Peer-reviewed version available at *Diagnostic Microbiology and Infectious Disease* **2020**; doi:10.1016/j.diagmicrobio.2020.115094

Clinical, molecular and epidemiological characterization of the SARS-CoV2 virus and the Coronavirus disease 2019 (COVID-19), a comprehensive literature review

Esteban Ortiz-Prado*[1], Katherine Simbaña-Rivera[1], Lenin Gómez- Barreno[1], Mario Rubio-Neira[2], Linda P. Guaman[3], Nikolaos kyriakidis[4], Claire Muslin[1], Ana María Gómez Jaramillo[5], Carlos Barba[1], Doménica Cevallos[6], Hugo Sanches-SanMiguel[1], Luis Unigarro[7], Rasa Zalakeviciute[8,9], Naomi Gadian[10], Andrés López-Cortés[11,12]

[1] One Health Research Group, Faculty of Medicine, Universidad de Las Americas (UDLA), Quito, Ecuador

[2] Hospital Baca Ortiz, Pediatric and Cardiology Department, Quito, Ecuador

[3] Centro de Investigación Biomédica, Facultad de Ciencias de la Salud Eugenio Espejo, Universidad UTE, Quito, Ecuador

[4] Grupo de Investigación en Biotecnología Aplicada a Biomedicina (BIOMED), Universidad de la Americas, Quito, Ecuador

[5] Faculty of Medicine, Pontificia Universidad Católica del Ecuador, Quito, Ecuador

[6] Intensive Care Unit, Hospital Quito Sur IESS, Quito, Ecuador

[7] Intensive Care Unit, Hospital SOLCA Quito., Quito, Ecuador

[8] Grupo de Biodiversidad Medio Ambiente y Salud (BIOMAS), Universidad de Las Americas, Quito, Ecuador.

[9] Intelligent and Interactive Systems Lab (SI2 Lab) Universidad de Las Americas (UDLA), Quito, Ecuador

[10] University of Southampton, Department of Public Health, Southampton, United Kingdome

[11] Centro de Investigación Genética y Genómica, Facultad de Ciencias de la Salud Eugenio Espejo, Universidad UTE, Quito, Ecuador

[12] Red Latinoamericana de Implementación y Validación de Guías Clínicas Farmacogenómicas (RELIVAF-CYTED), Quito, Ecuador

Esteban Ortiz-Prado: e.ortizprado@gmail.com

Katherine Simbaña-Rivera: ksimbanarivera@gmail.com

Lenin Gómez- Barreno: lsgomezbarreno@gmail.com

Mario Rubio: drrubio24@yahoo.com

Linda P. Guaman: linda.guaman@ute.edu.ec

Nikolaos kyriakidis: nikolaos.kyriakidis@udla.edu.ec

Claire Muslin: claire.muslin@udla.edu.ec

Carlos Barba: carlos.barba.ostria@udla.edu.ec

Ana María Gómez Jaramillo: mdanamagomez84@gmail.com

Carlos Barba: carlos.barba.ostria@udla.edu.ec

Doménica Cevallos: domecevallos.92@gmail.com

Hugo Sanches-SanMiguel: hmssanmiguel@icloud.com

Luis Unigarro: luisunigarro3@gmail.com

Rasa Zalakeviciute: rasa.zalakeviciute@udla.edu.ec

Naomi Gadian: naomigadian@hotmail.com

Andrés López-Cortés: aalc84@gmail.com

Corresponding author: Esteban Ortiz-Prado One Health Research Group, Universidad de las Américas, Quito, Ecuador Calle de los Colimes y Avenida De los Granados, Quito 170137, Ecuador. Email: e.ortizprado@gmail.com Phone: +593995760693

Abstract

Coronaviruses are an extensive family of viruses that can cause disease in both animals and humans. The current classification of coronaviruses recognizes 39 species in 27 subgenera that belong to the family Coronaviridae. From those, at least seven coronaviruses are known to cause respiratory infections in humans. Four of these viruses can cause common cold-like symptoms, while others that infect animals can evolve and become infectious to humans. Three recent examples of this viral jumps include SARS CoV, MERS-CoV and SARS CoV-2 virus. They are responsible for causing severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and the most recently discovered coronavirus disease during 2019 (COVID-19).

COVID-19, a respiratory disease caused by the SARS-CoV-2 virus, was declared a pandemic by the World Health Organization (WHO) on 11 March 2020. The rapid spread of the disease has taken the scientific and medical community by surprise. Latest figures from 14 April 2020 show more than 2 million people had been infected with the virus, causing more than 120,000 deaths in over 210 countries worldwide.

The large amount of information we receive every day concerning this new disease is so abundant and dynamic that medical staff, health authorities, academics and the media are not able to keep up with this new pandemic.

In order to offer a clear insight of the extensive literature available, we have conducted a comprehensive literature review of the SARS CoV-2 Virus and the Coronavirus Diseases 2019 (COVID-19).

Key words: Covid-19; Coronavirus; SARS-CoV-2; Review**;** Pandemic;

Background

The new COVID-19 disease is caused by a novel coronavirus (SARS-CoV2) probably originated in Wuhan, China. In mid- December 2019, the Wuhan health authorities detected few cases of an atypical pneumonia that eventually was discovered to be caused by a novel coronavirus that probably jumped from an animal reservoir to a human during the first week of November 2019 [1].

Subsequent investigations discovered that the etiological agent was a RNA virus related to same family of coronavirus that caused the Severe Acute Respiratory Syndrome (SARS) and to Respiratory Syndrome of Middle East (MERS) pandemic during 2003 and 2012 respectively[2].

The specific origin of this new pandemic is not totally understood. At the beginning of the outbrake it was believed that a viral jump occurred between a wild animal and a human being in one of the most populated *wet market* in Wuhan, China during the November 2019. Further investigations were focused in finding which animals were responsible for this new zoonotic diseases, although still unclear which animal is the intermediary host, is well-known that bats are the main reservoirs for these type of virus and they probably emerged in one of the local wild-animal farms[3, 4].

Chronology of the pandemic

The Center for Disease Control in China (CDCC) reported that during the last week of December 2019, the first cases of an atypical pneumonia were seen in Wuhan, the capital of Central China's Hubei province. Days later, after the first cases were reported, the Chinese health authorities decided to close the Huanan's "wet market" after some research suggested this place as the probable initial source of contagion[5].

During the first week of January, China's authorities announced that the new atypical pneumonia was not caused by either the SARS or the MERS coronavirus, but a new variant of the Coronaviriade family, a newly discovered virus called SARS-CoV2[5].

In January 11th the first SARS CoV-2 related death was reported and one day later, a group of Chinese researchers reveal the genome of the virus implicated in the Wuhan pneumonia outbreak.

From the initial case reported in China, the SARS-CoV-2 virus spread worldwide. At the beginning of the outbrake it started to move through Asia but only days later the first suspicious cases were reported in Europe and North America. In March 11th the World Health Organization (WHO) declare this disease a worldwide distributed pandemic. Since the first case and using the latest figures from April the 14th , 2020 more than 2 million people had been infected with the virus, causing more than 120,000 deaths in over 210 countries worldwide[6].

Structure and genome of the SARS-CoV-2 virion

The family *Coronaviridae* is a large group of viruses infecting animals and humans. There are seven types of human coronaviruses that are primarily respiratory pathogens: 229E, NL63, OC43, KHU1, Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). MERS-CoV, SARS-CoV and SARS-CoV-2 belong to genus *Betacoronavirus* and all have high mutation rates that result in viral genetic diversity, plasticity, and adaptability to invade a wide range of hosts[7].

