolt, Scott Lindquist, Kathy H. Lofy, John Wiesman, Hollianne Bruce, Christopher Spitters, Keith Ericson, Sara Wilkerson, Ahmet Tural, … Satish K. Pillai
New England Journal of Medicine (2020-01-31) https://doi.org/ggjvr6
DOI: 10.1056/nejmoa2001191 · PMID: 32004427 · PMCID: PMC7092802

137. **Compassionate Use of Remdesivir for Patients with Severe Covid-19**
Jonathan Grein, Norio Ohmagari, Daniel Shin, George Diaz, Erika Asperges, Antonella Castagna, Torsten Feldt, Gary Green, Margaret L. Green, François-Xavier Lescure, … Timothy Flanigan
New England Journal of Medicine (2020-04-10) https://doi.org/ggrm99
DOI: 10.1056/nejmoa2007016 · PMID: 32275812 · PMCID: PMC7169476

138. **A Phase 3 Randomized Study to Evaluate the Safety and Antiviral Activity of Remdesivir (GS-5734™) in Participants With Moderate COVID-19 Compared to Standard of Care Treatment**

Gilead Sciences
clinicaltrials.gov (2020-05-05) https://clinicaltrials.gov/ct2/show/NCT04292730

139. https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-000936-23/FR

140. **A Phase 3 Randomized, Double-blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Remdesivir in Hospitalized Adult Patients With Mild and Moderate COVID-19.**
Bin Cao
clinicaltrials.gov (2020-04-13) https://clinicaltrials.gov/ct2/show/NCT04252664

141. **A Phase 3 Randomized, Double-blind, Placebo-controlled, Multicenter Study to Evaluate the Efficacy and Safety of Remdesivir in Hospitalized Adult Patients With Severe COVID-19.**
Bin Cao
clinicaltrials.gov (2020-04-13) https://clinicaltrials.gov/ct2/show/NCT04257656

142. **Proteases Essential for Human Influenza Virus Entry into Cells and Their Inhibitors as Potential Therapeutic Agents**
Hiroshi Kido, Yuushi Okumura, Hiroshi Yamada, Trong Quang Le, Mihiro Yano
Current Pharmaceutical Design (2007-02-01) https://doi.org/bts3xp
DOI: 10.2174/138161207780162971 · PMID: 17311557

143. **Protease inhibitors targeting coronavirus and filovirus entry**
Yanchen Zhou, Punitha Vedantham, Kai Lu, Juliet Agudelo, Ricardo Carrion, Jerritt W. Nunneley, Dale Barnard, Stefan Pöhlmann, James H. McKerrow, Adam R. Renslo, Graham Simmons
Antiviral Research (2015-04) https://doi.org/ggr984
DOI: 10.1016/j.antiviral.2015.01.011 · PMID: 25666761 · PMCID: PMC4774534

144. **Design of Wide-Spectrum Inhibitors Targeting Coronavirus Main Proteases**
Haitao Yang, Weiqing Xie, Xiaoyu Xue, Kailin Yang, Jing Ma, Wenxue Liang, Qi Zhao, Zhe Zhou, Duanqing Pei, John Ziebuhr, … Zihe Rao
PLoS Biology (2005-09-06) https://doi.org/bcm9k7
DOI: 10.1371/journal.pbio.0030324 · PMID: 16128623 · PMCID: PMC1197287

145. **The newly emerged SARS-Like coronavirus HCoV-EMC also has an "Achilles' heel": current effective inhibitor targeting a 3C-like protease**
Zhilin Ren, Liming Yan, Ning Zhang, Yu Guo, Cheng Yang, Zhiyong Lou, Zihe Rao
Protein & Cell (2013-04-03) https://doi.org/ggr7vh
DOI: 10.1007/s13238-013-2841-3 · PMID: 23549610 · PMCID: PMC4875521

146. **Structure of Mpro from COVID-19 virus and discovery of its inhibitors**
Zhenming Jin, Xiaoyu Du, Yechun Xu, Yongqiang Deng, Meiqin Liu, Yao Zhao, Bing Zhang, Xiaofeng Li, Leike Zhang, Chao Peng, … Haitao Yang
Nature (2020-04-09) https://doi.org/ggrp42
DOI: 10.1038/s41586-020-2223-y · PMID: 32272481

147. **Ebselen, a promising antioxidant drug: mechanisms of action and targets of biological pathways**
Gajendra Kumar Azad, Raghuvir S. Tomar
Molecular Biology Reports (2014-05-28) https://doi.org/f6cnq3
DOI: 10.1007/s11033-014-3417-x · PMID: 24867080

148. **Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors**
Linlin Zhang, Daizong Lin, Xinyuanyuan Sun, Ute Curth, Christian Drosten, Lucie Sauerhering, Stephan Becker, Katharina Rox, Rolf Hilgenfeld
Science (2020-03-20) https://doi.org/ggp9sb
DOI: 10.1126/science.abb3405 · PMID: 32198291 · PMCID: PMC7164518

149. **Target discovery of ebselen with a biotinylated probe**
Zhenzhen Chen, Zhongyao Jiang, Nan Chen, Qian Shi, Lili Tong, Fanpeng Kong, Xiufen Cheng, Hao Chen, Chu Wang, Bo Tang
Chemical Communications (2018) https://doi.org/ggrtcm
DOI: 10.1039/c8cc04258f · PMID: 30091742

150. **Lysosomotropic agents as HCV entry inhibitors**
Usman A Ashfaq, Tariq Javed, Sidra Rehman, Zafar Nawaz, Sheikh Riazuddin
Virology Journal (2011-04-12) https://doi.org/dr5g4m
DOI: 10.1186/1743-422x-8-163 · PMID: 21481279 · PMCID: PMC3090357

151. **New concepts in antimalarial use and mode of action in dermatology**
Sunil Kalia, Jan P Dutz
Dermatologic Therapy (2007-07) https://doi.org/fv69cb
DOI: 10.1111/j.1529-8019.2007.00131.x · PMID: 17970883 · PMCID: PMC7163426

152.**:{unav)**
Martin J Vincent, Eric Bergeron, Suzanne Benjannet, Bobbie R Erickson, Pierre E Rollin, Thomas G Ksiazek, Nabil G Seidah, Stuart T Nichol
Virology Journal (2005) https://doi.org/dvbds4
DOI: 10.1186/1743-422x-2-69 · PMID: 16115318 · PMCID: PMC1232869

153. **In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)**
Xueting Yao, Fei Ye, Miao Zhang, Cheng Cui, Baoying Huang, Peihua Niu, Xu Liu, Li Zhao, Erdan Dong, Chunli Song, … Dongyang Liu
Clinical Infectious Diseases (2020-03-09) https://doi.org/ggpx7z
DOI: 10.1093/cid/ciaa237 · PMID: 32150618 · PMCID: PMC7108130

154. **Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19**
Yan Zhang, Meng Xiao, Shulan Zhang, Peng Xia, Wei Cao, Wei Jiang, Huan Chen, Xin Ding, Hua Zhao, Hongmin Zhang, … Shuyang Zhang
New England Journal of Medicine (2020-04-23) https://doi.org/ggrgz7
DOI: 10.1056/nejmc2007575 · PMID: 32268022 · PMCID: PMC7161262

155. **Mechanism of Action of Hydroxychloroquine in the Antiphospholipid Syndrome**
Nadine Müller-Calleja, Davit Manukyan, Wolfram Ruf, Karl Lackner
Blood (2016-12-02) https://doi.org/ggrm82
DOI: 10.1182/blood.v128.22.5023.5023

156. **14th International Congress on Antiphospholipid Antibodies Task Force Report on Antiphospholipid Syndrome Treatment Trends**
Doruk Erkan, Cassyanne L. Aguiar, Danieli Andrade, Hannah Cohen, Maria J. Cuadrado, Adriana Danowski, Roger A. Levy, Thomas L. Ortel, Anisur Rahman, Jane E. Salmon, … Michael D. Lockshin

Autoimmunity Reviews (2014-06) https://doi.org/ggp8r8
DOI: 10.1016/j.autrev.2014.01.053 · PMID: 24468415

157. **What is the role of hydroxychloroquine in reducing thrombotic risk in patients with antiphospholipid antibodies?**
Tzu-Fei Wang, Wendy Lim
Hematology (2016-12-02) https://doi.org/ggrn3k
DOI: 10.1182/asheducation-2016.1.714 · PMID: 27913551 · PMCID: PMC6142483

158. **Chloroquine diphosphate in two different dosages as adjunctive therapy of hospitalized patients with severe respiratory syndrome in the context of coronavirus (SARS-CoV-2) infection: Preliminary safety results of a randomized, double-blinded, phase IIb clinical trial (CloroCovid-19 Study)**
Mayla Gabriela Silva Borba, Fernando de Almeida Val, Vanderson Sousa Sampaio, Marcia Almeida Ara&uacutejo Alexandre, Gisely Cardoso Melo, Marcelo Brito, Maria Paula Gomes Mour&atildeo, José Diego Brito Sousa, Djane Clarys Baia-da-Silva, Marcus Vinitius Farias Guerra, … CloroCovid-19 Team
medRxiv (2020-04-16) https://doi.org/ggr3nj
DOI: 10.1101/2020.04.07.20056424

159. **Heart risk concerns mount around use of chloroquine and hydroxychloroquine for Covid-19 treatment**
Jacqueline Howard CNN Elizabeth Cohen, Nadia Kounang and Per Nyberg
CNN https://www.cnn.com/2020/04/13/health/chloroquine-risks-coronavirus-treatment-trials-study/index.html

160. **Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial**
Philippe Gautret, Jean-Christophe Lagier, Philippe Parola, Van Thuan Hoang, Line Meddeb, Morgane Mailhe, Barbara Doudier, Johan Courjon, Valérie Giordanengo, Vera Esteves Vieira, … Didier Raoult
International Journal of Antimicrobial Agents (2020-03) https://doi.org/dp7d
DOI: 10.1016/j.ijantimicag.2020.105949 · PMID: 32205204 · PMCID: PMC7102549

161. **Statement on IJAA paper | International Society of Antimicrobial Chemotherapy**
M. T. C. Media
https://www.isac.world/news-and-publications/official-isac-statement

162. **Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial**
Zhaowei Chen, Jijia Hu, Zongwei Zhang, Shan Jiang, Shoumeng Han, Dandan Yan, Ruhong Zhuang, Ben Hu, Zhan Zhang
medRxiv (2020-03-31) https://doi.org/ggqm4v
DOI: 10.1101/2020.03.22.20040758

163. **A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19)**
CHEN Jun, LIU Danping, LIU Li, LIU Ping, XU Qingnian, XIA Lu, LING Yun, HUANG Dan, SONG Shuli, ZHANG Dandan, … LU Hongzhou
Journal of Zhejiang University (Medical Sciences) (2020-03) https://doi.org/10.3785/j.issn.1008-9292.2020.03.03
DOI: 10.3785/j.issn.1008-9292.2020.03.03

164. **No Evidence of Rapid Antiviral Clearance or Clinical Benefit with the Combination of Hydroxychloroquine and Azithromycin in Patients with Severe COVID-19 Infection**
Jean Michel Molina, Constance Delaugerre, Jerome Le Goff, Breno Mela-Lima, Diane Ponscarme, Lauriane Goldwirt, Nathalie de Castro
Médecine et Maladies Infectieuses (2020-03) https://doi.org/ggqzrb
DOI: 10.1016/j.medmal.2020.03.006 · PMID: 32240719 · PMCID: PMC7195369

165. **Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies**
Jianjun Gao, Zhenxue Tian, Xu Yang
BioScience Trends (2020) https://doi.org/ggm3mv
DOI: 10.5582/bst.2020.01047 · PMID: 32074550

166. **Targeting the Endocytic Pathway and Autophagy Process as a Novel Therapeutic Strategy in COVID-19**
Naidi Yang, Han-Ming Shen
International Journal of Biological Sciences (2020) https://doi.org/ggqspm
DOI: 10.7150/ijbs.45498 · PMID: 32226290 · PMCID: PMC7098027

167. **SARS-CoV-2: an Emerging Coronavirus that Causes a Global Threat**
Jun Zheng
International Journal of Biological Sciences (2020) https://doi.org/ggqspr
DOI: 10.7150/ijbs.45053 · PMID: 32226285 · PMCID: PMC7098030

168. **Hydroxychloroquine in patients mainly with mild to moderate COVID-19: an open-label, randomized, controlled trial**
Wei Tang, Zhujun Cao, Mingfeng Han, Zhengyan Wang, Junwen Chen, Wenjin Sun, Yaojie Wu, Wei Xiao, Shengyong Liu, Erzhen Chen, … Qing Xie
medRxiv (2020-05-07) https://doi.org/ggr68m
DOI: 10.1101/2020.04.10.20060558

169. **Detection of SARS-CoV-2 in Different Types of Clinical Specimens**
Wenling Wang, Yanli Xu, Ruqin Gao, Roujian Lu, Kai Han, Guizhen Wu, Wenjie Tan
JAMA (2020-03-11) https://doi.org/ggpp6h
DOI: 10.1001/jama.2020.3786 · PMID: 32159775 · PMCID: PMC7066521

170. **Life Threatening Severe QTc Prolongation in Patient with Systemic Lupus Erythematosus due to Hydroxychloroquine**
John P. O'Laughlin, Parag H. Mehta, Brian C. Wong
Case Reports in Cardiology (2016) https://doi.org/ggqzrc
DOI: 10.1155/2016/4626279 · PMID: 27478650 · PMCID: PMC4960328

171. **Structural Design Principles for Delivery of Bioactive Components in Nutraceuticals and Functional Foods**
David Julian McClements, Eric Andrew Decker, Yeonhwa Park, Jochen Weiss
Critical Reviews in Food Science and Nutrition (2009-06-16) https://doi.org/dt68m4
DOI: 10.1080/10408390902841529 · PMID: 19484636

172. **Nutraceutical therapies for atherosclerosis**
Joe W. E. Moss, Dipak P. Ramji
Nature Reviews Cardiology (2016-07-07) https://doi.org/f9g389
DOI: 10.1038/nrcardio.2016.103 · PMID: 27383080 · PMCID: PMC5228762

173. **Potential interventions for novel coronavirus in China: A systematic review**
Lei Zhang, Yunhui Liu
Journal of Medical Virology (2020-03-03) https://doi.org/ggpx57
DOI: 10.1002/jmv.25707 · PMID: 32052466 · PMCID: PMC7166986

174. **Phospholipids of Animal and Marine Origin: Structure, Function, and Anti-Inflammatory Properties**
Ronan Lordan, Alexandros Tsoupras, Ioannis Zabetakis
Molecules (2017-11-14) https://doi.org/ggqmgq
DOI: 10.3390/molecules22111964 · PMID: 29135918 · PMCID: PMC6150200

175. **Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?**
Philip C. Calder
British Journal of Clinical Pharmacology (2013-03) https://doi.org/ggqmgg
DOI: 10.1111/j.1365-2125.2012.04374.x · PMID: 22765297 · PMCID: PMC3575932

176. **Proresolving Lipid Mediators and Mechanisms in the Resolution of Acute Inflammation**
Christopher D. Buckley, Derek W. Gilroy, Charles N. Serhan
Immunity (2014-03) https://doi.org/f5wntr
DOI: 10.1016/j.immuni.2014.02.009 · PMID: 24656045 · PMCID: PMC4004957

177. **Specialized pro-resolving mediators: endogenous regulators of infection and inflammation**
Maria C. Basil, Bruce D. Levy
Nature Reviews Immunology (2015-12-21) https://doi.org/f9fgtd
DOI: 10.1038/nri.2015.4 · PMID: 26688348 · PMCID: PMC5242505

178. **The Lipid Mediator Protectin D1 Inhibits Influenza Virus Replication and Improves Severe Influenza**
Masayuki Morita, Keiji Kuba, Akihiko Ichikawa, Mizuho Nakayama, Jun Katahira, Ryo Iwamoto, Tokiko Watanebe, Saori Sakabe, Tomo Daidoji, Shota Nakamura, … Yumiko Imai
Cell (2013-03) https://doi.org/f4rbgb
DOI: 10.1016/j.cell.2013.02.027 · PMID: 23477864

179. **Fish Oil-Fed Mice Have Impaired Resistance to Influenza Infection**
Nicole M. J. Schwerbrock, Erik A. Karlsson, Qing Shi, Patricia A. Sheridan, Melinda A. Beck
The Journal of Nutrition (2009-08) https://doi.org/dv45f4
DOI: 10.3945/jn.109.108027 · PMID: 19549756 · PMCID: PMC2709305

180. **Modulation of host defence against bacterial and viral infections by omega-3 polyunsaturated fatty acids**
Marie-Odile Husson, Delphine Ley, Céline Portal, Madeleine Gottrand, Thomas Hueso, Jean-Luc Desseyn, Frédéric Gottrand
Journal of Infection (2016-12) https://doi.org/f9pp2h
DOI: 10.1016/j.jinf.2016.10.001 · PMID: 27746159

181. **Zinc and immunity: An essential interrelation**
Maria Maares, Hajo Haase
Archives of Biochemistry and Biophysics (2016-12) https://doi.org/f9c9b5
DOI: 10.1016/j.abb.2016.03.022 · PMID: 27021581

182. **Zinc-Dependent Suppression of TNF-α Production Is Mediated by Protein Kinase A-Induced Inhibition of Raf-1, IκB Kinase β, and NF-κB**

Verena von Bülow, Svenja Dubben, Gabriela Engelhardt, Silke Hebel, Birgit Plümäkers, Holger Heine, Lothar Rink, Hajo Haase
The Journal of Immunology (2007-09-15) https://doi.org/f3vs45
DOI: 10.4049/jimmunol.179.6.4180 · PMID: 17785857

183. **Zinc activates NF-κB in HUT-78 cells**
Ananda S. Prasad, Bin Bao, Frances W. J. Beck, Fazlul H. Sarkar
Journal of Laboratory and Clinical Medicine (2001-10) https://doi.org/cnc6fr
DOI: 10.1067/mlc.2001.118108 · PMID: 11574819

184. **Innate or Adaptive Immunity? The Example of Natural Killer Cells**
E. Vivier, D. H. Raulet, A. Moretta, M. A. Caligiuri, L. Zitvogel, L. L. Lanier, W. M. Yokoyama, S. Ugolini
Science (2011-01-06) https://doi.org/ckzg9g
DOI: 10.1126/science.1198687 · PMID: 21212348 · PMCID: PMC3089969

185. **Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress**
Ananda S Prasad, Frances WJ Beck, Bin Bao, James T Fitzgerald, Diane C Snell, Joel D Steinberg, Lavoisier J Cardozo
The American Journal of Clinical Nutrition (2007-03) https://doi.org/ggqmgs
DOI: 10.1093/ajcn/85.3.837 · PMID: 17344507

186. **The Role of Zinc in Antiviral Immunity**
Scott A Read, Stephanie Obeid, Chantelle Ahlenstiel, Golo Ahlenstiel
Advances in Nutrition (2019-07) https://doi.org/ggqmgr
DOI: 10.1093/advances/nmz013 · PMID: 31305906 · PMCID: PMC6628855

187. **Efficacy of Zinc Against Common Cold Viruses: An Overview**
Darrell Hulisz
Journal of the American Pharmacists Association (2004-09) https://doi.org/cf6pmt
DOI: 10.1331/1544-3191.44.5.594.hulisz · PMID: 15496046 · PMCID: PMC7185598

188. **Zinc Lozenges May Shorten the Duration of Colds: A Systematic Review**
Harri Harri
The Open Respiratory Medicine Journal (2011-06-23) https://doi.org/bndmfq
DOI: 10.2174/1874306401105010051 · PMID: 21769305 · PMCID: PMC3136969

189. **Zn2+ Inhibits Coronavirus and Arterivirus RNA Polymerase Activity In Vitro and Zinc Ionophores Block the Replication of These Viruses in Cell Culture**
Aartjan J. W. te Velthuis, Sjoerd H. E. van den Worm, Amy C. Sims, Ralph S. Baric, Eric J. Snijder, Martijn J. van Hemert
PLoS Pathogens (2010-11-04) https://doi.org/d95x4g
DOI: 10.1371/journal.ppat.1001176 · PMID: 21079686 · PMCID: PMC2973827

190. **The SARS-coronavirus papain-like protease: Structure, function and inhibition by designed antiviral compounds**
Yahira M. Báez-Santos, Sarah E. St. John, Andrew D. Mesecar
Antiviral Research (2015-03) https://doi.org/f63hjp
DOI: 10.1016/j.antiviral.2014.12.015 · PMID: 25554382 · PMCID: PMC5896749

191. **Vitamin B12 May Inhibit RNA-Dependent-RNA Polymerase Activity of nsp12 from the COVID-19 Virus**
Naveen Narayanan, Deepak T. Nair

(2020-03-22) https://doi.org/ggqmjc
DOI: 10.20944/preprints202003.0347.v1

192. **Vitamin C Mitigates Oxidative Stress and Tumor Necrosis Factor-Alpha in Severe Community-Acquired Pneumonia and LPS-Induced Macrophages**
Yuanyuan Chen, Guangyan Luo, Jiao Yuan, Yuanyuan Wang, Xiaoqiong Yang, Xiaoyun Wang, Guoping Li, Zhiguang Liu, Nanshan Zhong
Mediators of Inflammation (2014) https://doi.org/f6nb5f
DOI: 10.1155/2014/426740 · PMID: 25253919 · PMCID: PMC4165740

193. **Intravenous infusion of ascorbic acid decreases serum histamine concentrations in patients with allergic and non-allergic diseases**
Alexander F. Hagel, Christian M. Layritz, Wolfgang H. Hagel, Hans-Jürgen Hagel, Edith Hagel, Wolfgang Dauth, Jürgen Kressel, Tanja Regnet, Andreas Rosenberg, Markus F. Neurath, … Martin Raithel
Naunyn-Schmiedeberg's Archives of Pharmacology (2013-05-11) https://doi.org/f48jsb
DOI: 10.1007/s00210-013-0880-1 · PMID: 23666445

194. **Vitamin C and Immune Function**
Anitra Carr, Silvia Maggini
Nutrients (2017-11-03) https://doi.org/gfzrjs
DOI: 10.3390/nu9111211 · PMID: 29099763 · PMCID: PMC5707683

195. **Changes in Leucocyte Ascorbic Acid during the Common Cold**
R. Hume, Elspeth Weyers
Scottish Medical Journal (2016-06-25) https://doi.org/ggqrfj
DOI: 10.1177/003693307301800102 · PMID: 4717661

196. **ASCORBIC ACID FUNCTION AND METABOLISM DURING COLDS**
C. W. M. Wilson
Annals of the New York Academy of Sciences (1975-09) https://doi.org/bjfdtb
DOI: 10.1111/j.1749-6632.1975.tb29312.x · PMID: 1106304

197. **Metabolism of ascorbic acid (vitamin C) in subjects infected with common cold viruses**
J. E. W. Davies, R. E. Hughes, Eleri Jones, Sylvia E. Reed, J. W. Craig, D. A. J. Tyrrell
Biochemical Medicine (1979-02) https://doi.org/fd22sv
DOI: 10.1016/0006-2944(79)90058-9

198. **Vitamin C and Infections**
Harri Hemilä
Nutrients (2017-03-29) https://doi.org/gfkb9n
DOI: 10.3390/nu9040339 · PMID: 28353648 · PMCID: PMC5409678

199. **Vitamin C and the common cold**
Harri Hemilä
British Journal of Nutrition (2007-03-09) https://doi.org/fszhc6
DOI: 10.1079/bjn19920004 · PMID: 1547201

200. **Vitamin C for preventing and treating the common cold**
Harri Hemilä, Elizabeth Chalker
Cochrane Database of Systematic Reviews (2013-01-31) https://doi.org/xz5
DOI: 10.1002/14651858.cd000980.pub4 · PMID: 23440782

201. **Vitamin C intake and susceptibility to pneumonia**
HARRI HEMILÄ
The Pediatric Infectious Disease Journal (1997-09) https://doi.org/fkvs9d
DOI: 10.1097/00006454-199709000-00003 · PMID: 9306475

202. **Vitamin C Can Shorten the Length of Stay in the ICU: A Meta-Analysis**
Harri Hemilä, Elizabeth Chalker
Nutrients (2019-03-27) https://doi.org/gfzscg
DOI: 10.3390/nu11040708 · PMID: 30934660 · PMCID: PMC6521194

203. **Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients With Sepsis and Severe Acute Respiratory Failure**
Alpha A. Fowler, Jonathon D. Truwit, R. Duncan Hite, Peter E. Morris, Christine DeWilde, Anna Priday, Bernard Fisher, Leroy R. Thacker, Ramesh Natarajan, Donald F. Brophy, … Matthew Halquist
JAMA (2019-10-01) https://doi.org/ggqmh8
DOI: 10.1001/jama.2019.11825 · PMID: 31573637 · PMCID: PMC6777268

204. **Vitamin C Infusion for the Treatment of Severe 2019-nCoV Infected Pneumonia: a Prospective Randomized Clinical Trial**
ZhiYong Peng
clinicaltrials.gov (2020-03-06) https://clinicaltrials.gov/ct2/show/NCT04264533

205. **A new clinical trial to test high-dose vitamin C in patients with COVID-19**
Anitra C. Carr
Critical Care (2020-04-07) https://doi.org/ggsbmg
DOI: 10.1186/s13054-020-02851-4 · PMID: 32264963 · PMCID: PMC7137406

206. **Use of Ascorbic Acid in Patients With COVID 19**
Salvatore Corrao MD
clinicaltrials.gov (2020-03-24) https://clinicaltrials.gov/ct2/show/NCT04323514

207. **Vitamin D and the Immune System**
Cynthia Aranow
Journal of Investigative Medicine (2015-12-15) https://doi.org/f3wh87
DOI: 10.2310/jim.0b013e31821b8755 · PMID: 21527855

208. **Vitamin D in the prevention of acute respiratory infection: Systematic review of clinical studies**
David A. Jolliffe, Christopher J. Griffiths, Adrian R. Martineau
The Journal of Steroid Biochemistry and Molecular Biology (2013-07) https://doi.org/ggqmh9
DOI: 10.1016/j.jsbmb.2012.11.017 · PMID: 23220552

209. **Vitamin D: modulator of the immune system**
Femke Baeke, Tatiana Takiishi, Hannelie Korf, Conny Gysemans, Chantal Mathieu
Current Opinion in Pharmacology (2010-08) https://doi.org/d43qtf
DOI: 10.1016/j.coph.2010.04.001 · PMID: 20427238

210. **Evidence That Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths**
William B. Grant, Henry Lahore, Sharon L. McDonnell, Carole A. Baggerly, Christine B. French, Jennifer L. Aliano, Harjit Pal Bhattoa
(2020-03-30) https://doi.org/ggqmjb
DOI: 10.20944/preprints202003.0235.v2

211. **Factors Affecting 25-Hydroxyvitamin D Concentration in Response to Vitamin D Supplementation**
Hajar Mazahery, Pamela von Hurst
Nutrients (2015-06-25) https://doi.org/f7sztz
DOI: 10.3390/nu7075111 · PMID: 26121531 · PMCID: PMC4516990

212. **Optimal Nutritional Status for a Well-Functioning Immune System is an Important Factor to Protect Against Viral Infections**
Philip C. Calder, Anitra C. Carr, Adrian F. Gombart, Manfred Eggersdorfer
(2020-03-12) https://www.preprints.org/manuscript/202003.0199/v1

213. **The frontier between nutrition and pharma: The international regulatory framework of functional foods, food supplements and nutraceuticals**
Laura Domínguez Díaz, Virginia Fernández-Ruiz, Montaña Cámara
Critical Reviews in Food Science and Nutrition (2019-03-29) https://doi.org/ggqs3w
DOI: 10.1080/10408398.2019.1592107 · PMID: 30924346

214. **Coronavirus Update: FDA and FTC Warn Seven Companies Selling Fraudulent Products that Claim to Treat or Prevent COVID-19**
Office of the Commissioner
FDA (2020-03-27) https://www.fda.gov/news-events/press-announcements/coronavirus-update-fda-and-ftc-warn-seven-companies-selling-fraudulent-products-claim-treat-or

215. **Development of therapeutic antibodies for the treatment of diseases**
Ruei-Min Lu, Yu-Chyi Hwang, I-Ju Liu, Chi-Chiu Lee, Han-Zen Tsai, Hsin-Jung Li, Han-Chung Wu
Journal of Biomedical Science (2020-01-02) https://doi.org/ggqbpx
DOI: 10.1186/s12929-019-0592-z · PMID: 31894001 · PMCID: PMC6939334

216. **Broadly Neutralizing Antiviral Antibodies**
Davide Corti, Antonio Lanzavecchia
Annual Review of Immunology (2013-03-21) https://doi.org/gf25g8
DOI: 10.1146/annurev-immunol-032712-095916 · PMID: 23330954

217. **Neutralizing Monoclonal Antibodies as Promising Therapeutics against Middle East Respiratory Syndrome Coronavirus Infection**
Hui-Ju Han, Jian-Wei Liu, Hao Yu, Xue-Jie Yu
Viruses (2018-11-30) https://doi.org/ggp87v
DOI: 10.3390/v10120680 · PMID: 30513619 · PMCID: PMC6315345

218. **Ibalizumab Targeting CD4 Receptors, An Emerging Molecule in HIV Therapy**
Simona A. Iacob, Diana G. Iacob
Frontiers in Microbiology (2017-11-27) https://doi.org/gcn3kh
DOI: 10.3389/fmicb.2017.02323 · PMID: 29230203 · PMCID: PMC5711820

219. **Product review on the monoclonal antibody palivizumab for prevention of respiratory syncytial virus infection**
Bernhard Resch
Human Vaccines & Immunotherapeutics (2017-06-12) https://doi.org/ggqbps
DOI: 10.1080/21645515.2017.1337614 · PMID: 28605249 · PMCID: PMC5612471

220. **Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus**
P.-R. Hsueh, L.-M. Huang, P.-J. Chen, C.-L. Kao, P.-C. Yang

Clinical Microbiology and Infection (2004-12) https://doi.org/cwwg87
DOI: 10.1111/j.1469-0691.2004.01009.x · PMID: 15606632 · PMCID: PMC7129952

221. **Neutralizing Antibodies in Patients with Severe Acute Respiratory Syndrome-Associated Coronavirus Infection**
Nie Yuchun, Wang Guangwen, Shi Xuanling, Zhang Hong, Qiu Yan, He Zhongping, Wang Wei, Lian Gewei, Yin Xiaolei, Du Liying, … Ding Mingxiao
The Journal of Infectious Diseases (2004-09) https://doi.org/cgqj5b
DOI: 10.1086/423286 · PMID: 15319862 · PMCID: PMC7199490

222. **Potent human monoclonal antibodies against SARS CoV, Nipah and Hendra viruses**
Ponraj Prabakaran, Zhongyu Zhu, Xiaodong Xiao, Arya Biragyn, Antony S Dimitrov, Christopher C Broder, Dimiter S Dimitrov
Expert Opinion on Biological Therapy (2009-04-08) https://doi.org/b88kw8
DOI: 10.1517/14712590902763755 · PMID: 19216624 · PMCID: PMC2705284

223. **Prior Infection and Passive Transfer of Neutralizing Antibody Prevent Replication of Severe Acute Respiratory Syndrome Coronavirus in the Respiratory Tract of Mice**
Kanta Subbarao, Josephine McAuliffe, Leatrice Vogel, Gary Fahle, Steven Fischer, Kathleen Tatti, Michelle Packard, Wun-Ju Shieh, Sherif Zaki, Brian Murphy
Journal of Virology (2004-04-01) https://doi.org/b8wr7c
DOI: 10.1128/jvi.78.7.3572-3577.2004 · PMID: 15016880 · PMCID: PMC371090

224. **The Effectiveness of Convalescent Plasma and Hyperimmune Immunoglobulin for the Treatment of Severe Acute Respiratory Infections of Viral Etiology: A Systematic Review and Exploratory Meta-analysis**
John Mair-Jenkins, Maria Saavedra-Campos, J. Kenneth Baillie, Paul Cleary, Fu-Meng Khaw, Wei Shen Lim, Sophia Makki, Kevin D. Rooney, Jonathan S. Nguyen-Van-Tam, Charles R. Beck, Convalescent Plasma Study Group
Journal of Infectious Diseases (2015-01-01) https://doi.org/f632n7
DOI: 10.1093/infdis/jiu396 · PMID: 25030060 · PMCID: PMC4264590

225. **Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution**
X.-C. Tang, S. S. Agnihothram, Y. Jiao, J. Stanhope, R. L. Graham, E. C. Peterson, Y. Avnir, A. S. C. Tallarico, J. Sheehan, Q. Zhu, … W. A. Marasco
Proceedings of the National Academy of Sciences (2014-04-28) https://doi.org/smr
DOI: 10.1073/pnas.1402074111 · PMID: 24778221 · PMCID: PMC4024880

226. **Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus**
Wenhui Li, Michael J. Moore, Natalya Vasilieva, Jianhua Sui, Swee Kee Wong, Michael A. Berne, Mohan Somasundaran, John L. Sullivan, Katherine Luzuriaga, Thomas C. Greenough, … Michael Farzan
Nature (2003-11) https://doi.org/bqvpjh
DOI: 10.1038/nature02145 · PMID: 14647384 · PMCID: PMC7095016

227. **The Role of ACE2 in Cardiovascular Physiology**
Gavin Y. Oudit, Michael A. Crackower, Peter H. Backx, Josef M. Penninger
Trends in Cardiovascular Medicine (2003-04) https://doi.org/bsbp49
DOI: 10.1016/s1050-1738(02)00233-5

228. **A human monoclonal antibody blocking SARS-CoV-2 infection**
Chunyan Wang, Wentao Li, Dubravka Drabek, Nisreen M. A. Okba, Rien van Haperen, Albert D. M.

E. Osterhaus, Frank J. M. van Kuppeveld, Bart L. Haagmans, Frank Grosveld, Berend-Jan Bosch
bioRxiv (2020-03-12) https://doi.org/ggnw4t
DOI: 10.1101/2020.03.11.987958

229. **Unexpected Receptor Functional Mimicry Elucidates Activation of Coronavirus Fusion**
Alexandra C. Walls, Xiaoli Xiong, Young-Jun Park, M. Alejandra Tortorici, Joost Snijder, Joel Quispe, Elisabetta Cameroni, Robin Gopal, Mian Dai, Antonio Lanzavecchia, … David Veesler
Cell (2019-02) https://doi.org/gft3jg
DOI: 10.1016/j.cell.2018.12.028 · PMID: 30712865 · PMCID: PMC6751136

230. ***In Vitro* Neutralization Is Not Predictive of Prophylactic Efficacy of Broadly Neutralizing Monoclonal Antibodies CR6261 and CR9114 against Lethal H2 Influenza Virus Challenge in Mice**
Troy C. Sutton, Elaine W. Lamirande, Kevin W. Bock, Ian N. Moore, Wouter Koudstaal, Muniza Rehman, Gerrit Jan Weverling, Jaap Goudsmit, Kanta Subbarao
Journal of Virology (2017-12-15) https://doi.org/ggqbpt
DOI: 10.1128/jvi.01603-17 · PMID: 29046448 · PMCID: PMC5709608

231. **Human neutralizing antibodies against MERS coronavirus: implications for future immunotherapy**
Xian-Chun Tang, Wayne A Marasco
Immunotherapy (2015-07) https://doi.org/ggqbpz
DOI: 10.2217/imt.15.33 · PMID: 26098703 · PMCID: PMC5068219

232. **A Potent and Broad Neutralizing Antibody Recognizes and Penetrates the HIV Glycan Shield**
R. Pejchal, K. J. Doores, L. M. Walker, R. Khayat, P.-S. Huang, S.-K. Wang, R. L. Stanfield, J.-P. Julien, A. Ramos, M. Crispin, … I. A. Wilson
Science (2011-10-13) https://doi.org/bzqv8c
DOI: 10.1126/science.1213256 · PMID: 21998254 · PMCID: PMC3280215

233. **Broadly Neutralizing Antibodies against HIV: Back to Blood**
Amir Dashti, Anthony L. DeVico, George K. Lewis, Mohammad M. Sajadi
Trends in Molecular Medicine (2019-03) https://doi.org/ggqbpr
DOI: 10.1016/j.molmed.2019.01.007 · PMID: 30792120 · PMCID: PMC6401214

234. **Importance of Neutralizing Monoclonal Antibodies Targeting Multiple Antigenic Sites on the Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein To Avoid Neutralization Escape**
Lingshu Wang, Wei Shi, James D. Chappell, M. Gordon Joyce, Yi Zhang, Masaru Kanekiyo, Michelle M. Becker, Neeltje van Doremalen, Robert Fischer, Nianshuang Wang, … Barney S. Graham
Journal of Virology (2018-04-27) https://doi.org/ggqbpv
DOI: 10.1128/jvi.02002-17 · PMID: 29514901 · PMCID: PMC5923077

235. **Anti–spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection**
Li Liu, Qiang Wei, Qingqing Lin, Jun Fang, Haibo Wang, Hauyee Kwok, Hangying Tang, Kenji Nishiura, Jie Peng, Zhiwu Tan, … Zhiwei Chen
JCI Insight (2019-02-21) https://doi.org/ggqbpw
DOI: 10.1172/jci.insight.123158 · PMID: 30830861 · PMCID: PMC6478436

236. **The antiviral effect of interferon-beta against SARS-Coronavirus is not mediated by MxA protein**

Martin Spiegel, Andreas Pichlmair, Elke Mühlberger, Otto Haller, Friedemann Weber
Journal of Clinical Virology (2004-07) https://doi.org/cmc3ds
DOI: 10.1016/j.jcv.2003.11.013 · PMID: 15135736 · PMCID: PMC7128634

237. **Coronavirus virulence genes with main focus on SARS-CoV envelope gene**
Marta L. DeDiego, Jose L. Nieto-Torres, Jose M. Jimenez-Guardeño, Jose A. Regla-Nava, Carlos Castaño-Rodriguez, Raul Fernandez-Delgado, Fernando Usera, Luis Enjuanes
Virus Research (2014-12) https://doi.org/f6wm24
DOI: 10.1016/j.virusres.2014.07.024 · PMID: 25093995 · PMCID: PMC4261026

238. **The COVID-19 vaccine development landscape**
Tung Thanh Le, Zacharias Andreadakis, Arun Kumar, Raúl Gómez Román, Stig Tollefsen, Melanie Saville, Stephen Mayhew
Nature Reviews Drug Discovery (2020-04-09) https://doi.org/ggrnbr
DOI: 10.1038/d41573-020-00073-5 · PMID: 32273591

239. **Developing Covid-19 Vaccines at Pandemic Speed**
Nicole Lurie, Melanie Saville, Richard Hatchett, Jane Halton
New England Journal of Medicine (2020-03-30) https://doi.org/ggq8bc
DOI: 10.1056/nejmp2005630 · PMID: 32227757

240. **Newer Vaccine Technologies Deployed to Develop COVID-19 Shot**
The Scientist Magazine®
https://www.the-scientist.com/news-opinion/newer-vaccine-technologies-deployed-to-develop-covid-19-shot-67152

241. **WHO | DNA vaccines**
WHO
https://www.who.int/biologicals/areas/vaccines/dna/en/

242. **Phase 1 Open-label Study to Evaluate the Safety, Tolerability and Immunogenicity of INO-4800, a Prophylactic Vaccine Against SARS-CoV-2, Administered Intradermally Followed by Electroporation in Healthy Volunteers**
Inovio Pharmaceuticals
clinicaltrials.gov (2020-04-21) https://clinicaltrials.gov/ct2/show/NCT04336410

243. **Electroporation delivery of DNA vaccines: prospects for success**
Niranjan Y Sardesai, David B Weiner
Current Opinion in Immunology (2011-06) https://doi.org/cq8b4p
DOI: 10.1016/j.coi.2011.03.008 · PMID: 21530212 · PMCID: PMC3109217

244. **Tolerability of intramuscular and intradermal delivery by CELLECTRA ® adaptive constant current electroporation device in healthy volunteers**
Malissa C Diehl, Jessica C Lee, Stephen E Daniels, Pablo Tebas, Amir S Khan, Mary Giffear, Niranjan Y Sardesai, Mark L Bagarazzi
Human Vaccines & Immunotherapeutics (2014-10-27) https://doi.org/ggrj7h
DOI: 10.4161/hv.24702 · PMID: 24051434 · PMCID: PMC3906411

245. **Advances in mRNA Vaccines for Infectious Diseases**
Cuiling Zhang, Giulietta Maruggi, Hu Shan, Junwei Li
Frontiers in Immunology (2019-03-27) https://doi.org/ggsnm7
DOI: 10.3389/fimmu.2019.00594 · PMID: 30972078 · PMCID: PMC6446947

246. **mRNA vaccine delivery using lipid nanoparticles**
Andreas M Reichmuth, Matthias A Oberli, Ana Jaklenec, Robert Langer, Daniel Blankschtein
Therapeutic Delivery (2016-05) https://doi.org/f8xfzc
DOI: 10.4155/tde-2016-0006 · PMID: 27075952 · PMCID: PMC5439223

247. **Mechanism of action of mRNA-based vaccines**
Carlo Iavarone, Derek T. O'hagan, Dong Yu, Nicolas F. Delahaye, Jeffrey B. Ulmer
Expert Review of Vaccines (2017-07-28) https://doi.org/ggsnm6
DOI: 10.1080/14760584.2017.1355245 · PMID: 28701102

248. **SARS-CoV-2 Vaccines: Status Report**
Fatima Amanat, Florian Krammer
Immunity (2020-04) https://doi.org/ggrdj4
DOI: 10.1016/j.immuni.2020.03.007 · PMID: 32259480 · PMCID: PMC7136867

249. **Evaluation of the Kinetics of mRNA Expression After Two Doses of GSK Biologicals' Candidate Tuberculosis (Tuberculosis) Vaccine GSK 692342 in Healthy Adults**
GlaxoSmithKline
clinicaltrials.gov (2019-03-20) https://clinicaltrials.gov/ct2/show/NCT01669096

250. **Nucleoside-modified mRNA immunization elicits influenza virus hemagglutinin stalk-specific antibodies**
Norbert Pardi, Kaela Parkhouse, Ericka Kirkpatrick, Meagan McMahon, Seth J. Zost, Barbara L. Mui, Ying K. Tam, Katalin Karikó, Christopher J. Barbosa, Thomas D. Madden, … Drew Weissman
Nature Communications (2018-08-22) https://doi.org/gd49qt
DOI: 10.1038/s41467-018-05482-0 · PMID: 30135514 · PMCID: PMC6105651

251. **RNA vaccines: an introduction**
PHG Foundation
https://www.phgfoundation.org/briefing/rna-vaccines

252. **T Follicular Helper Cell Differentiation, Function, and Roles in Disease**
Shane Crotty
Immunity (2014-10) https://doi.org/ggsp64
DOI: 10.1016/j.immuni.2014.10.004 · PMID: 25367570 · PMCID: PMC4223692

253. **mRNA vaccines — a new era in vaccinology**
Norbert Pardi, Michael J. Hogan, Frederick W. Porter, Drew Weissman
Nature Reviews Drug Discovery (2018-01-12) https://doi.org/gcsmgr
DOI: 10.1038/nrd.2017.243 · PMID: 29326426 · PMCID: PMC5906799

254. **Phase I, Open-Label, Dose-Ranging Study of the Safety and Immunogenicity of 2019-nCoV Vaccine (mRNA-1273) in Healthy Adults**
National Institute of Allergy and Infectious Diseases (NIAID)
clinicaltrials.gov (2020-04-30) https://clinicaltrials.gov/ct2/show/study/NCT04283461

255. **Design and Synthesis of Hydroxyferroquine Derivatives with Antimalarial and Antiviral Activities**
Christophe Biot, Wassim Daher, Natascha Chavain, Thierry Fandeur, Jamal Khalife, Daniel Dive, Erik De Clercq
Journal of Medicinal Chemistry (2006-05) https://doi.org/db4n83
DOI: 10.1021/jm0601856 · PMID: 16640347

256. **Sanofi and Regeneron begin global Kevzara® (sarilumab) clinical trial program in patients with severe COVID-19 - Mar 16, 2020** http://www.news.sanofi.us/2020-03-16-Sanofi-and-Regeneron-begin-global-Kevzara-R-sarilumab-clinical-trial-program-in-patients-with-severe-COVID-19

257. **An Adaptive Phase 2/3, Randomized, Double-blind, Placebo Controlled Study Assessing Efficacy and Safety of Sarilumab for Hospitalized Patients With COVID19**
Sanofi
clinicaltrials.gov (2020-05-06) https://clinicaltrials.gov/ct2/show/NCT04327388

258. **Prevention of infection caused by immunosuppressive drugs in gastroenterology**
Katarzyna Orlicka, Eleanor Barnes, Emma L. Culver
Therapeutic Advances in Chronic Disease (2013-04-22) https://doi.org/ggrqd3
DOI: 10.1177/2040622313485275 · PMID: 23819020 · PMCID: PMC3697844

259. **Immunosuppression for hyperinflammation in COVID-19: a double-edged sword?**
Andrew I Ritchie, Aran Singanayagam
The Lancet (2020-04) https://doi.org/ggq8hs
DOI: 10.1016/s0140-6736(20)30691-7 · PMID: 32220278 · PMCID: PMC7138169

260. **TNF-α inhibition for potential therapeutic modulation of SARS coronavirus infection**
Edward Tobinick
Current Medical Research and Opinion (2008-09-22) https://doi.org/bq4cx2
DOI: 10.1185/030079903125002757 · PMID: 14741070

261. **COVID-19: combining antiviral and anti-inflammatory treatments**
Justin Stebbing, Anne Phelan, Ivan Griffin, Catherine Tucker, Olly Oechsle, Dan Smith, Peter Richardson
The Lancet Infectious Diseases (2020-04) https://doi.org/dph5
DOI: 10.1016/s1473-3099(20)30132-8 · PMID: 32113509 · PMCID: PMC7158903

262. **Baricitinib as potential treatment for 2019-nCoV acute respiratory disease**
Peter Richardson, Ivan Griffin, Catherine Tucker, Dan Smith, Olly Oechsle, Anne Phelan, Justin Stebbing
The Lancet (2020-02) https://doi.org/ggnrsx
DOI: 10.1016/s0140-6736(20)30304-4 · PMID: 32032529 · PMCID: PMC7137985

263. **Lilly Begins Clinical Testing of Therapies for COVID-19**
Eli Lilly and Company
https://investor.lilly.com/news-releases/news-release-details/lilly-begins-clinical-testing-therapies-covid-19

264. **Baricitinib Combined With Antiviral Therapy in Symptomatic Patients Infected by COVID-19: an Open-label, Pilot Study**
Fabrizio Cantini
clinicaltrials.gov (2020-04-19) https://clinicaltrials.gov/ct2/show/NCT04320277

265. **Table 1, Cost-Comparison Table for Biologic Disease-Modifying Drugs for Rheumatoid Arthritis**
National Center for Biotechnology Information, U. S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 Usa
(2015-08) https://www.ncbi.nlm.nih.gov/books/NBK349513/table/T43/

266. https://escholarship.umassmed.edu/cgi/viewcontent.cgi?article

267. **An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice**
Timothy P. Sheahan, Amy C. Sims, Shuntai Zhou, Rachel L. Graham, Andrea J. Pruijssers, Maria L. Agostini, Sarah R. Leist, Alexandra Schäfer, Kenneth H. Dinnon, Laura J. Stevens, … Ralph S. Baric
Science Translational Medicine (2020-04-29) https://doi.org/ggrqd2
DOI: 10.1126/scitranslmed.abb5883 · PMID: 32253226 · PMCID: PMC7199910

268. **Antiviral Monoclonal Antibodies: Can They Be More Than Simple Neutralizing Agents?**
Mireia Pelegrin, Mar Naranjo-Gomez, Marc Piechaczyk
Trends in Microbiology (2015-10) https://doi.org/f7vzrf
DOI: 10.1016/j.tim.2015.07.005 · PMID: 26433697 · PMCID: PMC7127033

269. **Structure of M ^{pro} from COVID-19 virus and discovery of its inhibitors**
Zhenming Jin, Xiaoyu Du, Yechun Xu, Yongqiang Deng, Meiqin Liu, Yao Zhao, Bing Zhang, Xiaofeng Li, Leike Zhang, Chao Peng, … Haitao Yang
bioRxiv (2020-03-29) https://doi.org/ggqs5x
DOI: 10.1101/2020.02.26.964882

270. **Main protease structure and XChem fragment screen - - Diamond Light Source**https://www.diamond.ac.uk/covid-19/for-scientists/Main-protease-structure-and-XChem.html

271. **Using the MAARIE Framework To Read the Research Literature**
M. Corcoran
American Journal of Occupational Therapy (2006-07-01) https://doi.org/bqh97x
DOI: 10.5014/ajot.60.4.367 · PMID: 16915865

272. **Open collaborative writing with Manubot**
Daniel S. Himmelstein, Vincent Rubinetti, David R. Slochower, Dongbo Hu, Venkat S. Malladi, Casey S. Greene, Anthony Gitter
PLOS Computational Biology (2019-06-24) https://doi.org/c7np
DOI: 10.1371/journal.pcbi.1007128 · PMID: 31233491 · PMCID: PMC6611653

273. **Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody**
Xiaolong Tian, Cheng Li, Ailing Huang, Shuai Xia, Sicong Lu, Zhengli Shi, Lu Lu, Shibo Jiang, Zhenlin Yang, Yanling Wu, Tianlei Ying
bioRxiv (2020-01-28) https://doi.org/ggjqfd
DOI: 10.1101/2020.01.28.923011

274. **Integrative Bioinformatics Analysis Provides Insight into the Molecular Mechanisms of 2019-nCoV**
Xiang He, Lei Zhang, Qin Ran, Anying Xiong, Junyi Wang, Dehong Wu, Feng Chen, Guoping Li
medRxiv (2020-02-05) https://doi.org/ggrbd8
DOI: 10.1101/2020.02.03.20020206

275. **Diarrhea may be underestimated: a missing link in 2019 novel coronavirus**
Weicheng Liang, Zhijie Feng, Shitao Rao, Cuicui Xiao, Zexiao Lin, Qi Zhang, Wei Qi
medRxiv (2020-02-17) https://doi.org/ggrbdw
DOI: 10.1101/2020.02.03.20020289

276. **Specific ACE2 Expression in Cholangiocytes May Cause Liver Damage After 2019-nCoV Infection**
Xiaoqiang Chai, Longfei Hu, Yan Zhang, Weiyu Han, Zhou Lu, Aiwu Ke, Jian Zhou, Guoming Shi, Nan Fang, Jia Fan, … Fei Lan
bioRxiv (2020-02-04) https://doi.org/ggq626
DOI: 10.1101/2020.02.03.931766

277. **Recapitulation of SARS-CoV-2 Infection and Cholangiocyte Damage with Human Liver Organoids**
Bing Zhao, Chao Ni, Ran Gao, Yuyan Wang, Li Yang, Jinsong Wei, Ting Lv, Jianqing Liang, Qisheng Zhang, Wei Xu, … Xinhua Lin
bioRxiv (2020-03-17) https://doi.org/ggq648
DOI: 10.1101/2020.03.16.990317

278. **ACE2 expression by colonic epithelial cells is associated with viral infection, immunity and energy metabolism**
Jun Wang, Shanmeizi Zhao, Ming Liu, Zhiyao Zhao, Yiping Xu, Ping Wang, Meng Lin, Yanhui Xu, Bing Huang, Xiaoyu Zuo, … Yuxia Zhang
medRxiv (2020-02-07) https://doi.org/ggrfbx
DOI: 10.1101/2020.02.05.20020545

279. **The Pathogenicity of SARS-CoV-2 in hACE2 Transgenic Mice**
Linlin Bao, Wei Deng, Baoying Huang, Hong Gao, Jiangning Liu, Lili Ren, Qiang Wei, Pin Yu, Yanfeng Xu, Feifei Qi, … Chuan Qin
bioRxiv (2020-02-28) https://doi.org/dph2
DOI: 10.1101/2020.02.07.939389

280. **Caution on Kidney Dysfunctions of COVID-19 Patients**
Zhen Li, Ming Wu, Jiwei Yao, Jie Guo, Xiang Liao, Siji Song, Jiali Li, Guangjie Duan, Yuanxiu Zhou, Xiaojun Wu, … Anti-2019-nCoV Volunteers
medRxiv (2020-03-27) https://doi.org/ggq627
DOI: 10.1101/2020.02.08.20021212

281. **Acute renal impairment in coronavirus-associated severe acute respiratory syndrome**
Kwok Hong Chu, Wai Kay Tsang, Colin S. Tang, Man Fai Lam, Fernand M. Lai, Ka Fai To, Ka Shun Fung, Hon Lok Tang, Wing Wa Yan, Hilda W. H. Chan, … Kar Neng Lai
Kidney International (2005-02) https://doi.org/b7tgtx
DOI: 10.1111/j.1523-1755.2005.67130.x · PMID: 15673319 · PMCID: PMC7112337

282. **Single-cell Analysis of ACE2 Expression in Human Kidneys and Bladders Reveals a Potential Route of 2019-nCoV Infection**
Wei Lin, Longfei Hu, Yan Zhang, Joshua D. Ooi, Ting Meng, Peng Jin, Xiang Ding, Longkai Peng, Lei Song, Zhou Xiao, … Yong Zhong
bioRxiv (2020-02-18) https://doi.org/ggq629
DOI: 10.1101/2020.02.08.939892

283. **The immune vulnerability landscape of the 2019 Novel Coronavirus, SARS-CoV-2**
James Zhu, Jiwoong Kim, Xue Xiao, Yunguan Wang, Danni Luo, Ran Chen, Lin Xu, He Zhang, Guanghua Xiao, John W. Schoggins, … Yang Xie
bioRxiv (2020-03-23) https://doi.org/ggq628
DOI: 10.1101/2020.02.08.939553

284. **Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis**
I Hamming, W Timens, MLC Bulthuis, AT Lely, GJ Navis, H van Goor
The Journal of Pathology (2004-06) https://doi.org/bhpzc3
DOI: 10.1002/path.1570 · PMID: 15141377 · PMCID: PMC7167720

285. **Clinical Course and Outcomes of Critically Ill Patients With Middle East Respiratory Syndrome Coronavirus Infection**
Yaseen M. Arabi, Ahmed A. Arifi, Hanan H. Balkhy, Hani Najm, Abdulaziz S. Aldawood, Alaa Ghabashi, Hassan Hawa, Adel Alothman, Abdulaziz Khaldi, Basel Al Raiy
Annals of Internal Medicine (2014-03-18) https://doi.org/ggptxw
DOI: 10.7326/m13-2486 · PMID: 24474051

286. **Neutrophil-to-Lymphocyte Ratio Predicts Severe Illness Patients with 2019 Novel Coronavirus in the Early Stage**
Jingyuan Liu, Yao Liu, Pan Xiang, Lin Pu, Haofeng Xiong, Chuansheng Li, Ming Zhang, Jianbo Tan, Yanli Xu, Rui Song, … Xianbo Wang
medRxiv (2020-02-12) https://doi.org/ggrbdx
DOI: 10.1101/2020.02.10.20021584

287. **Dysregulation of immune response in patients with COVID-19 in Wuhan, China**
Chuan Qin, Luoqi Zhou, Ziwei Hu, Shuoqi Zhang, Sheng Yang, Yu Tao, Cuihong Xie, Ke Ma, Ke Shang, Wei Wang, Dai-Shi Tian
Clinical Infectious Diseases (2020-03-12) https://doi.org/ggpxcf
DOI: 10.1093/cid/ciaa248 · PMID: 32161940 · PMCID: PMC7108125

288. **Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP)**
Suxin Wan, Qingjie Yi, Shibing Fan, Jinglong Lv, Xianxiang Zhang, Lian Guo, Chunhui Lang, Qing Xiao, Kaihu Xiao, Zhengjun Yi, … Yongping Chen
medRxiv (2020-02-12) https://doi.org/ggq63b
DOI: 10.1101/2020.02.10.20021832

289. **Longitudinal Characteristics of Lymphocyte Responses and Cytokine Profiles in the Peripheral Blood of SARS-CoV-2 Infected Patients**
Jing Liu, Sumeng Li, Jia Liu, Boyun Liang, Xiaobei Wang, Wei Li, Hua Wang, Qiaoxia Tong, Jianhua Yi, Lei Zhao, … Xin Zheng
SSRN Electronic Journal (2020) https://doi.org/ggq655
DOI: 10.2139/ssrn.3539682

290. **Epidemiological and Clinical Characteristics of 17 Hospitalized Patients with 2019 Novel Coronavirus Infections Outside Wuhan, China**
Jie Li, Shilin Li, Yurui Cai, Qin Liu, Xue Li, Zhaoping Zeng, Yanpeng Chu, Fangcheng Zhu, Fanxin Zeng
medRxiv (2020-02-12) https://doi.org/ggq63c
DOI: 10.1101/2020.02.11.20022053

291. **ACE2 Expression in Kidney and Testis May Cause Kidney and Testis Damage After 2019-nCoV Infection**
Caibin Fan, Kai Li, Yanhong Ding, Wei Lu Lu, Jianqing Wang
medRxiv (2020-02-13) https://doi.org/ggq63d
DOI: 10.1101/2020.02.12.20022418

292. **Aberrant pathogenic GM-CSF $^{+}$ T cells and inflammatory CD14 $^{+}$ CD16 $^{+}$ monocytes in severe pulmonary syndrome patients of a new coronavirus**
Yonggang Zhou, Binqing Fu, Xiaohu Zheng, Dongsheng Wang, Changcheng Zhao, Yingjie qi, Rui Sun, Zhigang Tian, Xiaoling Xu, Haiming Wei
bioRxiv (2020-02-20) https://doi.org/ggq63f
DOI: 10.1101/2020.02.12.945576

293. **Clinical Characteristics of 2019 Novel Infected Coronavirus Pneumonia：A Systemic Review and Meta-analysis**
Kai Qian, Yi Deng, Yonghang Tai, Jun Peng, Hao Peng, Lihong Jiang
medRxiv (2020-02-17) https://doi.org/ggrgbq
DOI: 10.1101/2020.02.14.20021535

294. **Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients**
Jing Liu, Sumeng Li, Jia Liu, Boyun Liang, Xiaobei Wang, Hua Wang, Wei Li, Qiaoxia Tong, Jianhua Yi, Lei Zhao, … Xin Zheng
medRxiv (2020-02-22) https://doi.org/ggq63g
DOI: 10.1101/2020.02.16.20023671

295. **Clinical and immunologic features in severe and moderate forms of Coronavirus Disease 2019**
Guang Chen, Di Wu, Wei Guo, Yong Cao, Da Huang, Hongwu Wang, Tao Wang, Xiaoyun Zhang, Huilong Chen, Haijing Yu, … Qin Ning
medRxiv (2020-02-19) https://doi.org/ggq63h
DOI: 10.1101/2020.02.16.20023903

296. **SARS-CoV-2 and SARS-CoV Spike-RBD Structure and Receptor Binding Comparison and Potential Implications on Neutralizing Antibody and Vaccine Development**
Chunyun Sun, Long Chen, Ji Yang, Chunxia Luo, Yanjing Zhang, Jing Li, Jiahui Yang, Jie Zhang, Liangzhi Xie
bioRxiv (2020-02-20) https://doi.org/ggq63j
DOI: 10.1101/2020.02.16.951723

297. **Protection of Rhesus Macaque from SARS-Coronavirus challenge by recombinant adenovirus vaccine**
Yiyou Chen, Qiang Wei, Ruobing Li, Hong Gao, Hua Zhu, Wei Deng, Linlin Bao, Wei Tong, Zhe Cong, Hong Jiang, Chuan Qin
bioRxiv (2020-02-21) https://doi.org/ggq63k
DOI: 10.1101/2020.02.17.951939

298. **Reduction and Functional Exhaustion of T Cells in Patients with Coronavirus Disease 2019 (COVID-19)**
Bo Diao, Chenhui Wang, Yingjun Tan, Xiewan Chen, Ying Liu, Lifeng Ning, Li Chen, Min Li, Yueping Liu, Gang Wang, … Yongwen Chen
medRxiv (2020-02-20) https://doi.org/ggq63m
DOI: 10.1101/2020.02.18.20024364

299. **Clinical characteristics of 25 death cases infected with COVID-19 pneumonia: a retrospective review of medical records in a single medical center, Wuhan, China**
Xun Li, Luwen Wang, Shaonan Yan, Fan Yang, Longkui Xiang, Jiling Zhu, Bo Shen, Zuojiong Gong
medRxiv (2020-02-25) https://doi.org/ggq63n
DOI: 10.1101/2020.02.19.20025239

300. **SARS-CoV-2 infection does not significantly cause acute renal injury: an analysis of 116 hospitalized patients with COVID-19 in a single hospital, Wuhan, China**
Lunwen Wang, Xun Li, Hui Chen, Shaonan Yan, Yan Li, Dong Li, Zuojiong Gong
medRxiv (2020-02-23) https://doi.org/ggq63p
DOI: 10.1101/2020.02.19.20025288

301. **Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China**
Dawei Wang, Bo Hu, Chang Hu, Fangfang Zhu, Xing Liu, Jing Zhang, Binbin Wang, Hui Xiang, Zhenshun Cheng, Yong Xiong, … Zhiyong Peng
JAMA (2020-02-07) https://doi.org/ggkh48
DOI: 10.1001/jama.2020.1585 · PMID: 32031570 · PMCID: PMC7042881

302. **Clinical characteristics of 2019 novel coronavirus infection in China**
Wei-jie Guan, Zheng-yi Ni, Yu Hu, Wen-hua Liang, Chun-quan Ou, Jian-xing He, Lei Liu, Hong Shan, Chun-liang Lei, David SC Hui, … Nan-shan Zhong
medRxiv (2020-02-09) https://doi.org/ggkj9s
DOI: 10.1101/2020.02.06.20020974

303. **Potential T-cell and B-cell Epitopes of 2019-nCoV**
Ethan Fast, Russ B. Altman, Binbin Chen
bioRxiv (2020-03-18) https://doi.org/ggq63q
DOI: 10.1101/2020.02.19.955484

304. **Structure, function and antigenicity of the SARS-CoV-2 spike glycoprotein**
Alexandra C. Walls, Young-Jun Park, M. Alexandra Tortorici, Abigail Wall, Andrew T. McGuire, David Veesler
bioRxiv (2020-02-20) https://doi.org/ggrgbr
DOI: 10.1101/2020.02.19.956581

305. **Breadth of concomitant immune responses underpinning viral clearance and patient recovery in a non-severe case of COVID-19**
Irani Thevarajan, Thi HO Nguyen, Marios Koutsakos, Julian Druce, Leon Caly, Carolien E van de Sandt, Xiaoxiao Jia, Suellen Nicholson, Mike Catton, Benjamin Cowie, … Katherine Kedzierska
medRxiv (2020-02-23) https://doi.org/ggq63r
DOI: 10.1101/2020.02.20.20025841

306. **The landscape of lung bronchoalveolar immune cells in COVID-19 revealed by single-cell RNA sequencing**
Minfeng Liao, Yang Liu, Jin Yuan, Yanling Wen, Gang Xu, Juanjuan Zhao, Lin Chen, Jinxiu Li, Xin Wang, Fuxiang Wang, … Zheng Zhang
medRxiv (2020-02-26) https://doi.org/ggq63s
DOI: 10.1101/2020.02.23.20026690

307. **Influenza A Virus Infection Induces Hyperresponsiveness in Human Lung Tissue-Resident and Peripheral Blood NK Cells**
Marlena Scharenberg, Sindhu Vangeti, Eliisa Kekäläinen, Per Bergman, Mamdoh Al-Ameri, Niclas Johansson, Klara Sondén, Sara Falck-Jones, Anna Färnert, Hans-Gustaf Ljunggren, … Nicole Marquardt
Frontiers in Immunology (2019-05-17) https://doi.org/ggq656
DOI: 10.3389/fimmu.2019.01116 · PMID: 31156653 · PMCID: PMC6534051

308. **Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China**
Chaolin Huang, Yeming Wang, Xingwang Li, Lili Ren, Jianping Zhao, Yi Hu, Li Zhang, Guohui Fan, Jiuyang Xu, Xiaoying Gu, … Bin Cao
The Lancet (2020-02) https://doi.org/ggjfnn
DOI: 10.1016/s0140-6736(20)30183-5 · PMID: 31986264 · PMCID: PMC7159299

309. **Alveolar Macrophages in the Resolution of Inflammation, Tissue Repair, and Tolerance to Infection**
Benoit Allard, Alice Panariti, James G. Martin
Frontiers in Immunology (2018-07-31) https://doi.org/gd3bnz
DOI: 10.3389/fimmu.2018.01777 · PMID: 30108592 · PMCID: PMC6079255

310. **PPAR-γ in Macrophages Limits Pulmonary Inflammation and Promotes Host Recovery following Respiratory Viral Infection**
Su Huang, Bibo Zhu, In Su Cheon, Nick P. Goplen, Li Jiang, Ruixuan Zhang, R. Stokes Peebles, Matthias Mack, Mark H. Kaplan, Andrew H. Limper, Jie Sun
Journal of Virology (2019-04-17) https://doi.org/ggq652
DOI: 10.1128/jvi.00030-19 · PMID: 30787149 · PMCID: PMC6475778

311. **Can routine laboratory tests discriminate 2019 novel coronavirus infected pneumonia from other community-acquired pneumonia?**
Yunbao Pan, Guangming Ye, Xiantao Zeng, Guohong Liu, Xiaojiao Zeng, Xianghu Jiang, Jin Zhao, Liangjun Chen, Shuang Guo, Qiaoling Deng, … Xinghuan Wang
medRxiv (2020-02-25) https://doi.org/ggq63t
DOI: 10.1101/2020.02.25.20024711

312. **Correlation Analysis Between Disease Severity and Inflammation-related Parameters in Patients with COVID-19 Pneumonia**
Jing Gong, Hui Dong, Song Qing Xia, Yi Zhao Huang, Dingkun Wang, Yan Zhao, Wenhua Liu, Shenghao Tu, Mingmin Zhang, Qi Wang, Fuer Lu
medRxiv (2020-02-26) https://doi.org/ggq63v
DOI: 10.1101/2020.02.25.20025643

313. **An Effective CTL Peptide Vaccine for Ebola Zaire Based on Survivors' CD8+ Targeting of a Particular Nucleocapsid Protein Epitope with Potential Implications for COVID-19 Vaccine Design**
CV Herst, S Burkholz, J Sidney, A Sette, PE Harris, S Massey, T Brasel, E Cunha-Neto, DS Rosa, WCH Chao, … R Rubsamen
bioRxiv (2020-04-06) https://doi.org/ggq63x
DOI: 10.1101/2020.02.25.963546

314. **Epitope-based peptide vaccine design and target site characterization against novel coronavirus disease caused by SARS-CoV-2**
Lin Li, Ting Sun, Yufei He, Wendong Li, Yubo Fan, Jing Zhang
bioRxiv (2020-02-27) https://doi.org/ggnqwt
DOI: 10.1101/2020.02.25.965434

315. **The definition and risks of Cytokine Release Syndrome-Like in 11 COVID-19-Infected Pneumonia critically ill patients: Disease Characteristics and Retrospective Analysis**
Wenjun Wang, Jianxing He, puyi Lie, liyan Huang, Sipei Wu, yongping lin, xiaoqing liu
medRxiv (2020-02-27) https://doi.org/ggrgbs
DOI: 10.1101/2020.02.26.20026989

316. **Clinical characteristics of 36 non-survivors with COVID-19 in Wuhan, China**
Ying Huang, Rui Yang, Ying Xu, Ping Gong
medRxiv (2020-03-05) https://doi.org/ggq63z
DOI: 10.1101/2020.02.27.20029009

317. **Risk factors related to hepatic injury in patients with corona virus disease 2019**
Lu Li, Shuang Li, Manman Xu, Pengfei Yu, Sujun Zheng, Zhongping Duan, Jing Liu, Yu Chen, Junfeng Li
medRxiv (2020-03-10) https://doi.org/ggq632
DOI: 10.1101/2020.02.28.20028514

318. **Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely associated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients**
Xiaohua Chen, Binghong Zhao, Yueming Qu, Yurou Chen, Jie Xiong, Yong Feng, Dong Men, Qianchuan Huang, Ying Liu, Bo Yang, … Feng Li
medRxiv (2020-03-03) https://doi.org/ggq633
DOI: 10.1101/2020.02.29.20029520

319. **Prognostic factors in the acute respiratory distress syndrome**
Wei Chen, Lorraine B Ware
Clinical and Translational Medicine (2015-07-02) https://doi.org/ggq653
DOI: 10.1186/s40169-015-0065-2 · PMID: 26162279 · PMCID: PMC4534483

320. **Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study**
Li Tan, Qi Wang, Duanyang Zhang, Jinya Ding, Qianchuan Huang, Yi-Quan Tang, Qiongshu Wang, Hongming Miao
medRxiv (2020-03-03) https://doi.org/ggq634
DOI: 10.1101/2020.03.01.20029074

321. **The potential role of IL-6 in monitoring severe case of coronavirus disease 2019**
Tao Liu, Jieying Zhang, Yuhui Yang, Hong Ma, Zhengyu Li, Jiaoyue Zhang, Ji Cheng, Xiaoyun Zhang, Yanxia Zhao, Zihan Xia, … Jianhua Yi
medRxiv (2020-03-10) https://doi.org/ggq635
DOI: 10.1101/2020.03.01.20029769

322. **Clinical and Laboratory Profiles of 75 Hospitalized Patients with Novel Coronavirus Disease 2019 in Hefei, China**
Zonghao Zhao, Jiajia Xie, Ming Yin, Yun Yang, Hongliang He, Tengchuan Jin, Wenting Li, Xiaowu Zhu, Jing Xu, Changcheng Zhao, … Xiaoling Ma
medRxiv (2020-03-06) https://doi.org/ggq636
DOI: 10.1101/2020.03.01.20029785

323. **Exuberant elevation of IP-10, MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome**
Yang Yang, Chenguang Shen, Jinxiu Li, Jing Yuan, Minghui Yang, Fuxiang Wang, Guobao Li, Yanjie Li, Li Xing, Ling Peng, … Yingxia Liu
medRxiv (2020-03-06) https://doi.org/ggq637
DOI: 10.1101/2020.03.02.20029975

324. **Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019**
Juanjuan Zhao, Quan Yuan, Haiyan Wang, Wei Liu, Xuejiao Liao, Yingying Su, Xin Wang, Jing Yuan, Tingdong Li, Jinxiu Li, … Zheng Zhang

medRxiv (2020-03-02) https://doi.org/ggrbj6
DOI: 10.1101/2020.03.02.20030189

325. **Restoration of leukomonocyte counts is associated with viral clearance in COVID-19 hospitalized patients**
Xiaoping Chen, Jiaxin Ling, Pingzheng Mo, Yongxi Zhang, Qunqun Jiang, Zhiyong Ma, Qian Cao, Wenjia Hu, Shi Zou, Liangjun Chen, … Yong Xiong
medRxiv (2020-03-06) https://doi.org/ggq639
DOI: 10.1101/2020.03.03.20030437

326. **Effects of Systemically Administered Hydrocortisone on the Human Immunome**
Matthew J. Olnes, Yuri Kotliarov, Angélique Biancotto, Foo Cheung, Jinguo Chen, Rongye Shi, Huizhi Zhou, Ena Wang, John S. Tsang, Robert Nussenblatt, The CHI Consortium
Scientific Reports (2016-03-14) https://doi.org/f8dmvw
DOI: 10.1038/srep23002 · PMID: 26972611 · PMCID: PMC4789739

327. **Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis**
Giuseppe Lippi, Mario Plebani
Clinica Chimica Acta (2020-06) https://doi.org/ggpxp7
DOI: 10.1016/j.cca.2020.03.004 · PMID: 32145275 · PMCID: PMC7094472

328. **Clinical findings in critical ill patients infected with SARS-Cov-2 in Guangdong Province, China: a multi-center, retrospective, observational study**
Yonghao Xu, Zhiheng Xu, Xuesong Liu, Lihua Cai, Haichong Zheng, Yongbo Huang, Lixin Zhou, Linxi Huang, Yun Lin, Liehua Deng, … Yimin Li
medRxiv (2020-03-06) https://doi.org/ggq64b
DOI: 10.1101/2020.03.03.20030668

329. **Multi-epitope vaccine design using an immunoinformatics approach for 2019 novel coronavirus in China (SARS-CoV-2)**
Ye Feng, Min Qiu, Shengmei Zou, Yun Li, Kai Luo, Rongchang Chen, Yingqiang Sun, Kui Wang, Xinlei Zhuang, Shanshan Zhang, … Fan Mo
bioRxiv (2020-03-03) https://doi.org/ggq64c
DOI: 10.1101/2020.03.03.962332

330. **Clinical Features of Patients Infected with the 2019 Novel Coronavirus (COVID-19) in Shanghai, China**
Min Cao, Dandan Zhang, Youhua Wang, Yunfei Lu, Xiangdong Zhu, Ying Li, Honghao Xue, Yunxiao Lin, Min Zhang, Yiguo Sun, … Longping Peng
medRxiv (2020-03-06) https://doi.org/ggq64d
DOI: 10.1101/2020.03.04.20030395

331. **Serological detection of 2019-nCoV respond to the epidemic: A useful complement to nucleic acid testing**
Jin Zhang, Jianhua Liu, Na Li, Yong Liu, Rui Ye, Xiaosong Qin, Rui Zheng
medRxiv (2020-03-10) https://doi.org/ggq64f
DOI: 10.1101/2020.03.04.20030916

332. **Human Kidney is a Target for Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection**
Bo Diao, Chenhui Wang, Rongshuai Wang, Zeqing Feng, Yingjun Tan, Huiming Wang, Changsong Wang, Liang Liu, Ying Liu, Yueping Liu, … Yongwen Chen

medRxiv (2020-04-10) https://doi.org/ggq64g
DOI: 10.1101/2020.03.04.20031120

333. **COVID-19 early warning score: a multi-parameter screening tool to identify highly suspected patients**
Cong-Ying Song, Jia Xu, Jian-Qin He, Yuan-Qiang Lu
medRxiv (2020-03-08) https://doi.org/ggq64h
DOI: 10.1101/2020.03.05.20031906

334. **LY6E impairs coronavirus fusion and confers immune control of viral disease**
Stephanie Pfaender, Katrina B. Mar, Eleftherios Michailidis, Annika Kratzel, Dagny Hirt, Philip V'kovski, Wenchun Fan, Nadine Ebert, Hanspeter Stalder, Hannah Kleine-Weber, … Volker Thiel
bioRxiv (2020-03-07) https://doi.org/dpvn
DOI: 10.1101/2020.03.05.979260

335. **A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients**
Lei Liu, Wanbing Liu, Shengdian Wang, Shangen Zheng
medRxiv (2020-03-08) https://doi.org/ggq64j
DOI: 10.1101/2020.03.06.20031856

336. **Monoclonal antibodies for the S2 subunit of spike of SARS-CoV cross-react with the newly-emerged SARS-CoV-2**
Zhiqiang Zheng, Vanessa M. Monteil, Sebastian Maurer-Stroh, Chow Wenn Yew, Carol Leong, Nur Khairiah Mohd-Ismail, Suganya Cheyyatraivendran Arularasu, Vincent Tak Kwong Chow, Raymond Lin Tzer Pin, Ali Mirazimi, … Yee-Joo Tan
bioRxiv (2020-03-07) https://doi.org/ggrbj7
DOI: 10.1101/2020.03.06.980037

337. **Mortality of COVID-19 is Associated with Cellular Immune Function Compared to Immune Function in Chinese Han Population**
Qiang Zeng, Yong-zhe Li, Gang Huang, Wei Wu, Sheng-yong Dong, Yang Xu
medRxiv (2020-03-13) https://doi.org/ggq64k
DOI: 10.1101/2020.03.08.20031229

338. **Retrospective Analysis of Clinical Features in 101 Death Cases with COVID-19**
Jlan Chen, Hua Fan, Lin Zhang, Bin Huang, Muxin Zhu, Yong Zhou, WenHu Yu, Liping Zhu, Shaohui Cheng, Xiaogen Tao, Huan Zhang
medRxiv (2020-03-17) https://doi.org/ggq64n
DOI: 10.1101/2020.03.09.20033068

339. **Relationship between the ABO Blood Group and the COVID-19 Susceptibility**
Jiao Zhao, Yan Yang, Hanping Huang, Dong Li, Dongfeng Gu, Xiangfeng Lu, Zheng Zhang, Lei Liu, Ting Liu, Yukun Liu, … Peng George Wang
medRxiv (2020-03-27) https://doi.org/ggpn3d
DOI: 10.1101/2020.03.11.20031096

340. **The inhaled corticosteroid ciclesonide blocks coronavirus RNA replication by targeting viral NSP15**
Shutoku Matsuyama, Miyuki Kawase, Naganori Nao, Kazuya Shirato, Makoto Ujike, Wataru Kamitani, Masayuki Shimojima, Shuetsu Fukushi
bioRxiv (2020-03-12) https://doi.org/ggq64p
DOI: 10.1101/2020.03.11.987016

341. **Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19**
Bicheng Zhang, Xiaoyang Zhou, Chengliang Zhu, Fan Feng, Yanru Qiu, Jia Feng, Qingzhu Jia, Qibin Song, Bo Zhu, Jun Wang
medRxiv (2020-03-16) https://doi.org/ggq64q
DOI: 10.1101/2020.03.12.20035048

342. **Reinfection could not occur in SARS-CoV-2 infected rhesus macaques**
Linlin Bao, Wei Deng, Hong Gao, Chong Xiao, Jiayi Liu, Jing Xue, Qi Lv, Jiangning Liu, Pin Yu, Yanfeng Xu, … Chuan Qin
bioRxiv (2020-03-14) https://doi.org/ggn8r8
DOI: 10.1101/2020.03.13.990226

343. **A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV**
Meng Yuan, Nicholas C. Wu, Xueyong Zhu, Chang-Chun D. Lee, Ray T. Y. So, Huibin Lv, Chris K. P. Mok, Ian A. Wilson
bioRxiv (2020-03-14) https://doi.org/ggq64s
DOI: 10.1101/2020.03.13.991570

344. **Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR**
Lianhua Dong, Junbo Zhou, Chunyan Niu, Quanyi Wang, Yang Pan, Sitong Sheng, Xia Wang, Yongzhuo Zhang, Jiayi Yang, Manqing Liu, … Xiang Fang
medRxiv (2020-03-30) https://doi.org/ggqnqh
DOI: 10.1101/2020.03.14.20036129

345. **SARS-CoV-2 invades host cells via a novel route: CD147-spike protein**
Ke Wang, Wei Chen, Yu-Sen Zhou, Jian-Qi Lian, Zheng Zhang, Peng Du, Li Gong, Yang Zhang, Hong-Yong Cui, Jie-Jie Geng, … Zhi-Nan Chen
bioRxiv (2020-03-14) https://doi.org/ggq64t
DOI: 10.1101/2020.03.14.988345

346. **CD147 (EMMPRIN/Basigin) in kidney diseases: from an inflammation and immune system viewpoint**
Tomoki Kosugi, Kayaho Maeda, Waichi Sato, Shoichi Maruyama, Kenji Kadomatsu
Nephrology Dialysis Transplantation (2015-07) https://doi.org/ggq624
DOI: 10.1093/ndt/gfu302 · PMID: 25248362

347. **The roles of CyPA and CD147 in cardiac remodelling**
Hongyan Su, Yi Yang
Experimental and Molecular Pathology (2018-06) https://doi.org/ggq622
DOI: 10.1016/j.yexmp.2018.05.001 · PMID: 29772453

348. **Cancer-related issues of CD147.**
Ulrich H Weidle, Werner Scheuer, Daniela Eggle, Stefan Klostermann, Hannes Stockinger
Cancer genomics & proteomics https://www.ncbi.nlm.nih.gov/pubmed/20551248
PMID: 20551248

349. **Blood single cell immune profiling reveals the interferon-MAPK pathway mediated adaptive immune response for COVID-19**
Lulin Huang, Yi Shi, Bo Gong, Li Jiang, Xiaoqi Liu, Jialiang Yang, Juan Tang, Chunfang You, Qi Jiang, Bo Long, … Zhenglin Yang

medRxiv (2020-03-17) https://doi.org/ggq64v
DOI: 10.1101/2020.03.15.20033472

350. **Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections**
Huibin Lv, Nicholas C. Wu, Owen Tak-Yin Tsang, Meng Yuan, Ranawaka A. P. M. Perera, Wai Shing Leung, Ray T. Y. So, Jacky Man Chun Chan, Garrick K. Yip, Thomas Shiu Hong Chik, … Chris K. P. Mok
bioRxiv (2020-03-17) https://doi.org/ggq64w
DOI: 10.1101/2020.03.15.993097

351. **The feasibility of convalescent plasma therapy in severe COVID-19 patients: a pilot study**
Kai Duan, Bende Liu, Cesheng Li, Huajun Zhang, Ting Yu, Jieming Qu, Min Zhou, Li Chen, Shengli Meng, Yong Hu, … Xiaoming Yang
medRxiv (2020-03-23) https://doi.org/dqrs
DOI: 10.1101/2020.03.16.20036145

352. **Hydroxychloroquine and Azithromycin as a treatment of COVID-19: preliminary results of an open-label non-randomized clinical trial**
Philippe GAUTRET, Jean Christophe LAGIER, Philippe PAROLA, Van Thuan HOANG, Line MEDDED, Morgan MAILHE, Barbara DOUDIER, Johan COURJON, Valerie GIORDANENGO, Vera ESTEVES VIEIRA, … Didier RAOULT
medRxiv (2020-03-20) https://doi.org/dqbv
DOI: 10.1101/2020.03.16.20037135

353. **Chloroquine: Modes of action of an undervalued drug**
Rodolfo Thomé, Stefanie Costa Pinto Lopes, Fabio Trindade Maranhão Costa, Liana Verinaud
Immunology Letters (2013-06) https://doi.org/f5b5cr
DOI: 10.1016/j.imlet.2013.07.004 · PMID: 23891850

354. **Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy**
B. Lo, K. Zhang, W. Lu, L. Zheng, Q. Zhang, C. Kanellopoulou, Y. Zhang, Z. Liu, J. M. Fritz, R. Marsh, … M. B. Jordan
Science (2015-07-23) https://doi.org/f7kc8d
DOI: 10.1126/science.aaa1663 · PMID: 26206937

355. **The sequence of human ACE2 is suboptimal for binding the S spike protein of SARS coronavirus 2**
Erik Procko
bioRxiv (2020-04-06) https://doi.org/ggrbj8
DOI: 10.1101/2020.03.16.994236

356. **Comparative Pathogenesis Of COVID-19, MERS And SARS In A Non-Human Primate Model**
Barry Rockx, Thijs Kuiken, Sander Herfst, Theo Bestebroer, Mart M. Lamers, Dennis de Meulder, Geert van Amerongen, Judith van den Brand, Nisreen M. A. Okba, Debby Schipper, … Bart L. Haagmans
bioRxiv (2020-03-17) https://doi.org/ggq649
DOI: 10.1101/2020.03.17.995639

357. **Lethal Infection of K18-hACE2 Mice Infected with Severe Acute Respiratory Syndrome Coronavirus**
P. B. McCray, L. Pewe, C. Wohlford-Lenane, M. Hickey, L. Manzel, L. Shi, J. Netland, H. P. Jia, C. Halabi, C. D. Sigmund, … S. Perlman

Journal of Virology (2006-11-01) https://doi.org/b2dr3s
DOI: 10.1128/jvi.02012-06 · PMID: 17079315 · PMCID: PMC1797474

358. **Investigating the Impact of Asymptomatic Carriers on COVID-19 Transmission**
Jacob B Aguilar, Jeremy Samuel Faust, Lauren M. Westafer, Juan B. Gutierrez
medRxiv (2020-03-31) https://doi.org/ggqnvp
DOI: 10.1101/2020.03.18.20037994