Like other coronaviruses, SARS-CoV-2 is an enveloped virus with roughly spherical or moderately pleomorphic virions of approximately 60 to 140 nm in diameter (Figure 1a) [8].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2020 doi:10.20944/preprints202004.0283.v1

Peer-reviewed version available at *Diagnostic Microbiology and Infectious Disease* **2020**; doi:10.1016/j.diagmicrobio.2020.115094

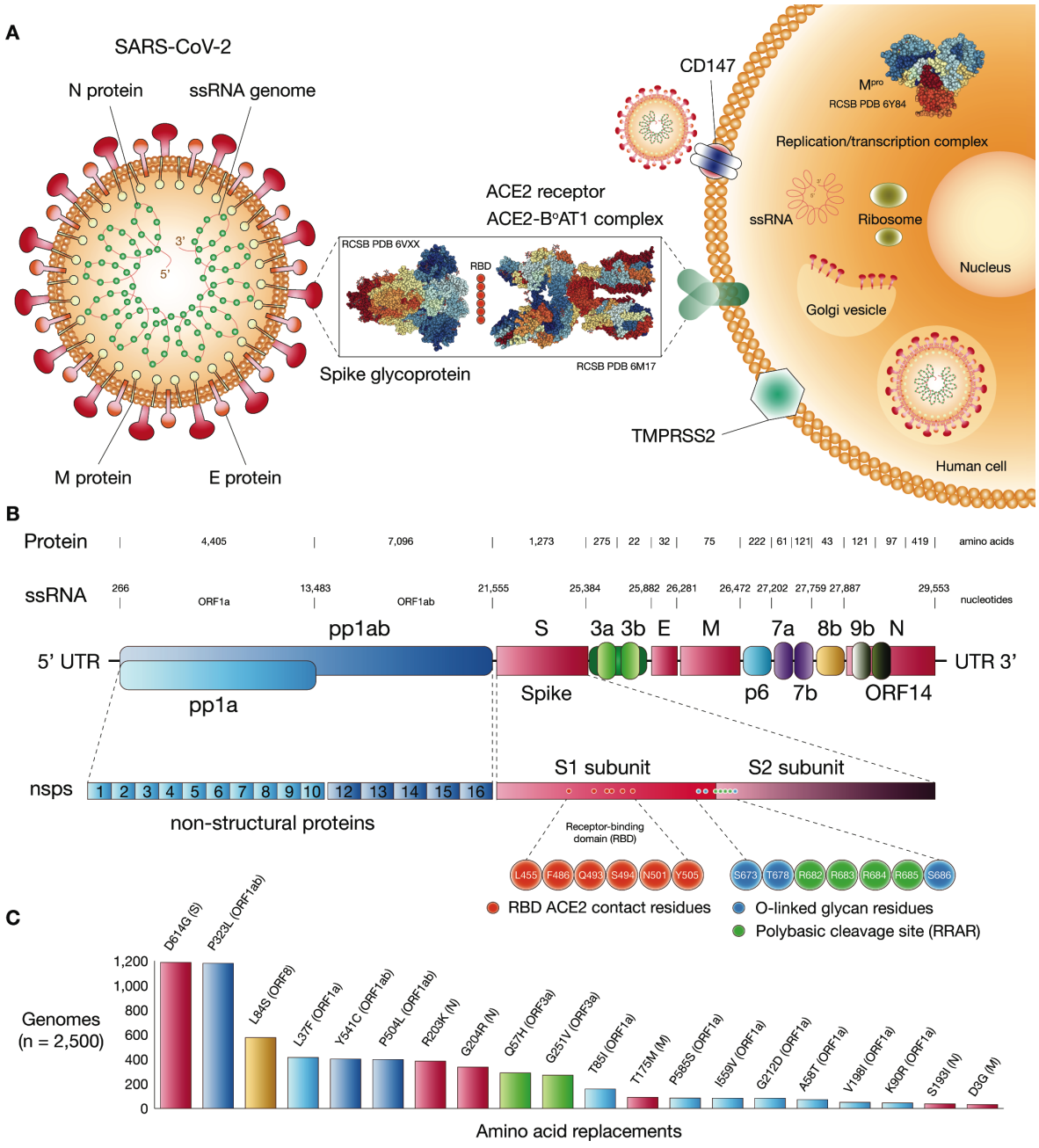

Figure 1 Overall structure and mechanism of infection of SARS-CoV-2. A) Structure and mechanism of infection of the novel coronavirus into human cells through the spike glycoprotein, the ACE2 receptor protein, and the CD147 receptor. The structure of the spike glycoprotein was taken from RCSB PDB 6VXX according to Walls et al. [9]; the structure of the ACE2-BoAT1 complex was taken from RCSB PDB 6M17 according to Yan et al. [10]; lastly, the structure of the main protease (Mpro) was taken from RCSB PDB 6Y84 according to Zhang et al. [11]. B) Genomic structure and proteins encoded by SARS-CoV-2. C) Genomic structure and proteins encoded by SARS-CoV-2. B) Most frequent amino acid replacements in genomes analyzed worldwide.

The viral membrane contains the spike (S) glycoprotein that forms the peplomers on the virion surface, giving the virus its 'corona'- or crown-like morphology in the electron microscope. The membrane (M) glycoprotein and the envelope (E) protein provide the ring

structure. Within the virion interior lies a helical nucleocapsid comprised of the nucleocapsid (N) protein complexed with a single positive-strand RNA genome of about 30 kb in length [12].

The first genome of SARS-CoV-2 named Wuhan-Hu-1 (NCBI reference sequence NC_045512) was isolated and sequenced in China in January 2020 [8, 12]. The SARS-CoV-2 genome has similarities to other viruses: approximately 96% similarity to the bat coronavirus BatCoV RaTH13; an estimated 80% similarity with SARS-CoV [12], and an estimated 50% identity with MERS-CoV [13, 14]. SARS-CoV-2 has a positive-sense single-stranded RNA genome. It is approximately 30,000 bases in length and comprises of a 5′ terminal cap structure and a 3′ poly A tail. According to Wu *et al*. [15], this novel coronavirus (IVDC-HB-01/2019 strain) has 14 open reading frames (ORFs) encoding 27 proteins. The 5' terminus of the genome contains the ORF1ab and ORF1a genes. ORF1ab is the largest gene and encodes the pp1ab protein that contains 15 non-structural proteins named nsps (nsp1-nsp10 and nsp12-nsp16). ORF1a encodes the pp1a protein and also has 10 nsps (nsp1-nsp10) [15]. The 3' terminus of the genome contains four structural proteins: spike (S) glycoprotein; envelope (E) protein; membrane (M) glycoprotein and nucleocapsid (N) phosphoprotein. It also contains 8 accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b and ORF14) [16] (Figure 1b).

The global scientific community from 52 countries have united to study this novel coronavirus by sequencing and submitting 2,500 SARS-CoV-2 genomes to the Global Initiative on Sharing All Influenza Data (GISAID) (https://www.gisaid.org/) between December 2019 and March 2020 [17, 18]. SARS-CoV-2 has accumulated mutations in its RNA genome as the outbreak progresses.