359. **Antibody responses to SARS-CoV-2 in COVID-19 patients: the perspective application of serological tests in clinical practice**
Quan-xin Long, Hai-jun Deng, Juan Chen, Jieli Hu, Bei-zhong Liu, Pu Liao, Yong Lin, Li-hua Yu, Zhan Mo, Yin-yin Xu, … Ai-long Huang
medRxiv (2020-03-20) https://doi.org/ggpvz3
DOI: 10.1101/2020.03.18.20038018

360. **Heat inactivation of serum interferes with the immunoanalysis of antibodies to SARS-CoV-2**
Xiumei Hu, Taixue An, Bo Situ, Yuhai Hu, Zihao Ou, Qiang Li, Xiaojing He, Ye Zhang, Peifu Tian, Dehua Sun, … Lei Zheng
medRxiv (2020-03-16) https://doi.org/ggq646
DOI: 10.1101/2020.03.12.20034231

361. **SARS-CoV-2 specific antibody responses in COVID-19 patients**
NISREEN M. A. OKBA, Marcel A Muller, Wentao Li, Chunyan Wang, Corine H. GeurtsvanKessel, Victor M. Corman, Mart M. Lamers, Reina S. Sikkema, Erwin de Bruin, Felicity D. Chandler, … Bart L. Haagmans
medRxiv (2020-03-20) https://doi.org/ggpvz2
DOI: 10.1101/2020.03.18.20038059

362. **A brief review of antiviral drugs evaluated in registered clinical trials for COVID-19**
Drifa Belhadi, Nathan Peiffer-Smadja, François-Xavier Lescure, Yazdan Yazdanpanah, France Mentré, Cédric Laouénan
medRxiv (2020-03-27) https://doi.org/ggq65b
DOI: 10.1101/2020.03.18.20038190

363. **ACE-2 Expression in the Small Airway Epithelia of Smokers and COPD Patients: Implications for COVID-19**
Janice M Leung, Chen Xi Yang, Anthony Tam, Tawimas Shaipanich, Tillie L Hackett, Gurpreet K Singhera, Delbert R Dorscheid, Don D Sin
medRxiv (2020-03-23) https://doi.org/dqx2
DOI: 10.1101/2020.03.18.20038455

364. **Dynamic profile of severe or critical COVID-19 cases**
Yang Xu
medRxiv (2020-03-20) https://doi.org/ggrbj9
DOI: 10.1101/2020.03.18.20038513

365. **Association between Clinical, Laboratory and CT Characteristics and RT-PCR Results in the Follow-up of COVID-19 patients**
Hang Fu, Huayan Xu, Na Zhang, Hong Xu, Zhenlin Li, Huizhu Chen, Rong Xu, Ran Sun, Lingyi Wen, Linjun Xie, … Yingkun Guo
medRxiv (2020-03-23) https://doi.org/ggq65c
DOI: 10.1101/2020.03.19.20038315

366. **An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 and multiple endemic, epidemic and bat coronavirus**
Timothy P. Sheahan, Amy C. Sims, Shuntai Zhou, Rachel L. Graham, Collin S. Hill, Sarah R. Leist, Alexandra Schäfer, Kenneth H. Dinnon, Stephanie A. Montgomery, Maria L. Agostini, … Ralph S. Baric
bioRxiv (2020-03-20) https://doi.org/ggrbkb
DOI: 10.1101/2020.03.19.997890

367. **Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs**
Sangeun Jeon, Meehyun Ko, Jihye Lee, Inhee Choi, Soo Young Byun, Soonju Park, David Shum, Seungtaek Kim
bioRxiv (2020-03-28) https://doi.org/ggq65h
DOI: 10.1101/2020.03.20.999730

368. **Respiratory disease and virus shedding in rhesus macaques inoculated with SARS-CoV-2**
Vincent J. Munster, Friederike Feldmann, Brandi N. Williamson, Neeltje van Doremalen, Lizzette Pérez-Pérez, Jonathan Schulz, Kimberly Meade-White, Atsushi Okumura, Julie Callison, Beniah Brumbaugh, … Emmie de Wit
bioRxiv (2020-03-21) https://doi.org/ggq65j
DOI: 10.1101/2020.03.21.001628

369. **Ocular conjunctival inoculation of SARS-CoV-2 can cause mild COVID-19 in Rhesus macaques**
Wei Deng, Linlin Bao, Hong Gao, Zhiguang Xiang, Yajin Qu, Zhiqi Song, Shunran Gong, Jiayi Liu, Jiangning Liu, Pin Yu, … Chuan Qin
bioRxiv (2020-03-30) https://doi.org/ggq64r
DOI: 10.1101/2020.03.13.990036

370. **ACE2 Expression is Increased in the Lungs of Patients with Comorbidities Associated with Severe COVID-19**
Bruna GG Pinto, Antonio ER Oliveira, Youvika Singh, Leandro Jimenez, Andre NA Goncalves, Rodrigo LT Ogava, Rachel Creighton, Jean PS Peron, Helder I Nakaya
medRxiv (2020-03-27) https://doi.org/ggq65k
DOI: 10.1101/2020.03.21.20040261

371. **Meplazumab treats COVID-19 pneumonia: an open-labelled, concurrent controlled add-on clinical trial**
Huijie Bian, Zhao-Hui Zheng, Ding Wei, Zheng Zhang, Wen-Zhen Kang, Chun-Qiu Hao, Ke Dong, Wen Kang, Jie-Lai Xia, Jin-Lin Miao, … Ping Zhu
medRxiv (2020-03-24) https://doi.org/ggq65m
DOI: 10.1101/2020.03.21.20040691

372. **CD147 facilitates HIV-1 infection by interacting with virus-associated cyclophilin A**
T. Pushkarsky, G. Zybarth, L. Dubrovsky, V. Yurchenko, H. Tang, H. Guo, B. Toole, B. Sherry, M. Bukrinsky
Proceedings of the National Academy of Sciences (2001-05-15) https://doi.org/cc4c7p
DOI: 10.1073/pnas.111583198 · PMID: 11353871 · PMCID: PMC33473

373. **CD147/EMMPRIN Acts as a Functional Entry Receptor for Measles Virus on Epithelial Cells**
Akira Watanabe, Misako Yoneda, Fusako Ikeda, Yuri Terao-Muto, Hiroki Sato, Chieko Kai
Journal of Virology (2010-05-01) https://doi.org/dpcsqg
DOI: 10.1128/jvi.02168-09 · PMID: 20147391 · PMCID: PMC2863760

374. **Basigin is a receptor essential for erythrocyte invasion by Plasmodium falciparum**
Cécile Crosnier, Leyla Y. Bustamante, S. Josefin Bartholdson, Amy K. Bei, Michel Theron, Makoto Uchikawa, Souleymane Mboup, Omar Ndir, Dominic P. Kwiatkowski, Manoj T. Duraisingh, … Gavin J. Wright
Nature (2011-11-09) https://doi.org/dm59hf
DOI: 10.1038/nature10606 · PMID: 22080952 · PMCID: PMC3245779

375. **Function of HAb18G/CD147 in Invasion of Host Cells by Severe Acute Respiratory Syndrome Coronavirus**
Zhinan Chen, Li Mi, Jing Xu, Jiyun Yu, Xianhui Wang, Jianli Jiang, Jinliang Xing, Peng Shang, Airong Qian, Yu Li, … Ping Zhu
The Journal of Infectious Diseases (2005-03) https://doi.org/cd8snd
DOI: 10.1086/427811 · PMID: 15688292 · PMCID: PMC7110046

376. **CD147 mediates intrahepatic leukocyte aggregation and determines the extent of liver injury**
Christine Yee, Nathan M. Main, Alexandra Terry, Igor Stevanovski, Annette Maczurek, Alison J. Morgan, Sarah Calabro, Alison J. Potter, Tina L. Iemma, David G. Bowen, … Nicholas A. Shackel
PLOS ONE (2019-07-10) https://doi.org/ggq654
DOI: 10.1371/journal.pone.0215557 · PMID: 31291257 · PMCID: PMC6619953

377. **Characterisation of the transcriptome and proteome of SARS-CoV-2 using direct RNA sequencing and tandem mass spectrometry reveals evidence for a cell passage induced in-frame deletion in the spike glycoprotein that removes the furin-like cleavage site**
Andrew D. Davidson, Maia Kavanagh Williamson, Sebastian Lewis, Deborah Shoemark, Miles W. Carroll, Kate Heesom, Maria Zambon, Joanna Ellis, Phillip A. Lewis, Julian A. Hiscox, David A. Matthews
bioRxiv (2020-03-24) https://doi.org/ggq65n
DOI: 10.1101/2020.03.22.002204

378. **Modifications to the Hemagglutinin Cleavage Site Control the Virulence of a Neurotropic H1N1 Influenza Virus**
X. Sun, L. V. Tse, A. D. Ferguson, G. R. Whittaker
Journal of Virology (2010-06-16) https://doi.org/drs2zt
DOI: 10.1128/jvi.00797-10 · PMID: 20554779 · PMCID: PMC2919019

379. **The architecture of SARS-CoV-2 transcriptome**
Dongwan Kim, Joo-Yeon Lee, Jeong-Sun Yang, Jun Won Kim, V. Narry Kim, Hyeshik Chang
bioRxiv (2020-03-14) https://doi.org/ggpx9q
DOI: 10.1101/2020.03.12.988865

380. **First Clinical Study Using HCV Protease Inhibitor Danoprevir to Treat Naive and Experienced COVID-19 Patients**
Hongyi Chen, Zhicheng Zhang, Li Wang, Zhihua Huang, Fanghua Gong, Xiaodong Li, Yahong Chen, Jinzi J. WU
medRxiv (2020-03-24) https://doi.org/ggrgbt
DOI: 10.1101/2020.03.22.20034041

381. **Preclinical Characteristics of the Hepatitis C Virus NS3/4A Protease Inhibitor ITMN-191 (R7227)**
S. D. Seiwert, S. W. Andrews, Y. Jiang, V. Serebryany, H. Tan, K. Kossen, P. T. R. Rajagopalan, S. Misialek, S. K. Stevens, A. Stoycheva, … L. M. Blatt

Antimicrobial Agents and Chemotherapy (2008-09-29) https://doi.org/btpg52
DOI: 10.1128/aac.00699-08 · PMID: 18824605 · PMCID: PMC2592891

382. **Efficacy and Safety of All-oral, 12-week Ravidasvir Plus Ritonavir-boosted Danoprevir and Ribavirin in Treatment-naïve Noncirrhotic HCV Genotype 1 Patients: Results from a Phase 2/3 Clinical Trial in China**
Xiaoyuan Xu, Bo Feng, Yujuan Guan, Sujun Zheng, Jifang Sheng, Xingxiang Yang, Yuanji Ma, Yan Huang, Yi Kang, Xiaofeng Wen, … Lai Wei
Journal of Clinical and Translational Hepatology (2019-09-30) https://doi.org/ggrbkd
DOI: 10.14218/jcth.2019.00033 · PMID: 31608212 · PMCID: PMC6783683

383. **Potentially highly potent drugs for 2019-nCoV**
Duc Duy Nguyen, Kaifu Gao, Jiahui Chen, Rui Wang, Guo-Wei Wei
bioRxiv (2020-02-13) https://doi.org/ggrbj5
DOI: 10.1101/2020.02.05.936013

384. **Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset**
Bin Lou, Tigndong Li, Shufa Zheng, Yingying Su, Zhiyong Li, Wei Liu, Fei Yu, Shengxiang Ge, Qianda Zou, Quan Yuan, … Yu Chen
medRxiv (2020-03-27) https://doi.org/ggrbkc
DOI: 10.1101/2020.03.23.20041707

385. **SARS-CoV-2 launches a unique transcriptional signature from in vitro, ex vivo, and in vivo systems**
Daniel Blanco-Melo, Benjamin E. Nilsson-Payant, Wen-Chun Liu, Rasmus Møller, Maryline Panis, David Sachs, Randy A. Albrecht, Benjamin R. tenOever
bioRxiv (2020-03-24) https://doi.org/ggq65q
DOI: 10.1101/2020.03.24.004655

386. **A New Predictor of Disease Severity in Patients with COVID-19 in Wuhan, China**
Ying Zhou, Zhen Yang, Yanan Guo, Shuang Geng, Shan Gao, Shenglan Ye, Yi Hu, Yafei Wang
medRxiv (2020-03-27) https://doi.org/ggq65r
DOI: 10.1101/2020.03.24.20042119

387. **Metabolic disturbances and inflammatory dysfunction predict severity of coronavirus disease 2019 (COVID-19): a retrospective study**
Shuke Nie, Xueqing Zhao, Kang Zhao, Zhaohui Zhang, Zhentao Zhang, Zhan Zhang
medRxiv (2020-03-26) https://doi.org/ggq65s
DOI: 10.1101/2020.03.24.20042283

388. **Viral Kinetics and Antibody Responses in Patients with COVID-19**
Wenting Tan, Yanqiu Lu, Juan Zhang, Jing Wang, Yunjie Dan, Zhaoxia Tan, Xiaoqing He, Chunfang Qian, Qiangzhong Sun, Qingli Hu, … Guohong Deng
medRxiv (2020-03-26) https://doi.org/ggq65t
DOI: 10.1101/2020.03.24.20042382

389. **Global profiling of SARS-CoV-2 specific IgG/ IgM responses of convalescents using a proteome microarray**
He-wei Jiang, Yang Li, Hai-nan Zhang, Wei Wang, Dong Men, Xiao Yang, Huan Qi, Jie Zhou, Sheng-ce Tao
medRxiv (2020-03-27) https://doi.org/ggq65g
DOI: 10.1101/2020.03.20.20039495

390. **COVID-19 infection induces readily detectable morphological and inflammation-related phenotypic changes in peripheral blood monocytes, the severity of which correlate with patient outcome**
Dan Zhang, Rui Guo, Lei Lei, Hongjuan Liu, Yawen Wang, Yili Wang, Tongxin Dai, Tianxiao Zhang, Yanjun Lai, Jingya Wang, ... Jinsong Hu
medRxiv (2020-03-26) https://doi.org/ggq65v
DOI: 10.1101/2020.03.24.20042655

391. **Correlation between universal BCG vaccination policy and reduced morbidity and mortality for COVID-19: an epidemiological study**
Aaron Miller, Mac Josh Reandelar, Kimberly Fasciglione, Violeta Roumenova, Yan Li, Gonzalo H Otazu
medRxiv (2020-03-28) https://doi.org/ggq65w
DOI: 10.1101/2020.03.24.20042937

392. **Non-specific effects of BCG vaccine on viral infections**
S. J. C. F. M. Moorlag, R. J. W. Arts, R. van Crevel, M. G. Netea
Clinical Microbiology and Infection (2019-12) https://doi.org/ggq62z
DOI: 10.1016/j.cmi.2019.04.020 · PMID: 31055165

393. **BCG vaccination to Reduce the impact of COVID-19 in Australian healthcare workers following Coronavirus Exposure (BRACE) Trial | Murdoch Children's Research Institute**https://www.mcri.edu.au/BRACE

394. **Non-neural expression of SARS-CoV-2 entry genes in the olfactory epithelium suggests mechanisms underlying anosmia in COVID-19 patients**
David H. Brann, Tatsuya Tsukahara, Caleb Weinreb, Darren W. Logan, Sandeep Robert Datta
bioRxiv (2020-03-28) https://doi.org/ggqr4m
DOI: 10.1101/2020.03.25.009084

395. **Cigarette smoke triggers the expansion of a subpopulation of respiratory epithelial cells that express the SARS-CoV-2 receptor ACE2**
Joan C Smith, Jason Meyer Sheltzer
bioRxiv (2020-03-31) https://doi.org/ggq65x
DOI: 10.1101/2020.03.28.013672

396. **The comparative superiority of IgM-IgG antibody test to real-time reverse transcriptase PCR detection for SARS-CoV-2 infection diagnosis**
Rui Liu, Xinghui Liu, Huan Han, Muhammad Adnan Shereen, Zhili Niu, Dong Li, Fang Liu, Kailang Wu, Zhen Luo, Chengliang Zhu
medRxiv (2020-03-30) https://doi.org/ggqtp5
DOI: 10.1101/2020.03.28.20045765

8 Appendix 1

This appendix contains reviews produced by the Immunology Institute of the Icahn School of Medicine

8.1 Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody

Tian et al. *Emerg Microbes Infect 2020* [273]

8.1.1 Keywords

- Monoclonal antibody
- Cross-reactivity
- receptor binding domain

8.1.2 Summary

Considering the relatively high identity of the receptor binding domain (RBD) of the spike proteins from 2019-nCoV and SARS-CoV (73%), this study aims to assess the cross-reactivity of several anti-SARS-CoV monoclonal antibodies with 2019-nCoV. The results showed that the SARS-CoV-specific antibody CR3022 can potently bind 2019-nCoV RBD.

8.1.3 Main Findings

The structure of the 2019-nCoV spike RBD and its conformation in complex with the receptor angiotensin-converting enzyme (ACE2) was modeled *in silico* and compared with the SARS-CoV RBD structure. The models predicted very similar RBD-ACE2 interactions for both viruses. The binding capacity of representative SARS-CoV-RBD specific monoclonal antibodies (m396, CR3014, and CR3022) to recombinant 2019-nCoV RBD was then investigated by ELISA and their binding kinetics studied using biolayer interferometry. The analysis showed that only CR3022 was able to bind 2019-nCoV RBD with high affinity (KD of 6.3 nM), however it did not interfere with ACE2 binding. Antibodies m396 and CR3014, which target the ACE2 binding site of SARS-CoV failed to bind 2019-nCoV spike protein.

8.1.4 Limitations

The 2019-nCoV RBD largely differ from the SARS-CoV at the C-terminus residues, which drastically impact the cross-reactivity of antibodies described for other B beta-coronaviruses, including SARS-CoV. This study claims that CR3022 antibody could be a potential candidate for therapy. However, none of the antibodies assayed in this work showed cross-reactivity with the ACE2 binding site of 2019-nCoV, essential for the replication of this virus. Furthermore, neutralization assays with 2019-nCoV virus or pseudovirus were not performed. Although the use of neutralizing antibodies is an interesting approach, these results suggest that it is critical the development of novel monoclonal antibodies able to specifically bind 2019-nCoV spike protein.

8.1.5 Credit

Review by D.L.O as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.2 Integrative Bioinformatics Analysis Provides Insight into the Molecular Mechanisms of 2019-nCoV

He et al. *medRxiv* [274]

8.2.1 Keywords

- ACE2
- lungs
- smoking
- COPD
- asthma
- SARS-Cov
- IL-1
- IL-10
- IL-6
- IL-8

8.2.2 Main Findings

The authors used bioinformatics tools to identify features of ACE2 expression in the lungs of different patent groups: healthy, smokers, patients with chronic airway disease (i.e., COPD) or asthma. They used gene expression data publicly available from GEO that included lung tissues, bronchoalveolar lavage, bronchial epithelial cells, small airway epithelial cells, or SARS-Cov infected cells.

The authors describe no significant differences in ACE2 expression in lung tissues of Healthy, COPD, and Asthma groups ($p=0.85$); or in BAL of Healthy and COPD ($p=0.48$); or in epithelial brushings of Healthy and Mild/Moderate/Severe Asthma ($p=0.99$). ACE2 was higher in the small airway epithelium of long-term smokers vs non-smokers ($p<0.001$). Consistently, there was a trend of higher ACE2 expression in the bronchial airway epithelial cells 24h post-acute smoking exposure ($p=0.073$). Increasing ACE2 expression at 24h and 48h compared to 12h post SARS-Cov infection ($p=0.026$; $n=3$ at each time point) was also detected.

15 lung samples' data from healthy participants were separated into high and low ACE2 expression groups. "High" ACE2 expression was associated with the following GO pathways: innate and adaptive immune responses, B cell mediated immunity, cytokine secretion, and IL-1, IL-10, IL-6, IL-8 cytokines. The authors speculate that a high basal ACE2 expression will increase susceptibility to SARS-CoV infection.

In 3 samples SARS-Cov infection was associated with IL-1, IL-10 and IL-6 cytokine production (GO pathways) at 24h. And later, at 48h, with T-cell activation and T-cell cytokine production. It is unclear whether those changes were statistically significant.

The authors describe a time course quantification of immune infiltrates in epithelial cells infected with SARS-Cov infection. They state that in healthy donors ACE2 expression did not correlate with the immune cell infiltration. However, in SARS-Cov samples, at 48h they found that ACE2 correlated with neutrophils, NK-, Th17-, Th2-, Th1- cells, and DCs. Again, while authors claim significance, the corresponding correlation coefficients and p-values are not presented in the text or figures. In addition, the source of the data for this analysis is not clear.

Using network analysis, proteins SRC, FN1, MAPK3, LYN, MBP, NLRC4, NLRP1 and PRKCD were found to be central (Hub proteins) in the regulating network of cytokine secretion after coronavirus

infection. Authors conclude this indicates that these molecules were critically important in ACE2-induced inflammatory response. Additionally, authors speculate that the increased expression of ACE2 affected RPS3 and SRC, which were the two hub genes involved in viral replication and inflammatory response.

8.2.3 Limitations

The methods section is very limited and does not describe any of the statistical analyses; and description of the construction of the regulatory protein networks is also limited. For the findings in Figures 2 authors claim significance, which is not supported by p-values or coefficients. For the sample selection, would be useful if sample sizes and some of the patients' demographics (e.g. age) were described.

For the analysis of high vs low ACE2 expression in healthy subjects, it is not clear what was the cut off for 'high' expression and how it was determined. Additionally, further laboratory studies are warranted to confirm that high ACE2 gene expression would have high correlation with the amount of ACE2 protein on cell surface. For the GO pathway analysis significance was set at $p<0.05$, but not adjusted for multiple comparisons.

There were no samples with SARS-CoV-2 infection. While SARS-Cov and SARS-CoV-2 both use ACE2 to enter the host cells, the analysis only included data on SARS-Cov and any conclusions about SARS-CoV2 are limited.

Upon checking GSE accession numbers of the datasets references, two might not be cited correctly: GSE37758 ("A spergillus niger: Control (fructose) vs. steam-exploded sugarcane induction (SEB)"" was used in this paper as "lung tissue" data) and GSE14700 ("Steroid Pretreatment of Organ Donors to Prevent Postischemic Renal Allograft Failure: A Randomized, Controlled Trial" – was used as SARS-Cov infection data).

8.2.4 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.3 Diarrhea may be underestimated: a missing link in 2019 novel coronavirus

Liang et al. *medRxiv* [275]

8.3.1 Keywords

- SARS-CoV-2
- diarrhea
- ACE2
- scRNA-seq

8.3.2 Main Findings

This study examined the incidence of diarrhea in patients infected with SARS-CoV-2 across three recently published cohorts and found that there are statistically significant differences by Fisher's exact test. They report that this could be due to subjective diagnosis criterion for diarrhea or from patients first seeking medical care from gastroenterologist. In order to minimize nosocomial

infections arising from unsuspected patients with diarrhea and gain comprehensive understanding of transmission routes for this viral pathogen, they compared the transcriptional levels of ACE2 of various human tissues from NCBI public database as well as in small intestine tissue from CD57BL/6 mice using single cell sequencing. They show that ACE2 expression is not only increased in the human small intestine, but demonstrate a particular increase in mice enterocytes positioned on the surface of the intestinal lining exposed to viral pathogens. Given that ACE2 is the viral receptor for SARS-CoV-2 and also reported to regulate diarrhea, their data suggests the small intestine as a potential transmission route and diarrhea as a potentially underestimated symptom in COVID19 patients that must be carefully monitored. Interestingly, however, they show that ACE2 expression level is not elevated in human lung tissue.

8.3.3 Limitations

Although this study demonstrates a statistical difference in the incidence of diarrhea across three separate COVID19 patient cohorts, their conclusions are limited by a small sample size. Specifically, the p-value computed by Fisher's exact test is based on a single patient cohort of only six cases of which 33% are reported to have diarrhea, while the remaining two larger cohorts with 41 and 99 cases report 3% and 2% diarrhea incidence, respectively. Despite showing significance, they would need to acquire larger sample sizes and cohorts to minimize random variability and draw meaningful conclusions. Furthermore, they do not address why ACE2 expression level is not elevated in human lung tissue despite it being a major established route of transmission for SARS-CoV-2. It could be helpful to validate this result by looking at ACE2 expression in mouse lung tissue. Finally, although this study is descriptive and shows elevated ACE2 expression in small intestinal epithelial cells, it does not establish a mechanistic link to SARS-CoV-2 infection of the host. Overall, their claim that infected patients exhibiting diarrhea pose an increased risk to hospital staff needs to be further substantiated.

8.3.4 Significance

This study provides a possible transmission route and a potentially underappreciated clinical symptom for SARS-CoV-2 for better clinical management and control of COVID19.

8.3.5 Credit

Summary generated as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.4 Specific ACE2 Expression in Cholangiocytes May Cause Liver Damage After 2019-nCoV Infection

Chai et al. *bioRxiv* [276]

8.4.1 Keywords

- ACE2
- Cholangiocytes
- COVID-associated Liver Damage

8.4.2 Summary

Using both publicly available scRNA-seq dataset of liver samples from colorectal patients and scRNA-sequencing of four liver samples from healthy volunteers, the authors show that ACE2 is significantly enriched in the majority of cholangiocytes (59.7 %) but not in hepatocytes (2.6%).

8.4.3 Main Findings

Using bioinformatics approaches of RNASeq analysis, this study reveals that ACE2 dominates in cholangiocytes and is present at very low levels in hepatocytes.

8.4.4 Limitations

The study does not provide mechanistic insights into how SARS-CoV-2 can infect and replicate in cholangiocytes and the types of intrinsic anti-viral responses induced by cholangiocytes when infected. In addition, because the study relies on the assumption that SARS-CoV-2 infects cells only through ACE2, it cannot discount the possibility that the virus can infect hepatocytes through mechanisms other than ACE2-mediated entry. Furthermore, because the scRNA-seq analysis were performed on healthy liver samples, one cannot draw any definitive conclusions about gene expression states (including ACE2 expression in liver cell types) in system-wide inflammatory contexts.

8.4.5 Significance

This article with other studies on liver damage in COVID patients suggests that liver damage observed in COVID patients is more due to inflammatory cytokines than direct infection of the liver. Even if cholangiocytes are infectable by SARS-CoV-2 (which was demonstrated by human liver ductal organoid study ([277]), published clinical data show no significant increase in bile duct injury related indexes (i.e. alkaline phosphatase, gamma-glutamyl transpeptidase and total bilirubin). In sum, it underscores the importance of future studies characterizing cellular responses of extra-pulmonary organs in the context of COVID or at least in viral lung infections..

8.4.6 Credit

Summary generated by Chang Moon as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.5 ACE2 expression by colonic epithelial cells is associated with viral infection, immunity and energy metabolism

Wang et al. *medRxiv*. [278]

8.5.1 Keywords

- single cell RNA seq
- ACE2 expression
- human colonic biopsy

8.5.2 Main Findings

Colonic enterocytes primarily express ACE2. Cellular pathways associated with ACE2 expression include innate immune signaling, HLA up regulation, energy metabolism and apoptotic signaling.

8.5.3 Limitations

This is a study of colonic biopsies taken from 17 children with and without IBD and analyzed using scRNAseq to look at ACE2 expression and identify gene families correlated with ACE2 expression. The authors find ACE2 expression to be primarily in colonocytes. It is not clear why both healthy and IBD patients were combined for the analysis. Biopsies were all of children so extrapolation to adults is

limited. The majority of genes found to be negatively correlated with ACE2 expression include immunoglobulin genes (IGs). IG expression will almost certainly be low in colonocytes irrespective of ACE2 expression.

8.5.4 Significance

This study performs a retrospective analysis of ACE2 expression using an RNAseq dataset from intestinal biopsies of children with and without IBD. The implications for the CoV-19 epidemic are modest, but do provide support that ACE2 expression is specific to colonocytes in the intestines. The ontological pathway analysis provides some limited insights into gene expression associated with ACE2.

8.5.5 Credit

Summary generated as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.6 The Pathogenicity of 2019 Novel Coronavirus in hACE2 Transgenic Mice

Bao et al. *bioRxiv* [279]

8.6.1 Keywords

- Covid-19 mouse model
- hACE2 mice
- 2019-nCoV model
- ACE2
- 2019-nCoV

8.6.2 Main Findings

Using a transgenic human Angiotensin-converting enzyme 2 (hACE2) mouse that has previously been shown susceptible to infection by SARS-CoV, Bao et al. create a model of pandemic 2019-nCoV strain coronavirus. The model includes interstitial hyperplasia in lung tissue, moderate inflammation in bronchioles and blood vessels, and histology consistent with viral pneumonia at 3 days post infection. Wildtype did not experience these symptoms. In addition, viral antigen and hACE2 receptor were found to co-localize the lung by immunofluorescence 3-10 days post infection only in the hACE2 infected mice.

8.6.3 Limitations

The characterization of the infection remains incomplete, as well as lacking characterization of the immune response other than the presence of a single antiviral antibody. Though they claim to fulfill Koch's postulates, they only isolate the virus and re-infect Vero cells, rather than naive mice.

8.6.4 Significance

This paper establishes a murine model for 2019-nCoV infection with symptoms consistent with viral pneumonia. Though not fully characterized, this model allows in vivo analysis of viral entry and pathology that is important for the development of vaccines and antiviral therapeutics.

8.6.5 Credit

Review by Dan Fu Ruan, Evan Cody and Venu Pothula as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.7 Caution on Kidney Dysfunctions of 2019-nCoV Patients

Li et al. *medRxiv*. [280]

8.7.1 Keywords

CoVID-19, 2019-nCoV, SARS-CoV-2, kidney, clinical, creatinine, proteinuria, albuminuria, CT

8.7.2 Main Findings

- Retrospective study of 59 patients assayed key function indicators of the kidney–including urine protein, blood urea nitrogen (BUN), plasma creatinine (Cre), and renal CT scan data.
- Found that 34% of patients developed massive albuminuria on the first day of admission, and 63% developed proteinuria during their stay in hospital; and 19% of patients had high plasma creatinine, especially the terminal cases.
- CT analyses of 27 patients showed all patients to have abnormal kidney damage; indicate that inflammation and edema of the renal parenchyma very common.

8.7.3 Limitations

- No analysis of immunity-dependent damage and cytokines in blood/plasma/urine. Will be worth correlating disease progression with cytokine production, immune activity and kidney function.
- Extrapolating to earlier SARS-CoV studies provides the only rationale for viral-damage in kidney and resultant pathologic immune response *(understandable for this clinical study)*.

8.7.4 Significance

- Multiple lines of evidence along this study's finding point to the idea that renal impairment/injury is a key risk factor in 2019-nCoV patients similar to what has been reported for SARS-CoV[281]; this may be one of the major causes of virally-induced damage and contribute to multiorgan failure.
- ACE2 expression in kidney proximal tubule epithelia and bladder epithelia [282] support these clinical findings.
- Study argues for closely monitoring kidney function, and applying potential interventions including continuous renal replacement therapies (CRRT) for protecting kidney functions as early as possible, particularly for those with rising plasma creatinine.

8.7.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.8 Profiling the immune vulnerability landscape of the 2019 Novel Coronavirus

Zhu et al. *bioRxiv* [283]

8.8.1 Keywords

- epitope prediction
- vaccine development.

8.8.2 Main Findings

This study harnesses bioinformatic profiling to predict the potential of COV2 viral proteins to be presented on MHC I and II and to form linear B-cell epitopes. These estimates suggest a T-cell antigenic profile distinct from SARS-CoV or MERS-CoV, identify focused regions of the virus with a high density of predicted epitopes, and provide preliminary evidence for adaptive immune pressure in the genetic evolution of the virus.

8.8.3 Limitations

While the study performs a comprehensive analysis of potential epitopes within the virus genome, the analysis relies solely on bioinformatic prediction to examine MHC binding affinity and B-cell epitope potential and does not capture the immunogenicity or recognition of these epitopes. Future experimental validation in data from patients infected with SARS-CoV-2 will be important to validate and refine these findings. Thus some of the potential conclusions stated, including viral evolution toward lower immunogenicity or a dominant role for CD4+ T-cells rather than CD8+ T-cells in viral clearance, require further valiadtion.

8.8.4 Significance

These findings may help direct peptide vaccine design toward relevant epitopes and provide intriguing evidence of viral evolution in response to immune pressure.

8.8.5 Credit

Summary generated as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.9 Single-cell Analysis of ACE2 Expression in Human Kidneys and Bladders Reveals a Potential Route of 2019-nCoV Infection

Lin et al. *bioRxiv* [282]

8.9.1 Keywords

- CoVID-19
- 2019-nCoV
- SARS-CoV-2
- ACE2
- scRNAseq
- kidney
- bladder
- public dataset

8.9.2 Main Findings

- To investigate the possible cause of kidney damage in 2019-nCoV patients, authors used published kidney and bladder cell atlas data (GSE131685, GSE108097; 3 healthy donors each) as well as an unpublished kidney single-cell RNA-Seq data (in-house from 2 transplant donors) to evaluate ACE2 gene expressions in all cell types of healthy kidneys and bladders.

- They find enriched expression of ACE2 transcript in all subtypes of proximal tubule cells of kidney, with 5%-15% of both straight and convoluted proximal tubule cells expressing ACE2.

- They also find detectable levels of ACE2 in bladder epithelial cells, noting expression from around 1.5% of cells in the outer layer umbrella cells of the bladder epithelium and decreasing in the basal cells.

- Importantly endothelial or immune cells in kidney/bladder do not express ACE2.

8.9.3 Limitations

- This study primarily characterizes ACE2 expression (amongst other genes) from a small healthy-donor dataset, and will benefit from supporting data in (expired) patient samples to show functional viral damage. ACE2 transcript does not necessarily translate to viral permissiveness in kidney/bladder epithelia or cytokine release.

- This study focuses on only healthy tissue; it will be useful to analyze kidney/bladder epithelial ACE2 expression under inflammatory conditions or in patients with underlying kidney conditions.

- Given what is known about protease TMPRSS2 expression during SARS-CoV-2 infection, ACE2+TMPRSS2+ double-positive cell identification would be useful in these datasets.

8.9.4 Significance

- ACE2 protein is spatially restricted to brush border of proximal tubules and in bladder umbrella cells [284], such cells in direct contact with viral particles are likely to be highly sensitive to viral-induced damage.

- SARS-CoV and MERS-CoV have been shown to be detected in urine of patients and associate with higher mortality [281,285], thus worth understanding kidney damage and resultant immune response in SARS-CoV-2 as well.

- This study argues for a potential mode of viral infectivity and resultant inflammatory responses in these tissue in addition to reported infectivity in the lung and digestive system, which is supported by clinical data showing acute and early kidney complications in 2019-nCoV patients [280].

- Clinically, thus very important to track urinary CoVID-19 shedding as well as study acute kidney injury-related co-morbidities.

8.9.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.10 Neutrophil-to-Lymphocyte Ratio Predicts Severe Illness Patients with 2019 Novel Coronavirus in the Early Stage

Liu et al. *medRxiv* [286]

8.10.1 Keywords

- severe disease
- pneumonia
- lymphocytes
- neutrophils

8.10.2 Main Findings

This study aimed to find prognostic biomarkers of COVID-19 pneumonia severity. Sixty-one (61) patients with COVID-19 treated in January at a hospital in Beijing, China were included. On average, patients were seen within 5 days from illness onset. Samples were collected on admission; and then patients were monitored for the development of severe illness with a median follow-up of 10 days].

Patients were grouped as “mild” (N=44) or “moderate/severe” (N=17) according to symptoms on admission and compared for different clinical/laboratory features. “Moderate/severe” patients were significantly older (median of 56 years old, compared to 41 years old). Whereas comorbidies rates were largely similar between the groups, except for hypertension, which was more frequent in the severe group (p= 0.056). ‘Severe’ patients had higher counts of neutrophils, and serum glucose levels; but lower lymphocyte counts, sodium and serum chlorine levels. The ratio of neutrophils to lymphocytes (NLR) was also higher for the ‘severe’ group. ‘Severe’ patients had a higher rate of bacterial infections (and antibiotic treatment) and received more intensive respiratory support and treatment.

26 clinical/laboratory variables were used to select NLR and age as the best predictors of the severe disease. Predictive cutoffs for a severe illness as NLR ≥ 3.13 or age ≥ 50 years.

8.10.3 Limitations

Identification of early biomarkers is important for making clinical decisions, but large sample size and validation cohorts are necessary to confirm findings. It is worth noting that patients classified as “mild” showed pneumonia by imaging and fever, and in accordance with current classifications this would be consistent with “moderate” cases. Hence it would be more appropriate to refer to the groups as “moderate” vs “severe/critical”. Furthermore, there are several limitations that could impact the interpretation of the results: e.g. classification of patients was based on symptoms presented on admission and not based on disease progression, small sample size, especially the number of ‘severe’ cases (with no deaths among these patients). Given the small sample size, the proposed NLR and age cut offs might not hold for a slightly different set of patients. For example, in a study of >400 patients, ‘non-severe’ and ‘severe’ NLR were 3.2 and 5.5, respectively [287].

8.10.4 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.11 Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP)

Wan et. al. *medRxiv* [288]

8.11.1 Keywords

- Cytokines
- lymphocyte subsets
- CD8 + T
- B cells
- NK cells,
- PBMCs
- IL-6
- IL-10

8.11.2 Main Findings

The authors analyzed lymphocyte subsets and cytokines of 102 patients with mild disease and 21 with severe disease. CD8+T cells and CD4+T cells were significantly reduced in both cohort. particularly in severe patients. The cytokines IL6 and IL10 were significantly elevated in severe patients as compared to mild. No significant differences were observed in frequency of B cells and NK cells.

The authors argue that the measurement of T cell frequencies and cytokine levels of IL6 and IL10 can be used to predict progression of disease from Mild to severe Cov-2 infection.

8.11.3 Limitations

The study demonstrates in a limited cohort similar associations to several other reported studies. The authors didn't compare the changes in lymphocyte and cytokine with healthy individual (Covid-19 Negative) rather used an internal standard value. The recently preprint in LANCET shows The degree of lymphopenia and a pro-inflammatory cytokine storm is higher in severe COVID-19 patients than in mild cases, and is associated with the disease severity [289].

8.11.4 Significance

This translational data identifies key cytokines and lymphopenia associated with disease severity although mechanism and key cellular players are still unknown. Higher level IL-6 production in severe patient suggests potential role of Tocilizumab (anti-IL6R) biologic although clinical trial will be necessary.

8.11.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.12 Epidemiological and Clinical Characteristics of 17 Hospitalized Patients with 2019 Novel Coronavirus Infections Outside Wuhan, China

Li et al. *medRxiv* [290]

8.12.1 Keywords

- epidemiology
- clinical characteristics

8.12.2 Major Findings

These authors looked at 17 hospitalized patients with COVID-19 confirmed by RT-PCR in Dazhou, Sichuan. Patients were admitted between January 22 and February 10 and the final data were collected on February 11. Of the 17 patients, 12 remained hospitalized while 5 were discharged after meeting national standards. The authors observed no differences based on the sex of the patients but found that the discharged patients were younger in age ($p = 0.026$) and had higher lymphocyte counts ($p = 0.005$) and monocyte counts ($p = 0.019$) upon admission.

8.12.3 Limitations

This study is limited in the sample size of the study and the last data collection point was only one day after some of the patients were admitted.

8.12.4 Significance

These findings have been somewhat supported by subsequent studies that show that older age and an immunocompromised state are more likely to result in a more severe clinical course with COVID-19. However, other studies have been published that report on larger numbers of cases.

8.12.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.13 ACE2 Expression in Kidney and Testis May Cause Kidney and Testis Damage After 2019-nCoV Infection

[291]

8.13.1 Keywords

- CoVID-19
- 2019-nCoV
- SARS-CoV-2
- kidney
- testis
- ACE2
- scRNAseq

8.13.2 Main Findings

- Study used online datasets (scRNAseq GSE131685, scRNAseq GSE107585, Human Protein Atlas, GTEx portal, CCLE) to analyze ACE2 expression in different human organs.

- Study re-analyzed three clinical datasets (n=6, n=99, and n=41) to show 3~10% of 2019-nCoV patients present with abnormal renal function.

- results indicate ACE2 highly expressed in renal tubular cells, Leydig cells and seminiferous ductal cells of testis.

8.13.3 Limitations

- Very preliminary transcript/protein dataset analysis in healthy cohorts; does not necessarily translate to actual viral tropism and permissiveness.

- Clinically, would be important to determine with larger longitudinal dataset if SARS-CoV-2 infection changes sperm quality or testicular inflammation.

- Similarly, would be important to determine if simultaneous HBV or syphilis infection and orchitis impacts SARS-CoV-2 severity.

- Examination and follow-up of renal function and viral orchitis/sperm quality of CoVID-19 patients not done in this preliminary study.

8.13.4 Significance

- Kidney ACE2 result supports other concurrent sequencing studies [282] and clinical reports of abnormal renal function or even kidney damage in patients infected with 2019-nCoV [280].

- High ACE2 expression in testis suggests potential tropism of the virus to testicular tissues and indicates potential risks for male fertility. Viral orchitis reported for SARS-CoV previously [1], but no clear evidence so far of infertility in SARS, MERS or CoVID-19 patients.

8.13.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.14 Aberrant pathogenic GM-CSF+ T cells and inflammatory CD14+CD16+ monocytes in severe pulmonary syndrome patients of a new coronavirus

[292]

8.14.1 Keywords

- immunopathology
- Th1
- inflammatory monocytes
- GM-CSF
- IFN-γ
- IL-6

8.14.2 Main Findings

The authors of this study sought to characterize the immune mechanism causing severe pulmonary disease and mortality in 2019-nCoV (COVID-19) patients. Peripheral blood was collected from hospitalized ICU (n=12) and non-ICU (n=21) patients with confirmed 2019-nCoV and from healthy controls (n=10) in The First Affiliated Hospital of University of Science and Technology China (Hefei, Anhui). Immune analysis was conducted by flow cytometry. 2019-nCoV patients had decreased lymphocyte, monocyte, and CD4 T cell counts compared to healthy controls. ICU patients had fewer lymphocytes than non-ICU patients. CD4 T cells of 2019-nCoV patients expressed higher levels of activation markers (OX40, CD69, CD38, CD44) and exhaustion markers (PD-1 and Tim3) than those of healthy controls. CD4 cells of ICU patients expressed significantly higher levels of OX40, PD-1, and Tim3 than those of non-ICU patients. 2019-nCoV patients had higher percentages of CD4 T cells co-

expressing GM-CSF and IL-6 compared to healthy controls, while ICU patients had a markedly higher percentage of GM-CSF+ IFN-γ+ CD4 T cells than non-ICU patients. The CD4 T cells of nCoV patients and healthy controls showed no differences in TNF-α secretion.

The CD8 T cells of 2019-nCoV patients also showed higher expression of activation markers CD69, CD38, and CD44, as well as exhaustion markers PD-1 and Tim3, compared to healthy controls. CD8 T cells of ICU patients expressed higher levels of GM-CSF than those of non-ICU patients and healthy controls. No IL-6 or TNF-α was found in the CD8 T cells of any group. There were no differences in numbers of NK cells or B cells in 2019-nCoV patients and healthy controls, nor was there any GM-CSF or IL-6 secretion from these cells in either group.

Percentages of CD14+ CD16+ GM-CSF+ and CD14+ CD16+ IL-6+ inflammatory monocytes were significantly increased in nCoV patients compared to healthy controls; in particular, patients in the ICU had greater percentages of CD14+ CD16+ IL-6+ monocytes than non-ICU patients. The authors suggest that in 2019-nCoV patients, pathogenic Th1 cells produce GM-CSF, recruiting CD14+ CD16+ inflammatory monocytes that secrete high levels of IL-6. These may enter pulmonary circulation and damage lung tissue while initiating the cytokine storm that causes mortality in severe cases. This is consistent with the cytokine storm seen in similar coronaviruses, as IL-6, IFN-γ, and GM-CSF are key inflammatory mediators seen in patients with SARS-CoV-1 and MERS-CoV.

8.14.3 Limitations

Though the results of this study open questions for further investigation, this is an early study on a small cohort of patients, and as such there are a number of limitations. The study included only 12 ICU patients and 21 non-ICU patients, and ideally would be repeated with a much larger patient cohort. Though the authors make claims about differences in lymphocyte and monocyte counts between patients and healthy controls, they did not report baseline laboratory findings for the control group. Additionally, severity of disease was classified based on whether or not patients were in the ICU. It would be interesting to contextualize the authors' immunological findings with more specific metrics of disease severity or time course. Noting mortality, time from disease onset, pre-existing conditions, or severity of lung pathology in post-mortem tissue samples would paint a fuller picture of how to assess risk level and the relationship between severity of disease and immunopathology. Another limitation is the selection of cytokines and immune markers for analysis, as the selection criteria were based on the cell subsets and cytokine storm typically seen in SARS-CoV-1 and MERS-CoV patients. Unbiased cytokine screens and immune profiling may reveal novel therapeutic targets that were not included in this study.

8.14.4 Significance

This study identifies potential therapeutic targets that could prevent acute respiratory disease syndrome (ARDS) and mortality in patients most severely affected by COVID-19. The authors propose testing monoclonal antibodies against IL6-R or GM-CSF to block recruitment of inflammatory monocytes and the subsequent cytokine storm in these patients.

8.14.5 Credit

Review by Gabrielle Lubitz as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.15 Clinical Characteristics of 2019 Novel Infected Coronavirus Pneumonia：A Systemic Review and Meta-analysis

Qian et al. *medRxiv*. [293]

8.15.1 Keywords

- White Blood Cells
- Lymphocytes
- Neutrophils

8.15.2 Main Findings

The authors performed a meta analysis of literature on clinical, laboratory and radiologic characteristics of patients presenting with pneumonia related to SARSCoV2 infection, published up to Feb 6 2020. They found that symptoms that were mostly consistent among studies were sore throat, headache, diarrhea and rhinorrhea. Fever, cough, malaise and muscle pain were highly variable across studies. Leukopenia (mostly lymphocytopenia) and increased white blood cells were highly variable across studies. They identified three most common patterns seen on CT scan, but there was high variability across studies. Consistently across the studies examined, the authors found that about 75% of patients need supplemental oxygen therapy, about 23% mechanical ventilation and about 5% extracorporeal membrane oxygenation (ECMO). The authors calculated a staggering pooled mortality incidence of 78% for these patients.

8.15.3 Limitations

The authors mention that the total number of studies included in this meta analysis is nine, however they also mentioned that only three studies reported individual patient data. It is overall unclear how many patients in total were included in their analysis. This is mostly relevant as they reported an incredibly high mortality (78%) and mention an absolute number of deaths of 26 cases overall. It is not clear from their report how the mortality rate was calculated.

The data is based on reports from China and mostly from the Wuhan area, which somewhat limits the overall generalizability and applicability of these results.

8.15.4 Significance

This meta analysis offers some important data for clinicians to refer to when dealing with patients with COVID-19 and specifically with pneumonia. It is very helpful to set expectations about the course of the disease.

8.15.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.16 Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients

Liu et al. *medRxiv* [294]

8.16.1 Keywords

- Lymphopenia
- Neutrophil to CD8 T cell ratio (N8R)

- inflammatory cytokines

8.16.2 Main Findings

Liu et al. enrolled a cohort of 40 patients from Wuhan including 27 mild cases and 13 severe cases of COVID-19. They performed a 16-day kinetic analysis of peripheral blood from time of disease onset. Patients in the severe group were older (medium age of 59.7, compared to 48.7 in mild group) and more likely to have hypertension as a co-morbidity. Lymphopenia was observed in 44.4% of the mild patients and 84.6% of the severe patients. Lymphopenia was due to low T cell count, specially CD8 T cells. Severe patients showed higher neutrophil counts and an increase of cytokines in the serum (IL2, IL6, IL10 and IFNγ). The authors measured several other clinical laboratory parameters were also higher in severe cases compared to mild, but concluded that neutrophil to CD8 T cell ratio (N8R) as the best prognostic factor to identify the severe cases compared to other receiver operating characteristic (ROC).

8.16.3 Limitations

This was a small cohort (N=40), and two of the patients initially included in the severe group (N=13) passed away and were excluded from the analysis due to lack of longitudinal data. However, it would be most important to be able to identify patients with severe disease with higher odds of dying. It seems that the different time points analyzed relate to hospital admission, which the authors describe as disease onset. The time between first symptoms and first data points is not described. It would have been important to analyze how the different measured parameters change according to health condition, and not just time (but that would require a larger cohort). The predictive value of N8R compared to the more commonly used NLR needs to be assessed in other independent and larger cohorts. Lastly, it is important to note that pneumonia was detected in patients included in the “mild” group, but according to the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition) this group should be considered “moderate”.

8.16.4 Significance

Lymphopenia and cytokine storm have been described to be detrimental in many other infections including SARS-CoV1 and MERS-CoV. However, it was necessary to confirm that this dramatic immune response was also observed in the SARS-CoV2 infected patients. These results and further validation of the N8R ratio as a predictor of disease severity will contribute for the management of COVID19 patients and potential development of therapies.

8.16.5 Credit

Review by Pauline Hamon as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.17 Clinical and immunologic features in severe and moderate forms of Coronavirus Disease 2019

Chen et al. *medRxiv* [295]

8.17.1 Keywords

- severe disease
- lymphocytes
- cytokines

- IFNy
- CD4 Tcells
- HLA-DR CD8
- Tcells

8.17.2 Main Findings

This study retrospectively evaluated clinical, laboratory, hematological, biochemical and immunologic data from 21 subjects admitted to the hospital in Wuhan, China (late December/January) with confirmed SARS-CoV-2 infection. The aim of the study was to compare 'severe' (n=11, ~64 years old) and 'moderate' (n=10, ~51 years old) COVID-19 cases. Disease severity was defined by patients' blood oxygen level and respiratory output. They were classified as 'severe' if SpO2 93% or respiratory rates 30 per min.

In terms of the clinical laboratory measures, 'severe' patients had higher CRP and ferritin, alanine and aspartate aminotransferases, and lactate dehydrogenase but lower albumin concentrations.

The authors then compared plasma cytokine levels (ELISA) and immune cell populations (PBMCs, Flow Cytometry). 'Severe' cases had higher levels of IL-2R, IL-10, TNFa, and IL-6 (marginally significant). For the immune cell counts, 'severe' group had higher neutrophils, HLA-DR+ CD8 T cells and total B cells; and lower total lymphocytes, CD4 and CD8 T cells (except for HLA-DR+), CD45RA Tregs, and IFNy-expressing CD4 T cells. No significant differences were observed for IL-8, counts of NK cells, CD45+RO Tregs, IFNy-expressing CD8 T and NK cells.

8.17.3 Limitations

Several potential limitations should be noted: 1) Blood samples were collected 2 days post hospital admission and no data on viral loads were available; 2) Most patients were administered medications (e.g. corticosteroids), which could have affected lymphocyte counts. Medications are briefly mentioned in the text of the manuscript; authors should include medications as part of Table 1. 3) 'Severe' cases were significantly older and 4/11 'severe' patients died within 20 days. Authors should consider a sensitivity analysis of biomarkers with the adjustment for patients' age.

8.17.4 Significance

Although the sample size was small, this paper presented a broad range of clinical, biochemical, and immunologic data on patients with COVID-19. One of the main findings is that SARS-CoV-2 may affect T lymphocytes, primarily CD4+ T cells, resulting in decreased IFNy production. Potentially, diminished T lymphocytes and elevated cytokines can serve as biomarkers of severity of COVID-19.

8.17.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.18 SARS-CoV-2 and SARS-CoV Spike-RBD Structure and Receptor Binding Comparison and Potential Implications on Neutralizing Antibody and Vaccine Development

Sun et al. *bioRxiv* [296]

8.18.1 Keywords

- SARS-CoV
- SARS-CoV-2
- ACE2
- Spike (S) protein
- receptor binding domain (RBD)
- receptor binding motif (RBM)
- neutralizing antibody

8.18.2 Main Findings

This study compared the structure of SARS-CoV and SARS-CoV-2 Spike (S) protein receptor binding domain (RBD) and interactions with ACE2 using computational modeling, and interrogated cross-reactivity and cross-neutralization of SARS-CoV-2 by antibodies against SARS-CoV. While SARS-CoV and SARS-CoV-2 have over 70 % sequence homology and share the same human receptor ACE2, the receptor binding motif (RBM) is only 50% homologous.

Computational prediction of the SARS-CoV-2 and ACE2 interactions based on the previous crystal structure data of SARS-CoV, and measurement of binding affinities against human ACE2 using recombinant SARS-CoV and SARS-CoV-2 S1 peptides, demonstrated similar binding of the two S1 peptides to ACE2, explaining the similar transmissibility of SARS-CoV and SARS-CoV-2 and consistent with previous data (Wall et al Cell 2020).

The neutralization activity of SARS-CoV-specific rabbit polyclonal antibodies were about two-order of magnitude less efficient to neutralize SARS-CoV-2 than SARS-CoV, and four potently neutralizing monoclonal antibodies against SARS-CoV had poor binding and neutralizing activity against SARS-CoV-2. In contrast, 3 poor SARS-CoV-binding monoclonal antibodies show some efficiency to bind and neutralize SARS-CoV-2. The results suggest that that antibodies to more conserved regions outside the RBM motif might possess better cross-protective neutralizing activities between two strains.

8.18.3 Limitations

It would have been helpful to show the epitopes recognized by the monoclonal antibodies tested on both SARS-CoV, SARS-CoV-2 to be able to make predictions for induction of broadly neutralizing antibodies. The data on monoclonal antibody competition with ACE2 for binding to SARS-CoV RBD should have also included binding on SARS-CoV2, especially for the three monoclonal antibodies that showed neutralization activity for SARS-CoV2. Because of the less homology in RBM sequences between viruses, it still may be possible that these antibodies would recognize the ACE2 RBD in SARS-CoV-2.

8.18.4 Significance

It is noteworthy that immunization to mice and rabbit with SARS-CoV S1 or RBD protein could induce monoclonal antibodies to cross-bind and cross-neutralize SARS-CoV-2 even if they are not ACE2-blocking. If these types of antibodies could be found in human survivors or in the asymptomatic populations as well, it might suggest that exposure to previous Coronavirus strains could have induced cross-neutralizing antibodies and resulted in the protection from severe symptoms in some cases of SARS-CoV2.

8.18.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.19 Protection of Rhesus Macaque from SARS-Coronavirus challenge by recombinant adenovirus vaccine

Chen et al. *bioRxiv* [297]

8.19.1 Keywords

- SARS-CoV-1
- rhesus macaque
- recombinant adenovirus vaccine

8.19.2 Main Findings

Rhesus macaques were immunized intramuscularly twice (week 0 and week 4) with SV8000 carrying the information to express a S1-orf8 fusion protein and the N protein from the BJ01 strain of SARS-CoV-1. By week 8, immunized animals had signs of immunological protection (IgG and neutralization titers) against SARS-CoV-1 and were protected against challenge with the PUMC-1 strain, with fewer detectable symptoms of respiratory distress, lower viral load, shorter periods of viral persistence, and less pathology in the lungs compared to non-immunized animals.

8.19.3 Limitations

The authors should write clearer descriptions of the methods used in this article. They do not describe how the IgG titers or neutralization titers were determined. There are some issues with the presentation of data, for example, in Figure 1a, y-axis should not be Vmax; forming cells and 1d would benefit from showing error bars. Furthermore, although I inferred that the animals were challenged at week 8, the authors did not explicitly detail when the animals were challenged. The authors should explain the design of their vaccine, including the choice of antigens and vector. The authors also do not include a description of the ethical use of animals in their study.

8.19.4 Significance

The authors describe a vaccine for SARS-CoV-1 with no discussion of possible implications for the current SARS-CoV-2 pandemic. Could a similar vaccine be designed to protect against SARS-CoV-2 and would the concerns regarding emerging viral mutations that the authors describe as a limitation for SARS-CoV-1 also be true in the context of SARS-CoV-2?

8.19.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.20 Reduction and Functional Exhaustion of T cells in Patients with Coronavirus Disease 2019 (COVID-19)

[298]

8.20.1 Keywords

- T cell exhaustion
- T cell lymphopenia
- IL-6
- IL-10
- TNF-α

8.20.2 Main Findings

Based on a retrospective study of 522 COVID patients and 40 healthy controls from two hospitals in Wuhan, China, authors show both age-dependent and clinical severity-dependent decrease in T cell numbers with elderly patients and patients who are in ICU-care showing the most dramatic decrease in T cell counts. Cytokine profiling of COVID patients reveal that TNF-α, IL-6 and IL-10 are increased in infected patients with patients in the ICU showing the highest levels. Interestingly, these three cytokine levels were inversely correlated with T cell counts and such inverse relationship was preserved throughout the disease progression. Surface staining of exhaustion markers (PD-1 and Tim-3) and flow cytometry of stained peripheral blood of 14 patients and 3 healthy volunteers demonstrate that T cells of COVID patients have increased expression of PD-1 with patients in ICU having the highest number of $CD8^{+}PD\text{-}1^{+}$ cells than their counterparts in non-ICU groups.

8.20.3 Limitations

Compared to the number of patients, number of control (n= 40) is small and is not controlled for age. Additional data linking inflammatory cytokines and the quality of the adaptive response including humoral and antigen specific T cell response is much needed. T cell exhaustion study relies on marker-dependent labeling of T cell functionality of a very limited sample size (n=17)—a functional/mechanistic study of these T cells from PBMCs would have bolstered their claims.

8.20.4 Significance

Limited but contains interesting implications. It is already known in literature that in the context of acute respiratory viral infections CD8 T cells exhibit exhaustion-like phenotypes which further underscores the importance of mechanistic studies that can elucidate how COVID infection leads to lymphopenia and T cell exhaustion-like phenotype.

However, as authors have noted, the data does point to an interesting question: How these inflammatory cytokines (TNF-α, IL-6 and IL-10) correlate with or affect effective viral immunity and what types of cells produce these cytokines? Answering that question will help us refine our targets for immune-modulatory therapies especially in patients suffering from cytokine storms.

8.20.5 Credit

This review by Chang Moon was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.21 Clinical Characteristics of 25 death cases infected with COVID-19 pneumonia: a retrospective review of medical records in a single medical center, Wuhan, China

[299]

8.21.1 Keywords

- COVID-19
- pneumonia
- hypertension
- diabetes
- biomarker
- neutrophilia
- lymphopenia

8.21.2 Main Findings

Most common chronic conditions among 25 patients that died from COVID-19 related respiratory failure were hypertension (64%) and diabetes (40%). Disease progression was marked by progressive organ failure, starting first with lung dysfunction, then heart (e.g. increased cTnI and pro-BNP), followed by kidney (e.g. increased BUN, Cr), and liver (e.g. ALT, AST). 72% of patients had neutrophilia and 88% also had lymphopenia. General markers of inflammation were also increased (e.g. PCT, D-Dimer, CRP, LDH, and SAA).

8.21.3 Limitations

The limitations of this study include small sample size and lack of measurements for some tests for several patients. This study would also have been stronger with comparison of the same measurements to patients suffering from less severe disease to further validate and correlate proposed biomarkers with disease severity.

8.21.4 Significance

This study identifies chronic conditions (i.e. hypertension and diabetes) that strongly correlates with disease severity. In addition to general markers of inflammation, the authors also identify concomitant neutrophilia and lymphopenia among their cohort of patients. This is a potentially interesting immunological finding because we would typically expect increased lymphocytes during a viral infection. Neutrophilia may also be contributing to cytokine storm. In addition, PCT was elevated in 90.5% of patients, suggesting a role for sepsis or secondary bacterial infection in COVID-19 related respiratory failure.

8.21.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.22 SARS-CoV-2 infection does not significantly cause acute renal injury: an analysis of 116 hospitalized patients with COVID-19 in a single hospital, Wuhan, China

[300]

8.22.1 Keywords

- CoVID-19
- 2019-nCoV
- SARS-CoV-2
- kidney
- clinical

- longitudinal

8.22.2 Main Findings

- Clinical data from 116 hospitalized CoVID-19 patients analyzed over 4 weeks for correlation with renal injury. Comorbidities included chronic renal failure (CRF) in 5 patients (4.3%).

- 10.8% of patients with no prior kidney disease showed elevations in blood urea or creatinine, and 7.2% of patients with no prior kidney disease showed albuminuria.

- Patients with pre-existing CRF underwent continuous renal replacement therapy (CRRT) alongside CoVID-19 treatment. Renal functions remained stable in these patients.

- All 5 patients with CRF survived CoVID-19 therapy without progression to ARDS or worsening of CRF.

8.22.3 Limitations

- Renal injury biomarkers in patients with incipient kidney abnormalities not tabulated separately, making overall data hard to interpret. It will be critical to separately examine kidney function (BUN, urine creatinine and eGFR) in patients that developed any kidney abnormalities (7.2~10.8% of cohort).

- No information on type of CoVID-19 therapy used across cohort; will be useful to correlate how treatment modality influences kidney function (and other parameters).

- Invokes previous clinical-correlation studies that indicate low instances of kidney damage[301,302], but those studies did not track longitudinal urine samples for acute renal injury markers and viral shedding.

- CRRT in patients with CRF is standard therapy irrespective of CoVID-19 status; it will be important to compare clinical parameters of these patients (n=5) with virus-naïve CRF patients (none in this study) to make any meaningful conclusions.

8.22.4 Significance

- This study argues that renal impairment is uncommon in CoVID-19 and not associated with high mortality, in stark contrast with a concurrent study [280]. If supported by further studies, this argues kidney impairment is secondary to cytokine storm/inflammation-induced organ failure, and not due to direct viral replication.

- Will be important to comprehensively characterize large-datasets of CoVID-19 patients to conclude if kidney function actively disrupted due to viral infection.

8.22.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.23 Potential T-cell and B-Cell Epitopes of 2019-nCoV

[303]

8.23.1 Keywords

- COVID-19
- vaccine
- epitopes
- spike protein
- MHC-I
- MHC-II
- neutralizing antibodies
- ACE2

8.23.2 Main Findings

The authors use 2 neural network algorithms, NetMHCpan4 and MARIA, to identify regions within the COVID-19 genome that are presentable by HLA. They identify 405 viral epitopes that are presentable on MHC-I and MHC-II and validate using known epitopes from SARS-CoV. To determine whether immune surveillance drives viral mutations to evade MHC presentation, the authors analyzed 68 viral genomes from 4 continents. They identified 93 point mutations that occurred preferentially in regions predicted to be presented by MHC-I ($p=0.02$) suggesting viral evolution to evade CD8 T-cell mediated killing. 2 nonsense mutations were also identified that resulted in loss of presentation of an associated antigen (FGDSVEEVL) predicted to be good antigen for presentation across multiple HLA alleles.

To identify potential sites of neutralizing antibody binding, the authors used homology modeling to the SARS-CoV's spike protein (S protein) to determine the putative structure of the CoV2 spike protein. They used Discotope2 to identify antibody binding sites on the protein surface in both the down and up conformations of the S protein. The authors validate this approach by first identifying antibody binding site in SARS-CoV S protein. In both the down and up conformation of the CoV2 S protein, the authors identified a potential antibody binding site on the S protein receptor binding domain (RBD) of the ACE2 receptor (residues 440-460, 494-506). While RBDs in both SARS-CoV and CoV2 spike proteins may be important for antibody binding, the authors note that SARS-CoV has larger attack surfaces than CoV2. These results were later validated on published crystal structures of the CoV2 S protein RBD and human ACE2. Furthermore, analysis of 68 viral genomes did not identify any mutations in this potential antibody binding site in CoV2.

Finally, the authors compile a list of potential peptide vaccine candidates across the viral genome that can be presented by multiple HLA alleles. Several of the peptides showed homology to SARS-CoV T-cell and B-cell epitopes.

8.23.3 Limitations

While the authors used computational methods of validation, primarily through multiple comparisons to published SARS-CoV structures and epitopes, future work should include experimental validation of putative T-cell and B-cell epitopes.

8.23.4 Significance

The authors identified potential T-cell and B-cell epitopes that may be good candidates for peptide based vaccines against CoV2. They also made interesting observations in comparing SARS-CoV and CoV2 potential antibody binding sites, noting that SARS-CoV had larger attack surfaces for potential neutralizing antibody binding. One of the highlights of this paper was the authors' mutation analysis of 68 viral genomes from 4 continents. This analysis not only validated their computational method for identifying T-cell epitopes, but showed that immune surveillance likely drives viral mutation in

MHC-I binding peptides. The smaller attack surface may point to potential mechanisms of immune evasion by CoV2. However, absence of mutations in the RBD of CoV2 and the small number of mutations in peptides presentable to T cells suggests that vaccines against multiple epitopes could still elicit robust immunity against CoV2.

8.23.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.24 Structure, Function, and Antigenicity of the SARSCoV-2 Spike Glycoprotein

Walls et al. *bioRxiv*. [304] now [24]

8.24.1 Keywords

- binding affinity
- antigenicity
- neutralizing antibody

8.24.2 Main Findings

The authors highlight a human angiotensin-converting enzyme 2 (hACE2), as a potential receptor used by the current Severe Acute respiratory syndrome coronavirus-2 (SARS-CoV-2) as a host factor that allows the virus target human cells. This virus-host interaction facilitates the infection of human cells with a high affinity comparable with SARS-CoV. The authors propose this mechanism as a probable explanation of the efficient transmission of SARS-CoV-2 between humans. Besides, Walls and colleagues described SARS-CoV-2 S glycoprotein S by Cryo-EM along with neutralizing polyclonal response against SAR-CoV-2 S from mice immunized with SAR-CoV and blocking SAR-CoV-2 S-mediated entry into VeroE6 infected cells.**

8.24.3 Limitations

The SARS-CoV-2 depends on the cell factors ACE2 and TMPRSS2, this last, according to a recent manuscript by Markus Hoffman et al., *Cell*, 2020. The authors used green monkey (VeroE6) and hamster (BHK) cell lines in the experiments to drive its conclusions to humans; however, it is well known the caucasian colon adenocarcinoma human cell line (CaCo-2), highly express the hACE2 receptor as the TMPRSS2 protease as well. In humans, ACE2 protein is highly expressed in the gastrointestinal tract, which again, makes the CaCo-2 cell line suitable for the following SARS-CoV-2 studies.

8.24.4 Significance

The results propose a functional receptor used by SARS-CoV-2 to infect humans worldwide and defining two distinct conformations of spike (S) glycoprotein by cryogenic electron microscopy (Cryo-EM). This study might help establish a precedent for initial drug design and treatment of the current global human coronavirus epidemic.

8.24.5 Credit

Review by postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.25 Breadth of concomitant immune responses underpinning viral clearance and patient recovery in a non-severe case of COVID-19

Thevarajan et al. *medRxiv* [305]

8.25.1 Keywords

- IgG
- IgM
- TfH cells
- NK cells
- SNP

8.25.2 Main Findings

The authors characterized the immune response in peripheral blood of a 47-year old COVID-19 patient.

SARS-CoV2 was detected in nasopharyngeal swab, sputum and faeces samples, but not in urine, rectal swab, whole blood or throat swab. 7 days after symptom onset, the nasopharyngeal swab test turned negative, at day 10 the radiography infiltrates were cleared and at day 13 the patient became asymptomatic.

Immunofluorescence staining shows from day 7 the presence of **COVID-19-binding IgG and IgM** antibodies in plasma, that increase until day 20.

Flow cytometry on whole blood reveals a plasmablast peak at day 8, a gradual increase in T follicular helper cells, stable HLA-DR$^+$ NK frequencies and decreased monocyte frequencies compared to healthy counterparts. The expression of CD38 and HLA-DR peaked on T cells at D9 and was associated with higher production of cytotoxic mediators by CD8$^+$ T cells.

IL-6 and IL-8 were undetectable in plasma.

The authors further highlight the presence of the **IFITM3 SNP-rs12252-C/C variant** in this patient, which is associated with higher susceptibility to influenza virus.

8.25.3 Limitations

These results need to be confirmed in additional patients.

COVID-19 patients have increased infiltration of macrophages in their lungs [306]. Monitoring monocyte proportions in blood earlier in the disease might help to evaluate their eventual migration to the lungs.

The stable concentration of HLA-DR$^+$ NK cells in blood from day 7 is not sufficient to rule out NK cell activation upon SARS-CoV2 infection. In response to influenza A virus, NK cells express higher levels of activation markers CD69 and CD38, proliferate better and display higher cytotoxicity [307]. Assessing these parameters in COVID-19 patients is required to better understand NK cell role in clearing this infection.

Neutralization potential of the COVID-19-binding IgG and IgM antibodies should be assessed in future studies.

This patient was able to clear the virus, while presenting a SNP associated with severe outcome following influenza infection. The association between this SNP and outcome upon SARS-CoV2 infection should be further investigated.

8.25.4 Significance

This study is among the first to describe the appearance of COVID-19-binding IgG and IgM antibodies upon infection. The emergence of new serological assays might contribute to monitor more precisely the seroconversion kinetics of COVID-19 patients [84]. Further association studies between IFITM3 SNP-rs12252-C/C variant and clinical data might help to refine the COVID-19 outcome prediction tools.

8.25.5 Credit

Review by Bérengère Salomé as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.26 The landscape of lung bronchoalveolar immune cells in COVID-19 revealed by single-cell RNA sequencing

Liao et al. *medRxiv* [306]

8.26.1 Keywords

- COVID-19
- SARS-CoV-2
- Broncho-alveolar lavage
- macrophages
- NK cells
- T cells
- cytokine storm
- scRNAseq

8.26.2 Main Findings

The authors performed single-cell RNA sequencing (scRNAseq) on bronchoalveolar lavage fluid (BAL) from 6 COVID-19 patients (n=3 mild cases, n=3 severe cases). Data was compared to previously generated scRNAseq data from healthy donor lung tissue (n=8).

Clustering analysis of the 6 patients revealed distinct immune cell organization between mild and severe disease. Specifically, they found that transcriptional clusters annotated as tissue resident alveolar macrophages were strongly reduced while monocytes-derived $FCN1^{+}SPP1^{+}$ inflammatory macrophages dominated the BAL of patients with severe COVID19 diseases. They show that inflammatory macrophages upregulated interferon-signaling genes, monocytes recruiting chemokines including CCL2, CCL3, CCL4 as well as IL-6, TNF, IL-8 and profibrotic cytokine TGF-β, while alveolar macrophages expressed lipid metabolism genes, such as PPARG.

The lymphoid compartment was overall enriched in lungs from patients. Clonally expanded CD8 T cells were enriched in mild cases suggesting that CD8 T cells contribute to viral clearance as in Flu infection, whereas proliferating T cells were enriched in severe cases.

SARS-CoV-2 viral transcripts were detected in severe patients, but considered here as ambient contaminations.

8.26.3 Limitations

These results are based on samples from 6 patients and should therefore be confirmed in the future in additional patients. Longitudinal monitoring of BAL during disease progression or resolution would have been most useful.

The mechanisms underlying the skewing of the macrophage compartment in patients towards inflammatory macrophages should be investigated in future studies.

Deeper characterization of the lymphoid subsets is required. The composition of the “proliferating” cluster and how these cells differ from conventional T cell clusters should be assessed. NK and CD8 T cell transcriptomic profile, in particular the expression of cytotoxic mediator and immune checkpoint transcripts, should be compared between healthy and diseased lesions.

8.26.4 Significance

COVID-19 induces a robust inflammatory cytokine storm in patients that contributes to severe lung tissue damage and ARDS [308]. Accumulation of monocyte-derived inflammatory macrophages at the expense of Alveolar macrophages known to play an anti-inflammatory role following respiratory viral infection, in part through the PPARγ pathway [309,310] are likely contributing to lung tissue injuries. These data suggest that reduction of monocyte accumulation in the lung tissues could help modulate COVID-19-induced inflammation. Further analysis of lymphoid subsets is required to understand the contribution of adaptive immunity to disease outcome.

8.26.5 Credit

Review by Bérengère Salomé and Assaf Magen as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.27 Can routine laboratory tests discriminate 2019 novel coronavirus infected pneumonia from other community-acquired pneumonia?

Pan et al. *medRxiv* [311]

8.27.1 Keywords

- Routine laboratory testing

8.27.2 Main Findings

In an attempt to use standard laboratory testing for the discrimination between “Novel Coronavirus Infected Pneumonia” (NCIP) and a usual community acquired pneumonia (CAP), the authors compared laboratory testing results of 84 NCIP patients with those of a historical group of 316 CAP patients from 2018 naturally COVID-19 negative. The authors describe significantly lower white blood- as well as red blood- and platelet counts in NCIP patients. When analyzing differential blood counts, lower absolute counts were measured in all subsets of NCIP patients. With regard to clinical chemistry parameters, they found increased AST and bilirubin in NCIP patients as compared to CAP patients.

8.27.3 Limitations

The authors claim to describe a simple method to rapidly assess a pre-test probability for NCIP. However, the study has substantial weakpoints. The deviation in clinical laboratory values in NCIP patients described here can usually be observed in severely ill patients. The authors do not comment on how severely ill the patients tested here were in comparison to the historical control. Thus, the conclusion that the tests discriminate between CAP and NCIP lacks justification.

8.27.4 Significance

The article strives to compare initial laboratory testing results in patients with COVID-19 pneumonia as compared to patients with a usual community acquired pneumonia. The implications of this study for the current clinical situation seem restricted due to a lack in clinical information and the use of a control group that might not be appropriate.

8.27.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.28 Correlation Analysis Between Disease Severity and Inflammation-related Parameters in Patients with COVID-19 Pneumonia

[312]

8.28.1 Keywords

- cytokine
- COVID-19 pneumonia
- severity
- disease progression

8.28.2 Main Findings

This study is a cross-sectional analysis of 100 patients with COVID-19 pneumonia, divided into mild (n = 34), severe (n = 34), and critical (n = 32) disease status based on clinical definitions.

The criteria used to define disease severity are as follows:

1. *Severe* – any of the following: respiratory distress or respiratory rate ≥ 30 respirations/minute; oxygen saturation ≤ 93% at rest; oxygen partial pressure (PaO2)/oxygen concentration (FiO2) in arterial blood ≤ 300mmHg, progression of disease on imaging to >50% lung involvement in the short term.

2. *Critical* – any of the following: respiratory failure that requires mechanical ventilation; shock; other organ failure that requires treatment in the ICU.

3. Patients with pneumonia who test positive for COVID-19 who do not have the symptoms delineated above are considered *mild*.

Peripheral blood inflammatory markers were correlated to disease status. Disease severity was significantly associated with levels of IL-2R, IL-6, IL-8, IL-10, TNF-α, CRP, ferroprotein, and procalcitonin. Total WBC count, lymphocyte count, neutrophil count, and eosinophil count were also significantly correlated with disease status. Since this is a retrospective, cross-sectional study of clinical laboratory

values, these data may be extrapolated for clinical decision making, but without studies of underlying cellular causes of these changes this study does not contribute to a deeper understanding of SARS-CoV-2 interactions with the immune system.

It is also notable that the mean age of patients in the mild group was significantly different from the mean ages of patients designated as severe or critical ($p < 0.001$). The mean patient age was not significantly different between the severe and critical groups. However, IL-6, IL-8, procalcitonin (Table 2), CRP, ferroprotein (Figure 3A, 3B), WBC count, and neutrophil count (Figure 4A, 4B) were all significantly elevated in the critical group compared to severe. These data suggest underlying differences in COVID-19 progression that is unrelated to age.

8.28.3 Significance

Given the inflammatory profile outlined in this study, patients who have mild or severe COVID-19 pneumonia, who *also* have any elevations in the inflammatory biomarkers listed above, should be closely monitored for potential progression to critical status.

8.28.4 Credit

This review by JJF was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.29 An Effective CTL Peptide Vaccine for Ebola Zaire Based on Survivors' CD8+ Targeting of a Particular Nucleocapsid Protein Epitope with Potential Implications for COVID-19 Vaccine Design

Herst et al. *bioRxiv* [313]

8.29.1 Keywords

- Peptide vaccine
- Ebolavirus
- nucleocapsid
- epitope
- vaccine design
- microsphere

8.29.2 Main Findings

Vaccination of mice with a single dose of a 9-amino-acid peptide NP44-52 located in a conserved region of ebolavirus (EBOV) nucleocapsid protein (NP) confers CD8+ T-cell-mediated immunity against mouse adapted EBOV (maEBOV). Bioinformatic analyses predict multiple conserved CD8+ T cell epitopes in the SARS-CoV-2 NP, suggesting that a similar approach may be feasible for vaccine design against SARS-CoV-2.

The authors focus on a site within a 20-peptide region of EBOV NP which was commonly targeted by CD8+ T cells in a group of EBOV survivors carrying the HLA-A*30:01:01 allele. To justify the testing of specific vaccine epitopes in a mouse challenge setting, the authors cite known examples of human pathogen-derived peptide antigens that are also recognized by C57BL/6 mice, as well as existing data surrounding known mouse immunogenicity of peptides related to this EBOV NP region. Testing 3 distinct 9mer peptides over an 11 amino-acid window and comparing to vaccination with the 11mer

with a T-cell reactivity readout demonstrated that optimizing peptide length and position for immunogenicity may be crucial, likely due to suboptimal peptide processing and MHC-class-I loading.

Vaccines for maEBOV challenge studies were constructed by packaging NP44-52 in d,l poly(lactic-co-glycolic) acid microspheres. CpG was also packaged within the microspheres, while Monophosphoryl Lipid A (a TLR4 ligand) was added to the injectate solution. A second peptide consisting of a predicted MHC-II epitope from the EBOV VG19 protein was added using a separate population of microspheres, and the formulation was injected by intraperitoneal administration. The vaccine was protective against a range of maEBOV doses up to at least 10,000 PFU. Survival was anticorrelated with levels of IL6, MCP-1 (CCL2), IL9, and GM-CSF, which recapitulated trends seen in human EBOV infection.

While HLA-A*30:01:01 is only present in a minority of humans, the authors state that MHC binding algorithms predict NP44-52 to be a strong binder of a set of more common HLA-A*02 alleles. The authors predict that a peptide vaccine based on the proposed formulation could elicit responses in up to 50% of people in Sudan or 30% of people in North America.

SARS-CoV-2 NP, meanwhile, has conserved regions which may provide peptide-vaccine candidates. Scanning the SARS-CoV-2 NP sequence for HLA-binding 9mers identified 53 peptides with predicted binding affinity < 500nM, including peptides that are predicted to bind to HLA-class-I alleles of 97% of humans, 7 of which have previously been tested *in-vitro*.

The results support previously appreciated correlations between certain cytokines and disease severity, specifically IL6 which relates to multiple trial therapies. Prediction of HLA-class-I binding of SARS-CoV-2 NP peptides suggests the plausibility of a peptide vaccine targeting conserved regions of SARS-CoV-2 NP although further validation in previously infected patient samples will be essential.

8.29.3 Credit

Review by Andrew M. Leader as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.30 Epitope-based peptide vaccines predicted against novel coronavirus disease caused by SARS-CoV-2

Li et al. *bioRxiv*. [314]

8.30.1 Keywords

- SARS-CoV-2
- immune-informatics
- vaccine design
- T cell epitope
- B cell epitope

8.30.2 Main Findings

This study employs a series of bioinformatic pipelines to identify T and B cell epitopes on spike (S) protein of SARS-CoV-2 and assess their properties for vaccine potential. To identify B cell epitopes, they assessed structural accessibility, hydrophilicity, and beta-turn and flexibility which are all factors that promote their targeting by antibodies. To identify T cell epitopes, they filtered for peptides with high antigenicity score and capacity to bind 3 or more MHC alleles. Using the protein digest server, they also demonstrated that their identified T and B cell epitopes are stable, having multiple non-

digesting enzymes per epitope. Epitopes were also determined to be non-allergenic and non-toxin as assessed by Allergen FP 1.0 and ToxinPred, respectively. For T cell epitopes, they assessed the strength of epitope-HLA interaction via PepSite. Overall, they predict four B cell and eleven T cell epitopes (two MHC I and nine MHC II binding) to pass stringent computational thresholds as candidates for vaccine development. Furthermore, they performed sequence alignment between all identified SARS-CoV-2 S protein mutations and predicted epitopes, and showed that the epitopes are conserved across 134 isolates from 38 locations worldwide. However, they report that these conserved epitopes may soon become obsolete given the known mutation rate of related SARS-CoV is estimated to be 4x10-4/site/year, underscoring the urgency of anti-viral vaccine development.

8.30.3 Limitations

While spike (S) protein may have a critical role in viral entry into host cells and their epitope prediction criterion were comprehensive, this study did not examine other candidate SARS-CoV-2 proteins. This point is particularly important given that a single epitope may not be sufficient to induce robust immune memory, and recent approaches involve multi-epitope vaccine design. Furthermore, their study only included a direct implementation of various published methods, but did not validate individual bioinformatic tools with controls to demonstrate robustness. Finally, it is critical that these predicted epitopes are experimentally validated before any conclusions can be drawn about their potential as vaccine candidates or their clinical efficacy.

8.30.4 Significance

This study provides a computational framework to rapidly identify epitopes that may serve as potential vaccine candidates for treating SARS-CoV-2.

8.30.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.31 The definition and risks of Cytokine Release Syndrome-Like in 11 COVID-19-Infected Pneumonia critically ill patients: Disease Characteristics and Retrospective Analysis

Wang Jr. et al. *medRxiv*. [315]

8.31.1 Keywords

- Cytokine release syndrome (CRS)
- biomarkers
- ARDS
- IL-6
- lymphopenia

8.31.2 Main Findings

This study describes the occurrence of a cytokine release syndrome-like (CRSL) toxicity in ICU patients with COVID-19 pneumonia. The median time from first symptom to acute respiratory distress syndrome (ARDS) was 10 days. All patients had decreased CD3, CD4 and CD8 cells, and a significant increase of serum IL-6. Furthermore, 91% had decreased NK cells. The changes in IL-6 levels preceded those in CD4 and CD8 cell counts. All of these parameters correlated with the area of pulmonary

inflammation in CT scan images. Mechanical ventilation increased the numbers of CD4 and CD8 cells, while decreasing the levels of IL-6, and improving the immunological parameters.

8.31.3 Limitations

The number of patients included in this retrospective single center study is small (n=11), and the follow-up period very short (25 days). Eight of the eleven patients were described as having CRSL, and were treated by intubation (7) or ECMO (2). Nine patients were still in the intensive care unit at the time of publication of this article, so their disease outcome is unknown.

8.31.4 Significance

The authors define a cytokine release syndrome-like toxicity in patients with COVID-19 with clinical radiological and immunological criteria: 1) decrease of circulating CD4, CD8 and NK cells; 2) substantial increase of IL-6 in peripheral blood; 3) continuous fever; 4) organ and tissue damage. This event seems to occur very often in critically ill patients with COVID-19 pneumonia. Interestingly, the increase of IL-6 in the peripheral blood preceded other laboratory alterations, thus, IL-6 might be an early biomarker for the severity of COVID-19 pneumonia. The manuscript will require considerable editing for organization and clarity.

8.31.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.32 Clinical characteristics of 36 non-survivors with COVID-19 in Wuhan, China

Huang et al. *medRxiv*. [316]

8.32.1 Keywords

- Clinical Characteristics
- Non Survivors
- retrospective study

8.32.2 Main Findings

This is a simple study reporting clinical characteristics of patients who did not survive COVID-19. All patients (mean age=69.22 years) had acute respiratory distress syndrome (ARDS) and their median time from onset to ARDS was 11 days. The median time from onset to death was 17 days. Most patients were older male (70% male) with co-morbidities and only 11 % were smokers. 75% patients showed bilateral pneumonia. Many patients had chronic diseases, including hypertension (58.33%). cardiovascular disease (22.22%) and diabetes (19.44%). Typical clinical feature measured in these patients includes lymphopenia and elevated markers of inflammation.

8.32.3 Limitations

As noted by the authors, the conclusions of this study are very limited because this is single-centered study focusing on a small cohort of patients who did not survive. Many clinical parameters observed by the authors (such* as increase levels of serum CRP, PCT, IL-6) have also been described in other COVID19 patients who survived the infection

8.32.4 Significance

This study is essentially descriptive and may be useful for clinical teams monitoring COVID19 patients.