From the 2,500 viral genomes of SARS-CoV02 sequences analyzed to date in the outbreak, the CoV-GLUE project (http://cov-glue.cvr.gla.ac.uk/#/home) has identified 1,539 amino acid replacements, 3 insertions, and 18 coding region deletions.. Regarding amino acid replacements, 206 mutations were found in nsp3 (ORF1a) corresponding to the papain-like protease (PL^{pro}) / transmembrane domain 1; 146 were found in the S glycoprotein; 89 were found in nsp2 (ORF1a); 61 were found in the N phosphoprotein; 59 were found in nsp12

(ORF1ab) corresponding to the RNA-dependent RNA polymerase (RdRp); 56 were found in nsp14 (ORF1ab) corresponding to the 3'-5' exonuclease; 50 were found in nsp4 (ORF1a) corresponding to the transmembrane domain 2; 48 were found in nsp13 (ORF1ab) corresponding to the Zinc-binding domain / helicase domain; 43 were found in nsp15 (ORF1ab) corresponding to the endoRNAse; 39 were found in ORF3a, 41 were found in nsp16 (ORF1ab) corresponding to the 2'-O-ribose methyltransferase; 41 were found in nsp6 (ORF1a) corresponding to the putative transmembrane domain; 38 were found in ORF7a; 27 were found in nsp5 (ORF1a) corresponding to the 3C-like proteinase; 29 were found in nsp1 (ORF1a); 26 were found in the M glycoprotein; 22 were found in ORF8; 17 were found in nsp10 (ORF1a); 15 were found in ORF10 and ORF6; 14 were found in nsp8 corresponding to the putative primase; 13 were found in the E protein; 9 were found in nsp9 corresponding to the ssRNA-binding domain; 8 were found in nsp7; 7 were found in ORF7b; and 2 were found in nsp11. The most prevalent amino acid replacements were D614G (S glycoprotein) in 1,188 genomes, P323L (RdRp) in 1,181 genomes, L84S (ORF8) in 576 genomes, L37F (putative transmembrane domain) in 415 genomes, and Y541C (Zinc-binding domain) in 402 genomes (Figure 1c and Supplementary Table 1).

SARS-CoV-2 replication cycle

As an intracellular obligate microorganism, the coronavirus exploits the host cell machinery for its own replication and spread. Since virus–host interactions form the basis of diseases, knowledge about their interplay is of great importance, particularly when identifying key targets for antivirals.

SARS-CoV-2 entry into host cells is mediated by the transmembrane S glycoprotein that forms homotrimers protrunding from the viral surface (Figure 1a) [9]. Coronavirus S protein consists of two functional subunits: S1 subunit, where the receptor-binding domain (RBD) is found and is responsible for binding host cell surface receptors and S2 subunit, which mediates subsequent fusion between the viral and host cellular membranes [19, 20].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2020 doi:10.20944/preprints202004.0283.v1

Peer-reviewed version available at *Diagnostic Microbiology and Infectious Disease* **2020**; doi:10.1016/j.diagmicrobio.2020.115094

SARS-CoV-2 RBD directly binds to the peptide domain of angiotensin-covering enzyme 2 (ACE2), which is also the cellular receptor for SARS-CoV [9, 10, 21, 22]. RBD is the most variable part of SARS-CoV-2 genome [12, 23]. Six RBD amino acids (L455, F486, Q493, S494, N501 and Y505) are involved in the binding to ACE2 receptors [24], and five of these six residues differ between SARS-CoV and SARS-CoV-2 [25] (Figures 1a and 1b).

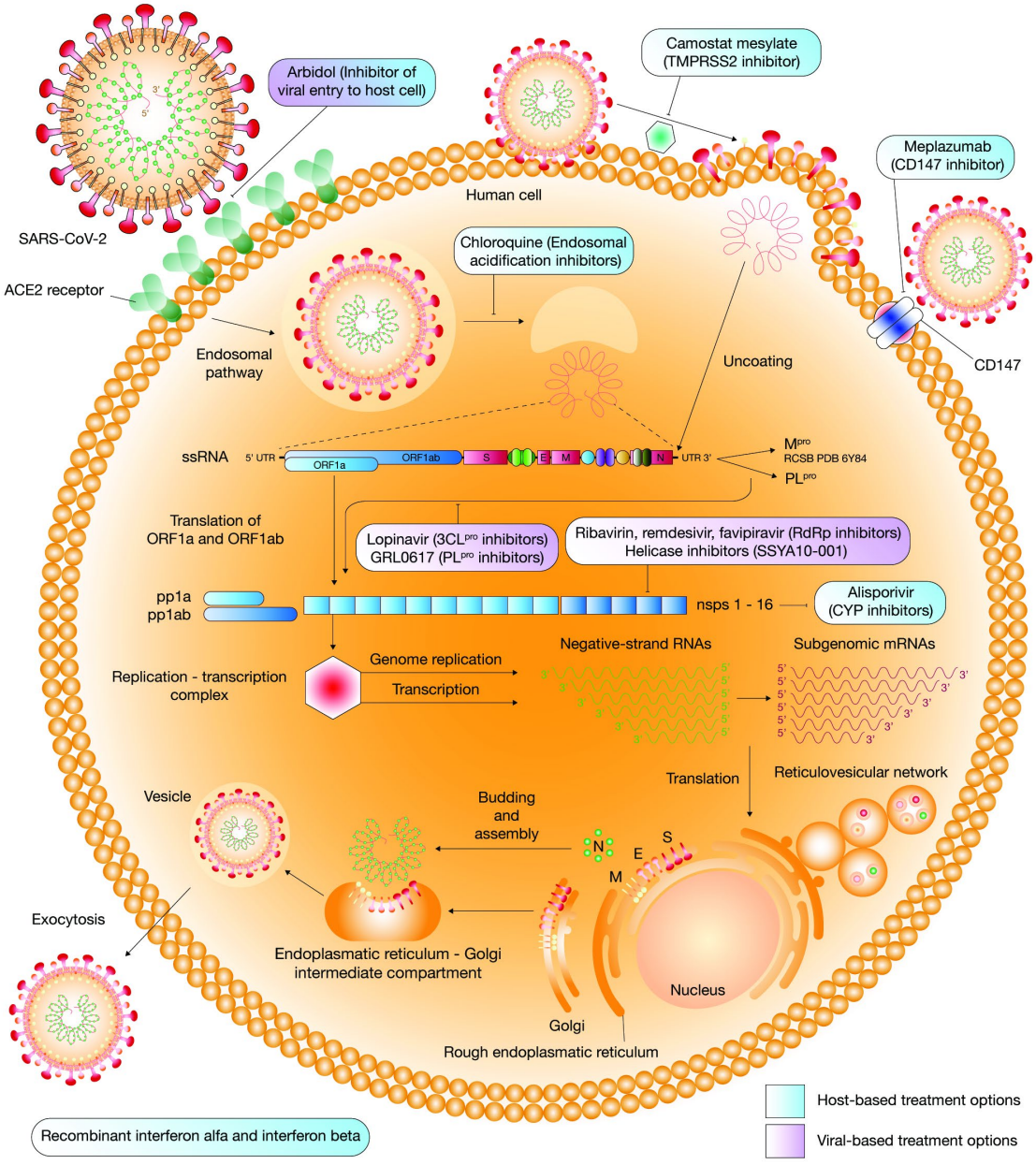

Figure 2 . SARS-CoV-2 replication cycle and its inhibitors. SARS-CoV-2 infection begins with the attachment of the spike (S) protein with the host cell receptor. Two cellular receptors have been identified for SARS-CoV-2 so far: angiotensin-

converting enzyme 2 (ACE2) and CD147. After receptor interaction, the cleavage of S protein by the cell surface-associated transmembrane protease serine 2 TMPRSS2 promotes the fusion of viral and cell membranes. Following the release of the nucleocapsid to the cytoplasm, the viral genomic RNA is translated through ribosomal frameshifting to produce polyproteins pp1a and pp1ab, which undergo cotranslational proteolytic processing into the 15 non-structural proteins (nsp1-nsp10 and nsp12-nsp16) that form the replication-transcription complex (RTC). The RTC is involved in the genomic RNA replication and in the transcription of a set of nested subgenomics mRNAs required to express the structural and accessory protein genes. New virions are assembled by budding into the intracellular membranes of the ER - Golgi intermediate compartment membranes and released through exocytosis. Additionally, there are detailed host-based treatment options in blue and viral-based treatment options in pink.