8.32.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.33 Risk Factors Related to Hepatic Injury in Patients with Corona Virus Disease 2019

[317]

8.33.1 Keywords

- COVID-related Hepatic Injury

8.33.2 Main Findings

Based on a retrospective study of 85 hospitalized COVID patients in a Beijing hospital, authors showed that patients with elevated ALT levels (n = 33) were characterized by significantly higher levels of lactic acid and CRP as well as lymphopenia and hypoalbuminemia compared to their counterparts with normal ALT levels. Proportion of severe and critical patients in the ALT elevation group was significantly higher than that of normal ALT group. Multivariate logistic regression performed on clinical factors related to ALT elevation showed that CRP $\geq$ 20mg/L and low lymphocyte count (<1.1*10^9 cells/L) were independently related to ALT elevation—a finding that led the authors to suggest cytokine storm as a major mechanism of liver damage.

8.33.3 Limitations

The article's most attractive claim that liver damage seen in COVID patients is caused by cytokine storm (rather than direct infection of the liver) hinges solely on their multivariate regression analysis. Without further mechanistic studies a) demonstrating how high levels of inflammatory cytokines can induce liver damage and b) contrasting types of liver damage incurred by direct infection of the liver vs. system-wide elevation of inflammatory cytokines, their claim remains thin. It is also worth noting that six of their elevated ALT group (n=33) had a history of liver disease (i.e. HBV infection, alcoholic liver disease, fatty liver) which can confound their effort to pin down the cause of hepatic injury to COVID.

8.33.4 Significance

Limited. This article confirms a rich body of literature describing liver damage and lymphopenia in COVID patients.

8.33.5 Credit

Review by Chang Moon as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.34 Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely associated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients

[318]

8.34.1 Keywords

- ARDS
- interleukin-6 (IL-6)
- procalcitonin (PCT)
- pro-inflammatory cytokines
- SARS-CoV-2 RNAaemia

8.34.2 Main Findings

48 adult patients diagnosed with Covid19 according to Chinese guidelines for Covid19 diagnosis and treatment version 6 were included in this study. Patients were further sub-divided into three groups based on clinical symptoms and disease severity: (1) mild, positive Covid19 qPCR with no or mild clinical symptoms (fever; respiratory; radiological abnormalities); (2) severe, at least one of the following: shortness of breath/respiratory rate >30/min, oxygen saturation SaO_2<93%, Horowitz index paO2/FiO_2 < 300 mmHg (indicating moderate pulmonary damage); and (3) critically ill, at least one additional complicating factor: respiratory failure with need for mechanical ventilation; systemic shock; multi-organ failure and transfer to ICU. Serum samples and throat-swaps were collected from all 48 patients enrolled. SARS-CoV-2 RNA was assessed by qPCR with positive results being defined as Ct values < 40, and serum interleukin-6 (IL-6) was quantified using a commercially available detection kit. Briefly, patient characteristics in this study confirm previous reports suggesting that higher age and comorbidities are significant risk factors of clinical severity. Of note, 5 out of 48 of patients (10.41%), all in the critically ill category, were found to have detectable serum SARS-CoV-2 RNA levels, so-called RNAaemia. Moreover, serum IL-6 levels in these patients were found to be substantially higher and this correlated with the presence of detectable SARS-CoV-2 RNA levels. The authors hypothesize that viral RNA might be released from acutely damages tissues in moribund patients during the course of Covid19 and that RNaemia along with IL-6 could potentially be used as a prognostic marker.

8.34.3 Limitations

While this group's report generally confirms some of the major findings of a more extensive study, published in early February 2020, [308], there are limitations that should be taken into account. First, the number of patients enrolled is relatively small; second, interpretation of these data would benefit from inclusion of information about study specifics as well as providing relevant data on the clinical course of these patients other than the fact that some were admitted to ICU (i.e. demographics on how many patients needed respiratory support, dialysis, APACHE Ii/III or other standard ICU scores as robust prognostic markers for mortality etc). It also remains unclear at which time point the serum samples were taken, i.e. whether at admission, when the diagnosis was made or during the course of the hospital stay (and potentially after onset of therapy, which could have affected both IL-6 and RNA levels). The methods section lacks important information on the qPCR protocol employed, including primers and cycling conditions used. From a technical point of view, Ct values >35 seem somewhat non-specific (although Ct <40 was defined as the CDC cutoff as well) indicating that serum RNA levels are probably very low, therefore stressing the need for highly specific primers and high qPCR efficiency. In addition, the statistical tests used (t-tests, according to the methods section) do not seem appropriate as the organ-specific data such as BUN and troponin T values seem to be not normally

distributed across groups (n= 5 RNAaemia+ vs. n= 43 RNAaemia-). Given the range of standard deviations and the differences in patient sample size, it is difficult to believe that these data are statistically significantly different.

8.34.4 Significance

This study is very rudimentary and lacks a lot of relevant clinical details. However, it corroborates some previously published observations regarding RNAemia and IL-6 by another group. Generally, regarding future studies, it would be important to address the question of IL-6 and other inflammatory cytokine dynamics in relation to Covid19 disease kinetics (high levels of IL-6, IL-8 and plasma leukotriene were shown to have prognostic value at the onset of ARDS ; serum IL-2 and IL-15 have been associated with mortality; reviewed by Chen W & Ware L, Clin Transl Med. 2015 [319]).

8.34.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.35 Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study

[320]

8.35.1 Keywords

- Lymphopenia

8.35.2 Main Findings

Based on a retrospective study of 162 COVID patients from a local hospital in Wuhan, China, the authors show an inverse correlation between lymphocyte % (LYM%) of patients and their disease severity. The authors have also tracked LYM% of 70 cases (15 deaths; 15 severe; 40 moderate) throughout the disease progression with fatal cases showing no recovery of lymphocytes (<5%) even after 17-19 days post-onset. The temporal data of LYM % in COVID patients was used to construct a Time-Lymphocyte% model which is used to categorize and predict patients' disease severity and progression. The model was validated using 92 hospitalized cases and kappa statistic test was used to assess agreement between predicted disease severity and the assigned clinical severity (k = 0.49).

8.35.3 Limitations

Time-Lymphocyte % Model (TLM) that authors have proposed as a predictive model for clinical severity is very simple in its construction and derives from correlative data of 162 patients. In order for the model to be of use, it needs validation using a far more robust data set and possibly a mechanistic study on how COVID leads to lymphopenia in the first place. In addition, it should be noted that no statistical test assessing significance of LYM % values between disease severities was performed.

8.35.4 Significance

This article is of limited significance as it simply reports similar descriptions of COVID patients made in previous literature that severe cases are characterized by lymphopenia.

8.35.5 Credit

Review by Chang Moon as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.36 The potential role of IL-6 in monitoring severe case of coronavirus disease 2019

Liu et al. *medRxiv*. [321]

8.36.1 Keywords

- Cytokine Release Syndrome
- lymphocytopenia
- IL-6
- CRP
- COVID19
- pneumonia

8.36.2 Main Findings

Study on blood biomarkers on 80 COVID19 patients (69 severe and 11 non-severe). Patients with severe symptoms at admission (baseline) showed obvious lymphocytopenia and significantly increased interleukin-6 (IL-6) and CRP, which was positively correlated with symptoms severity. IL-6 at baseline positively correlates with CRP, LDH, ferritin and D-Dimer abundance in blood.

Longitudinal analysis of 30 patients (before and after treatment) showed significant reduction of IL-6 in remission cases.

8.36.3 Limitations

Limited sample size at baseline, especially for the non-severe leads to question on representativeness. The longitudinal study method is not described in detail and suffers from non-standardized treatment. Limited panel of pro-inflammatory cytokine was analyzed. Patients with severe disease show a wide range of altered blood composition and biomarkers of inflammation, as well as differences in disease course (53.6% were cured, about 10% developed acute respiratory distress syndrome). The authors comment on associations between IL-6 levels and outcomes, but these were not statistically significant (maybe due to the number of patients, non-standardized treatments, etc.) and data is not shown. Prognostic biomarkers could have been better explored. Study lacks multivariate analysis.

8.36.4 Significance

IL-6 could be used as a pharmacodynamic marker of disease severity. Cytokine Release Syndrome (CRS) is a well-known side effect for CAR-T cancer therapy and there are several effective drugs to manage CRS. Drugs used to manage CRS could be tested to treat the most severe cases of COVID19.

8.36.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.37 Clinical and Laboratory Profiles of 75 Hospitalized Patients with Novel Coronavirus Disease 2019 in Hefei, China

Zhao et al. *medRxiv*. [322]

8.37.1 Keywords

- Routine laboratory testing

8.37.2 Main Findings

The authors of this study provide a comprehensive analysis of clinical laboratory assessments in 75 patients (median age 47 year old) hospitalized for Corona virus infection in China measuring differential blood counts including T-cell subsets (CD4, CD8), coagulation function, basic blood chemistry, of infection-related biomarkers including CRP, Procalcitonin (PCT) (Precursor of calcitonin that increases during bacterial infection or tissue injury), IL-6 and erythrocyte sedimentation rate as well as clinical parameters. Among the most common hematological changes they found increased neutrophils, reduced CD4 and CD8 lymphocytes, increased LDH, CRP and PCT

When looking at patients with elevated IL-6, the authors describe significantly reduced CD4 and CD8 lymphocyte counts and elevated CRP and PCT levels were significantly increased in infected patients suggesting that increased IL-6 may correlate well with disease severity in COVID-19 infections

8.37.3 Limitations

The authors performed an early assessment of clinical standard parameters in patients infected with COVID-19. Overall, the number of cases (75) is rather low and the snapshot approach does not inform about dynamics and thus potential relevance in the assessment of treatment options in this group of patients.

8.37.4 Significance

The article summarizes provides a good summary of some of the common changes in immune cells inflammatory cytokines in patients with a COVID-19 infection and. Understanding how these changes can help predict severity of disease and guide therapy including IL-6 cytokine receptor blockade using Tocilizumab or Sarilumab will be important to explore.

8.37.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.38 Exuberant elevation of IP-10, MCP-3 and IL-1ra during SARS-CoV-2 infection is associated with disease severity and fatal outcome

Yang et al. *medRxiv* [323]

8.38.1 Keywords

- cytokine
- IP-10

- MCP-3
- IL-1Ra
- lymphocyte
- neutrophil
- stratification
- disease severity
- viral load
- lung function
- complications
- clinical data

8.38.2 Summary

Plasma cytokine analysis (48 cytokines) was performed on COVID-19 patient plasma samples, who were sub-stratified as severe (N=34), moderate (N=19), and compared to healthy controls (N=8). Patients were monitored for up to 24 days after illness onset: viral load (qRT-PCR), cytokine (multiplex on subset of patients), lab tests, and epidemiological/clinical characteristics of patients were reported.

8.38.3 Main Findings

- Many elevated cytokines with COVID-19 onset compared to healthy controls (IFNy, IL-1Ra, IL-2Ra, IL-6, IL-10, IL-18, HGF, MCP-3, MIG, M-CSF, G-CSF, MIG-1a, and IP-10).
- IP-10, IL-1Ra, and MCP-3 (esp. together) were associated with disease severity and fatal outcome.
- IP-10 was correlated to patient viral load ($r=0.3006$, $p=0.0075$).
- IP-10, IL-1Ra, and MCP-3 were correlated to loss of lung function (PaO_2/FaO_2 (arterial/atmospheric O2) and Murray Score (lung injury) with MCP-3 being the most correlated ($r=0.4104$ $p<0.0001$ and $r=0.5107$ $p<0.0001$ respectively).
- Viral load (Lower Ct Value from qRT-PCR) was associated with upregulated IP-10 only (not IL-1Ra or MCP-3) and was mildly correlated with decreased lung function: PaO_2/FaO_2 (arterial/atmospheric O2) and Murray Score (lung injury).
- Lymphopenia (decreased CD4 and CD8 T cells) and increased neutrophil correlated w/ severe patients.
- Complications were associated with COVID severity (ARDS, hepatic insufficiency, renal insufficiency).

8.38.4 Limitations

Collection time of clinical data and lab results not reported directly (likely 4 days (2,6) after illness onset), making it very difficult to determine if cytokines were predictive of patient outcome or reflective of patient compensatory immune response (likely the latter). Small N for cytokine analysis (N=2 fatal and N=5 severe/critical, and N=7 moderate or discharged). Viral treatment strategy not clearly outlined.

8.38.5 Expanded Results

NOTE: Moderate COVID-19 was classified by fever, respiratory manifestations, and radiological findings consistent with pneumonia while severe patients had one or more of the following: 1)

respiratory distraction, resting O2 saturation, or 3) arterial PaO2/FiO2 < 300 mmHg.

Cytokine Results (Human Cytokine Screening Panel, Bio-Rad):

- **Significant elevation of cytokines observed in COVID patients compared to healthy controls: IFNy, IL-1Ra, IL-2Ra, IL-6, IL-10, IL-18, HGF, MCP-3, MIG, M-CSF, G-CSF, MIG-1a, and IP-10.**
- Severity was correlated **with increase in measured IP-10, MCP-3, and IL-Ra** as measure by area under the curve analysis during sample timecourse (2-24 days after illness onset).
- IL-1Ra incr. significant 0-7 days after onset, MCP-3 signif. upregulated throughout observation timecourse, and IP-10 increased and upregulated throughout (trending downwards over time).
- **The three cytokines together (IP-10, IL-1Ra, and MCP-3 AUC) served as the best predictors of disease deterioration and fatal outcome.**
- No significant differences between moderate/severe observed between groups in IL-2Ra, IL-6, IL-10, IL-18, CTACK, G-CSF, HGF, M-CSF, MIP-1a, MIG, and IFNy at any timepoints.
- **Viral load (Lower Ct Value from qRT-PCR) was associated with upregulated IP-10 only (not IL-1Ra or MCP-3) and was highly correlated with decreased lung function: PaO_2/FaO_2 (arterial/atmospheric O2) and Murray Score (lung injury).**
- **Antibodies against these cytokines (esp. anti-IP-10) may serve as a potential treatment for amelioration of COVID-19 (and associated ARDS).**

Lab results:

- **Decreased lymphocytes (%) in all patients – lymphopenia corr. w/ severe patients**
    - **Decreased CD4 and CD8 T cells** – no monocyte or eosino/basophil % measured
- **Increased neutrophils (%)**
- Increased BUN (mmol/L) – other kidney markers, liver markers, and LDH were not significantly different between groups and were not compared to healthy controls.

Clinical features (between moderate vs. severe patient groups):

- **Complications were associated with severity (ARDS, hepatic insufficiency, renal insufficiency).**
- Coexisting conditions between groups were not significantly different (chronic heart/lung/renal/liver disease, diabetes, or cancer) and patient time courses (onset to admission and onset to viral tx) also not significantly different – 4 days (2, 6) on average for admission and 4 (3,7) for antiviral.
- Increased corticosteroids and mechanical/ invasive mechanical ventilation in severe patients.
- Increased median age in severe group (Median (Range = 63.5 (42-74) vs. 51 (22-78)) and patients > 60 yrs had higher ratio of severe patients as compared patients 16-59 yrs.

- Higher incidence of fever is severe patients (91.2 vs. 68.4%), myalgia (57.7 vs. 48.1%), and chill (17.6% vs. 0%).

- No differences in cough, headache, nausea/vomiting, or diarrhea.

8.38.6 Significance

Outline of pathological time course (implicating innate immunity esp.) and identification key cytokines associated with disease severity and prognosis (+ comorbidities). Anti-IP-10 as a possible therapeutic intervention (ex: Eldelumab).

8.38.7 Credit

Review by Natalie Vaninov as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.39 Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019

Zhao Jr. et al. *medRxiv*. [324]

8.39.1 Keywords

- SARS-CoV-2 IgG
- seroconversion rate
- total Ab
- Ig
- IgM

8.39.2 Main Findings

This study examined antibody responses in the blood of COVID-19 patients during the early SARS CoV2 outbreak in China. Total 535 plasma samples were collected from 173 patients (51.4% female) and were tested for seroconversion rate using ELISA. Authors also compared the sensitivity of RNA and antibody tests over the course of the disease . The key findings are:

- Among 173 patients, the seroconversion rates for total antibody (Ab), IgM and IgG were 93.1% (161/173), 82.7% (143/173) and 64.7% (112/173), respectively.

- The seroconversion sequentially appeared for Ab, IgM and then IgG, with a median time of 11, 12 and 14 days, respectively. Overall, the seroconversion of Ab was significantly quicker than that of IgM ($p = 0.012$) and IgG ($p < 0.001$). Comparisons of seroconversion rates between critical and non-critical patients did not reveal any significant differences.

- RNA tests had higher sensitivity in early phase and within 7 days of disease onset than antibody assays (66.7% Vs 38.3% respectively).

- The sensitivity of the Ab assays was higher 8 days after disease onset, reached 90% at day 13 and 100% at later time points (15-39 days). In contrast, RNA was only detectable in 45.5% of samples at days 15-39.

- In patients with undetectable RNA in nasal samples collected during day 1-3, day 4-7, day 8-14 and day 15-39 since disease onset, 28.6% (2/7), 53.6% (15/28), 98.2% (56/57) and 100% (30/30) had detectable total Ab titers respectively Combining RNA and antibody tests significantly raised the sensitivity for detecting COVID-19 patients in different stages of the disease ($p < 0.001$).

- There was a strong positive correlation between clinical severity and antibody titer 2-weeks after illness onset.

- Dynamic profiling of viral RNA and antibodies in representative COVID-19 patients (n=9) since onset of disease revealed that antibodies may not be sufficient to clear the virus. It should be noted that increases in of antibody titers were not always accompanied by RNA clearance.

8.39.3 Limitations

Because different types of ELISA assays were used for determining antibody concentrations at different time points after disease onset, sequential seroconversion of total Ab, IgM and IgG may not represent actual temporal differences but rather differences in the affinities of the assays used. Also, due to the lack of blood samples collected from patients in the later stage of illness, how long the antibodies could last remain unknown. For investigative dynamics of antibodies, more samples were required.

8.39.4 Significance

Total and IgG antibody titers could be used to understand the epidemiology of SARS CoV-2 infection and to assist in determining the level of humoral immune response in patients.

The findings provide strong clinical evidence for routine serological and RNA testing in the diagnosis and clinical management of COVID-19 patients. The understanding of antibody responses and their half-life during and after SARS CoV2 infection is important and warrants further investigations.

8.39.5 Credit

This review was undertaken by Zafar Mahmood and edited by K Alexandropoulos as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.40 Restoration of leukomonocyte counts is associated with viral clearance in COVID-19 hospitalized patients

Chen et al. *medRxiv* [325]

8.40.1 Keywords

- COVID-19
- T cell
- B cell
- NK cell
- IL-6
- pro-calcitonin
- cytokine storm

8.40.2 Main Findings

The authors collected data on 25 COVID-19 patients (n=11 men, n=14 women) using standard laboratory tests and flow cytometry. All patients were treated with antibiotics. Twenty-four of the 25 patients were also treated with anti-viral Umefinovir and 14 of the patients were treated with corticosteroids. 14 patients became negative for the virus after 8-14 days of treatment. The same treatment course was extended to 15-23 days for patients who were still positive for the virus at day 14.

The authors found a negative association between age and resolution of infection. Patients with hypertension, diabetes, malignancy or chronic liver disease were all unable to clear the virus at day 14, though not statistically significant.

Elevated procalcitonin and a trend for increased IL-6 were also found in peripheral blood prior to the treatment.

A trend for lower NK cell, T cell and B cell counts in patients was also reported. B cell, CD4 and CD8 T cell counts were only increased upon treatment in patients who cleared the virus. NK cell frequencies remained unchanged after treatment in all the patients.

8.40.3 Limitations

73% of the patients who remained positive for SARS-CoV2 after the 1st treatment, and 43% of all patients who cleared the virus were treated with corticosteroids. Corticosteroids have strong effects on the immune compartment in blood [326]. The authors should have accounted for corticosteroid treatment when considering changes in T, NK and B cell frequencies.

Assessing if IL-6 concentrations were back to baseline levels following treatment would have provided insights into the COVID-19 cytokine storm biology. Patients with higher baseline levels of IL-6 have been reported to have lower CD8 and CD4 T cell frequencies [322]. Correlating IL-6 with cell counts before and after treatment would thus have also been of interest. The report of the laboratory measures in table 2 is incomplete and should include the frequencies of patients with increased/decreased levels for each parameter.

Correction is needed for the 1st paragraph of the discussion as data does not support NK cell restoration upon treatment in patients who cleared the virus. NK cells remain unchanged after the 1st treatment course and only seem to increase in 2 out of 6 donors after the 2nd treatment course in those patients.

8.40.4 Significance

Previous reports suggest an association between disease severity and elevated IL-6 or pro-calcitonin concentrations in COVID-19 patients [318,327]. IL-6 receptor blockade is also being administered to patients enrolled in clinical trials (NCT04317092). This report thus contributes to highlight elevated concentrations of these analytes in COVID-19 patients. Mechanisms underlying the association between viral clearance and restoration of the T cell and B cell frequencies suggests viral-driven immune dysregulation, which needs to be investigated in further studies.

8.40.5 Credit

Review by Bérengère Salomé as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.41 Clinical findings in critically ill patients infected with SARS-CoV-2 in Guangdong Province, China: a multi-center, retrospective, observational study

Xu et al. *medRxiv*. [328]

8.41.1 Keywords

- clinical outcomes
- prognosis
- critically ill patients
- ICU
- lymphopenia
- LDH

8.41.2 Main Findings:

This work analyses laboratory and clinical data from 45 patients treated in the in ICU in a single province in China. Overall, 44% of the patients were intubated within 3 days of ICU admission with only 1 death.

Lymphopenia was noted in 91% of patient with an inverse correlation with LDH.

Lymphocyte levels are negatively correlated with Sequential Organ Failure Assessment (SOFA) score (clinical score, the higher the more critical state), LDH levels are positively correlated to SOFA score. Overall, older patients (>60yo), with high SOFA score, high LDH levels and low lymphocytes levels at ICU admission are at higher risk of intubation.

Of note, convalescent plasma was administered to 6 patients but due to limited sample size no conclusion can be made.

8.41.3 Limitations

While the study offers important insights into disease course and clinical lab correlates of outcome, the cohort is relatively small and is likely skewed towards a less-severe population compared to other ICU reports given the outcomes observed. Analysis of laboratory values and predictors of outcomes in larger cohorts will be important to make triage and treatment decisions. As with many retrospective analyses, pre-infection data is limited and thus it is not possible to understand whether lymphopenia was secondary to underlying comorbidities or infection.

Well-designed studies are necessary to evaluate the effect of convalescent plasma administration.

8.41.4 Significance

This clinical data enables the identification of at-risk patients and gives guidance for research for treatment options. Indeed, further work is needed to better understand the causes of the lymphopenia and its correlation with outcome.

8.41.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.42 Immune Multi-epitope vaccine design using an immunoinformatics approach for 2019 novel coronavirus in China (SARS-CoV-2)

[329]

8.42.1 Keywords

- Vaccine
- in silico
- B cell epitopes
- T cell epitopes

8.42.2 Main Findings

Using in silico bioinformatic tools, this study identified putative antigenic B-cell epitopes and HLA restricted T-cell epitopes from the spike, envelope and membrane proteins of SARS-CoV-2, based on the genome sequence available on the NCBI database. T cell epitopes were selected based on predicted affinity for the more common HLA-I alleles in the Chinese population. Subsequently, the authors designed vaccine peptides by bridging selected B-cell epitopes and adjacent T-cell epitopes. Vaccine peptides containing only T-cell epitopes were also generated.

From 61 predicted B-cell epitopes, only 19 were exposed on the surface of the virion and had a high antigenicity score. A total of 499 T-cell epitopes were predicted. Based on the 19 B-cell epitopes and their 121 adjacent T-cell epitopes, 17 candidate vaccine peptides were designed. Additionally, another 102 vaccine peptides containing T-cell epitopes only were generated. Based on the epitope counts and HLA score, 13 of those were selected. Thus, a total of 30 peptide vaccine candidates were designed.

8.42.3 Limitations

While this study provides candidates for the development of vaccines against SARS-CoV-2, in vitro and in vivo trials are required to validate the immunogenicity of the selected B and T cell epitopes. This could be done using serum and cells from CoV-2-exposed individuals, and in preclinical studies. The implication of this study for the current epidemic are thus limited. Nevertheless, further research on this field is greatly needed.

8.42.4 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.43 Clinical Features of Patients Infected with the 2019 Novel Coronavirus (COVID-19) in Shanghai, China

Cao et al. *medRxiv* [330]

8.43.1 Keywords

- Disease severity
- clinical features
- laboratory abnormalities

8.43.2 Main Findings

This single-center cohort study analyzes the clinical and laboratory features of 198 patients with confirmed COVID-19 infection in Shanghai, China and correlated these parameters with clinical disease severity, including subsequent intensive care unit (ICU) admission. 19 cases (9.5%) required ICU admission after developing respiratory failure or organ dysfunction. Age, male sex, underlying cardiovascular disease, and high symptom severity (high fever, dyspnea) were all significantly correlated with ICU admission. Additionally, ICU admission was more common in patients who presented with lymphopenia and elevated neutrophil counts, among other laboratory abnormalities. Flow cytometric analysis revealed that patients admitted to the ICU had significantly reduced circulating CD3+ T cell, CD4+ T cell, CD8+ T cell, and CD45+ leukocyte populations compared to the cohort of patients not requiring ICU admission.

8.43.3 Limitations

The limitations of this study include the relatively small sample size and lack of longitudinal testing. The authors also did not assess whether respiratory comorbidity – such as asthma or chronic obstructive lung disease – in addition to immunosuppression affected ICU admission likelihood.

8.43.4 Significance

COVID-19 has already sickened thousands across the globe, though the severity of these infections is markedly diverse, ranging from mild symptoms to respiratory failure requiring maximal intervention. Understanding what clinical, laboratory, and immunologic factors predict the clinical course of COVID-19 infection permits frontline providers to distribute limited medical resources more effectively.

8.43.5 Credit

Review by Andrew Charap as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine at Mount Sinai.

8.44 Serological detection of 2019-nCoV respond to the epidemic: A useful complement to nucleic acid testing

Zhang et al. *medRxiv*. [331]

8.44.1 Keywords

- Immunoassay
- serum IgM and IgG
- specific antibodies

8.44.2 Main Finding

This study showed that both anti-2019-nCov IgM and IgG were detected by automated chemiluminescent immunoassay in the patients who had been already confirmed as positive by nucleic acid detection, while single positivity of IgM or IgG were detected in a very few cases in the other population including 225 non-COVID-19 cases. In addition to the increase of anti-2019-nCov IgM 7-12 days after morbidity, the increase of IgG was detected in three patients with COVID-19 within a very short of time (0-1 day).

8.44.3 Limitations

The limitation of this study is only 3 confirmed COVID-19 cases were included, so that the relationship between anti-2019-nCov antibodies and disease progression might not be clearly defined. Another limitation is that they did not show the course of 2019-nCov specific antibodies in the cases with positive for COVID-19 but without clinical symptoms.

8.44.4 Significance

The detection of anti-2019-nCov antibodies can be an alternative method to diagnose and treat COVID-19 more comprehensively by distinguish non COVID-19 patients. It may be helpful to understand the course of individual cases with COVID-19 to predict the prognosis if more cases will be evaluated.

8.44.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.45 Human Kidney is a Target for Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection

[332]

8.45.1 Keywords

- Kidney/Renal Failure
- Macrophage Infiltration
- Complement Activation

8.45.2 Main Finding

Analyzing the eGFR (effective glomerular flow rate) of 85 Covid-19 patients and characterizing tissue damage and viral presence in post-mortem kidney samples from 6 Covid-19 patients, the authors conclude that significant damage occurs to the kidney, following Covid-19 infection. This is in contrast to the SARS infection from the 2003 outbreak. They determine this damage to be more prevalent in patients older than 60 years old, as determined by analysis of eGFR. H&E and IHC analysis in 6 Covid-19 patients revealed that damage was in the tubules, not the glomeruli of the kidneys and suggested that macrophage accumulation and C5b-9 deposition are key to this process.

8.45.3 Limitations

Severe limitations include that the H&E and IHC samples were performed on post-mortem samples of unknown age, thus we cannot assess how/if age correlates with kidney damage, upon Covid-19 infection. Additionally, eGFR was the only *in-vivo* measurement. Blood urea nitrogen and proteinuria are amongst other measurements that could have been obtained from patient records. An immune panel of the blood was not performed to assess immune system activation. Additionally, patients are only from one hospital.

8.45.4 Significance

This report makes clear that kidney damage is prevalent in Covid-19 patients and should be accounted for.

8.45.5 Credit

Review by Dan Fu Ruan, Evan Cody and Venu Pothula as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine at Mount Sinai.

8.46 COVID-19 early warning score: a multi-parameter screening tool to identify highly suspected patients

Song et al. *medRxiv*. [333]

8.46.1 Keywords

- retrospective
- electronic health records
- blood counts
- diagnostic
- prognostic
- modeling

8.46.2 Main Findings

The aim of this study was to identify diagnostic or prognostic criteria which could identify patients with COVID-19 and predict patients who would go on to develop severe respiratory disease. The authors use EMR data from individuals taking a COVID-19 test at Zhejiang hospital, China in late January/Early February. A large number of clinical parameters were different between individuals with COVID-19 and also between 'severe' and 'non-severe' infections and the authors combine these into a multivariate linear model to derive a weighted score, presumably intended for clinical use.

8.46.3 Limitations

Unfortunately, the paper is lacking a lot of crucial information, making it impossible to determine the importance or relevance of the findings. Most importantly, the timings of the clinical measurements are not described relative to the disease course, so it is unclear if the differences between 'severe' and 'non-severe' infections are occurring before progression to severe disease (which would make them useful prognostic markers), or after (which would not).

8.46.4 Significance

This paper is one of many retrospective studies coming from hospitals in China studying individuals with COVID-19. Because of the sparse description of the study design, this paper offers little new information. However, studies like this could be very valuable and we would strongly encourage the authors to revise this manuscript to include more information about the timeline of clinical measurements in relation to disease onset and more details of patient outcomes.

8.46.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.47 LY6E impairs coronavirus fusion and confers immune control of viral disease

Pfaender et al. *bioRxiv*. [334]

8.47.1 Keywords

- interferon-stimulated genes
- antiviral interferons
- human coronaviruses (CoV)
- murine hepatitis virus (MHV)

8.47.2 Main Findings

Screening a cDNA library of >350 human interferon-stimulated genes for antiviral activity against endemic human coronavirus HCoV-229E (associated with the common cold), Pfaender S & Mar K *et al.* identify lymphocyte antigen 6 complex, locus E (Ly6E) as an inhibitor of cellular infection of Huh7 cells, a human hepatoma cell line susceptible to HCoV-229E and other coronaviruses. In a series of consecutive *in vitro* experiments including both stable Ly6E overexpression and CRISPR-Cas9-mediated knockout the authors further demonstrate that Ly6E reduces cellular infection by various other coronaviruses including human SARS-CoV and SARS-CoV-2 as well as murine CoV mouse hepatitis virus (MHV). Their experiments suggest that this effect is dependent on Ly6E inhibition of CoV strain-specific spike protein-mediated membrane fusion required for viral cell entry.

To address the function of Ly6E *in vivo*, hematopoietic stem cell-specific Ly6E knock-out mice were generated by breeding Ly6E$^{fl/fl}$ mice (referred to as functional wild-type mice) with transgenic *Vav-iCre* mice (offspring referred to as Ly6E HSC ko mice); wild-type and Ly6E HSC ko mice of both sexes were infected intraperitoneally with varying doses of the natural murine coronavirus MHV, generally causing a wide range of diseases in mice including hepatitis, enteritis and encephalomyelitis. Briefly, compared to wild-type controls, mice lacking hematopoietic cell-expressed Ly6E were found to present with a more severe disease phenotype as based on serum ALT levels (prognostic of liver damage), liver histopathology, and viral titers in the spleen. Moreover, bulk RNAseq analysis of infected liver and spleen tissues indicated changes in gene expression pathways related to tissue damage and antiviral immune responses as well as a reduction of genes associated with type I IFN response and inflammation. Finally, the authors report substantial differences in the numbers of hepatic and splenic APC subsets between wild-type and knockout mice following MHV infection and show that Ly6E-deficient B cells and to a lesser extent also DCs are particularly susceptible to MHV infection *in vitro*.

8.47.3 Limitations

Experiments and data in this study are presented in an overall logical and coherent fashion; however, some observations and the conclusions drawn are problematic and should be further addressed & discussed by the authors. Methodological & formal limitations include relatively low replicate numbers as well as missing technical replicates for some *in vitro* experiments (*cf.* Fig. legend 1; Fig. legend 2e); the omission of “outliers” in Fig. legend 2 without an apparent rationale as to why this approach was chosen; the lack of detection of actual Ly6E protein levels in Ly6E HSC ko or wild-type mice; and most importantly, missing information on RNAseq data collection & analysis in the method section and throughout the paper. A more relevant concern though is that the interpretation of the experimental data presented and the language used tend to overrate and at times overgeneralize findings: for example, while the authors demonstrate statistically significant, Ly6E-mediated reduction of coronavirus titers in stable cells lines *in vitro*, it remains unclear whether a viral titer reduction by one log decade would be of actual biological relevance in face of high viral titers *in vivo*. After high-dose intraperitoneal MHV infection *in vivo*, early viral titers in Ly6E HSC knockout vs. wt mice only showed an elevation in the spleen (~1.5 log decades) but not liver of the ko mice (other tissue not evaluated), and while ko mice presented with only modestly increased liver pathology, both male and

female ko mice exhibited significantly higher mortality. Thus, the manuscript tile statement that “Ly6E … confers immune control of viral disease” is supported by only limited *in vivo* data, and gain-of-function experiments (eg. Ly6E overexpression) were not performed. Of additional note here, tissue tropism and virulence differ greatly among various MHV strains and isolates whereas dose, route of infection, age, genetic background and sex of the mice used may additionally affect disease outcome and phenotype (*cf.* Taguchi F & Hirai-Yuki A, https://doi.org/10.3389/fmicb.2012.00068; Kanolkhar A et al, https://jvi.asm.org/content/ 83/18/9258). Observations attributed to hematopoietic stem cell-specific Ly6E deletion could therefore be influenced by the different genetic backgrounds of floxed and cre mice used, and although it appears that littermates wt and ko littermates were used in the experiments, the potentially decisive impact of strain differences should at least have been discussed. Along these lines, it should also be taken into account that the majority of human coronaviruses cause respiratory symptoms, which follow a different clinical course engaging other primary cellular mediators than the hepatotropic murine MHV disease studied here. It therefore remains highly speculative how the findings reported in this study will translate to human disease and it would therefore be important to test other routes of MHV infection and doses that have been described to produce a more comparable phenotype to human coronavirus disease (*cf.* Kanolkhar A et al, https://jvi.asm.org/content/ 83/18/9258). Another important shortcoming of this study is the lack of any information on functional deficits or changes in Ly6E-deficient immune cells and how this might relate to the phenotype observed. Overall, the *in vitro* experiments are more convincing than the *in vivo* studies which appear somewhat limited.

8.47.4 Significance

Despite some shortcomings, the experiments performed in this study suggest a novel and somewhat unexpected role of Ly6E in the protection against coronaviruses across species. These findings are of relevance and should be further explored in ongoing research on potential coronavirus therapies. Yet an important caveat pertains to the authors’ suggestion that “therapeutic mimicking of Ly6E action” may constitute a first line of defense against novel coronaviruses since their own prior work demonstrated that Ly6E can enhance rather than curtail infection with influenza A and other viruses.

8.47.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.48 A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients

Liu et al. *medRxiv*. [335]

8.48.1 Keywords

- diagnosis
- serological assay
- ELISA
- RT-PCR

8.48.2 Main Findings

While RT-PCR is being used currently to routinely diagnose infection with SARS-CoV-2, there are significant limitations to the use of a nucleic acid test that lead to a high false-negative rate. This article describes ELISAs that can measure IgM and IgG antibodies against the N protein of SARS-CoV-2

to test samples from 238 patients (153 positive by RT-PCR and 85 negative by RT-PCR) at different times after symptom onset. The positivity rate of the IgM and/or IgG ELISAs was greater than that of the RT-PCR (81.5% compared to 64.3%) with similar positive rates in the confirmed and suspected cases (83% and 78.8%, respectively), suggesting that many of the suspected but RT-PCR-negative cases were also infected. The authors also found that the ELISAs have higher positive rates later after symptom onset while RT-PCR is more effective as a diagnostic test early during the infection.

8.48.3 Limitations

I cannot identify any limitations to this study.

8.48.4 Significance

The authors make a strong case for using a combination of ELISA and RT-PCR for diagnosis of infection with SARS-CoV-2, especially considering the dynamics of positivity rates of RT-PCR and ELISA. Fewer false-negative diagnoses would improve infection control and patient management.

8.48.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.49 Monoclonal antibodies for the S2 subunit of spike of SARS-CoV cross-react with the newly-emerged SARS-CoV-2

[336]

8.49.1 Keywords

- Coronavirus Disease 2019 (COVID-19)
- SARS-CoV-2
- Spike protein
- Cross- reactive antibodies

8.49.2 Main Findings

Whole genome sequencing-based comparisons of the 2003 Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and the 2019 SARS-CoV-2 revealed conserved receptor binding domain (RBD) and host cell receptor, angiotensin-converting enzyme 2 (ACE2). In line with this, the authors tested cross-reactivity of murine monoclonal antibodies (mAbs) previously generated against the SARS-CoV spike (S) glycoprotein involved in viral entry. One of the screened mAb, 1A9, was able to bind and cross-neutralize multiple strains of SARS-CoV, as well as, detect the S protein in SARS-CoV-2-infected cells. mAb 1A9 was generated using an immunogenic fragment in the S2 subunit of SARS-CoV and binds through a novel epitope within the S2 subunit at amino acids 1111-1130. It is important to note that CD8+ T lymphocyte epitopes overlap with these residues, suggesting that S2 subunit could be involved in inducing both, humoral and cell-mediated immunity.

8.49.3 Limitations

The authors used previously generated mouse mAbs against the S protein in SARS-CoV expressed in mammalian cell line. Future experimental validation using COVID-19 patient samples is needed to validate these findings. In addition, the results of these studies are predominantly based on in vitro

experiments and so, evaluating the effects of the mAb 1A9 in an animal model infected with this virus will help us better understand the host immune responses in COVID-19 and potential therapeutic vaccines.

8.49.4 Significance

This study identified mAbs that recognize the new coronavirus, SARS-Cov-2. These cross-reactive mAbs will help in developing diagnostic assays for COVID-19.

8.49.5 Credit

This review was undertaken by Tamar Plitt and Katherine Lindblad as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.50 Mortality of COVID-19 is Associated with Cellular Immune Function Compared to Immune Function in Chinese Han Population

Zeng et al. *medRxiv*. [337]

8.50.1 Keywords

- WBC
- peripheral blood
- CD4
- CD8 T cells

8.50.2 Main Findings

Retrospective study of the clinical characteristics of 752 patients infected with COVID-19 at Chinese PLA General Hospital, Peking Union Medical College Hospital, and affiliated hospitals at Shanghai University of medicine & Health Sciences. This study is the first one that compares PB from healthy controls from the same regions in Shanghai and Beijing, and infected COVID-19 patients to standardize a reference range of WBCs of people at high risk.

8.50.3 Limitations

Lower levels of leukocyte counts -B cells, CD4 and CD8 T cells- correlated with mortality (WBCs are significantly lower in severe or critical UCI patients vs mild ones). Based on 14,117 normal controls in Chinese Han population (ranging in age from 18-86) it is recommended that reference ranges of people at high risk of COVID-19 infection are CD3+ lymphocytes below 900 cells/mm3, CD4+ lymphocytes below 500 cells/mm3, and CD8+ lymphocytes below 300 cells/mm3. Importantly, this study also reported that the levels of D-dimer, C-reactive protein and IL-6 were elevated in COVID-19 pts., indicating clot formation, severe inflammation and cytokine storm.

8.50.4 Significance

This study sets a threshold to identify patients at risk by analyzing their levels of leukocytes, which is an easy and fast approach to stratify individuals that require hospitalization. Although the study is limited (only counts of WBC are analyzed and not its profile) the data is solid and statistically robust to correlate levels of lymphopenia with mortality.

8.50.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.51 Retrospective Analysis of Clinical Features in 101 Death Cases with COVID-19

Chen et al. *medRxiv*. [338]

8.51.1 Keywords

- death biomarkers
- cardiac damage
- Troponin
- Blood type
- respiratory failure
- hypertension

8.51.2 Main Findings

This is a retrospective study involving 101 death cases with COVID-19 in Wuhan Jinyintan Hospital. The aim was to describe clinical, epidemiological and laboratory features of fatal cases in order to identify the possible primary mortality causes related to COVID-19.

Among 101 death cases, 56.44% were confirmed by RT-PCR and 43.6% by clinical diagnostics. Males dominated the number of deaths and the average age was 65.46 years. All patients died of respiratory failure and multiple organs failure, except one (acute coronary syndrome). The predominant comorbidities were hypertension (42.57%) and diabetes (22.77%). 25.74% of the patients presented more than two underlying diseases. 82% of patients presented myocardial enzymes abnormalities at admission and further increase in myocardial damage indicators with disease progression: patients with elevated Troponin I progressed faster to death. Alterations in coagulation were also detected. Indicators of liver and kidney damage increased 48 hours before death. The authors studied the deceased patients' blood type and presented the following results: type A (44.44%), type B (29.29%), type AB (8.08%) and type O (18.19%), which is inconsistent with the distribution in Han population in Wuhan.

Clinical analysis showed that the most common symptom was fever (91.9%), followed by cough and dyspnea. The medium time from onset of symptoms to acute respiratory distress syndrome (ARDS) development was 12 days. Unlike SARS, only 2 patients with COVID-19 had diarrhea. 98% presented abnormal lung imaging at admission and most had double-lung abnormalities. Related to the laboratorial findings some inflammatory indicators gradually increased during the disease progression, such as IL-6 secretion in the circulation, procalcitonin (PCT) and C-reactive protein (CRP), while platelets numbers decreased. The authors also reported an initial lymphopenia that was followed by an increase in the lymphocytes numbers. Neutrophil count increased with disease progression.