ACE2 is a type I membrane protein that participates in the maturation of angiotensin, a peptide hormone that controls vasoconstriction and blood pressure [26]. In the respiratory tract, ACE2 is widely expressed on the epithelial cells of alveoli, trachea, bronchi, bronchial serous glands [27], and alveolar monocytes and macrophages [28]. Xu *et al*. reported the [29] RNA-seq profiling data of 13 organs with para-carcinoma normal tissues from The Cancer Genome Atlas (TCGA; https://www.cancer.gov/tcga) and 14 organs with normal tissue from FANTOM5 CAGE (https://fantom.gsc.riken.jp/). These were used to validate the expression of the human cell receptor ACE2 in the virus and may indicate the potential infection routes of SARS-CoV-2 [30]. Interestingly, the ACE2 receptor is expressed more in oral cavity than lung. This potentially could indicate that susceptibility and infectivity of SARS-CoV-2 is greater from oral mucosa surfaces. [29].

Following the binding of the RBD in the S1 subunit to the receptor ACE2, SARS-CoV-2 S protein is cleaved by the cell surface-associated transmembrane protease serine 2 TMPRSS2, which activates S2 domain for membrane fusion between the viral and cell membrane [31]. A functional polybasic (furin) cleavage site was found at the S1-S2 boundary through the insertion of 12 nucleotides [9, 25, 32]. The S673, T678 and S686 residues of O-linked glycans flank the cleavage site and are unique in SARS-CoV-2 [25].

In addition to the S glycoprotein - ACE2 receptor complex, Wang *et al*. reported an alternative route where SARS-CoV-2 invades host cell through the S glycoprotein – CD147 complex. These findings were validated using co-immunoprecipitation, ELISA, and *in vitro* antiviral tests with meplazumab. This anti-CD147 humanized antibody significantly inhibited the viruses from invading host cells (https://doi.org/10.1101/2020.03.14.988345) Paper: SARS-CoV-2 invades host cells via a novel route: CD147-spike protein.

Like SARS-CoV and other coronaviruses, SARS-CoV-2 likely enter target cells through receptor-mediated endocytosis, where fusion of the virus envelops the endosome membranes and leads to the release of the viral nucleocapsid into the cytosol of the infected cell [33].

Following the release and uncoating of viral RNA to the cytoplasm, coronavirus replication starts with the translation of ORF1a and ORF1b into polyproteins pp1a and pp1ab via a frameshifting mechanism (Figure 2) [34]. Subsequently, polyproteins pp1a and pp1ab are processed by internal viral proteases, including the main protease M^{pro}, a potential drug target whose crystal structure was recently determined for SARS-CoV-2 [11]. Polyprotein cleavage yields 15 mature replicase proteins, which assemble into a replication-transcription complex that engages in negative-strand RNA synthesis. Both full-length and multiple subgenomic negative-strand RNAs are produced. The former serves as template for new full-length genomic RNAs and the latter template the synthesis of the subgenomic mRNAs required to express the structural and accessory protein genes residing in the 3′-proximal quarter of the genome [33]. Coronavirus RNA replication occurs on a virus-induced reticulovesicular network of modified endoplasmic reticulum (ER) membranes [35].

The assembly of virions is quickly ensued with the accumulation of new genomic RNA and structural components. The N protein complexes with genome RNA, forming helical structures. Then, the transmembrane M protein, localized to the intracellular membranes of the ER - Golgi intermediate compartment (ERGIC), interacts with the other viral structural proteins (S, E and N proteins) to allow the budding of virions [36, 37]. Following assembly and budding, virions are transported in vesicles and eventually released by exocytosis.

SARS CoV-2 and human immune responses

Normal immune responses against the majority of viruses involves a rapid containment response mediated by innate immunity components. These include antiviral Type I IFNs, pro-inflammatory cytokine production and NK cells, and a delayed virus-tailored adaptive

immune response aiming to eradicate the pathogen and produce long-lasting memory. The latter involves antigen specific CD8+ cytotoxic T cells (CTLs), the Th1 subset of CD4+ T helper cells that orchestrates the immune response against viruses and other intracellular pathogens, specific antibody producing plasma cells, and finally the production of memory T and B cell subsets.

Immune system responses following SARS-CoV-2 infection can be a double-edged sword. The response can lead to virus clearance and immune memory or, for others, cause severe pathology that can lead to pneumonia, ARDS, septic shock, multi-organ failure and, eventually, death.

Accordingly, patients who have immune system is weakened or otherwise dysregulated, such as older men with comorbidities severe COVID-19 is clearly more likely to occur [38–40].

Innate immunity:

Type I IFNs are mainly produced by plasmacytoid dendritic cells (pDCs) and have a plethora of antiviral effects such as blocking cell entry and trafficking of viral particles, inducing RNase and DNase expression to degrade virus genetic material, enhancing presentation of viral antigens by MHC-I, inhibiting protein synthesis and inducing apoptosis of infected cells [41].

Pathogen recognition receptors like cytosolic RIG-I and MDA-5 [42, 43] or endosomal Toll like receptors (TLRs) 7 and 8 that recognize viral RNA [44] are responsible for the activation of signaling cascades that activate the transcription factors NF-kB, interferon regulatory factor (IRF) 3 and IRF7 that translocate to the nucleus and induce proinflammatory cytokines and Type I interferon (IFN) production. In turn, Type I IFNs activate the downstream JAK-STAT signal pathway resulting in expression of IFN-stimulated genes (ISGs) [45, 46].

Our experience from SARS-CoV and MERS-CoV infection has shown that delayed type I IFN production and excessive recruitment and activation of infiltrating proinflammatory cells

(neutrophils and monocytes-macrophages) are possible mediators of lung dysfunction and bad prognostic factors for the outcome of the infection. Delayed type I IFN production allows for highly efficient viral replication that, in turn, results in recruitment of hyperinflammatory neutrophils and monocytes. Therefore, the pathogen recognition receptors (PRRs) of these proinflammatory cells recognize high numbers of their ligands and subsequently secrete excessive amounts of proinflammatory cytokines that lead to septic shock, lung pathology, pneumonia or acute respiratory distress syndrome [47–49].

It has been shown that in severe cases both SARS-CoV and MERS-CoV fruitfully employ an immune evasion mechanism whereby early type I IFN responses to viral infection are dampened [48]. This can be achieved by blocking signaling both upstream, as well as downstream of type I IFN expression. SARS-CoV can inhibit IRF3 nuclear translocation, whereas MERS-CoV can impede histone modification [50]. Additionally, both viruses can inhibit IFN signaling by decreasing STAT1 phosphorylation [51]. Due to the many sequence similarities of SARS-CoV-2 with SARS-CoV and MERS-CoV it would be enticing to speculate that similar mechanisms are also present, however further studies are needed to shed light to this hypothesis.

Hyperactivated neutrophils and monocytes-macrophages are the usual source of the cytokine storm. In this aspect, absolute neutrophil counts and neutrophil to lymphocyte ratio (NLR) were strongly associated with disease severity in a large cohort of COVID-19 patients and were proposed as markers of adverse disease prognosis [52].

Interestingly, the increased amounts of proinflammatory cytokines in serum associated with pulmonary inflammation and extensive lung damage described both in SARS [53] and MERS diseases [54] were also reported in the early study of 41 patients with COVID-19 in Wuhan [39]. Evidence shows that the leading cause of COVID-19 mortality is respiratory failure caused by acute respiratory distress syndrome (ARDS). There is an association with a cytokine storm mediated by high-levels of proinflammatory cytokines including IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A and TNF-α. ARDS was associated with increased fatality and subsequent studies confirmed IL-6 and C-reactive protein are significantly upregulated in patients that died compared to convalescent patients [50]. Moreover, a recent

study of 452 patients in Wuhan identified that severe cases showed significantly higher cytokines and chemokines such as tumor necrosis factor-α (TNF-α), IL-2, IL-6, IL-8 and IL-10 expressed [52].

In accordance with these findings, therapeutic strategies are being tested. A phase 3 randomized controlled trial of IL-1 blockade (anakinra) in sepsis has shown significant survival benefit in patients with hyperinflammation, without apparent increased adverse events [55]. Currently, a multicenter, randomized controlled trial of tocilizumab (IL-6 receptor blockade, licensed for cytokine release syndrome), is being trialled in patients with COVID-19 pneumonia presenting with high levels of IL-6 in China (ChiCTR2000029765) [56]. Moreover, several clinical trials are exploring if the well-established antiviral [57] and anti-inflammatory effects of hydroxychloroquine will be effective in treating patients with COVID-19 as has previously been suggested for SARS-CoV infection [58]. This has also been demonstrated *in vitro* for SARS-CoV-2 [59]. In contrast, Janus kinase (JAK) inhibition has been proposed as a potential treatment in order to reduce both inflammation and cellular viral entry in COVID-19 [60]. Thus, it comes as no surprise that in a recent correspondence, Lancet authors have identified the following potential therapeutic options for cytokine storm syndrome including ARDS the use of corticosteroids, selective cytokine blockade (eg, anakinra or tocilizumab) and JAK inhibition [61].

Adaptive immunity:

Virus presentation to the different T cell subsets stands on the crossroads between innate and adaptive immune responses. Studies on SARS-CoV [62–65] and MERS-CoV [66] presentation have identified several susceptibility and protection conferring HLA alleles. The dearth of similar data regarding SARS-CoV-2 antigen presentation to T cells and possible virus evasion mechanisms of this process suggests it is a virgin investigation field to be explored.

Apart from the sustained inflammation and cytokine storm, lymphopenia has been implicated as a major risk factor for ARDS and mortality in the context of COVID-19 [67]. Similar findings were described for SARS-CoV infected patients who had considerable decreases of CD4+ T and CD8+ T cells [63]. However, in convalescent patients specific T-cell memory responses to SARS-CoV were still found six years post infection [68]. Though it is still very early to trace memory responses against SARS-CoV-2, the observations linking lymphopenia with severe pathology are similar to patients diagnosed with severe acute respiratory syndrome (SARS) during the 2003 epidemic.

In a study of 452 Chinese patients in Wuhan, severe cases tended to have lower lymphocyte counts. This dearth of lymphocytes was mainly attributed to significantly lower T cell counts in severe cases. Numbers of CD8+ T lymphocytic cells responsible for recognizing and killing infected cells were found to be significantly lower in patients with severe manifestations of COVID-19. Additionally, severely affected patients presented with a higher naïve CD4+ to memory T cells ratio, suggesting that the adaptive immune system in the severe infection subgroup was less activated. Furthermore, these patients had less numbers of regulatory T cells (Tregs), especially induced Tregs. Tregs form the T cell subset responsible for controlling excessive inflammatory responses and their absence can lead to production of cytokine storm and enhancement of tissue pathology. Overall, this data suggest that dysregulation of T cell mediated immune responses may play a pivotal role in COVID-19 pathogenesis and severity [52].

Production of protective antiviral antibodies and long-lived memory B cells are fundamental for avoiding reinfection with the virus and form the basic principles behind vaccination. Less research has been completed relating to humoral immunity compared to than cellular against coronaviruses. However, in view of COVID-19 patient sera portraying some cross-reactivity with SARS-CoV, but not with other coronavirus, it might imply that similar mounting of humoral responses could be expected [8]. Studies conducted during the SARS epidemic have revealed that seroconversion is induced as early as day 4 after disease onset and that IgG protective antibodies lasted for as long as 2 years after infection [69] Anti-SARS-CoV IgM in turn disappeared after 12 weeks [70].

Preliminary data suggests that humoral responses are robust and follow a similar pattern. A study including 173 COVID-19 positive Chinese patients showed that 93.1% of the patients demonstrated anti-SARS-CoV-2 seroconversion. There was no late stage data available for the remainder of patients. Anti-SARS-CoV-2 antibodies were detected as early as 4 days' post disease onset, with a median time of positivity for IgM and IgG seroconversion being 11- and 14-days after disease onset, respectively. Interestingly, high antibody levels were not always found to be enough to clear the virus, as critically ill patients were found to have significantly higher virus specific antibody titers. However, the authors argue that combining viral nucleic acid and seroconversion detection significantly raised the detection sensitivity for patients [71]. Another recent study where a new ELISA assay for anti-SARS-CoV-2 specific antibody detection was developed reported the existence of IgA specific antibody in patients' serum apart from the expected IgM and IgG isotypes. Notably, among IgG subtypes tested IgG3 exhibited the highest reactivity followed by IgG1, while IgG4 showed no reactivity with viral antigens. However, the small number of sera used (n=4) implies that further investigation is needed to corroborate these results [72]. Nonetheless, since we are currently in early stages of SARS-CoV-2 pandemic more studies need to be carried out to shed light on antibody persistence (both IgM and IgG) and protective effects.

Recently, macaques re-challenged with SARS-CoV-2 after a primary infection did not show signs of re-infection, suggesting that protective immunity and memory responses were fruitfully mounted. This finding can also impact vaccine production strategies [73].

Importantly, COVID-19 convalescent sera was shown to hold promise as a passive immune therapy alternative to facilitate disease containment [74]. To the best of our knowledge, at least one pharmaceutical company, Takeda, is preparing to purify antibody preparations from COVID-19 convalescent sera against SARS-CoV-2 [75].

A recently published case report of a patient with mild-to-moderate COVID-19 revealed the presence of an increased activated CD4+ T cells and CD8+ T cells, antibody-secreting cells (ASCs), follicular helper T cells (TFH cells), and anti-SARS-CoV-2 IgM and IgG antibodies,

suggesting that both cellular and humoral responses are important in containing the virus and inhibiting severe pathology [76].

Antibody dependent enhancement (ADE) is a mechanism whereby non-protective antibodies produced during an infection with an agent cross-recognize a different pathogen and facilitate its entrance to target cells [77]. Evidence emerging over the past two decades suggests that antibodies against different coronavirus can cross-react to some extent and mediate ADE [78]. ADE in the context of SARS-CoV was thought to be mediated by antibodies produced against 229E-CoV [79] and was contemplated as contributing to high mortality rates in China [80]. The described mechanism suggests that anti-Spike protein antibodies mediate the infection of immune cells, further aggravating the dysregulation of anti-SARS-CoV immune responses [81]. Indeed, *in vitro* as well *as in vivo* experimental models have shown that ADE hinders the ability to manage inflammation in the lung and elsewhere. This may lead to ARDS and other hyperinflammation-induced clinical manifestations also observed in several of the documented cases of severe COVID-19 [82, 83]. While the molecular and immunological host response to SARS-CoV-2 infection has not yet been fully elucidated to confirm ADE is occurring, anti-SARS-CoV-2 have been shown to partially cross-react with SARS-CoV, suggesting enhancement is a possibility. With this in mind, ADE in populations previously exposed to other coronavirus can partially explain the geographic discrepancies observed in COVID-19 pathogenesis and severity.