The patients received different treatments such as antiviral drugs (60.40%), glucocorticoids, thymosin and immunoglobulins. All patients received antibiotic treatment and some received antifungal drugs. All patients received oxygen therapy (invasive or non-invasive ones).

8.51.3 Limitations

This study involves just fatal patients, lacking comparisons with other groups of patients e.g. patients that recovered from COVID-19. The authors didn't discuss the different approaches used for treatments and how these may affect the several parameters measured. The possible relationship between the increase of inflammatory indicators and morbidities of COVID-19 are not discussed.

8.51.4 Significance

This study has the largest cohort of fatal cases reported so far. The authors show that COVID-19 causes fatal respiratory distress syndrome and multiple organ failure. This study highlights prevalent myocardial damage and indicates that cardiac function of COVID-19 patients should be carefully monitored. The data suggest that Troponin I should be further investigated as an early indicator of patients with high risk of accelerated health deterioration. Secondary bacterial and fungal infections were frequent in critically ill patients and these need to be carefully monitored in severe COVID-19 patients. Differences in blood type distribution were observed, suggesting that type A is detrimental while type O is protective – but further studies are needed to confirm these findings and elucidate if blood type influences infection or disease severity. Several inflammatory indicators (neutrophils, PCT, CRP and IL-6, D-dimer) increased according to disease severity and should be assessed as biomarkers and to better understand the biology of progression to severe disease.

8.51.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.52 Relationship between the ABO Blood Group and the COVID-19 Susceptibility

Zhao et al. *medRxiv*. [339]

8.52.1 Keywords

- ABO blood group
- COVID-19 susceptibility

8.52.2 Main Findings

These authors compared the ABO blood group of 2,173 patients with RT-PCR-confirmed COVID-19 from hospitals in Wuhan and Shenzhen with the ABO blood group distribution in unaffected people in the same cities from previous studies (2015 and 2010 for Wuhan and Shenzhen, respectively). They found that people with blood group A are statistically over-represented in the number of those infected and who succumb to death while those with blood group O are statistically underrepresented with no influence of age or sex.

8.52.3 Limitations

This study compares patients with COVID-19 to the general population but relies on data published 5 and 10 years ago for the control. The mechanisms that the authors propose may underlie the differences they observed require further study.

8.52.4 Significance

Risk stratification based on blood group may be beneficial for patients and also healthcare workers in infection control. Additionally, investigating the mechanism behind these findings could lead to better developing prophylactic and therapeutic targets for COVID-19.

8.52.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.53 The inhaled corticosteroid ciclesonide blocks coronavirus RNA replication by targeting viral NSP15

Matsuyama et al. *bioRxiv* [340]

8.53.1 Keywords

- Corticosteroids
- ciclesonide
- mometasone
- NSP15
- MERS-CoV

8.53.2 Main Findings

This study reconsiders the use of inhaled corticosteroids in the treatment of pneumonia by coronavirus. Corticosteroids were associated with increased mortality for SARS in 2003 and for MERS in 2013, probably due to that fact that systemic corticosteroids suppress the innate immune system, resulting in increased viral replication. However, some steroid compounds might block coronavirus replication. The authors screened steroids from a chemical library and assessed the viral growth suppression and drug cytotoxicity. Ciclesonide demonstrated low cytotoxicity and potent suppression of MERS-CoV viral growth. The commonly used systemic steroids cortisone, prednisolone and dexamethasone did not suppress viral growth, nor did the commonly used inhaled steroid fluticasone. To identify the drug target of virus replication, the authors conducted 11 consecutive MERS-CoV passages in the presence of ciclesonide or mometasone, and they could generate a mutant virus that developed resistance to ciclesonide, but not to mometasone. Afterwards, they performed next-generation sequencing and identified an amino acid substitution in nonstructural protein 15 (NSP15) as the predicted mechanism for viral resistance to ciclesonide. The authors were able to successfully generate a recombinant virus carrying that amino acid substitution, which overcome the antiviral effect of ciclesonide, suggesting that ciclosenide interacts with NSP15. The mutant virus was inhibited by mometasone, suggesting that the antiviral target of mometasone is different from that of ciclesonide. Lastly, the effects of ciclesonide and mometason on suppressing the replication of SARS-CoV-2 were evaluated. Both compounds were found to suppress viral replication with a similar efficacy to lopinavir.

8.53.3 Limitations

Most of the experiments, including the identification of the mutation in NSP15 were conducted with MERS-CoV. This is not the closest related virus to SARS-CoV-2, as that would be SARS-CoV. Thus, to repeat the initial experiments with SARS-CoV, or preferably SARS-CoV-2, is essential. The manuscript should address this and, therefore, it will require considerable editing for organization and clarity. Also, in terms of cell immunogenic epitopes, while SARS-CoV-2 spike protein contains several predicted B and T cell immunogenic epitopes that are shared with other coronaviruses, some studies

have shown critical differences between MERS-CoV, SARS-CoV and SARS-CoV-2. A main criticism is that the authors only used VeroE6/TMPRSS2 cells to gauge the direct cytotoxic effects of viral replication. To evaluate this in other cell lines, including human airway epithelial cells, is crucial, as the infectivity of coronavirus strains greatly varies in different cell lines,

8.53.4 Significance

Nevertheless, these findings encourage evaluating ciclesonide and mometasone as better options for patients with COVID-19 in need of inhaled steroids, especially as an alternative to other corticosteroids that have been shown to increase viral replication in vitro. This should be evaluated in future clinical studies.

8.53.5 Credit

This review was undertaken by Alvaro Moreira, MD as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.54 A human monoclonal antibody blocking SARS-CoV-2 infection **

Wang et al. *bioRxiv*. [228]

8.54.1 Keywords

- Monoclonal antibodies
- SARS-CoV2
- cross-neutralization
- potential treatment
- spike receptor

8.54.2 Main Findings

The authors reported a human monoclonal antibody that neutralizes SARS-CoV-2 and SARS-Cov which belong to same family of corona viruses. For identifying mAbs, supernatants of a collection of 51 hybridomas raised against the spike protein of SARS-CoV (SARS-S) were screened by ELISA for cross-reactivity against the spike protein of SARS-CoN2 (SARS2-S). Hybridomas were derived from immunized transgenic H2L2 mice (chimeric for fully human VH-VL and rat constant region). Four SARS-S hybridomas displayed cross-reactivity with SARS2-S, one of which (47D11) exhibited cross-neutralizing activity for SARS-S and SARS2-S pseudotyped VSV infection. A recombinant, fully human IgG1 isotype antibody was generated and used for further characterization.

The humanized 47D11 antibody inhibited infection of VeroE6 cells with SARS-CoV and SARS-CoV-2 with IC50 values of 0.19 and 0.57 µg/ml respectively. 47D11 mAb bound a conserved epitope on the spike receptor binding domain (RBD) explaining its ability to cross-neutralize SARS-CoV and SARS-CoV-2. 47D11 was shown to target the S1B RBD of SARS-S and SARS2-S with similar affinities. Interestingly, binding of 47D11 to SARS-S1B and SARS2-S1B did not interfere with S1B binding to ACE2 receptor-expressing cells assayed by flow cytometry.

8.54.3 Limitations

These results show that the human 47D11 antibody neutralizes SARS-CoV and SARS-Cov2 infectivity via an as yet unknown mechanism that is different from receptor binding interference. Alternative mechanisms were proposed but these as yet remain to be tested in the context of SARS-CoV2. From a

therapeutic standpoint and in the absence of in vivo data, it is unclear whether the 47D11 ab can alter the course of infection in an infected host through virus clearance or protect an uninfected host that is exposed to the virus. There is a precedent for the latter possibility as it relates to SARS-CoV that was cited by the authors and could turn out to be true for SARS-CoV2.

8.54.4 Significance

This study enabled the identification of novel neutralizing antibody against COV-that could potentially be used as first line of treatment in the near future to reduce the viral load and adverse effects in infected patients. In addition, neutralizing antibodies such as 47D11 represent promising reagents for developing antigen-antibody-based detection test kits and assays.

8.54.5 Credit

This review was edited by K. Alexandropoulos as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

Heat inactivation of serum interferes with the immunoanalysis of antibodies to SARS-CoV-2

Heat inactivation, immunochromatography, diagnosis, serum antibodies, IgM, IgG

Summary

The use of heat inactivation to neutralize pathogens in serum samples collected from suspected COVID-19 patients reduces the sensitivity of a fluorescent immunochromatographic assay to detect anti-SARS-CoV-2 IgM and IgG.

Major findings

Coronaviruses can be killed by heat inactivation, and this is an important safety precaution in laboratory manipulation of clinical samples. However, the effect of this step on downstream SARS-CoV-2-specific serum antibody assays has not been examined. The authors tested the effect of heat inactivation (56 deg C for 30 minutes) versus no heat inactivation on a fluorescence immunochromatography assay. Heat inactivation reduced all IgM measurements by an average of 54% and most IgG measurements (22/36 samples, average reduction of 50%), consistent with the lower thermal stability of IgM than that of IgG. Heat inactivation caused a subset of IgM but not IgG readings to fall below a specified positivity threshold.

Limitations

Limitations included the use of only one type of assay for testing heat inactivated vs non-inactivated sera, and the use of the same baseline for heat inactivated and non-inactivated sera. The results indicate that heat inactivation affects the quantification of SARS-CoV-2-antibody response, specially IgM, but still allows to distinguish positive specific IgG. Therefore, the effect of heat inactivation should be studied when designing assays that quantitatively associate immunoglobulin levels (especially IgM) to immune state.

Review by Andrew M. Leader as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn school of medicine, Mount Sinai.

8.55 Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19

Zhang et al. *medRxiv* [341]

8.55.1 Keywords

- Biomarkers
- cytokines
- IgG
- immune cells

8.55.2 Main Findings

In a cohort of 222 patients, anti-SARS-CoV-2 IgM and IgG levels were analyzed during acute and convalescent phases (up to day 35) and correlated to the diseases' severity. The same was done with neutrophil-to-lymphocyte ratio. High IgG levels and high neutrophil-to-lymphocyte ratio in convalescence were both independently associated to the severity of the disease. The simultaneous occurrence of both of these laboratory findings correlated even stronger to the diseases' severity.

Severe cases with high neutrophil-to-lymphocyte ratios had clearly higher levels of IL-6. The authors propose that a robust IgG response leads to immune-mediated tissue damage, thus explaining the worse outcome in patients with overexuberant antibody response.

8.55.3 Limitations

A main criticism is that the criteria for stratifying patients in severe vs. non-severe are not described. The only reference related to this is the difference between the percentage of patients who needed mechanical ventilation, which was greater in patients with both high IgG levels and high neutrophil-to-lymphocyte ratio. No patient with both low IgG levels and low neutrophil-to-lymphocyte ratio was treated with mechanical ventilation.

The proposed correlation of severity with IL-2 and IL-10 levels is not very strong.

Furthermore, although mostly ignored in the paper's discussion, one of the most interesting findings is that an early increase in anti-SARS-CoV-2 IgM levels also seems to correlate with severe disease. However, as only median values are shown for antibody kinetics curves, the extent of variation in acute phase cannot be assessed.

8.55.4 Significance

Anti-SARS-CoV-2 IgG levels and with neutrophil-to-lymphocyte ratio predict severity of COVID-19 independently of each other. An additive predictive value of both variables is noticeable. Importantly, an early-on increase in anti-SARS-CoV-2 IgM levels also seem to predict outcome.

8.55.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.56 Reinfection could not occur in SARS-2 CoV-2 infected rhesus macaques

Bao et al. *bioRxiv* [342]

8.56.1 Keywords

- SARS-CoV-2
- viral load
- reinfection
- relapse
- non-human primate model

8.56.2 Main Findings

This study addresses the issue or acquired immunity after a primary COVID-19 infection in rhesus monkeys. Four Chinese rhesus macaques were intratracheally infected with SARS-CoV-2 and two out of the four were re-infected at 28 days post initial infection (dpi) with the same viral dose after confirming the recovery by the absence of clinical symptoms, radiological abnormalities and viral detection (2 negative RT-PCR tests). While the initial infection led the viral loads in nasal and pharyngeal swabs that reach approximately 6.5 $\log_{10}$ RNA copies/ml at 3 dpi in all four monkeys, viral loads in the swabs tested negative after reinfection in the two reinfected monkeys. In addition, the necropsies from a monkey (M1) at 7 days after primary infection, and another monkey (M3) at 5 days post reinfection, revealed the histopathological damages and viral replication in the examined tissues from M1, while no viral replication as well as no histological damages were detected in the tissues from M3. Furthermore, sera from three monkeys at 21 and 28 dpi exhibited neutralizing activity against SARS-CoV-2 in vitro, suggesting the production of protective neutralizing antibodies in these monkeys. Overall, this study indicates that primary infection with SARS-CoV-2 may protect from subsequent exposure to the same virus.

8.56.3 Limitations

In human, virus has been detected by nasopharyngeal swabs until 9 to 15 days after the onset of symptoms. In the infected monkeys in this study, virus were detected from day 1 after the infection, declining to undetectable level by day 15 post infection. It may suggest that there is a faster viral clearance mechanism in monkeys, therefore the conclusions of reinfection protection for humans need to be carefully considered. In addition, only two monkeys were re-infected in this study and the clinical signs of these monkeys were not similar: M3 did not show weight loss and M4 showed relatively higher fever on the day of infection and the day of re-challenge.

8.56.4 Significance

This study showed clear viral clearance and no indications of relapse or viremia after a secondary infection with SARS-CoV-2 in a Chinese rhesus macaque model. These results support the idea that patients with full recovery (two negative RT-PCR results) may also be protected from secondary SARS-CoV-2 infection. Recovered patients may be able to reintegrate to normal public life and provide protective serum perhaps even if having had a mild infection. The results are also encouraging for successful vaccine development against SARS-CoV-2.

8.56.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.57 A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV

[343]

8.57.1 Keywords

- neutralizing antibody
- cross-reactivity

8.57.2 Main Findings

Given the sequence similarity of the surface spike glycoprotein (S) of SARS-CoV-2 and SAR-CoV, Yuan et al. (2020) propose that neutralizing antibodies isolated from convalescent SARS-CoV patients may offer insight into cross-reactive antibodies targeting SARS-CoV-2. In particular, they find that the receptor-binding domain (RBD) of SARS-CoV-2 S protein shares 86% sequence similarity with the RBD of SARS-CoV S protein that binds to the CR3022 neutralizing antibody. CR3022 also displays increased affinity for the "up" conformation of the SARS-CoV-2 S protein compared to the "down" conformation as it does for the SARS-CoV S protein. Therefore, the authors propose that this cross-reactive antibody may confer some degree of protection *in vivo* even if it fails to neutralize *in vitro*.

8.57.3 Limitations

Although the authors offer a logical rationale for identifying cross-reactive neutralizing antibodies derived from SARS-CoV, their study using only CR3022 failed to demonstrate whether this approach will be successful. After all, CR3022 failed to neutralize *in vitro* despite the binding affinity to a similar epitope on SARS-CoV-2. They would benefit from testing more candidates and using an *in vivo* model to demonstrate their claim that protection may be possible in the absence neutralization if combinations are used *in vivo.*

8.57.4 Significance

The ability to make use of previously characterized neutralizing antibodies for conserved epitopes can expedite drug design and treatment options.

8.57.5 Credit

This review was undertaken by Dan Fu Ruan, Evan Cody and Venu Pothula as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.58 Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR

Dong et al. *medRxiv* [344]

8.58.1 Keywords

- Diagnosis
- digital PCR

8.58.2 Main Findings

The authors present a digital PCR (dPCR) diagnostic test for SARS-CoV-2 infection. In 103 individuals that were confirmed in a follow-up to be infected, the standard qPCR test had a positivity rate of 28.2% while the dPCR test detected 87.4% of the infections by detecting an additional 61 positive cases. The authors also tested samples from close contacts (early in infection stage) and convalescing

individuals (late in infection stage) and were able to detect SARS-CoV-2 nucleic acid in many more samples using dPCR compared to qPCR.

8.58.3 Limitations

I did not detect limitations.

8.58.4 Significance

The authors make a strong case for the need for a highly sensitive and accurate confirmatory method for diagnosing COVID-19 during this outbreak and present a potential addition to the diagnostic arsenal. They propose a dPCR test that they present has a dramatically lower false negative rate than the standard RT-qPCR tests and can be especially beneficial in people with low viral load, whether they are in the earlier or later stages of infection.

8.58.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.59 SARS-CoV-2 invades host cells via a novel route: CD147-spike protein

Wang et al. *bioRxiv* [345]

8.59.1 Keywords

- spike protein
- viral entry
- CD147
- SARS-CoV-2

8.59.2 Main Findings

The authors propose a novel mechanism of SARS-CoV-2 viral entry through the interaction of the viral spike protein (SP) and the immunoglobulin superfamily protein CD147 (also known as Basigin). Using an in-house developed humanized antibody against CD147 (maplazumab), they show that blocking CD147 decreases viral replication in Vero E6 cells. Using surface plasmon resonance (SPR), ELISA, and Co-IP assays, they show that the spike protein of SARS-CoV-2 directly interacts with CD147. Lastly, they utilize immune-election microscopy to show spike protein and CD147 localize to viral inclusion bodies of Vero E6 cells.

8.59.3 Limitations

The authors claim that an anti-CD147 antibody (Meplazumab) inhibits SARS-CoV-2 replication by testing cell growth and viral load in cells infected with SARS-CoV-2, however there are key pieces of this experiment that are missing. First, the authors fail to use a non-specific antibody control. Second, the authors claim that viral replication is inhibited, and that they test this by qPCR, however this data is **not shown.** To further prove specificity, the authors should introduce CD147 to non-susceptible cells and show that they become permissive.

The authors claim that there is a direct interaction between CD147 and SP through SPR, ELISA, and Co-IP, and this data seems generally convincing. The electron microscopy provides further correlative

evidence that SARS-CoV-2 may interact with CD147 as they are both found in the same viral inclusion body. A quantification of this data would make the findings more robust.

Finally, the data in this paper lacks replicates, error bars, and statistics to show that the data are reproducible and statistically significant.

8.59.4 Significance

It has been shown in various studies that SARS-CoV-2 binds to the cell surface protein ACE2 for cell entry, yet ACE2 is highly expressed in heart, kidney, and intestinal cells, raising the concern that blocking ACE2 would result in harmful side effects [346] CD147 on the other hand is highly expressed in various tumor types, inflamed tissues, and pathogen infected cells, suggesting that the inhibition of CD147 would not result in major side effects [347,348] The research in this paper has resulted in an ongoing clinical trail in China to test the safety and efficacy of anti-CD147 Meplazumab to treat COVID-19. (ClinicalTrails.gov identifier NCT04275245).

8.59.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.60 Blood single cell immune profiling reveals that interferon-MAPK pathway mediated adaptive immune response for COVID-19

Huang et al. *medRxiv* [349]

8.60.1 Keywords

- COVID-19
- SARS-CoV-2
- PBMC
- single cell
- MAPK

8.60.2 Main Findings

The authors performed single-cell RNA sequencing (scRNAseq) of peripheral blood mononuclear cells isolated from whole blood samples of COVID-19 patients (n=10). Data was compared to scRNAseq of samples collected from patients with influenza A (n=1), acute pharyngitis (n=1), and cerebral infarction (n=1), as well as, three healthy controls. COVID-19 patients were categorized into those with moderate (n=6), severe (n=1), critical (n=1), and cured (n=2) disease. Analysis across all COVID-19 disease levels revealed 56 different cellular subtypes, among 17 immune cell types; comparisons between each category to the normal controls revealed **increased proportions of $CD1c^+$ dendritic cells, $CD8^+$ CTLs, and plasmacytoid dendritic cells and a decrease in proportions of B cells and $CD4^+$ T cells**.

TCR sequencing revealed that greater clonality is associated with milder COVID-19 disease; BCR sequencing revealed that COVID-19 patients have circulating antibodies against known viral antigens, including EBV, HIV, influenza A, and other RNA viruses. This may suggest that the immune response to SARS-CoV-2 infection elicits production of antibodies against known RNA viruses.

Excluding enriched pathways shared by COVID-19 patients and patients with other conditions (influenza A, acute pharyngitis, and cerebral infarction), the authors identified the **interferon-MAPK**

signaling pathway as a major response to SARS-CoV-2 infection. The authors performed quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) for interferon-MAPK signaling genes: *IRF27, BST2,* and *FOS*. These samples were collected from a separate cohort of COVID-19 patients (critical, n=3; severe, n=3; moderate, n=19; mild, n=3; and cured, n=10; and healthy controls, n=5). Notably, consistent with the original scRNAseq data, *FOS* showed up-regulation in COVID-19 patients and down-regulation in cured patients. **The authors propose that *FOS* may be a candidate marker gene for curative COVID-19 disease**.

8.60.3 Limitations

The sample size of this study is limited. To further delineate differences in the immune profile of peripheral blood of COVID-19 patients, a greater sample size is needed, and longitudinal samples are needed, as well. A better understanding of the immunological interactions in cured patients, for example, would require a profile before and after improvement.

Moreover, the conclusions drawn from this scRNAseq study point to potential autoimmunity and immune deficiency to distinguish different severities of COVID-19 disease. However, this requires an expanded number of samples and a more robust organization of specific immune cell subtypes that can be compared across different patients. Importantly, this criterion is likely needed to ensure greater specificity in identifying markers for COVID-19 infection and subsequent immune response.

8.60.4 Significance

At the single-cell level, COVID-19 disease has been characterized in the lung, but a greater understanding of systemic immunological responses is furthered in this study. Type I interferon is an important signaling molecule for the anti-viral response. The identification of the interferon-MAPK signaling pathway and the differential expression of MAPK regulators between patients of differing COVID-19 severity and compared to cured patients may underscore the importance of either immune deficiency or autoimmunity in COVID-19 disease.

8.60.5 Credit

This review was undertaken by Matthew D. Park as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.61 Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infection.

Lv et al. *bioRxiv* [350]

8.61.1 Keywords

SARS-CoV-2, SARS-CoV, spike protein, RBD, cross-reactivity, cross-neutralization, antibody, human patients, mouse

8.61.2 Main Findings

The authors explore the antigenic differences between SARS-CoV-2 and SARS-CoV by analyzing plasma samples from SARS-CoV-2 (n = 15) and SARS-CoV (n = 7) patients. Cross-reactivity in antibody binding to the spike protein between SARS-CoV-2 and SARS-CoV was found to be common, mostly targeting non-RBD regions in plasma from SARS-CoV-2 patients. Only one SARS-CoV-2 plasma sample was able

to cross-neutralize SARS-CoV, with low neutralization activity. No cross-neutralization response was detected in plasma from SARS-CoV patients.

To further investigate the cross-reactivity of antibody responses to SARS-CoV-2 and SARS-CoV, the authors analyzed the antibody response of plasma collected from mice infected or immunized with SARS-CoV-2 or SARS-CoV (n = 5 or 6 per group). Plasma from mice immunized with SARS-CoV-2 displayed cross-reactive responses to SARS-CoV S ectodomain and, to a lesser extent, SARS-CoV RBD. Similarly, plasma from mice immunized with SARS-CoV displayed cross-reactive responses to SARS-CoV-2 S ectodomain. Cross-neutralization activity was not detected in any of the mouse plasma samples.

8.61.3 Limitations

The size of each patient cohort is insufficient to accurately determine the frequency of cross-reactivity and cross-neutralization in the current SARS-CoV-2 pandemic. Recruitment of additional patients from a larger range of geographical regions and time points would also enable exploration into the effect of the genetic diversity and evolution of the SARS-CoV-2 virus on cross-reactivity. This work would also benefit from the mapping of specific epitopes for each sample. Future studies may determine whether the non-neutralizing antibody responses can confer *in vitro* protection or lead to antibody-dependent disease enhancement.

8.61.4 Significance

The cross-reactive antibody responses to S protein in the majority of SARS-CoV-2 patients is an important consideration for development of serological assays and vaccine development during the current outbreak. The limited extent of cross-neutralization demonstrated in this study indicates that vaccinating to cross-reactive conserved epitopes may have limited efficacy, presenting a key concern for the development of a more universal coronavirus vaccine to address the global health risk of novel coronavirus outbreaks.

8.61.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.62 The feasibility of convalescent plasma therapy in severe COVID-19 patients: a pilot study

Duan et al. *medRxiv* [351]

8.62.1 Keywords

- COVID-19
- SARS-CoV-2
- convalescent plasma
- treatment outcome
- pilot
- therapy
- transfusion

8.62.2 Main Findings

This is the first report to date of convalescent plasma therapy as a therapeutic against COVID-19 disease. This is a feasibility pilot study. The authors report the administration and clinical benefit of 200 mL of convalescent plasma (CP) (1:640 titer) derived from recently cured donors (CP selected among 40 donors based on high neutralizing titer and ABO compatibility) to 10 severe COVID-19 patients with confirmed viremia. The primary endpoint was the safety of CP transfusion. The secondary endpoint were clinical signs of improvement based on symptoms and laboratory parameters.

The authors reported use of methylene blue photochemistry to inactivate any potential residual virus in the plasma samples, without compromising neutralizing antibodies, and no virus was detected before transfusion.

The authors report the following:

- No adverse events were observed in all patients, except 1 patient who exhibited transient facial red spotting.
- All patients showed significant improvement in or complete disappearance of clinical symptoms, including fever, cough, shortness of breath, and chest pain after 3 days of CP therapy.
- Reduction of pulmonary lesions revealed by chest CT.
- Elevation of lymphocyte counts in patients with lymphocytopenia.
- Increase in SaO_2 in all patients, indicative of recuperating lung function.
- Resolution of SARS-CoV-2 viremia in 7 patients and increase in neutralizing antibody titers in 5 patients. Persistence of neutralizing antibody levels in 4 patients.

8.62.3 Limitations

It is important to note that most recipients had high neutralization titers of antibodies before plasma transfusion and even without transfusion it would be expected to see an increase in neutralizing antibodies over time. In addition to the small sample set number (n=10), there are additional limitations to this pilot study:

1. All patients received concurrent therapy, in addition to the CP transfusion. Therefore, it is unclear whether a combinatorial or synergistic effect between these standards of care and CP transfusion contributed to the clearance of viremia and improvement of symptoms in these COVID-19 patients.
2. The kinetics of viral clearance was not investigated, with respect to the administration of CP transfusion. So, the definitive impact of CP transfusion on immune dynamics and subsequent viral load is not well defined.
3. Comparison with a small historical control group is not ideal.

8.62.4 Significance

For the first time, a pilot study provides promising results involving the use of convalescent plasma from cured COVID-19 patients to treat others with more severe disease. The authors report that the administration of a single, high-dose of neutralizing antibodies is safe. In addition, there were encouraging results with regards to the reduction of viral load and improvement of clinical outcomes. It is, therefore, necessary to expand this type of study with more participants, in order to determine

optimal dose and treatment kinetics. It is important to note that CP has been studied to treat H1N1 influenza, SARS-CoV-1, and MERS-CoV, although it has not been proven to be effective in treating these infections.

8.62.5 Credit

Review by Matthew D. Park and revised by Alice O. Kamphorst and Maria A. Curotto de Lafaille as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.63 Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open label non-randomized clinical trial

[352]

8.63.1 Keywords

- hydroxychloroquine
- clearance
- viral load
- clinical trial

8.63.2 Main Findings

This study was a single-arm, open label clinical trial with 600 mg hydroxychloroquine (HCQ) in the treatment arm (n = 20). Patients who refused participation or patients from another center not treated with HCQ were included as negative controls (n = 16). Among the patients in the treatment arm, 6 received concomitant azithromycin to prevent superimposed bacterial infection. The primary endpoint was respiratory viral loads on day 6 post enrollment, measured by nasopharyngeal swab followed by real-time reverse transcription-PCR.

HCQ alone was able to significantly reduce viral loads by day 6 (n = 8/14, 57.1% complete clearance, $p < 0.001$); azithromycin appears to be synergistic with HCQ, as 6/6 patients receiving combined treatment had complete viral clearance ($p < 0.001$).

8.63.3 Limitations

Despite what is outlined above, this study has a number of limitations that must be considered. First, there were originally n = 26 patients in the treatment arm, with 6 lost to follow up for the following reasons: 3 transferred to ICU, 1 discharge, 1 self-discontinued treatment d/t side effects, and 1 patient expired. Total length of clinical follow up was 14 days, but the data beyond day 6 post-inclusion are not shown.

Strikingly, in supplementary table 1, results of the real-time RT-PCR are listed for the control and treatment arms from D0 – D6. However, the data are not reported in a standard way, with a mix of broadly positive or negative result delineation with Ct (cycle threshold) values, the standard output of real time PCR. It is impossible to compare what is defined as a positive value between the patients in the control and treatment arms without a standardized threshold for a positive test. Further, the starting viral loads reported at D0 in the groups receiving HCQ or HCQ + azithromycin were significantly different (ct of 25.3 vs 26.8 respectively), which could explain in part the differences observed in the response to treatment between 2 groups. Finally, patients in the control arm from outside the primary medical center in this study (Marseille) did not actually have samples tested by

PCR daily. Instead, positive test results from every other day were extrapolated to mean positive results on the day before and after testing as well (Table 2, footnote [a]).

Taken together, the results of this study suggest that HCQ represents a promising treatment avenue for COVID-19 patients. However, the limited size of the trial, and the way in which the results were reported does not allow for other medical centers to extrapolate a positive or negative result in the treatment of their own patients with HCQ +/- azithromycin. Further larger randomized clinical trials will be required to ascertain the efficacy of HCQ +/- azithromycin in the treatment of COVID-19.

8.63.4 Significance

Chloroquine is thought to inhibit viral infection, including SARS-Cov-2, by increasing pH within endosomes and lysosomes, altering the biochemical conditions required for viral fusion [131,353]. However, chloroquine also has immuno-modulatory effects that I think may play a role. Chloroquine has been shown to increase CTLA-4 expression at the cell surface by decreasing its degradation in the endo-lysosome pathway; AP-1 traffics the cytoplasmic tail of CTLA-4 to lysosomes, but in conditions of increased pH, the protein machinery required for degradation is less functional [354]. As such, more CTLA-4 remains in endosomes and is trafficked back to the cell surface. It is possible that this may also contribute to patient recovery via reduction of cytokine storm, in addition to the direct anti-viral effects of HCQ.

8.63.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.64 Recapitulation of SARS-CoV-2 Infection and Cholangiocyte Damage with Human Liver Organoids

[277]

8.64.1 Keywords

- CoVID-19
- 2019-nCoV
- SARS-CoV-2
- liver
- organoids
- Cholangiocyte

8.64.2 Main Findings

- Used human liver ductal organoids to determine ACE2+ cholangiocytes in healthy liver (2.92% of all cells) are infectable and support SARS-CoV-2 viral replication.

- Plaque-purified SARS-CoV-2 viral infection disrupted organoid barrier and bile transporting functions of cholangiocytes through dysregulation of genes involved in tight junction formation (CLDN1) and bile acid transportation (ASBT and CFTR).

8.64.3 Limitations:

- Unclear if liver damage observed in patients due to direct cholangiocyte infection or due to secondary immune/cytokine effects. This study argues for direct damage as it lacks immune contexture; but further studies needed with autopsy samples or organoid-immune cell co-culture to conclude strongly.

- Would be important to measure cholangiocyte-intrinsic anti-viral response and alarmins secreted upon infection, and furthermore study tropism of various immune cells to conditioned media from organoids infected with SARS-CoV-2.

- Does not address how cirrhotic liver or alcohol/smoking/obesity-associated liver organoids respond to SARS-CoV-2 infectivity and replication, worth pursuing to experimentally address clinical data indicating co-morbidities.

8.64.4 Significance

- Useful model to rapidly study drug activity against SARS-CoV-2 infection in liver, while monitoring baseline liver damage.

- Liver abnormality observed in >50% of CoVID-19 patients; the results from this study could explain the bile acid accumulation and consequent liver damage observed.

8.64.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.65 The sequence of human ACE2 is suboptimal for binding the S spike protein of SARS coronavirus 2

[355]

8.65.1 Keywords

- Coronavirus Disease 2019 (COVID-19)
- SARS-CoV-2
- Spike protein S
- ACE2

8.65.2 Main Findings

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infects cells through S spike glycoprotein binding angiotensin-converting enzyme (ACE2) on host cells. S protein can bind both membrane-bound ACE2 and soluble ACE2 (sACE2), which can serve as a decoy that neutralizes infection. Recombinant sACE2 is now being tested in clinical trials for COVID-19. To determine if a therapeutic sACE2 with higher affinity for S protein could be designed, authors generated a library containing every amino acid substitution possible at the 117 sites spanning the binding interface with S protein. The ACE2 library was expressed in human Expi293F cells and cells were incubated with medium containing the receptor binding domain (RBD) of SARS-CoV-2 fused to GFP. Cells with high or low affinity mutant ACE2 receptor compared to affinity of wild type ACE2 for the RBD were FACS sorted and transcripts from these sorted populations were deep sequenced. Deep mutagenesis identified numerous mutations in ACE2 that enhance RBD binding. This work serves to identify putative high affinity ACE2 therapeutics for the treatment of CoV-2.

8.65.3 Limitations

The authors generated a large library of mutated ACE2, expressed them in human Expi293F cells, and performed deep mutagenesis to identify enhanced binders for the RBD of SARS-CoV-2 S protein. While these data serve as a useful resource, the ability of the high affinity ACE2 mutants identified to serve as therapeutics needs further validation in terms of conformational stability when purified as well as efficacy/safety both *in vitro* and *in vivo*. Additionally, authors mentioned fusing the therapeutic ACE2 to Fc receptors to elicit beneficial host immune responses, which would need further design and validation.

8.65.4 Significance

This study identified structural ACE2 mutants that have potential to serve as therapeutics in the treatment of SARS-CoV-2 upon further testing and validation.

8.65.5 Credit

This review was undertaken by Katherine Lindblad and Tamar Plitt as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

Title: A serological assay to detect SARS-Cov-2 seroconversion in humans

Immunology keywords: specific serological assay - ELISA - seroconversion - antibody titers

Note: the authors of this review work in the same institution as the authors of the study

Main findings:

Production of recombinant whole Spike (S) protein and the smaller Receptor Binding Domain (RBD) based on the sequence of Wuhan-Hu-1 SARS-CoV-2 isolate. The S protein was modified to allow trimerization and increase stability. The authors compared the antibody reactivity of 59 banked human serum samples (non-exposed) and 3 serum samples from confirmed SARS-CoV-2 infected patients. All Covid-19 patient sera reacted to the S protein and RBD domain compared to the control sera.

The authors also characterized the antibody isotypes from the Covid-19 patients, and observed stronger IgG3 response than IgG1. IgM and IgA responses were also prevalent.

Limitations of the study:

The authors analyzed a total of 59 control human serum samples, and samples from only three different patients to test for reactivity against the RBD domain and full-length spike protein. It will be important to follow up with a larger number of patient samples to confirm the data obtained. Furthermore, it would be interesting to assess people at different age groups and determine whether unexposed control kids have a higher “background”.

Applications of the assay described in this study in diagnosis are limited, since antibody response should start to be detectable only one to two weeks after infection. Future studies will be required to assess how long after infection this assay allow to detect anti-CoV2 antibodies. Finally, while likely, the association of seroconversion with protective immunity against SARS-Cov-2 infection still needs to be fully established.

Relevance:

This study has strong implications in the research against SARS-Cov-2. First, it is now possible to perform serosurveys and determine who has been infected, allowing a more accurate estimate of infection prevalence and death rate. Second, if it is confirmed that re-infection does not happen (or is rare), this assay can be used as a tool to screen healthcare workers and prioritize immune ones to work with infected patients. Third, potential convalescent plasma donors can now be screened to help treating currently infected patients. Of note, this assay does not involve live virus handling. experimentally, this is an advantage as the assay does not require the precautions required by manipulation of live virus. Finally, the recombinant proteins described in this study represent new tools that can be used for further applications, including vaccine development.

8.66 COMPARATIVE PATHOGENESIS OF COVID-19, MERS AND SARS IN A NON-HUMAN PRIMATE MODEL

[356]

8.66.1 Keywords

- SARS-CoV2
- cynomolgus macaque
- SARS-CoV

8.66.2 Main Findings

This work assesses SARS-CoV-2 infection in young adult and aged cynomolgus macaques. 4 macaques per age group were infected with low-passage clinical sample of SARS-CoV-2 by intranasal and intratracheal administration. Viral presence was assessed in nose, throat and rectum through RT-PCR and viral culture. SARS-CoV-2 replication was confirmed in the respiratory track (including nasal samples), and it was also detected in ileum. Viral nucleocapsid detection by IHC showed infection of type I and II pneumocytes and epithelia. Virus was found to peak between 2 and 4 days after administration and reached higher levels in aged vs. young animals. The early peak is consistent with data in patients and contrasts to SARS-CoV replication. SARS-CoV-2 reached levels below detection between 8 and 21 days after inoculation and macaques established antibody immunity against the virus by day 14. There were histopathological alteration in lung, but no overt clinical signs. At day 4 post inoculation of SARS-CoV-2, two of four animals presented foci of pulmonary consolidation, with limited areas of alveolar edema and pneumonia, as well as immune cell infiltration. In sum, cynomolgus macaques are permissive to SARS- CoV-2 and develop lung pathology (less severe than SARC-CoV, but more severe than MERS-CoV).

8.66.3 Limitations

Even though cynomolgus macaques were permissive to SARS-CoV-2 replication, it is unclear if the viral load reaches levels comparable to humans and there wasn't overt clinical pathology.

8.66.4 Significance

The development of platforms in which to carry out relevant experimentation on SARS-CoV-2 pathophysiology is of great urgency. Cynomolgus macaques offer an environment in which viral replication can happen, albeit in a limited way and without translating into clinically relevant symptoms. Other groups are contributing to SARS-CoV2 literature using this animal model [342], potentially showing protection against reinfection in cured macaques. Therefore, this platform could

be used to examine SARS-CoV2 pathophysiology while studies in other animal models are also underway [279,357].

8.66.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.67 Investigating the Impact of Asymptomatic Carriers on COVID-19 Transmission

[358]

8.67.1 Keywords

- Coronavirus Disease 2019 (COVID-19)
- SARS-CoV-2
- asymptomatic carriers
- mathematical model
- transmission

8.67.2 Main Findings

Multiple studies reported the same level of infectiousness between symptomatic and asymptomatic carriers of SARS-CoV-2. Given that asymptomatic and undocumented carriers escape public health surveillance systems, a better mathematical model of transmission is needed to determine a more accurate estimate of the basic reproductive number (R_0) of the virus to assess the contagiousness of virus. The authors developed a SEYAR dynamical model for transmission of the new coronavirus that takes into account asymptomatic and undocumented carriers. The model was validated using data reported from thirteen countries during the first three weeks of community transmission. While current studies estimate R_0 to be around 3, this model indicates that the value could range between 5.5 to 25.4.

8.67.3 Limitations

The SEYAR model realistically depicts transmission of the virus only during the initial stages of the disease. More data is necessary to better fit the model with current trends. In addition, multiple factors (e.g. behavioral patterns, surveillance capabilities, environmental and socioeconomic factors) affect transmission of the virus and so, these factors must be taken into consideration when estimating the R_0.

8.67.4 Significance

Public health authorities use the basic reproductive number to determine the severity of disease. An accurate estimate of R_0 will inform intervention strategies. This model can be applied to different locations to assess the potential impact of COVID-19.

8.67.5 Credit

This review was undertaken by Tamar Plitt and Katherine Lindblad as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.68 Antibody responses to SARS-CoV-2 in COVID-19 patients: the perspective application of serological tests in clinical practice

Long et al. *medRxiv* [359]

8.68.1 Keywords

- Serum antibodies
- IgM
- IgG
- immunoassay
- diagnosis
- seroconversion

8.68.2 Main Findings

This study investigated the profile of the acute antibody response against SARS-CoV-2 and provided proposals for serologic tests in clinical practice. Magnetic Chemiluminescence Enzyme Immunoassay was used to evaluate IgM and IgG seroconversion in 285 hospital admitted patients who tested positive for SARS-CoV-2 by RT-PCR and in 52 COVID-19 suspected patients that tested negative by RT-PCR. A follow up study with 63 patients was performed to investigate longitudinal effects. In addition, IgG and IgM titers were evaluated in a cohort of close contacts (164 persons) of an infected couple.

The median day of seroconversion for both IgG and IgM was 13 days after symptom onset. Patients varied in the order of IgM/ IgG seroconversion and there was no apparent correlation of order with age, severity, or hospitalization time. This led the authors to conclude that for diagnosis IgM and IgG should be detected simultaneously at the early phase of infection.

IgG titers, but not IgM titers were higher in severe patients compared to non-severe patients after controlling for days post-symptom onset. Importantly, 12% of COVID-19 patients (RT-PCR confirmed) did not meet the WHO serological diagnosis criterion of either seroconversion or > 4-fold increase in IgG titer in sequential samples. This suggests the current serological criteria may be too stringent for COVID-19 diagnosis.

Of note, 4 patients from a group of 52 suspects (negative RT-PCR test) had anti-SARS-Cov-2 IgM and IgG. Similarly, 4.3% (7/162) of “close contacts” who had negative RT-PCR tests were positive for IgG and/or IgM. This highlights the usefulness of a serological assay to identify asymptomatic infections and/or infections that are missed by RT-PCR.

8.68.3 Limitations

This group’s report generally confirms the findings of others that have evaluated the acute antibody response to SARS-Cov-2. However, these data would benefit from inclusion of data on whether the participants had a documented history of viral infection. Moreover, serum samples that were collected prior to SARS-Cov-2 outbreak from patients with other viral infections would serve as a useful negative control for their assay. Methodological limitations include that only one serum sample per case was tested as well as the heat inactivation of serum samples prior to testing. It has previously been reported that heat inactivation interferes with the level of antibodies to SARS-Cov-2 and their protocol may have resulted in diminished quantification of IgM, specifically [360].