Molecular diagnosis methods to detect COVID-19

RT-qPCR:

Detection methods based on nucleic amplification tests (NAT) are usually preferred in the case of MERS-CoV and other viruses, because they have demonstrated the highest sensitivity at the earliest time point in the acute phase of infection [84]. Detection and surveillance of COVID-19 spread is currently carried out by one-step quantitative RT-PCR (RT-qPCR) targeting SARS-CoV-2 sequences. Recently, the WHO compiled a list of various protocols for detection of SARS-CoV-2, developed by researchers in China, Germany, Hong Kong,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2020 doi:10.20944/preprints202004.0283.v1

Peer-reviewed version available at Diagnostic Microbiology and Infectious Disease 2020; doi:10.1016/j.diagmicrobio.2020.115094

Japan, Thailand, France, and USA (WHO 2020). Relative positions of RT-qPCR primer-probe sets on the SARS-CoV-2 genome are shown in Figure 3 and detailed in Table 4.

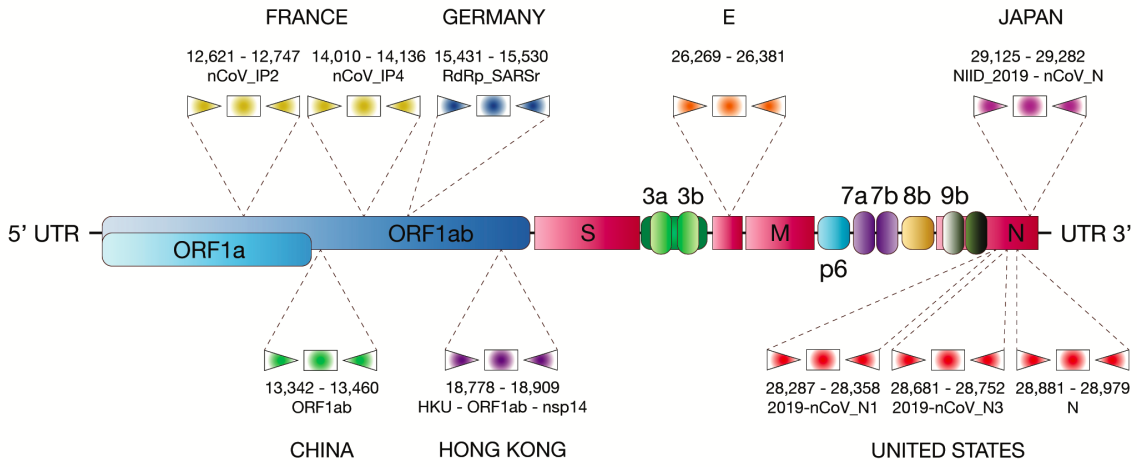

Figure 3 Relative positions of qRT-PCR primer-probe set on the SARS-CoV-2 listed by WHO. Institut Pasteur, Paris, France (nCoV_IP2, IP4 and E), China CDC (Orf1ab and N), Charité universitätsmedizin Berlin institute of virology in Germany (RdRp_SARSr and E), the University of Hong Kong (HKU-ORF1b_nsp14 and HKU-N), USA CDC (2019-nCoV_N1, N2, and N3), National Institute of Health in Thailand (WH-NIC N), National Institute of Infectious Disease in Japan (NIID_2019-nCoV_N). Orf1: open reading frame 1; RdRp: RNA-dependent RNA polymerase gene; Nsp14: non-structural protein 14 gene; S: spike protein gene; E: envelope protein gene, N: nucleocapsid protein gene. The number below amplicons are genome positions according to SARS-CoV-2, GenBank MN908947.3

Although RT-qPCR assay is considered the gold-standard method to detect viruses such as SARS-CoV and MERS-CoV [85, 86], currently available RT-qPCR assays targeting SARS-CoV-2 have important considerations. Firstly, due to the genome similarity of SARS-CoV-2 to SARS-CoV (82% of nucleotide identity [87]), some of the primer-probe sets described by different groups and listed in the WHO Coronavirus disease (COVID-19) technical guidance [88], have cross-reaction with SARS-CoV and other bat-associated SARS-related viruses, therefore, it is important to run confirmatory tests.

Table 1 Information of primers and probes recommended by WHO.

Target	Country	Institute	Name	Position	Reference
RdRp/Orf 1	China	China CDC	ORF1ab - F	13342 - 13362	[89]
			ORF1ab - R	13442 - 13460	
			ORF1ab - P	13377 - 13404	
	Germany	Charité	RdRp_SARSr - F	15431 - 15452	[90]
			RdRp_SARSr - R	15505 - 15530	
			RdRp_SARSr - P2	15470 - 15494	
	Hong Kong	HKU	HKU - ORF1b - nsp14F	18778 - 18797	[91]
			HKU - ORF1b - nsp14R	18889 - 18909	

			HKU - ORF1b - nsp14P	18849 - 18872	
	France	Institut Pasteur, Paris	nCoV_IP2-12669F nCoV_IP2-12759R nCoV_IP2-12696bP nCoV_IP4-14059F nCoV_IP4-14146R nCoV_IP4-14084P	12621- 12641 12727 - 12747 12696 - 12716 14010 - 14030 14116 - 14136 14084 - 14104	[92]
N	Japan	National Institute of Infectious Diseases	NIID_2019 - nCOV_N_F2 NIID_2019 - nCOV_N_R2 NIID_2019 - nCOV_N_P2	29125 - 29144 29263 - 29282 29222 - 29241	[93]
	Thailand	National Institute of Health	WH - NIC N - F WH - NIC N - R WH - NIC N - P	28320 - 28339 28358 - 28376 28341 - 28356	[94]
	USA	CDC	2019 - nCoV_N1 - F 2019 - nCoV_N1 - R 2019 - nCoV_N1 - P 2019 - nCoV_N2 - F 2019 - nCoV_N2 - R 2019 - nCoV_N2 - P 2019 - nCoV_N3 - F 2019 - nCoV_N3 - R 2019 - nCoV_N3 - P	28287 - 28306 28335 - 28358 28309 - 28332 29164 - 29183 29213 - 29230 29188 – 29210 28681 - 28702 28732 - 28752 28704 - 28727	[95]

Most of the tests enlisted in this review are currently available for use under an EUA by the FDA, a policy that aims to quicken the approval process for US labs developing tests for COVID-19. The approval is part of a concerted effort to make up for a lost time after delays and then a global shortage of the essential chemicals needed to make new tests (Table 5).