8.68.4 Significance

Understanding the features of the antibody responses against SARS-CoV is useful in the development of a serological test for the diagnosis of COVID-19. This paper addresses the need for additional screening methods that can detect the presence of infection despite lower viral titers. Detecting the production of antibodies, especially IgM, which are produced rapidly after infection can be combined with PCR to enhance detection sensitivity and accuracy and map the full spread of infection in communities, Moreover, serologic assays would be useful to screen health care workers in order to identify those with immunity to care for patients with COVID19.

8.68.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.69 SARS-CoV-2 specific antibody responses in COVID-19 patients

[361]

8.69.1 Keywords

- immunoassay
- antibody specificity
- serology
- cross-reactivity

8.69.2 Main findings

Antibodies specific to SARS-CoV-2 S protein, the S1 subunit and the RBD (receptor-binding domain) were detected in all SARS-CoV-2 patient sera by 13 to 21 days post onset of disease. Antibodies specific to SARS-CoV N protein (90% similarity to SARS-CoV-2) were able to neutralize SARS-CoV-2 by PRNT (plaque reduction neutralizing test). SARS-CoV-2 serum cross-reacted with SARS-CoV S and S1 proteins, and to a lower extent with MERS-CoV S protein, but not with the MERS-CoV S1 protein, consistent with an analysis of genetic similarity. No reactivity to SARS-CoV-2 antigens was observed in serum from patients with ubiquitous human CoV infections (common cold) or to non-CoV viral respiratory infections.

8.69.3 Limitations

Authors describe development of a serological ELISA based assay for the detection of neutralizing antibodies towards regions of the spike and nucleocapsid domains of the SARS-CoV-2 virus. Serum samples were obtained from PCR-confirmed COVID-19 patients. Negative control samples include a cohort of patients with confirmed recent exposure to non-CoV infections (i.e. adenovirus, bocavirus, enterovirus, influenza, RSV, CMV, EBV) as well as a cohort of patients with confirmed infections with ubiquitous human CoV infections known to cause the common cold. The study also included serum from patients with previous MERS-CoV and SARS-CoV zoonotic infections. This impressive patient cohort allowed the authors to determine the sensitivity and specificity of the development of their in-house ELISA assay. Of note, seroconversion was observed as early as 13 days following COVID-19 onset but the authors were not clear how disease onset was determined.

8.69.4 Significance

Validated serological tests are urgently needed to map the full spread of SARS-CoV-2 in the population and to determine the kinetics of the antibody response to SARS-CoV-2. Furthermore, clinical trials are

ongoing using plasma from patients who have recovered from SARS-CoV-2 as a therapeutic option. An assay such as the one described in this study could be used to screen for strong antibody responses in recovered patients. Furthermore, the assay could be used to screen health care workers for antibody responses to SARS-CoV-2 as personal protective equipment continues to dwindle. The challenge going forward will be to standardize and scale-up the various in-house ELISA's being developed in independent laboratories across the world.

8.70 A brief review of antiviral drugs evaluated in registered clinical trials for COVID-19

Belhadi et al. [362]

8.70.1 Keywords

- Clinical trials
- COVID-19
- SARS CoV-2
- 2019-nCoV
- SARS Cov-2
- Hcov-19
- novel coronal virus
- new corona virus
- antiviral drugs

8.70.2 Main Findings

Summary of clinical trials registered as of March7, 2020 from U.S, Chinese, Korean, Iranian and European registries. Out of the 353 studies identified, 115 were selected for data extraction. 80% of the trials were randomized with parallel assignment and the median number of planned inclusions was 63 (IRQ, 36-120). Most frequent therapies in the trials included; 1) antiviral drugs [lopinavir/ritonavir (n-15); umifenovir (n=9); favipiravir (n=7); redmesivir (n=5)]; 2) anti-malaria drugs [chloroquine (n-11); hydroxychloroquine (n=7)}; immunosuppressant drugs [methylprednisolone (n=5)]; and stem cell therapies (n=23). Medians of the total number of planned inclusions per trial for these therapies were also included. Stem cells and lopunavir/ritonavir were the most frequently evaluated candidate therapies (23 and 15 trials respectively), whereas remdesivir was only tested in 5 trials but these trials had the highest median number of planned inclusions per trial (400, IQR 394-453). Most of the agents used in the different trials were chosen based on preclinical assessments of antiviral activity against SARS CoV and MERS Cov corona viruses.

The primary outcomes of the studies were clinical (66%); virological (23%); radiological (8%); or immunological (3%). The trials were classified as those that included patients with severe disease only; trials that included patients with moderate disease; and trials that included patients with severe or moderate disease.

8.70.3 Limitations

The trials evaluated provided incomplete information: 23% of these were phase IV trials but the bulk of the trials (54%) did not describe the phase of the study. Only 52% of the trials (n=60) reported treatment dose and only 34% (n=39) reported the duration. A lot of the trials included a small number of patients and the trials are still ongoing, therefore no insight was provided on the outcome of the trials.

8.70.4 Significance

Nonetheless, this review serves as framework for identifying COVID-19 related trials, which can be expanded upon as new trials begin at an accelerated rate as the disease spreads around the world.

8.70.5 Credit

This review was undertaken by K Alexandropoulos as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.71 ACE-2 Expression in the Small Airway Epithelia of Smokers and COPD Patients: Implications for COVID-19

Leung et al. *medRxiv*. [363]

8.71.1 Keywords

- chronic obstructive pulmonary disease
- COPD
- smokingE-2
- risk factors

8.71.2 Main Findings

In bronchial epithelial samples from 3 different cohorts of individuals, ACE-2 gene expression was found to be significantly increased in both COPD patients and smokers relative to healthy controls. Across all test subjects, ACE-2 gene expression was also highly correlated with decreased forced expiratory volume in 1 second (FEV1), which may explain the increased COVID-19 disease severity in COPD patients. Former smokers were also found to show decreased ACE2 expression relative to current smokers and had no significant difference when compared to non-smokers.

8.71.3 Limitations

While the upregulation of ACE-2 is an interesting hypothesis for COVID-19 disease severity in COPD patients, this study leaves many more unanswered questions than it addresses. Further studies are required to show whether the specific cell type isolated in these studies is relevant to the pathophysiology of COVID-19. Furthermore, there is no attempt to show whether that increased ACE-2 expression contributes to greater disease severity. Does the increased ACE-2 expression lead to greater infectivity with SARS-CoV-2? There is no mechanistic explanation for why ACE-2 levels are increased in COPD patients. The authors could also have considered the impact of co-morbidities and interventions such as corticosteroids or bronchodilators on ACE-2 expression. Finally, given the extensive sequencing performed, the authors could have conducted significantly more in-depth analyses into gene signature differences.

8.71.4 Significance

This study attempts to address an important clinical finding that both smokers and COPD patients show increased mortality from COVID-19. The novel finding that ACE-2 expression is induced in smokers and COPD patients suggests not only a mechanism for the clinical observation, but also highlights the potential benefit of smoking cessation in reducing the risk of severe COVID-19 disease.

8.71.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.72 Dynamic profile of severe or critical COVID-19 cases

[364]

8.72.1 Keywords

- Coronavirus Disease 2019 (COVID-19)
- SARS-CoV-2
- progressive lymphopenia (PLD)
- T-lymphocytes
- clinical data
- co-infection
- influenza A

8.72.2 Main Findings

Authors evaluate clinical correlates of 10 patients (6 male and 4 female) hospitalized for severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). All patients required oxygen support and received broad spectrum antibiotics and 6 patients received anti-viral drugs. Additionally, 40% of patients were co-infected with influenza A. All 10 patients developed lymphopenia, two of which developed progressive lymphopenia (PLD) and died. Peripheral blood (PB) lymphocytes were analyzed – low CD4 and CD8 counts were noted in most patients, though CD4:CD8 ratio remained normal.

8.72.3 Limitations

The authors evaluated a small cohort of severe SARS-CoV-2 cases and found an association between T cell lymphopenia and adverse outcomes. However, this is an extremely small and diverse cohort (40% of patients were co-infected with influenza A). These findings need to be validated in a larger cohort. Additionally, the value of this data would be greatly increased by adding individual data points for each patient as well as by adding error bars to each of the figures.

8.72.4 Significance

This study provides a collection of clinical data and tracks evolution of T lymphocyte in 10 patients hospitalized for SARS-CoV-2, of which 4 patients were co-infected with influenza A.

8.72.5 Credit

This review was undertaken by Katherine Lindblad and Tamar Plitt as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.73 Association between Clinical, Laboratory and CT Characteristics and RT-PCR Results in the Follow-up of COVID-19 patients

Fu et al. *medRxiv*. [365]

8.73.1 Keywords

- COVID-19
- clinical
- lymphocyte
- CRP
- LDH
- HSST TNT
- PCR test
- readmission
- CT
- GGO
- disease progression

8.73.2 Study description

Data analyzed from 52 COVID-19 patients admitted and then discharged with COVID-19. Clinical, laboratory, and radiological data were longitudinally recorded with illness timecourse (PCR + to PCR-) and 7 patients (13.5%) were readmitted with a follow up positive test (PCR+) within two weeks of discharge.

8.73.3 Main Findings

- At admission:
    - The majority of patients had increased CRP at admission (63.5%).
    - LDH, and HSST TNT were significantly increased at admission.
    - Radiographic signs via chest CT showed increased involvement in lower lobes: right lower lobe (47 cases, 90.4%), left lower lobe (37 cases, 71.2%).
    - GGO (90.4%), interlobular septal thickening (42.3%), vascular enlargement (42.3%), and reticulation (11.5%) were most commonly observed.
- After negative PCR test (discharge):
    - CRP levels decreased lymphocyte counts (#/L) increased significantly (CD3+, CD3+/8+ and CD3+/4+) after negative PCR.
    - Consolidation and mixed GGO observed in longitudinal CT imaging w different extents of inflammatory exudation in lungs, with overall tendency for improvement (except 2/7 patients that were readmitted after discharge with re-positive test) after negative PCR.
- Seven patients repeated positive RT-PCR test and were readmitted to the hospital (9 to 17 day after initial discharge).
    - Follow up CT necessary to monitor improvement during recovery and patients with lesion progression should be given more attention.
    - Dynamic CT in addition to negative test essential in clinical diagnosis due to nasal swab PCR sampling bias (false-negatives).
    - Increase in CRP occurred in 2 readmitted patients (and decr. in lymphocytes in one patient), but was not correlated with new lesions or disease progression vs. improvement (very low N).

- Patients readmitted attributed to false-negative PCR vs. re-exposure.

8.73.4 Limitations

Patients sampled in this study were generally younger (65.4% < 50 yrs) and less critically ill/all discharged. Small number of recovered patients (N=18). Time of follow up was relatively short.. Limited clinical information available about patients with re-positive test (except CRP and lymph tracking).

8.73.5 Extended Results

NOTE: Patients sampled in this study were generally younger (65.4% < 50 yrs) and less critically ill/all discharged. After two consecutive negative PCR tests, patients were discharged.

Clinical Results at Admission

- Median interval disease onset to admission (5 days, IQR: 3-7)
- Most common symptoms included fever, fatigue, dry cough, and expectoration.
- Fifteen patients had reduced lymphocyte counts (28.8%).
- No change in WBC or Neutrophil counts.
- **The majority of patients had increased CRP at admission (63.5%).**
- **LDH, and HSST TNT were significantly increased at admission.**
- Fibrinogen was trending high though not significant.
- No major changes in liver function observed.
- **Radiographic signs via chest CT showed increased involvement in lower lobes: right lower lobe (47 cases, 90.4%), left lower lobe (37 cases, 71.2%).**
- **GGO (90.4%), interlobular septal thickening (42.3%), vascular enlargement (42.3%)**, and reticulation (11.5%) were most commonly observed.

Change in Clinical Results following Negative Test

- **CRP levels decreased after negative PCR.**
- **Lymphocyte counts (#/L) increased significantly (CD3+, CD3+/8+ and CD3+/4+).**
- No significant change to CD4/8 ratio.
- LDH, HSST TNT, and Fibronegin remained high throughout, though range observed decreased over time.
- **Consolidation and mixed GGO observed in longitudinal CT imaging.**

- **Patients showed different extents of inflammatory exudation in lungs, with overall tendency for improvement (except 2/7 patients that were readmitted after discharge with re-positive test).**

Patients Readmitted with PCR+ test

- **Seven patients repeated positive RT-PCR test and were readmitted to the hospital (9 to 17 day after initial discharge).**
- Improvement during readmission in 4 patients and observation of segmental progression CT in 2 patients (2/18 or 11.1% - re-positive 9 and 10 days post-discharge).
- Two patients showed new GGO, while others improved greatly.
- **Follow up CT necessary to monitor improvement during recovery and patients with lesion progression should be given more attention.**
- **Dynamic CT in addition to negative test essential in clinical diagnosis due to nasal swab PCR sampling bias (false-negatives).**
- **Increase in CRP occurred in 2 readmitted patients (and decr. in lymphocytes in one patient), but was not correlated with new lesions or disease progression vs. improvement (very low N).**

8.73.6 Significance

Study tracked key clinical features associated with disease progression, recovery, and determinants of clinical diagnosis/management of COVID-19 patients.

8.73.7 Credit

This review was undertaken by Natalie Vaninov as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.74 An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 and multiple 2 endemic, epidemic and bat coronavirus

Sheahan et al. *bioRxiv*. [366]

8.74.1 Keywords

- Treatment
- Antiviral
- Broad spectrum antiviral
- ribonucleoside analog β-D-N4 30 hydroxycytidine (NHC)
- Remdesivir

8.74.2 Main Findings

β-D-N4 30 –hydroxycytidine (NHC, EIDD-1931) is an orally bioavailable ribonucleoside with antiviral activity against various RNA viruses including Ebola, Influenza and CoV. NHC activity introduces mutations in the viral (but not cellular) RNA in a dose dependent *manner* that directly correlated with

a decrease in viral titers. Authors show that NHC inhibited multiple genetically distinct Bat-CoV viruses in human primary epithelial cells *without affecting cell viability even at high concentrations (100 μM)*. Prophylactic oral administration of NHC in C57BL/6 mice reduce lung titers of SARS-CoV and prevented weight loss and hemorrhage. Therapeutic administration of NHC in C57BL/6 mice 12 hours post infected with SARS-CoV reduced acute lung injury, viral titer, and lung hemorrhage. The degree of clinical benefit was dependent on the time of treatment initiation post infection. The authors also demonstrate that NHC reduces MERS-CoV infection titers, pathogenesis, and viral RNA in prophylactic and therapeutic settings.

8.74.3 Limitations

Most of the experiments were conducted using MERS-CoV, and SARS-CoV and a few experiments were conducted using other strains of CoV as opposed to SARS-CoV-2. The authors note the core residues that make up the RNA interaction sites (which constitutes the NHC interaction sites) are highly conserved among CoV and because of this conservation their understanding is that NHC can inhibit a broad-spectrum of CoV including SARS-CoV-2.

The increased viral mutation rates associated with NHC activity may have adverse effects if mutations cause the virus to become drug resistant, more infectious or speed-up immune evasion. *In addition, the temporal diminishing effectiveness of NHC on clinical outcome when NHC was used therapeutically is concerning. However, the longer window (7-10 days) for clinical disease onset in human patients from the time of infection compared to that of mice (24-48 hours), may associate with increased NHC effectiveness in the clinic.*

8.74.4 Significance

Prophylactic or therapeutic oral administration of NHC reduces lung titers and prevents acute lung failure in C57B\6 mice infected with CoV. Given its *broad-spectrum antiviral activity, NHC could turn out to be a useful drug for treating current, emerging and future corona virus outbreaks.* ####
Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.75 Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs

Sangeun Jeon et al. [367]

8.75.1 Keywords

- COVID-19
- SARS CoV-2
- antiviral drugs
- niclosamide
- ciclesonide

8.75.2 Main Findings

A panel of ~3,000 FDA- and IND-approved antiviral drugs were previously screened for inhibitory efficacy against SARS CoV, a coronavirus related to the novel coronavirus SARS CoV-2 (79.5%) homology. 35 of these drugs along with another 15 (suggested by infectious disease specialists) were

tested in vitro for their ability to inhibit SARS CoV-2 infectivity of Vero cells while preserving cell viability. The infected cells were scored by immunofluorescence analysis using an antibody against the N protein of SARS CoV-2. Chloroquine, lopinavir and remdesivir were used as reference drugs.

Twenty four out of 50 drugs exhibited antiviral activity with IC50 values ranging from 0.1-10μM. Among these, two stood ou: 1) the-anti helminthic drug niclosamide which exhibited potent antiviral activity against SARS CoV-2 (IC50=0.28 μM). The broad-spectrum antiviral effect of niclosamide against SARS and MERS-CoV have been previously documented and recent evidence suggests that in may inhibit autophagy and reduce MERS C0V replication. 2) Ciclesonide, a corticosteroid used to treat asthma and allergic rhinitis, also exhibited antiviral efficacy but with a lower IC50 (4.33μM) compared to niclosamide. The antiviral effects of ciclesonide were directed against NSP15, a viral riboendonuclease which is the molecular target of this drug.

8.75.3 Limitations

The drugs were tested against SARS CoV-2 infectivity in vitro only, therefore preclinical studies in animals and clinical trials in patients will be need for validation of these drugs as therapeutic agents for COVID-19. In addition, niclosamide exhibits low adsorption pharmatokinetically which could be alleviated with further development of drug formulation to increase effective delivery of this drug to target tissues. Nonetheless, niclosamide and ciclesonide represent promising therapeutic agents against SARS CoV-2 given that other compounds tested in the same study including favipiravir (currently used in clinical trials) and atazanavir (predicted as the most potent antiviral drug by AI-inference modeling) did not exhibit antiviral activity in the current study.

8.75.4 Credit

This review was undertaken by K Alexandropoulos as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.76 Respiratory disease and virus shedding in rhesus macaques inoculated with SARS-CoV-2

Munster et al. *bioRxiv*. [368]

8.76.1 Keywords

animal model, pulmonary infiltrates, dynamic of antibody response, cytokine

8.76.2 Main Findings

Inoculation of 8 Resus macaques with SARS-Cov-2 , which all showed clinical signs of infection (respiratory pattern, reduced appetite, weight loss, elevated body temperature) resulting in moderate, transient disease. Four animals were euthanized at 3dpi, the 4 others at 21 dpi. Study of viral loads in different organs showed that nose swab and throat swabs were the most sensitive, with bronchio-alveolar lavage. Interstitial pneumonia was visible in radiographies and at the histological scale too. Clinically, the macaques had similar symptoms as described in human patients with moderate disease.

Viral shedding was consistently detected in nose swabs and throat swabs immediately after infection but less consistent thereafter which could reflect virus administration route (intranasal, oral). Bronchoalveolar lavages performed as a measure of virus replication in the lower respiratory tract on animals maintained for 21 days, contained high viral loads in 1 and 3dpi. The majority of the animals

exhibited pulmonary edema and mild to moderate interstitial pneumonia on terminal bronchioles. In addition to the lung, viral RNA could also be detected throughout the respiratory track where viral replication mainly occurred.

Immunologic responses included leukocytosis, neutrophilia, monocytosis and lymphopenia in the majority of the animals at 1dpi. Lymphocytes and monocytes re-normalized at 2dpi. Neutrophils declined after 3dpi and through 10dpi after which they started to recover. After infection, serum analysis revealed significant increases in **IL1ra, IL6, IL10, IL15, MCP-1, MIP-1b, but quick normalization** (3dpi). **Antibody response started around 7dpi, and the antibody titers stayed elevated until 21dpi** (day of animal euthanasia).

8.76.3 Limitations

The macaques were inoculated via a combination of intratracheal, intranasal, ocular and oral routes, which might not reproduce how humans get infected. Maybe this can lead to different dynamics in the host immune response. Also, the authors noted that the seroconversion was not directly followed by a decline in viral loads, as observed in covid19 patients.

8.76.4 Significance

This work confirms that rhesus macaques can be a good model to study Covid-19, as it has been shown by other groups [342,356,369]. While these experiments recapitulate moderate COVID-19 in humans, the mode of inoculation via a combination of intratracheal, intranasal, ocular and oral routes, might not reproduce how humans get infected and may lead to different dynamics in the host immune response. For example, the authors noted that the seroconversion was not directly followed by a decline in viral loads, as observed in COVID-19 patients. Therefore, it will be interesting to follow their antibody titers longer and further assess the possibility/effect of reinfection in these macaques. It is essential to be able to understand the dynamic of the disease and associated immune responses, and to work on vaccine development and antiviral drug testing.

8.76.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.77 ACE2 Expression is Increased in the Lungs of Patients with Comorbidities Associated with Severe COVID-19

[370]

8.77.1 Keywords

- CoVID-19
- 2019-nCoV
- SARS-CoV-2
- lung
- comorbidities
- histone
- epigenetics

8.77.2 Main Findings

- Transcriptomic analysis using systems-level meta-analysis and network analysis of existing literature to determine ACE2 regulation in patients who have frequent COVID-19 comorbidities [eg- cardiovascular diseases, familial pulmonary hypertension, cancer].

- Enrichment analyses indicated pathways associated with inflammation, metabolism, macrophage autophagy, and ER stress.

- ACE2 higher in adenocarcinoma compared to adjacent normal lung; ACE2 higher in COPD patients compared to normal.

- Co-expression analysis identified genes important to viral entry such as RAB1A, ADAM10, HMGBs, and TLR3 to be associated with ACE2 in diseased lungs.

- ACE2 expression could be potentially regulated by enzymes that modify histones, including HAT1, HDAC2, and KDM5B.

8.77.3 Limitations:

- Not actual CoVID-19 patients with co-morbidities, so interpretations in this study need to be confirmed by analyzing upcoming transcriptomics from CoVID-19 patients having co-morbidity metadata.

- As mentioned by authors, study does not look at diabetes and autoimmunity as risk factors in CoVID-19 patients due to lack of data; would be useful to extend such analyses to those datasets when available.

- Co-expression analysis is perfunctory and needs validation-experiments especially in CoVID-19 lung samples to mean anything.

- Epigenomic analyses are intriguing but incomplete, as existence of histone marks does not necessarily mean occupancy. Would be pertinent to check cell-line data (CCLE) or actual CoVID-19 patient samples to confirm ACE2 epigenetic control.

8.77.4 Significance

- Study implies vulnerable populations have ACE2 upregulation that could promote CoVID-19 severity. Shows important data-mining strategy to find gene-networks associated with ACE2 upregulation in co-morbid patients.

- Several of the genes co-upregulated with ACE2 in diseased lung might play an important role in CoVID-19 and can be preliminary targets for therapeutics

- If in silico findings hold true, epigenetic control of ACE2 expression could be a new target for CoVID-19 therapy with strategies such as KDM5 demethylases.

8.77.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.78 Meplazumab treats COVID-19 pneumonia: an open-labelled, concurrent controlled add-on clinical trial

Bian et al. *medRxiv*. [371]

8.78.1 Keywords

- Meplazumab
- CD147
- humanized antibody
- clinical trial

8.78.2 Main Findings

This work is based on previous work by the same group that demonstrated that SARS-CoV-2can also enter host cells via CD147 (also called Basigin, part of the immunoglobulin superfamily, is expressed by many cell types) consistent with their previous work with SARS-CoV-1. [1] A prospective clinical trial was conducted with 17 patients receiving Meplazumab, a humanized anti-CD147 antibody, in addition to all other treatments. 11 patients were included as a control group (non-randomized).

They observed a faster overall improvement rate in the Meplazumab group (e.g. at day 14 47% vs 17% improvement rate) compared to the control patients. Also, virological clearance was more rapid with median of 3 days in the Meplazumab group vs 13 days in control group. In laboratory values, a faster normalization of lymphocyte counts in the Meplazumab group was observed, but no clear difference was observed for CRP levels.

8.78.3 Limitations

While the results from the study are encouraging, this study was non-randomized, open-label and on a small number of patients, all from the same hospital. It offers evidence to perform a larger scale study. Selection bias as well as differences between treatment groups (e.g. age 51yo vs 64yo) may have contributed to results. The authors mention that there was no toxic effect to Meplazumab injection but more patient and longer-term studies are necessary to assess this.

8.78.4 Significance

These results seem promising as for now there are limited treatments for Covid-19 patients, but a larger cohort of patient is needed. CD147 has already been described to facilitate HIV [372], measles virus [373], and malaria [374] entry into host cells. This group was the first to describe the CD147-spike route of SARS-Cov-2 entry in host cells [345] p147. Indeed, they had previously shown in 2005 that SARS-Cov could enter host cells via this transmembrane protein [375]. Further biological understanding of how SARS-CoV-2 can enter host cells and how this integrates with ACE2R route of entry is needed. Also, the specific cellular targets of the anti-CD147 antibody need to be assessed, as this protein can be expressed by many cell types and has been shown to involved in leukocytes aggregation [376]. Lastly, Meplazumab is not a commercially-available drug and requires significant health resources to generate and administer which might prevent rapid development and use.

8.78.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.79 Potent human neutralizing antibodies elicited 1 by SARS-CoV-2 infection

Ju et al. *bioRxiv*. [61]

8.79.1 Keywords

- monoclonal antibodies
- neutralization
- antibody cross-reactivity
- Receptor Binding Domain

8.79.2 Main Findings

In this study the authors report the affinity, cross reactivity (with SARS-CoV and MERS-CoV virus) and viral neutralization capacity of 206 monoclonal antibodies engineered from isolated IgG memory B cells of patients suffering from SARS-CoV-2 infection in Wuhan, China. All patients but one recovered from disease. Interestingly, the patient that did not recover had less SARS-CoV-2 specific B cells circulating compared to other patients.

Plasma from all patients reacted to trimeric Spike proteins from SARS-CoV-2, SARS-CoV and MERS-CoV but no HIV BG505 trimer. Furthermore, plasma from patients recognized the receptor binding domain (RBD) from SARS-CoV-2 but had little to no cross-reactivity against the RBD of related viruses SARS-CoV and MERS-CoV, suggesting significant differences between the RBDs of the different viruses. Negligible levels of cross-neutralization using pseudoviruses bearing Spike proteins of SARS-CoV-2, SARS-CoV or MERS-CoV, were observed, corroborating the ELISA cross-reactivity assays on the RBDs.

SARS-CoV-2 RBD specific B cells constituted 0.005-0.065% of the total B cell population and 0.023-0.329% of the memory subpopulation. SARS-CoV specific IgG memory B cells were single cell sorted to sequence the antibody genes that were subsequently expressed as recombinant IgG1 antibodies. From this library, 206 antibodies with different binding capacities were obtained. No discernible patterns of VH usage were found in the 206 antibodies suggesting immunologically distinct responses to the infection. Nevertheless, most high-binding antibodies were derived by clonal expansion. Further analyses in one of the patient derived clones, showed that the antibodies from three different timepoints did not group together in phylogenetic analysis, suggesting selection during early infection.

Using surface plasmon resonance (SPR) 13 antibodies were found to have 10^{-8} tp 10^{-9} dissociation constants (Kd). Of the 13 antibodies, two showed 98-99% blocking of SARS-CoV-2 RBD-ACE2 receptor binding in competition assays. Thus, low Kd values alone did not predict ACE2 competing capacities. Consistent with competition assays the two antibodies that show high ACE2 blocking (P2C-2F6 and P2C-1F11) were the most capable of neutralizing pseudoviruses bearing SARS-CoV-2 spike protein (IC_{50} of 0.06 and 0.03 μg/mL, respectively). Finally, using SPR the neutralizing antibodies were found to recognize both overlapping and distinct epitopes of the RBD of SARS-CoV-2.

8.79.3 Limitations

1. Relatively low number of patients

    a. No significant conclusion can be drawn about the possible > correlation between humoral response and disease severity

2. *In vitro* Cytopathic Effect Assay (CPE) for neutralization activity

    a. Huh7 cells were used in neutralization assays with > pseudoviruses, and they may not entirely mimic what happens in > the upper respiratory tract

b. CPE assay is not quantitative

3. Duplicated panel in Figure 4C reported (has been fixed in version 2)

8.79.4 Significance

This paper offers an explanation as to why previously isolated antibodies against SARS-CoV do not effectively block SARS-CoV-2. Also, it offers important insight into the development of humoral responses at various time points during the first weeks of the disease in small but clinically diverse group of patients. Furthermore, it provides valuable information and well characterized antibody candidates for the development of a recombinant antibody treatment for SARS-CoV-2. Nevertheless, it also shows that plasmapheresis might have variability in its effectiveness, depending on the donor's antibody repertoire at the time of donation.

8.79.5 Credit

Review by Jovani Catalan-Dibene as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.80 Characterisation of the transcriptome and proteome of SARS-CoV-2 using direct RNA sequencing and tandem mass spectrometry reveals evidence for a cell passage induced in-frame deletion in the spike glycoprotein that removes the furin-like cleavage site.

Davidson et al. [377]

8.80.1 Keywords

- Transcription
- RNA-seq
- proteomics
- mass spec
- furin cleavage site
- mutation
- pathogenicity

8.80.2 Main Findings

The authors performed long read RNA sequencing using an Oxford Nanopore MinION as well as tandem mass spec (MS) on Vero cells (a cell line derived from kidney cells of the African green monkey that is deficient in interferon) infected with SARS-CoV-2.

The authors found that passage of the virus in Vero cells gave rise to a spontaneous 9 amino acid deletion (679-NSPRRARSV-687 to I) in the spike (S) protein. The deleted sequence overlaps a predicted furin cleavage site at the S1 / S2 domain boundary that is present in SARS-CoV-2 but not SARS-CoV or the closely related bat coronavirus RaTG13, which are cleaved at S1 / S2 by other proteases [33]. Furin cleavage sites at similar positions in other viruses have been linked to increased pathogenicity and greater cell tropism [378]. Loss of this site in SARS-CoV-2 has also already been shown to increase viral entry into Vero but not BHK cells (which are also interferon deficient) [24]. The authors therefore make an important contribution in demonstrating that passage in Vero cells may lead to spontaneous loss of a key pathogenicity-conferring element in SARS-CoV-2.

8.80.3 Limitations

As the authors note, a similar study posted earlier by Kim et al., which also passaged SARS-CoV-2 in Vero cells, did not identify any loss in the S protein furin cleavage site [379]. It therefore remains to be determined how likely it is that this mutation spontaneously arises. A quantitative investigation using multiple experimental replicas to understand the spontaneous viral mutation rate at this site and elsewhere would be informative. Also, the mechanistic basis for the higher viral fitness conferred by loss of the furin cleavage site in Vero cells – but, evidently, not in vivo in humans, as this site is maintained in all currently sequenced circulating isolates - remains to be understood.

Due to the high base-call error rate of MinION sequencing, the authors' bioinformatic pipeline required aligning transcripts to a reference to correct sequencing artifacts. This presumably made it difficult or impossible to identify other kinds of mutations, such as single nucleotide substitutions, which may occur even more frequently than the deletions identified in this work. Pairing long read sequencing with higher-accuracy short-read sequencing may be one approach to overcome this issue.

8.80.4 Significance

As the authors suggest, animal studies using live virus challenge may need to periodically verify the genomic integrity of the virus, or potentially risk unknowingly using a likely less-pathogenic variant of the virus.

More broadly, the results emphasize the complexity and plasticity of the SARS-CoV-2 viral transcriptome and proteome. For example, the authors found multiple versions of transcripts encoding the nucleocapsid (N) protein, each with different small internal deletions, some of which were verified for translation by MS. A number of peptides arising from translation of unexpected rearrangements of transcripts were also detected. Additionally, the authors identified phosphorylation of a number of viral proteins (N, M, ORF 3a, nsp3, nsp9, nsp12 and S). For any cases where these have functional consequences, targeting the kinases responsible could be an avenue for drug development. Understanding the functional consequences of the mutations, transcript variations, and post translational modifications identified in this study will be important future work.

8.80.5 Credit

This review was undertaken by Tim O'Donnell, Maria Kuksin as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.81 A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug- Repurposing

Gordon et al. *bioRxiv* [64]

8.81.1 Keywords

- protein-protein interactions
- mass spectrometry
- drug targets

8.81.2 Main Findings

Gordon et al cloned, tagged and expressed 26 of the 29 SARS-CoV-2 proteins individually in HEK293T cells and used mass spectrometry to identify protein-protein interactions. They identified 332 viral-

host protein-protein interactions. Furthermore, they used these interactions to identify 66 existing drugs known to target host proteins or host pathways (eg SARS-CoV-2 N and Orf8 proteins interact with proteins regulated by the mTOR pathway, so mTOR inhibitors Silmitasertib and Rapamycin are possible drug candidates).

8.81.3 Limitations

The main limitation of the study stems from the reductionist model: overexpression of plasmids encoding individual viral proteins in HEK293T cells. This precludes any interactions between the viral proteins, or the combined effects of multiple proteins on the host, as they are expressed individually. Moreover, HEK293T cells come from primary embryonic kidney and therefore might not reflect how SARS-CoV-2 interacts with its primary target, the lung. However, the authors found that the proteins found to interact with viral proteins in their experiments are enriched in lung tissue compared to HEK293Ts.

8.81.4 Significance

The authors provide a "SARS-CoV-2 interaction map," which may provide potential hypotheses as to how the virus interacts with the host. Further, they identified existing drugs that could disrupt these host-viral interactions and curb SARS-CoV-2 infection. Although these interactions have not been validated, this paper acts as a valuable resource.

8.81.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.82 First Clinical Study Using HCV Protease Inhibitor Danoprevir to Treat Naïve and Experienced COVID-19 Patients

Chen et al. *medRxiv*. [380]

8.82.1 Keywords

- Clinical study
- HCV protease inhibitor
- Danoprevir
- Ritonavir
- Covid19 treatment

8.82.2 Main Findings

The authors treated 11 Covid-19 patients with Danoprevir, a commercialized HCV protease inhibitor [381][(p4)], boosted by ritonavir [382], a CYP3A4 inhibitor (which enhances the plasma concentration and bioavailabilty of Danoprevir). Two patients had never received anti-viral therapy before (=naïve), whereas nine patients were on Lopinavir/Ritonavir treatment before switching to Danoprevir/Ritonavir (=experienced). The age ranged from 18 to 66yo.

Naïve patients that received Danoprevir/Ritonavir treatment had a decreased hospitalization time. Patients treated with Lopinavir/Ritonavir did not have a negative PCR test, while after switching to Danoprevir/Ritonavir treatment, the first negative PCR test occurred at a median of two days.

8.82.3 Limitations

The results of the study are very hard to interpret as there is no control group not receiving Danoprevir/Ritonavir treatment. This was especially true in naïve patients who seemed to have more mild symptoms before the start of the study and were younger (18 and 44yo) compared to the experienced patients (18 to 66yo). The possibility that the patients would have recovered without Danoprevir/Ritonavir treatment cannot be excluded.

8.82.4 Significance

The authors of the study treated patients with Danoprevir, with the rational to that this is an approved and well tolerated drug for HCV patients [382], and that it could also target the protease from SARS-CoV-2 (essential for viral replication and transcription). Indeed, homology modelling data indicated that HCV protease inhibitors have the highest binding affinity to Sars-Cov2 protease among other approved drugs [383].

While this study shows that the combination of Danoprevir and Ritonavir might be beneficial for Covid-19 patients, additional clinical trials with more patients and with better methodology (randomization and control group) are needed to make further conclusions.

8.82.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.83 Efficacy of hydroxychloroquine in patients with COVID-19: results of a randomized clinical trial

[162]

8.83.1 Keywords

- hydroxychloroquine

8.83.2 Study Description

This is a randomized clinical trial of hydroxychloroquine (HCQ) efficacy in the treatment of COVID-19. From February 4 – February 28, 2020 142 COVID-19 positive patients were admitted to Renmin Hospital of Wuhan University. 62 patients met inclusion criteria and were enrolled in a double blind, randomized control trial, with 31 patients in each arm.

Inclusion criteria:

1. Age ≥ 18 years
2. Positive diagnosis COVID-19 by detection of SARS-CoV-2 by RT-PCR
3. Diagnosis of pneumonia on chest CT
4. Mild respiratory illness, defined by SaO_2/SPO_2 ratio > 93% or PaO_2/FIO_2 ratio > 300 mmHg in hospital room conditions (Note: relevant clinical references described below.)

a. Hypoxia is defined as an SpO2 of 85-94%; severe hypoxia < 85%.

b. The PaO_2/FIO_2 (ratio of arterial oxygen tension to fraction of inspired oxygen) is used to classify the severity of acute respiratory distress syndrome (ARDS). Mild ARDS has a PaO_2/FIO_2 of 200-300 mmHg, moderate is 100-200, and severe < 100.

5. Willing to receive a random assignment to any designated treatment group; not participating in another study at the same time

Exclusion criteria:

1. Severe or critical respiratory illness (not explicitly defined, presumed to be respiratory function worse than outlined in inclusion criteria); or participation in trial does not meet patient's maximum benefit or safe follow up criteria

2. Retinopathy or other retinal diseases

3. Conduction block or other arrhythmias

4. Severe liver disease, defined by Child-Pugh score ≥ C or AST > twice the upper limit

5. Pregnant or breastfeeding

6. Severe renal failure, defined by eGFR ≤ 30 mL/min/1.73m^2, or on dialysis

7. Potential transfer to another hospital within 72h of enrollment

8. Received any trial treatment for COVID-19 within 30 days before the current study

All patients received the standard of care: oxygen therapy, antiviral agents, antibacterial agents, and immunoglobulin, with or without corticosteroids. Patients in the HCQ treatment group received additional oral HCQ 400 mg/day, given as 200 mg 2x/day. HCQ was administered from days 1-5 of the trial. The primary endpoint was 5 days post enrollment or a severe adverse reaction to HCQ. The primary outcome evaluated was time to clinical recovery (TTCR), defined as return to normal body temperature and cough cessation for > 72h. Chest CT were imaged on days 0 and 6 of the trial for both groups; body temperature and patient reports of cough were collected 3x/day from day 0 – 6. The mean age and sex distribution between the HCQ and control arms were comparable.

8.83.3 Main Findings

There were 2 patients showing mild secondary effects of HCQ treatment. More importantly, while 4 patients in the control group progressed to severe disease, none progressed in the treatment group.

TTCR was significantly decreased in the HCQ treatment arm; recovery from fever was shortened by one day (3.2 days control vs. 2.2 days HCQ, p = 0.0008); time to cessation of cough was similarly reduced (3.1 days control vs. 2.0 days HCQ, p = 0.0016).

Overall, it appears that HCQ treatment of patients with mild COVID-19 has a modest effect on clinical recovery (symptom relief on average 1 day earlier) but may be more potent in reducing the progression from mild to severe disease.

8.83.4 Limitations

This study is limited in its inclusion of only patients with mild disease, and exclusion of those on any treatment other than the standard of care. It would also have been important to include the laboratory values of positive RT-PCR detection of SARS-CoV-2 to compare the baseline and evolution of the patients' viral load.

8.83.5 Limitations

Despite its limitations, the study design has good rigor as a double blind RCT and consistent symptom checks on each day of the trail. Now that the FDA has approved HCQ for treatment of COVID-19 in the USA, this study supports the efficacy of HCQ use early in treatment of patients showing mild symptoms, to improve time to clinical recovery, and possibly reduce disease progression. However, most of the current applications of HCQ have been in patients with severe disease and for compassionate use, which are out of the scope of the findings presented in this trial. Several additional clinical trials to examine hydroxychloroquine are now undergoing; their results will be critical to further validate these findings.

8.83.6 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

Structure-based modeling of SARS-CoV-2 peptide/HLA-A02 antigens

https://doi.org/10.1101/2020.03.23.004176

Immunology keywords:

CoVID-19, 2019-nCoV, SARS-CoV-2, comparative, homology, peptide, modeling, simulation, HLA-A, antigen

Summary of Findings:

- The authors utilize homology modeling to identify peptides from the SARS-CoV-2 proteome that potentially bind HLA-A*02:01.
- They utilize high-resolution X-ray structures of peptide/MHC complexes on Protein Data Bank, substitute homologous peptides with SARS-CoV-2 peptides, and calculate MHC/SARS-CoV-2 peptide Rosetta binding energy.
- They select MHC/SARS-CoV-2 complex models with highest binding energy for further study and publish models in an online database (https://rosettamhc.chemistry.ucsc.edu).

Limitations:

- The authors only utilize computational methods and predicted SARS-CoV-2 peptides must be validated experimentally for immunogenicity and clinical response.
- Due to computational burden and limited availability of high resolution X-ray structures on PDB, authors only simulate 9-mer and 10-mer peptide binding to HLA-A*02:01.
- Since the authors compare select existing X-ray structures as a starting point, backbone conformations that deviate significantly between test and template peptides are not captured.

Furthermore, Rosetta modeling protocols do not capture all possible structures and binding energy scoring does not fully recapitulate fundamental forces.[1,2]

Importance/Relevance:

- The authors identify and publish high-scoring SARS-CoV-2 peptides that may direct a targeted, experimental validation approach toward a COVID-19 vaccine.
- The authors utilize Rosetta simulation to further filter results from NetMHCpan 4.0, supporting machine learning prediction with structural analysis.
- The authors develop RosettaMHC, a computationally efficient method of leveraging existing X-ray structures for identification of immunogenic peptides.

References:

1. Bender, B. J., Cisneros, A., 3rd, Duran, A. M., Finn, J. A., Fu, D., Lokits, A. D., . . . Moretti, R. (2016). Protocols for Molecular Modeling with Rosetta3 and RosettaScripts. Biochemistry, 55(34), 4748-4763. doi:10.1021/acs.biochem.6b00444

2. Alford, R. F., Leaver-Fay, A., Jeliazkov, J. R., O'Meara, M. J., DiMaio, F. P., Park, H., . . . Gray, J. J. (2017). The Rosetta All-Atom Energy Function for Macromolecular Modeling and Design. J Chem Theory Comput, 13(6), 3031-3048. doi:10.1021/acs.jctc.7b00125

Review by Jonathan Chung as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn school of medicine, Mount Sinai.