Table 2 Commercially Available COVID-19 Diagnostic Tests with EUA status

Company/ Organization	Test Name	Instrument	Test type	Time	Ref.
Carbon Health	COVID-19 Home Test Kits	NA	PCR	3 hours	[96]
IDbyDNA	Explify Platform for respiratory diseases	NA	NGS	24 hours	[97]
Cepheid	Xpert® SARS-CoV-2	GeneXpert® System	PCR	45 minutes	[98]
Roche	cobas SARS-CoV-2 Test	Cobas 6800 and 8800	PCR	4 hours	[99]
Abbott	Abbott RealTime SARS-CoV-2 EUA test	m2000 RealTime system	PCR	1200 in 24 hours	[100]
CDC USA	CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (CDC)	NA	PCR	4 hours	[101]
DiaSorin Molecular	Simplexa COVID-19 Direct	LIAISON® MDX	PCR	6 hours	[102]
Thermo Fisher	TaqPath COVID-19 Combo Kit	Applied Biosystems 7500	PCR	3.5 hours	[103]
Hologic	Panther Fusion® SARS-CoV-2 test,	Panther Fusion ® System,	PCR	1150 in 24 hours	[104]
Quidel	Lyra SARS-CoV-2	Applied Biosystems 7500 Fast DX	PCR	75 minutes	[105]
GenMark Diagnostics.	ePlex SARS-CoV-2 Test	ePlex system	PCR	2 hours	[106]
Integrated DNA Technologies	IDT 2019-novel coronavirus kit	NA	PCR	5 hours	[107]
LGC, Biosearch Technologies	2019-nCoV CDC-qualified Probe and Primer Kits for SARS-CoV-2	NA	PCR	-	[108]
Wadsworth Center	New York SARS-CoV-2 Real-time RT-PCR Diagnostic Panel	NA	PCR	-	[109]
Quest Diagnostics	Coronavirus Disease 2019 (COVID-19) Test	NA	PCR	4 days	[107]

BioMérieux/BioFire Defense	BioFire COVID-19 test	Filmarray® 2.0 and Torch	PCR	45min	[107]
Laboratory Corporation of America	LabCorp 2019 Novel Coronavirus test	NA	PCR	4 hours	[110]
Novacyt/Primerdesign	COVID-19 Genesig Real-Time PCR assay	NA	PCR	-	[111]
PerkinElmer	PerkinElmer New Coronavirus Nucleic Acid Detection Kit	NA	PCR	-	[112]
Abbot	ID NOW™ COVID-19 test	ID NOW platform	Isothermal amplification	5 min.	[100]
BGI	Real-Time Fluorescent RT-PCR kit for detecting SARS-2019-nCoV	NA	PCR	3 hours	[111]
Cellex	qSARS-CoV-2 IgG/IgM Rapid Test	NA	Serological	10 min.	[113]
Ipsum Diagnostics	COV-19 IDx assay	NA	PCR	4 hours	[114]
Luminex Molecular Diagnostics	NxTAGCoV Extended Panel Assa	ARIES® M1 Systems	PCR	4 hours	[115]
Mesa Biotech	Accula SARS-CoV-2 test	Accula System	PCR	30 min.	[116]
NeuMoDx Molecular	NeuMoDx SARS-CoV-2 Assay	NeuMoDx™ Molecular Systems	PCR	80 min.	[117]
Qiagen	QiaStat-Dx Respiratory SARS-CoV-2 Panel	QIAstat-Dx Analyzer,	PCR	1 hour	[118]

Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP)

Loop-mediated isothermal amplification (LAMP) is a one-step isothermal amplification reaction that couples amplification of a target sequence with four to six primers, to ensure high sensitivity and specificity, under isothermal conditions (63-65°C), using a polymerase with high strand displacement activity[119]. In the case of an RNA sample, LAMP, is preceded by the reverse transcription of the sample RNA. RT-LAMP has been used before for the detection of various pathogens[120]. including SARS-CoV-2 [53,54] and other respiratory viruses[121, 122]. Recently, it received emergency use authorization (EUA) from the U.S. Food and Drug Administration (FDA) for a point-of-care test for the detection of novel coronavirus (COVID-19), delivering positive results in as little as five minutes and negative results in 13 minutes[100].

Serological tests:

Serological tests also, called immunoassays, are rapid and simple alternatives for screening of SARS-CoV-2 infected individuals based on the qualitative or quantitative detection of SARS-CoV-2 antigens and/or anti-SARS-CoV-2 antibodies. There are several types of serological tests available, including ELISA (enzyme-linked immunosorbent assay), IIFT

(indirect immunofluorescence test) and neutralization tests. Immunoassays assays are very useful because they allow us to study the immune response(s) to SARS-CoV-2 in a qualitative and quantitative manner. In addition, help us to determine the precise rate of infection [72, 123], and to determine the fatality rate of the infection [72]. Several SARS-CoV-2 targeted serological tests are commercially available or in development [124]. A recently developed kit, reported a sensitivity of 88.66% and specificity of 90.63% [125] using SARS-CoV-2 IgG-IgM combined antibody rapid (within 15 minutes) test [125]. Despite their simple and fast readout and their potential for being used outside laboratory environments (bedside, small clinics, airports, train stations, etc.), serological tests have a critical disadvantage; given the fact that antibodies specifically targeting the virus would normally appear after 6 days or longer [126] after the illness onset [127], tests based on this principle have a lag period of approximately 4 to 7 days post-infection. During this lag period, infected and non-infected individuals will both result in a negative output. In addition, it is important to highlight that because serological tests depend on the ability to produce antibodies, intrinsic immunological differences and/or responses between individuals, can significantly affect the outcome of these tests. Recently, some commercially available immunoassays received CE Mark for professional use [128, 129], and therefore are registered as in vitro diagnostic devices.

Alternative methods:

Even though COVID-19 can be diagnosed using qPCR as the gold standard, inadequate access to reagents and equipment has slowed disease detection even in developed countries such as the US. Several low cost and rapid tests using different approaches have been described.

The CRISPR-based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique for the detection of COVID-19 and the DETECTR (developed by Mammoth Biosciences) prototype rapid detection diagnosis kit using CRISPR to detect the SARS-COV-2 in human samples have been described[130].

The use of RNA aptamers, have recently emerged as a powerful background-free technology for live-cell RNA imaging due to their fluorogenic properties upon ligand binding, a technology that could be use to diagnose SARS-CoV-2 infection [131].

Finally the use of next generation sequence (Explify®) might be used to detect and identify bacterial, viral, fungal, and parasitic pathogens by their unique genome sequences[97].

Clinical features of COVID-19

In COVID-19 symptomatic infection, the clinical presentation can range from mild to critical scenarios. The symptoms of a lower respiratory infection, pneumonia, is the most serious manifestation of COVID-19 infection.

Studies derived from the Wuhan population have established the most common clinical characteristics at the beginning of the disease: fever, fatigue and cough [132]. Other descriptive studies of Wuhan patients with confirmed COVID-19 have reported a similar range of clinical findings. In cohorts of patients outside of Wuhan, this clinical behavior is similar. At Zhejian province cohort of 62 people, only 1 case required mechanical ventilation assistance [133].

Evolution of the disease: spectrum of clinical manifestations

The spectrum of symptoms of COVID19 infection are characteristic of a mild disease in most of the cases, however, there is important to point that the progression could lead to a severe respiratory distress.

Asymptomatic infection:

Asymptomatic infection (while incubation occurs) was described both in the first cases in Wuhan and in other cohorts. A group of isolated patients were screened for SARS-CoV-2, where 17% (629 cases) were positive for the test, and half of these cases had no symptoms. On the other hand, there are reports of cases without overt symptoms in which there were ground glass images in the chest tomography in up to 50% of patients [134].

Of the asymptomatic cases studied in Wuhan city, the 2.5% of people exposed developed specific symptoms in 2.2 days, and the remaining 97.5% were symptomatic in the following 11.5 days (CI, 8.2 to 15.6 days). The median estimated incubation period was 5.1 days (95% CI, 4.5 to 5.8 days) [135].