8.84 Serology characteristics of SARS-CoV-2 infection since the exposure and post symptoms onset

Lou et al. *medRxiv*. [384]

8.84.1 Keywords

- Seroconversion rate
- Total Antibody
- Ab
- IgG and IgM
- antibody

8.84.2 Main Findings

Currently, the diagnosis of SARS-CoV-2 infection entirely depends on the detection of viral RNA using polymerase chain reaction (PCR) assays. False negative results are common, particularly when the samples are collected from upper respiratory. Serological detection may be useful as an additional testing strategy. In this study the authors reported that a typical acute antibody response was induced during the SARS-CoV-2 infection, which was discuss earlier[1]. The seroconversion rate for Ab, IgM and IgG in COVID-19 patients was 98.8% (79/80), 93.8% (75/80) and 93.8% (75/80), respectively. The first detectible serology marker was total antibody followed by IgM and IgG, with a median seroconversion time of 15, 18 and 20 days-post exposure (d.p.e) or 9, 10- and 12-days post-onset (d.p.o). Seroconversion was first detected at day 7d.p.e in 98.9% of the patients. Interestingly they found that viral load declined as antibody levels increased. This was in contrast to a previous study [324],

showing that increased antibody titers did not always correlate with RNA clearance (low number of patient sample).

8.84.3 Limitations

Current knowledge of the antibody response to SAR-CoV-2 infection and its mechanism is not yet well elucidated. Similar to the RNA test, the absence of antibody titers in the early stage of illness could not exclude the possibility of infection. A diagnostic test, which is the aim of the authors, would not be useful at the early time points of infection but it could be used to screen asymptomatic patients or patients with mild disease at later times after exposure.

8.84.4 Significance

Understanding the antibody responses against SARS-CoV2 is useful in the development of a serological test for the diagnosis of COVID-19. This manuscript discussed acute antibody responses which can be deducted in plasma for diagnostic as well as prognostic purposes. Thus, patient-derived plasma with known antibody titers may be used therapeutically for treating COVID-19 patients with severe illness.

8.84.5 Credit

This review was undertaken and edited by Konstantina A as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.85 SARS-CoV-2 launches a unique transcriptional signature from in vitro, ex vivo, and in vivo systems

Blanco-Melo et al. *bioRxiv*. [385]

8.85.1 Keywords

- host cellular response
- host-pathogen interaction
- type I interferon
- type III interferon
- inflammation
- RNA-seq
- comparative analysis

8.85.2 Main Findings

Given the high mortality rate of SARS-CoV-2 relative to other respiratory viruses such seasonal IAV and RSV, there may be underlying host-pathogen interactions specific to SARS-CoV-2 that predispose to a worse clinical outcome. Using *in vivo*, *ex vivo*, and *in vitro* systems, the authors profiled host cell transcriptional responses to SARS-CoV-2 and to other common respiratory viruses (seasonal IAV and RSV). SARS-CoV-2 infection *in vitro* led to an induction of type I interferon response signaling and the upregulation of cytokine/chemokines transcripts. In comparison with IAV and RSV infection, SARS-CoV-2 *in vitro* appears to uniquely induce less type I and type III interferon expression and higher levels of two cytokines previously implicated in respiratory inflammation. Lastly, *in vivo* data from ferrets showed a reduced induction of cytokines and chemokines by SARS-CoV-2 infection relative to IAV infection.

8.85.3 Limitations

While these results are promising, there are several key weaknesses of this paper. 1) As the authors point out, there is an undetectable level of SARS-CoV-2 putative receptor (ACE2) and protease (TMPRSS2) expression in the lung epithelial cell line used for the *in vitro* studies. This raises the important question of whether viral replication actually occurs in any of the models used, which may explain the lack of interferon production observed *in vitro* in SARS-CoV-2 treated cells. Further studies characterizing viral titers across timepoints are needed. 2) Furthermore, these studies only characterize the host response at a single dose and timepoint per virus, and it is unclear why these doses/timepoints were chosen. This leaves open the possibility that the observed differences between viruses could be due to differences in dose, timing, host response, or a combination of all of these. 3) It is unclear whether ferrets are productively infected, which cell types are infected, and the extent/timing of the clinical course of infection. Moreover, the *in vitro* and *in vivo* data do not strongly correlate and the reasons for this are unclear.

8.85.4 Significance

This paper describes potentially unique transcriptional signatures of host cells exposed to SARS-CoV-2. If validated, these findings may help explain clinical outcomes and could be targeted in future therapeutic interventions.

8.85.5 Potential Conflicts of Interest Disclosure

The reviewers are also researchers at the Icahn School of Medicine at Mount Sinai.

8.85.6 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.86 A New Predictor of Disease Severity in Patients with COVID-19 in Wuhan, China

Zhou et al. *bioRxiv*. [386]

8.86.1 Keywords

- disease severity
- clinical data
- Neutrophils/lymphocytes ratio
- CRP
- D-dimer

8.86.2 Main Findings

377 hospitalized patients were divided into two groups: severe and non-severe pneumonia. The laboratory results of their first day of admission were retrospectively analyzed to identify predictors of disease severity.

After adjusting for confounding factors from chronic comorbidities (such as high blood pressure, type 2 diabetes, coronary heart disease, and chronic obstructive pulmonary disease), the independent risk

factors identified for severe pneumonia were **age**, the **ratio of neutrophil/lymphocytes counts**, **CRP** and **D-dimer** levels.

To further increase the specificity and sensibility of these markers, they showed that their multiplication **[(Neutrophil/lymphocyte count) * CRP * D-dimer]** was a better predictor of disease severity, with higher sensitivity (95.7%) and specificity (63.3%), with a cutoff value of 2.68.

8.86.3 Limitations

This study included 377 hospitalized patients. Among them, 45.6% patients tested positive for SARS-Cov-2 nucleic acid test results, and others were included in the study based on clinically diagnosis even if the molecular diagnosis was negative. Thus, additional studies are needed to verify this on a larger number of covid-19 certified patients and the cutoff value might be adjusted. Also, all the patients that did not have the clinical characteristics of severe pneumonia were included in the non-severe pneumonia group, but usually patients are also divided into moderate and mild disease.

Also, studying different subset of lymphocytes could lead to a more specific predictor. Another study showed that the neutrophils to CD8+ T cells ratio was a strong predictor of disease severity [294]. Another more precise study showed that the percentage of helper T cells and regulatory T cells decrease but the percentage of naïve helper T cells increases in severe cases [287]. Taking these subpopulations into account might make the predictor more powerful.

Other studies also noted an inverse correlation between disease severity and LDH [328] or IL6 [337] levels, but the authors here do not discuss LDH nor IL6 levels, although this could help to strengthen the predictor.

The study is based on the results obtained on the first day of admission, studying the dynamic of the changes in patients might also be interesting to better predict disease severity.

8.86.4 Significance

This study confirms that the neutrophil to lymphocyte ratio can be a predictor of disease severity as shown by many others [286,287,301]. The novelty here is that they show that a combination with other markers can enhance the specificity and sensibility of the predictor, although the study could be improved by taking into account sub-populations of lymphocytes and more biological factors from patients such as LDH and IL6.

8.86.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.87 Metabolic disturbances and inflammatory dysfunction predict severity of coronavirus disease 2019 (COVID-19): a retrospective study

Shuke Nie et al. *medRxiv*. [387]

8.87.1 Keywords

- metabolism
- fasting blood glucose
- serum total protein

- albumin
- blood lipid
- HDL-C
- APOA1
- lymphocytopenia
- IL-6
- CRP
- severity prediction of COVID19

8.87.2 Main Findings

Retrospective Study on 97 COVID-19 hospitalized patients (25 severe and 72 non-severe) analyzing clinical and laboratory parameter to predict transition from mild to severe disease based on more accessible indicators (such as fasting blood glucose, serum protein or blood lipid) than inflammatory indicators. In accordance with other studies, age and hypertension were risk factors for disease severity, and lymphopenia and increased IL-6 was observed in severe patients. The authors show that fasting blood glucose (FBG) was altered and patients with severe disease were often hyperglycemic. Data presented support that hypoproteinaemia, hypoalbuminemia, and reduction in high-densitylipoprotein (HDL-C) and ApoA1 were associated with disease severity.

8.87.3 Limitations

In this study non-severe patients were divided in two groups based on average course of the disease: mild group1 (14 days, n=28) and mild group 2 (30 days, n=44). However mild patients with a longer disease course did not show an intermediate phenotype (between mild patients with shorter disease course and severe patients), hence it is unclear whether this was a useful and how it impacted the analysis. Furthermore, the non-exclusion of co-morbidity factors in the analysis may bias the results (e.g. diabetic patients and glucose tests) It is not clear at what point in time the laboratory parameters are sampled. In table 3, it would have been interesting to explore a multivariate multiple regression. The correlation lacks of positive control to assess the specificity of the correlation to the disease vs. correlation in any inflammatory case. The dynamic study assessing the predictability of the laboratory parameter is limited to 2 patients. Hence there are several associations with disease severity, but larger studies are necessary to test the independent predictive value of these potential biomarkers.

8.87.4 Significance

As hospital are getting overwhelmed a set of easily accessible laboratory indicators (such as serum total protein) would potentially provide a triage methodology between potentially severe cases and mild ones. This paper also opens the question regarding metabolic deregulation and COVID-19 severity.

8.87.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.88 Viral Kinetics and Antibody Responses in Patients with COVID-19

[388]

8.88.1 Keywords

- CoVID-19
- 2019-nCoV
- SARS-CoV-2
- IgG
- IgM
- clinical
- kinetics
- antibodies

8.88.2 Main Findings

- Prospective cohort of 67 patients, clinical specimens taken and follow-up conducted.

- Viral shedding, serum IgM, IgG antibody against NP evaluated and correlated to disease severity and clinical outcome

- Viral RNA levels peaked at 1 week from febrile/cough symptom onset in sputum, nasal swabs, and stool samples. Shedding ranged from 12-19 days (median ranges) and was longer in severe patients.

- IgM and IgG titers stratified patients into three archetypes as 'strong vs weak vs non-responders'. Strong responders (with higher IgM/IgG titers) were significantly higher in severe patients.

8.88.3 Limitations

Specific for immune monitoring.

- Not clear if stool RNA captured from live infection in intestine/liver or from swallowed sputum. Transmission electron microscopy (TEM) carried out on sputum samples as proof of concept, but not stools. TEM unreasonable for actual clinical diagnosis.

- Several patients had co-morbidities (such as pulmonary and liver disease) that were not accounted for when tracking antibody responses. Viral kinetics and IgM/IgG titers in subsets of patients with underlying conditions/undergoing certain medication would be informative.

8.88.4 Significance

- Three archetypes of antibody response to SARS-CoV-2 with different disease progression and kinetics is useful to stratify patients, and for future serological tests.

- Strong spike-IgG levels often correlate with lymphopenia and CoVID-19 disease severity [389], similar to macaque studies in SARS [235]. It would be critical to see if anti-NP or anti-Spike IgG antibodies for SARS-CoV-2 also elicit similar detrimental effects before clinical use.

8.88.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.89 COVID-19 infection induces readily detectable morphological and inflammation-related phenotypic changes in peripheral blood monocytes, the severity of which correlate with patient outcome

[390]

8.89.1 Keywords

- Monocytes
- FSC-high
- PBMC
- ACE2
- inflammatory cytokines

8.89.2 Main Findings

This study is based on flow cytometry immunophenotyping of PBMCs from 28 patients diagnosed positive for SARS-Cov2 (COVID19). The authors identify a population of abnormally large (FSC-hi) monocytes, present in COVID19 patients, but absent in PBMCs of healthy volunteers (n=16) or patients with different infections (AIDS, malaria, TB). This FSC-hi monocytic population contains classical, intermediate and non-classical (monocytes (based on CD14 and CD16 expression) that produce inflammatory cytokines (IL-6, TNF and IL-10). The authors suggest an association of FSC-hi monocytes with poor outcome and correlate a high percentage of FSC-low monocytes, or higher ratio of FSC-low/hi monocytes, with faster hospital discharge.

8.89.3 Limitations

While identification of the monocytic population based on FSC is rather robust, the characterization of these cells remains weak. A comprehensive comparison of FSC-hi monocytes with FSC-low monocytes from patients and healthy controls would be of high value. It is unclear if percentages in blood are among CD45+ cells. Furthermore, it would have been important to include absolute numbers of different monocytic populations (in table 1 there are not enough samples and it is unclear what the authors show).

The authors show expression of the ACE2 receptor on the surface of the monocytes, and highlight these cells as potential targets of SARS-Cov2. However, appropriate controls are needed. CD16 has high affinity to rabbit IgG and it is unclear whether the authors considered unspecific binding of rabbit anti-ACE2 to Fc receptors. Gene expression of ACE-2 on monocytes needs to be assessed. Furthermore, it would be important to confirm infection of monocytes by presence of viral proteins or viral particles by microscopy.

Considering the predictive role of FSC-hi monocytes on the development of the disease and its severity, some data expected at this level are neither present nor addressed. Although the cohort is small, it does include 3 ICU patients. What about their ratio of FSC-low vs FSC-hi monocytes in comparison to other patients? Was this apparent early in the disease course? Does this population of FSC-hi monocytes differ between ICU patients and others in terms of frequency, phenotype or cytokine secretion?

In general, figures need to revised to make the data clear. For example, in Fig. 5, according to the legend it seems that patients with FSC-high monocytes are discharged faster from the hospital. However according to description in the text, patients were grouped in high or low levels of FSC-low monocytes.

8.89.4 Significance

Despite the limitations of this study, the discovery of a FSC-high monocyte population in COVID-19 patients is of great interest. With similar implication, a the recent study by Zhou et al. [292] identified a connection between an inflammatory CD14+CD16+ monocyte population and pulmonary immunopathology leading to deleterious clinical manifestations and even acute mortality after SARS-CoV-2 infections. Although the presence of these monocytes in the lungs has yet to be demonstrated, such results support the importance of monocytes in the critical inflammation observed in some COVID19 patients.

8.89.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.90 Correlation between universal BCG vaccination policy and reduced morbidity and mortality for COVID-19: an epidemiological study

Miller et al. *medRxiv*. [391]

8.90.1 Keywords

- BCG vaccine
- epidemiology
- vaccination policy

8.90.2 Main Findings

The authors compared middle and high income countries that never had a universal BCG vaccination policy (Italy, Lebanon, Nederland, Belgium) and countries with a current policy (low income countries were excluded from the analysis as their number of cases and deaths might be underreported for the moment). **Countries that never implement BCG vaccination have a higher mortality rate than countries which have a BCG vaccination policy** (16.38 deaths per million people vs 0.78). Next, **the authors show that an earlier start of vaccination correlates with a lower number of deaths per million inhabitants**. They interpret this as the vaccine protecting a larger fraction of elderly people, which are usually more affected by COVID-19. Moreover, higher number of COVID-19 **cases** were presented in countries that never implemented a universal BCG vaccination policy.

8.90.3 Limitations

While this study aims to test an intriguing hypothesis unfortunately, the data is not sufficient at this time to accurately make any determinations. Several caveats must be noted including: not all countries are in the same stage of the pandemic, the number of cases/deaths is still changing very rapidly in a lot of countries and thus the association may only reflect exposure to the virus. This analysis would need to be re-evaluated when all the countries are passed the pandemic and more accurate numbers are available. Additionally, very few middle and high-income countries ever implemented universal BCG vaccination, which can be a source of bias (5 countries, vs 55 that have a BCG vaccine policy). Effective screening and social isolation policies also varied considerable across the countries tested and may reflect another important confounder. The authors could consider analyzing the Case Fatality Rate (CFR, % of patients with COVID-19 that die), to more correct for exposure although testing availability will still bias this result. Variability in mortality within countries or cities with variable vaccination and similar exposure could also be appropriate although confounders will still be present.

8.90.4 Significance

BCG vaccine is a live attenuated strain derived from *Mycobacterium bovis* and used for a vaccine for tuberculosis (TB). This vaccine has been proven to be efficient in preventing childhood meningitis TB, but doesn't prevent adult TB as efficiently. For this reason, several countries are now only recommending this vaccine for at-risk population only.

This study shows that there is a correlation between BCG vaccination policy and reduced mortality for Covid-19. Indeed, BCG vaccine has been shown to protect against several viruses and enhance innate immunity [392], which could explain why it could protect against SARS-CoV-2 infection, but the exact mechanism is still unknown. **Moreover, the efficiency of adult/older people vaccination and protection against Covid-19 still needs to be assessed.** Regarding this, Australian researchers are starting a clinical trial of BCG vaccine for healthcare workers [393], to assess if it can protect them against Covid-19.

8.90.5 Credit

This review was undertaken as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.91 Non-neural expression of SARS-CoV-2 entry genes in the olfactory epithelium

[394]

8.91.1 Keywords

- CoVID-19
- 2019-nCoV
- SARS-CoV-2
- anosmia
- olfaction
- scRNAseq

8.91.2 Main Findings

- Study analyzed bulk and scRNAseq data of olfactory cell types from publicly-available mouse, nonhuman primate and human datasets.

- show that *ACE2* and *TMPRSS2* (genes involved in SARS-CoV-2 entry) are expressed in olfactory epithelial (OE) cells, basal stem cells and respiratory epithelium (RE), but not sensory neurons.

- Comparison of human RE and OE datasets (Deprez et al. 2019; Durante et al. 2020) revealed that *ACE2* and *TMPRSS2* expression in OE sustentacular cells was similar to expression in the remainder of the non-nasal respiratory tract.

8.91.3 Limitations

- Transcript data alone from healthy respiratory/olfactory cells is not sufficient to confirm infectivity of nasal passage, or to indicate damage to epithelia.

- No mechanism defined for anosmia; it is not clear if epithelial injury leads to reduced sensitivity or increased inflammation and altered immune contexture drives neural/epithelial dysfunction. Will be critical to test this in CoVID-19 patient samples or mouse models.

8.91.4 Significance

- Study provides possible rationale for anosmia observed in several CoVID-19 patients.

- Raises possibility that nasal respiratory goblet, ciliated cells, and olfactory epithelia may serve as a viral reservoir after initial SARS-CoV-2 infection.

- Human olfactory sensory neurons express several other molecules important to CoV (not CoV-19) entry such as *FURIN, ST6GAL1, ST3GAL4*; this suggests wider mechanism of neuronal infectivity in other coronaviruses.

8.91.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

Title:

SARS-CoV-2 proteome microarray for mapping COVID-19 antibody interactions at amino acid resolution

Immunology keywords: SARS-CoV-2, COVID-19, high throughput, peptide microarray, antibody epitope screening

The main finding of the article:

This study screened the viral protein epitopes recognized by antibodies in the serum of 10 COVID-19 patients using a new SARS-CoV-2 proteome peptide microarray. The peptide library was constructed with 966 linear peptides, each 15 amino acids long with a 5 amino acid overlap, based on the protein sequences encoded by the genome of the Wuhan-Hu-1 strain.

To investigate crossreactivity between SARS-CoV-1 and SARS-CoV-2, they tested rabbit monoclonal and polyclonal antibodies against SARS-CoV-1 nucleocapsid (N) in the microarray. Antibodies against SARS-CoV-1 N displayed binding to the SARS-CoV-2 nucleocapsid (N) peptides. Polyclonal antibodies showed some crossreactivity to other epitopes from membrane (M), spike (S), ORF1ab and ORF8. This suggests that previous exposure to SARS-CoV-1 may induced antibodies recognizing both viruses.

Screening of IgM and IgG antibodies from 10 COVID-19 patients showed that many antibodies targeted peptides on M, N, S, Orf1ab, Orf3a, Orf7a, and Orf8 from SARS-CoV-2, while immunodominant epitopes with antibodies in more than 80 % COVID-19 patients were present in N, S and Orf3. It is shown that the receptor binding domain (RBD) resides on S protein and RBD is important for SARS-CoV-2 to enter the host cells via ACE2. Among six epitopes on S protein, structural analysis predicted that three epitopes were located at the surface and three epitopes were located inside of the protein. Furthermore, some IgM antibodies from 1 patient and IgG antibodies from 2 patients bound to the same epitope (residue 456-460, FRKSN) which resided within the RBD, and structural analysis determined that this epitope was located in the region of the RBD loop that engages with ACE2.

Critical analysis of the study:

In addition to the limitations mentioned in the manuscript, it would have been informative to do the analysis over the course of the disease. The pattern of antibody recognition, especially on S protein, and the course of antibodies of different isotypes recognizing the same peptide might correlate to the clinical course in these patients. It would alos have been informative to analyze the presence of cross-reactive antibodies from pateints previously exposed to SARS-CoV-1.

The importance and implications for the current epidemics:

This study identified linear immunodominant epitopes on SARS-CoV-2, Wuhan-Hu-1 strain. This is a valuable information to design vaccines that will elicit desirable immune responses.

The Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Directly Decimates Human Spleens and Lymph Nodes

Review by Matthew D. Park

Revised by Miriam Merad

Keywords: COVID-19, SARS-CoV-2, spleen, lymph node, ACE2, macrophage

Main findings

It has been previously reported that COVID-19 patients exhibit severe lymphocytopenia, but the mechanism through which this depletion occurs has not been described. In order to characterize the cause and process of lymphocyte depletion in COVID-19 patients, the authors performed gross anatomical and *in situ* immune-histochemical analyses of spleens and lymph nodes (hilar and subscapular) obtained from post-mortem autopsies of 6 patients with confirmed positive viremia and 3 healthy controls (deceased due to vehicle accidents).

Primary gross observations noted significant splenic and LN atrophy, hemorrhaging, and necrosis with congestion of interstitial blood vessels and large accumulation of mononuclear cells and massive lymphocyte death. They found that $CD68^+$ $CD169^+$ cells in the spleens, hilar and subscapular LN, and capillaries of these secondary lymphoid organs expressed the ACE2 receptor and stain positive for the SARS-CoV-2 nucleoprotein (NP) antigen, while $CD3^+$ T cells and $B220^+$ B cells lacked both the ACE2 receptor and SARS-CoV-2 NP antigen. $ACE2^+$ NP^+ $CD169^+$ macrophages were positioned in the splenic marginal zone (MZ) and in the marginal sinuses of LN, which suggests that these macrophages were positioned to encounter invading pathogens first and may contribute to virus dissemination.

Since SARS-CoV-2 does not directly infect lymphocytes, the authors hypothesized that the NP^+ $CD169^+$ macrophages are responsible for persistent activation of lymphocytes via Fas::FasL interactions that would mediate activation-induced cell death (AICD). Indeed, the expression of Fas was significantly higher in virus-infected tissue than that of healthy controls, and TUNEL staining showed significant lymphocytic apoptosis. Since pro-inflammatory cytokines like IL-6 and TNF-α can also engage cellular apoptosis and necrosis, the authors interrogated the cytokine expression of the secondary lymphoid organs from COVID-19 patients; IL-6, not TNF-α, was elevated in virus-infected splenic and lymph node tissues, compared to those of healthy controls, and immunofluorescent staining showed that IL-6 is primarily produced by the infected macrophages. *In vitro* infection of THP1 cells with SARS-CoV-2 spike protein resulted in selectively increased *Il6* expression, as opposed to *Il1b* and *Tnfa* transcription. Collectively, the authors concluded that a combination of Fas up-regulation and IL-6 production by NP^+ $CD169^+$ macrophages induce AICD in lymphocytes in secondary lymphoid organs, resulting in lymphocytopenia.

In summary, this study reports that CD169^{+} macrophages in the splenic MZ, subscapular LN, and the lining capillaries of the secondary lymphoid tissues express ACE2 and are susceptible to SARS-CoV-2 infection. The findings point to the potential role of these macrophages in viral dissemination, immunopathology of these secondary lymphoid organs, hyperinflammation and lymphopenia.

Limitations

Technical

A notable technical limitation is the small number of samples (n=6); moreover, the analysis of these samples using multiplexed immunohistochemistry and immunofluorescence do not necessarily provide the depth of unbiased interrogation needed to better identify the cell types involved.

Biological

The available literature and ongoing unpublished studies, including single-cell experiments of spleen and LN from organ donors, do not indicate that ACE2 is expressed by macrophages; however, it remains possible that ACE2 expression may be triggered by type I IFN in COVID-19 patients. Importantly, the SARS-CoV-2 NP staining of the macrophages does not necessarily reflect direct infection of these macrophages; instead, positive staining only indicates that these macrophages carry SARS-CoV-2 NP as antigen cargo, which may have been phagocytosed. Direct viral culture of macrophages isolated from the secondary lymphoid organs with SARS-CoV-2 is required to confirm the potential for direct infection of macrophages by SARS-CoV-2. Additionally, it is important to note that the low to negligible viremia reported in COVID-19 patients to-date does not favor a dissemination route via the blood, as suggested by this study, which would be necessary to explain the presence of virally infected cells in the spleen.

Relevance

Excess inflammation in response to SARS-CoV-2 infection is characterized by cytokine storm in many COVID-19 patients. The contribution of this pathology to the overall fatality rate due to COVID-19, not even necessarily directly due to SARS-CoV-2 infection, is significant. A better understanding of the full effect and source of some of these major cytokines, like IL-6, as well as the deficient immune responses, like lymphocytopenia, is urgently needed. In this study, the authors report severe tissue damage in spleens and lymph nodes of COVID-19 patients and identify the role that CD169^{+} macrophages may play in the hyperinflammation and lymphocytopenia that are both characteristic of the disease. It may, therefore, be important to note the effects that IL-6 inhibitors like Tocilizumab and Sarilumab may specifically have on splenic and LN function. It is important to note that similar observations of severe splenic and LN necrosis and inflammation in patients infected with SARS-CoV-1 further support the potential importance and relevance of this study.

8.92 Cigarette smoke triggers the expansion of a subpopulation of respiratory epithelial cells that express the SARS-CoV-2 receptor ACE2

[395]

8.92.1 Keywords

- CoVID-19
- 2019-nCoV
- SARS-CoV-2
- respiration

- cigarette
- ACE2
- lung

8.92.2 Main Findings

- Study uses scRNAseq, bulk seq data and air-liquid interface culture experiments to show that cigarette smoke causes a dose-dependent upregulation of ACE2 in mouse and human lungs (transplantation, tumor resection, or IPF datasets).

- ACE2 was not up-regulated in patients with asthma or lung-sarcoidosis or in mouse models of cystic fibrosis or carcinogen exposure.

- Cathepsin B (alternate protease involved in viral entry) is increased in smoke-exposed mouse or human lungs.

- Smoke triggers a protective expansion of mucus-secreting MUC5AC+ goblet and SCGB1A1+ club cells; ACE2 presence in these cells is increased upon smoke exposure.

8.92.3 Limitations:

- Long-term smokers usually have several co-morbidities including immune dysfunction, which can affect interpretation of CoV-2 susceptibility in these datasets. Ideally, analyses can control for major co-morbidities across smokers and non-smokers (immune suppression, cardiovascular disease and atherosclerosis).

- Hyperplasia of ACE2+ goblet cells upon smoking needs to be separated from ACE2 upregulation in existing goblet cells.

- ACE2 expression increase alone does not confirm increased viral entry into goblet cells; future studies with air-liquid interface cultures testing CoV-2 infectivity in *ex vivo* epithelial cells from human epithelial lines, ex vivo samples or hACE2 mice will be very informative.

8.92.4 Significance

- This study may partially explain why smokers are more likely to develop severe SARS-CoV-2 infections. Also, the reversibility of ACE2 expression upon smoking cessation suggests that quitting smoking could lessen CoV-2 susceptibility.

- Absence of ACE2 upregulation in other lung inflammation pathologies implies CoV-2 susceptibility might be smoking-specific, and not fibrosis-specific.

- Another preprint showed ACE2 expression increases in lung of patients with CoV-2 co-morbidities such as hypertension [370]; these studies collectively paint a better picture of CoV-2 susceptibility before actual experiments can be carried out.

8.92.5 Credit

Review by Samarth Hegde as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

8.93 The comparative superiority of IgM-IgG antibody test to real-time reverse transcriptase PCR detection for SARS-CoV-2 infection diagnosis

Liu et al. *medRxiv*. [396]

8.93.1 Keywords

- IgM/IgG antibody test
- nucleic acid test
- SARS-CoV-2 detection

8.93.2 Main Findings

The study compares IgM and IgG antibody testing to RT-PCR detection of SARS-CoV-2 infection. 133 patients diagnosed with SARS-CoV-2 in Renmin Hospital (Wuhan University, China) were analyzed. The positive ratio was 78.95% (105/133) in IgM antibody test (SARS-CoV-2 antibody detection kit from YHLO Biotech) and 68.42% (91/133) in RT-PCR (SARS-CoV-2 ORF1ab/N qPCR detection kit). There were no differences in the sensitivity of SARS-CO-V2 diagnosis in patients grouped according to disease severity. For example, IgG responses were detected in 93.18% of moderate cases, 100% of severe cases and 97.3% of critical cases. In sum, positive ratios were higher in antibody testing compared to RT-PCR detection, demonstrating a higher detection sensitivity of IgM-IgG testing for patients hospitalized with COVID-19 symptoms.

8.93.3 Limitations

This analysis only included one-time point of 133 hospitalized patients, and the time from symptom onset was not described. There was no discussion about specificity of the tests and no healthy controls were included. It would be important to perform similar studies with more patients, including younger age groups and patients with mild symptoms as well as asymptomatic individuals. It is critical to determine how early after infection/symptom onset antibodies can be detected and the duration of this immune response.

8.93.4 Significance

The IgM-IgG combined testing is important to improve clinical sensitivity and diagnose COVID-19 patients. The combined antibody test shows higher sensitivity than individual IgM and IgG tests or nucleic acid-based methods, at least in patients hospitalized with symptoms.

8.93.5 Credit

Review by Erica Dalla as part of a project by students, postdocs and faculty at the Immunology Institute of the Icahn School of Medicine, Mount Sinai.

Title: Lectin-like Intestinal Defensin Inhibits 2019-nCoV Spike binding to ACE2

Immunology keywords: defensins, spike protein, intestinal Paneth cells, ACE2 binding

Main Findings:

Human ACE2 was previously identified as the host receptor for SARS-CoV-2. Despite ACE2 being expressed in both lung alveolar epithelial cells and small intestine enterocytes, respiratory problems are the most common symptom after viral infection while intestinal symptoms are much less

frequent. Thus, the authors here investigate the biology behind the observed protection of the intestinal epithelium from SARS-CoV-2. Human defensin 5 (HD5), produced by Paneth cells in the small intestine, was shown to interact with human ACE2, with a binding affinity of 39.3 nM by biolayer interferometry (BLI). A blocking experiment using different doses of HD5 coating ACE2 showed that HD5 lowered viral spike protein S1 binding to ACE2. Further, a molecular dynamic simulation demonstrated a strong intermolecular interaction between HD5 and the ACE2 ligand binding domain. To test HD5 inhibitory effect on S1 binding to ACE2, human intestinal epithelium Caco-2 cells were preincubated with HD5. Preincubation strongly reduced adherence of S1 to surface of cells. HD5 was effective at a concentration as low as 10 μg/mL, comparable to the concentration found in the intestinal fluid.

Limitations:

The study focuses exclusively on intestinal cells. However, HD5 could have been tested to block ACE2-S1 binding in human lung epithelial cells as a potential treatment strategy. It would be useful to know whether HD5 could also prevent viral entry in lung cells.

Relevance:

This work provides the first understanding of the different efficiency of viral entry and infection among ACE2-expressing cells and tissues. Specifically, the authors show that human defensin 5 produced in the small intestine is able to block binding between S1 and ACE2 necessary for viral entry into cells. The study provides a plausible explanation on why few patients show intestinal symptoms and suggests that patients with intestinal disease that decrease defensins' production may be more susceptible to SARS-CoV-2. It also indicates that HD5 could be used as a molecule to be exogenously administered to patients to prevent viral infection in lung epithelial cells.

Title:

Susceptibility of ferrets, cats, dogs and different domestic animals to SARS-coronavirus-2

Immunology keywords: SARS-CoV-2, ferret, cat, laboratory animal, domestic animals

The main finding of the article:

This study evaluated the susceptibility of different model laboratory animals (ferrets), as well as companion (cats and dogs), and domestic animals (pigs, chickens and ducks) to SARS-CoV-2. They tested infection with two SARS-CoV2 isolates, one from an environmental sample collected in the Huanan Seafood Market in Wuhan (F13-E) and the other from a human patient in Wuhan (CTan-H).

Ferrets were inoculated with either of the two viruses by intranasal route with 10^5 pfu, and the viral replication was evaluated. Two ferrets from each group were euthanized on day 4 post infection (p.i.). AT day 4 p.i., viral RNA and infectious viruses were detected only in upper respiratory tract (nasal turbinate, upper palate, tonisls, but not in the trachea, lungs or other tissues. Viral RNA and virus titer in the remaining ferrets were monitored in nasal washes and rectal swabs on days 2, 4, 6, 8 and 10 p.i. Viral RNA and infectious viruses were detected in nasal washes until day 8 p.i. One ferret in each group developed fever and loss of appetite on days 10 and 12 p.i., however, viral RNA was practically undetactable. These two ferrets showed severe lymphoplasmacytic perivasculitis and vasculitis in the lungs and lower antibody titers compare to other 4 ferrets.

Cats. Five *s*ubadult 8-month-old domestic cats were inoculated with CTan-h virus and three uninfected cats were placed in a cage adjacent to each of the infected cats to monitor respiratory droplet transmission. Viral RNA was detected in the upper respiratory organs from all infected cats and in one

out of three exposed cats. All infected (inoculated and exposed) cats developed elevated antibodies against SARS-CoV2. Viral replication studies with juvenile cats (70-100 days) revealed massive lesions in the nasal and tracheal mucosa epithelium and lungs of two inoculated cats which died or were euthanized on day 3 p.i., and infection in one out of three exposed cats. These results indicated SARS-CoV2 could replicate in cats, that juvenile cats were more susceptible that adults, and theat SARS-CoV2 could be transmit via respiratory droplets between cats.

Dogs and others. Five 3-month-old beagle dogs were inoculated and housed with two uninoculated beagles in a room. Two virus inoculated dogs seroconverted, but others including two contact dogs were all seronegative for SARS-CoV2 and infectious virus was not detected in any swabs collected. Viral RNA was not detected in swabs from pigs, chickens, and ducks inoculated or contacted. These results indicated that dogs, pigs, chickens, and ducks might have low or no susceptibility to SARS-CoV2.

Critical analysis of the study:

This manuscript describes the viral replication and clinical symptoms of SARS-CoV2 infection in ferrets, and the SARS-CoV2 infection and transmission in cats. Clinical and pathological analysis was not performed in cats, therefore the correlation of virus titer with symptoms severity in the adult and juvenile cats could not be determined.

The importance and implications for the current epidemics:

SARS-CoV-2 transmission to tigers, cats and dogs has been previously reported. It should be noted that this manuscript did not evaluate the transmission from cats to human. Nevertheless, it clearly showed higher susceptibility of ferrets and domestic cats to SARS-CoV-2. This data strongly indicates the need for surveillance of possible infection and transmission of SARS-CoV-2 by domestic cats.

8.94 Virus-host interactome and proteomic survey of PMBCs from COVID-19 patients reveal potential virulence factors influencing SARS-CoV-2 pathogenesis

Li et al. *bioRxiv*. [65]

8.94.1 Keywords

- PBMC
- virulence factors – interaction network – nsp9
- nsp10 – NKRF

8.94.2 Main findings

The authors identified **intra-viral protein-protein interactions** (PPI) with two different approaches: genome wide yeast-two hybrid (Y2H) and co-immunoprecipitation (co-IP). A total of 58 distinct PPI were characterized. A screen of **viral-host PPI** was also established by overexpressing all the SARS-CoV-2 genes with a Flag epitope into HEK293 cells and purifying each protein complex. Interacting host proteins were then identified by liquid chromatography and tandem mass spectrometry. 251 cellular proteins were identified, such as subunits of ATPase, 40S ribosomal proteins, T complex proteins and proteasome related proteins, for a total of 631 viral-host PPI. Several interactions suggesting protein-mediated modulation of the immune response were identified, highlighting the multiple ways SARS-CoV-2 might reprogram infected cells.

Subsequently, the authors compared global proteome profiles of PBMCs from healthy donors (n=6) with PBMC from COVID-19 patients with mild (n=22) or severe (n=13) symptoms. 220 proteins were found to be differentially expressed between *healthy donors and mild COVID-19 patients*, and a pathway analysis showed **a general activation of the innate immune response.** 553 proteins were differentially expressed between the PBMC of *mild and severe COVID-19 patients*, most of them (95%) being downregulated in severe patients. Functional pathway analysis indicated a defect of T cell activation and function in severe COVID-19. There was also evidence suggesting reduced antibody secretion by B cells. Together, these results suggest a **functional decline of adaptive immunity**. A FACS analysis of PBMC from severe patients indicated higher levels of IL6 and IL8 but not IL17 compared to mild patients.

Finally, the authors focused on NKRF, an endogenous repressor of IL8/IL6 synthesis that was previously identified as interacting with SARS-Cov-2 nsp9,10,12,13 and 15. Individually expressed nsp9 and nsp10 (but not nsp12, nsp13, nsp15) induced both IL6 and IL8 in lung epithelial A459 cells, indicating that nsp9 and nsp10 may be directly involved in the induction of these pro-inflammatory cytokines. The authors finally argue that nsp9 and nsp10 represent potential drug targets to prevent over-production of IL6 and IL8 in infected cells, and reducing the over-activation of neutrophils.

8.94.3 Limitations

First, the authors seem to have forgotten to include the extended data in the manuscript, and their proteomic data does not seem to be publicly available for the moment, which limits greatly our analysis of their results.

While this work provides important data on host and viral PPI, only 19 interactions were identified by Y2H system but 52 with co-IP. The authors do not comment about what could lead to such differences between the two techniques and they don't specify whether they detected the same interactions using the two techniques.

Moreover, the PBMC protein quantification was performed comparing bulk PBMC. Consequently, protein differences likely reflect differences in cell populations rather than cell-intrinsic differences in protein expression. While this analysis is still interesting, a similar experiment performed on pre-sorted specific cell populations would allow measuring proteome dynamics at a higher resolution.

Finally, the authors did not discussed their results in regards to another SARS-CoV-2 interactome of host-viral PPI that had been published previously[1]. This study reported 332 host-virus PPI, but no interaction of viral proteins with NKRF was found. Some interactions were found in both studies (eg. N and G3BP1, Orf6 and RAE1). However, the time point used to lyse the cells were different (40h previously vs 72h here), which could explain some of the differences.

8.94.4 Relevance

The identification of many interactions between intra-viral and host-virus PPI provides an overview of host protein and pathways that are modulated by SARS-CoV-2, which can lead to the identification of potential targets for drug development.

In the model proposed by the authors, nsp9 and nsp10 from SARS-Cov-2 induce an over-expression of IL6 and IL8 by lung epithelial cells, which recruits neutrophils and could lead to an excess in lung infiltration. Nsp9 has been shown to be essential for viral replication for SARS-Cov-1[2], and shares a 97% homology with nsp9 from SARS-Cov-2[3]. Further, nsp9 crystal structure was recently solved[3], which can help to develop drug inhibitors if this protein is further confirmed as being important for the virulence of SARS-Cov-2.

1. Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing. *bioRxiv*. March 2020:2020.03.22.002386. doi:10.1101/2020.03.22.002386

2. Miknis ZJ, Donaldson EF, Umland TC, Rimmer RA, Baric RS, Schultz LW. Severe acute respiratory syndrome coronavirus nsp9 dimerization is essential for efficient viral growth. *J Virol*. 2009;83(7):3007-3018. doi:10.1128/JVI.01505-08

3. Littler DR, Gully BS, Colson RN, Rossjohn J. *Crystal Structure of the SARS-CoV-2 Non-Structural Protein 9, Nsp9*. Molecular Biology; 2020. doi:10.1101/2020.03.28.013920

Title: Prediction and Evolution of B Cell Epitopes of Surface Protein in SARS-CoV-2

Keywords: SARS-CoV-2; Epitopes; Bioinformatics; Evolution

Summary/Main findings:

Lon et al. used a bioinformatic analysis of the published SARS-CoV-2 genomes in order to identify conserved linear and conformational B cell epitopes found on the spike (S), envelope (E), and membrane (M) proteins. The characterization of the surface proteins in this study began with an assessment of the peptide sequences in order to identify hydrophilicity indices and protein instability indices using the Port-Param tool in ExPASy. All three surface proteins were calculated to have an instability score under 40 indicating that they were stable. Linear epitopes were identified on the basis of surface probability and antigenicity, excluding regions of glycosylation. Using BepiPred 2.0 (with a cutoff value of 0.35) and ABCpred (with a cutoff value of 0.51), 4 linear B cell epitopes were predicted for the S protein, 1 epitope for the E protein, and 1 epitope for the M protein. For structural analysis, SARS-CoV assemblies published in the Protein Data Bank (PDB) acting as scaffolds for the SARS-CoV-2 S and E amino acid sequences were used for input into the SWISS-MODEL server in order to generate three-dimensional structural models for the assessment of conformational epitopes. Using Ellipro (cutoff value of 0.063) and SEPPA (cutoff value of 0.5), 1 conformational epitope was identified for the S protein and 1 epitope was identified for the E protein, both of which are accessible on the surface of the virus. Finally, the Consurf Server was used to assess the conservation of these epitopes. All epitopes were conserved across the published SARS-CoV-2 genomes and one epitope of the spike protein was predicted to be the most stable across coronavirus phylogeny.

Critical Analysis/Limitations:

While this study provides a preliminary identification of potential linear and conformational B cell epitopes, the translational value of the epitopes described still needs extensive experimental validation to ascertain whether these elicit a humoral immune response. The conformational epitope analyses are also limited by the fact that they are based off of predicted 3D structure from homology comparisons and not direct crystal structures of the proteins themselves. Additionally, since there was not a published M protein with a high homology to SARS-CoV-2, no conformational epitopes were assessed for this protein. Finally, while evolutionary conservation is an important consideration in understanding the biology of the virus, conservation does not necessarily imply that these sites neutralize the virus or aid in non-neutralizing *in vivo* protection.

Relevance/Implications:

With further experimental validation that confirms that these epitopes induce effective antibody responses to the virus, the epitopes described can be used for the development of treatments and vaccines as well as better characterize the viral structure to more deeply understand pathogenesis.