Acute infection: mild and moderate

Some patients with initially mild symptoms had symptom progression over the course of one week [136]. The descriptive studies available so far have concluded that the majority of cases are mild infections (more than 80% of cases); with up to 15% of patients being sever in most cohorts, and less than 5% have been considered as critical cases with high vital risk [137].

In a study describing 138 patients with COVID-19 pneumonia in Wuhan, the most common clinical characteristics at the onset of the disease were described. This is consistent with other international cohorts (Table 1) [132].

Table 3 Clinical Manifestations of COVID 19 infection.

Clinical manifestations	Presentation n=138 n (%)	ICU* n=36 n (%)	Non-ICU n=102 n (%)
Fever	136 (98.6)	36 (100)	100 (98)
Fatigue	96 (70)	29 (80.6)	67 (65.7)
Dry Cough	82 (59.4)	21 (58.3)	61 (59.8)
Anorexia	55 (40)	24 (66.7)	31 (30.4)
Myalgia	48 (34.8)	12 (35.3)	36 (35.3)
Dyspnea	43 (31.2)	23 (63.9)	20 (19.6)
Sputum production	37 (27)	8 (22.2)	29 (28.4)
Pharyngalgia	24 (17.4)	12 (33.3)	12 (11.8)
Diarrhea	14 (10.1)	6 (16.7)	8 (7.8)
Nausea	14 (10.1)	4 (11.1)	10 (9.8)
Dizziness	13 (9.4)	8 (22.2)	5 (4.9)
Headache	9 (6.5)	3 (8.3)	6 (5.9)
Abdominal pain	5 (3.6)	3 (8.3)	0 (0)
Vomiting	5 (3.6)	3 (8.3)	2 (2.0)

*ICU: intensive care unit

Source: Wang D et al., 2020 [132].

It is important to note that fever is not always present and up to 20% of patients could had a low grade temperature between 37.5 to 38 degrees Celsius or normal temperature. [138] If these patients required hospitalization, 89% developed a fever during the course of the illness. Rarer accompanying symptoms included headache without warning signs, odynophagia and rhinorrhea. Gastrointestinal symptoms such as nausea and watery diarrhea were relatively rare [133].

Dyspnea develops after a median of 5 to 8 days from the onset of symptoms. It is important to notice that, if dyspnea is an important clinical finding, not all the patients with this symptom will develop respiratory distress ore require oxygen supplementation [132].

According to World Health Organization (WHO) guidelines, COVID-19 infection can present as pneumonia without signs of severity, and could be managed in the outpatient setting; this applies to those patients who do not need supplemental oxygen [139].

Severe infection and critical state:

As previously mentioned, the most serious manifestation of COVID 19 infection is pneumonia, characterized by cough, dyspnea, and infiltrates on chest images; the latter is indistinguishable from other viral lung infections.

Acute respiratory distress syndrome (ARDS) is a major complication of COVID pneumonia in patients with severe disease. This develops in 20% after a median of eight days.Mechanical ventilation is implemented in 12.3% of cases [140].

In different case reports, the need for supplemental oxygen via the nasal cannula was required in approximately 50% of hospitalized patients. 30% required non-invasive mechanical ventilation, and less than 3% required invasive mechanical ventilation with or without Extracorporeal Membrane Oxygenation (ECMO) [141].

It is important to mention that the proportion of severe cases is highly dependent on the study population and may be related to the epidemiological behavior of the infection in each country. Additionally, the number of people tested will influence the denominator. In Italy, the average age of people infected with COVID-19 is between 60 and 65 years, and 16% of those hospitalized require admission to the intensive care unit (ICU) [142].

The WHO recommendations had stablished that severe COVID-19 disease could be defined by the following parameters in table 2 [139].

Table 4 Severe COVID-19 disease definitions in adults

Clinical scenario	Criteria	
Adolescent or adult: fever or suspected respiratory infection, plus one of:	Respiratory rate > 30 breaths/min	
	Severe respiratory distress; or	
	SpO2 ≤ 93% on room air.	
Acute respiratory distress syndrome (ARDS): Onset: within 1 week of a known clinical insult or new or worsening respiratory symptoms.	Chest imaging (radiograph, CT scan, or lung ultrasound): bilateral opacities, not fully explained by volume overload, lobar or lung collapse, or nodules.	
	Origin of pulmonary infiltrates: respiratory failure not fully explained by cardiac failure or fluid overload.	
	Need objective assessment (e.g. echocardiography) to exclude hydrostatic cause of infiltrates/edema if no risk factor is present.	
	Oxygenation impairment in adults	Mild ARDS: 200 mmHg < PaO2/FiO2 a ≤ 300 mmHg (with PEEP or CPAP ≥ 5 cmH2O, or non-ventilated)
		Moderate ARDS: 100 mmHg < PaO2/FiO2 ≤ 200 mmHg (with PEEP ≥ 5 cmH2O, or non-ventilated)
		Severe ARDS: PaO2/FiO2 ≤ 100 mmHg (with PEEP ≥ 5 cmH2O, or non-ventilated).
		When PaO2 is not available, SpO2/FiO2 ≤ 315 suggests ARDS (including in non-ventilated patients).

Adapted from: WHO, 2020. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected [143].

The Surviving Sepsis Campaign (SSC) has directed some recommendations to the population with COVID 19. This guideline focuses on the critical management of severe cases and makes recommendations through an exhaustive review of the literature. For more details, the clinical algorithm includes those recommendations in the critical scenario [144].

Risk factors for severe disease:

Among the established risk factors for the development of ARDS is age greater than 65 years,

diabetes mellitus and hypertension, in at least 40% of patients [133].

It should be clarified that, although advanced age is identified as a risk factor for a severe infection, those of any age may suffer from severe illness from COVID-19. The descriptions made so far of the patients from China have determined that almost 90% of the patients were between the ages of 30 and 79 years (cohort of 44,500 cases) [137].

In other population settings, such as in the United States, more than 60% of confirmed patients were older than 45 years. (CDC, et al. 2020) In most of the described cohorts, mortality was associated with age, with 80% of the deceased in China being over 65 years old, and in the USA the case fatality rate was up to 15% in adults over 70 years.

The Massachusetts General Hospital has suggested additional factors that can be considered risk for severe COVID 19 infection, detailed in Table 3 [145].

Table 5 Risk factors for severe COVID-19 infection Adapted from: Ginsberg, L. E. (2010). "If clinically indicated:" Is it? Radiology, 254(2), 324–325. https://doi.org/10.1148/radiol.09091736

Epidemiological - Category 1	Vital signs – Category 2	Laboratory – Category 3
Age > 55 years	Respiratory rate > 24 breaths/min	D-Dimer > 1000ng/mL
Diabetes Mellitus	Heart rate > 125 beats/min	CPK > 2 folds over upper limit
Hypertension and high cardiovascular risk	Spo_2 < 90% at room air	LDH > 245 U/L
Immunosuppression and use of biological drugs		Elevated troponin
HIV patients regardless CD4 count		High Troponin
		Lymphocyte count < 0.8
		Ferritin > 300ug/L

The document was developed by the Infectious Diseases division in conjunction with the front-line support departments. Their recommendations are continually updated as more data comes out.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2020 doi:10.20944/preprints202004.0283.v1

Peer-reviewed version available at *Diagnostic Microbiology and Infectious Disease* **2020**; doi:10.1016/j.diagmicrobio.2020.115094

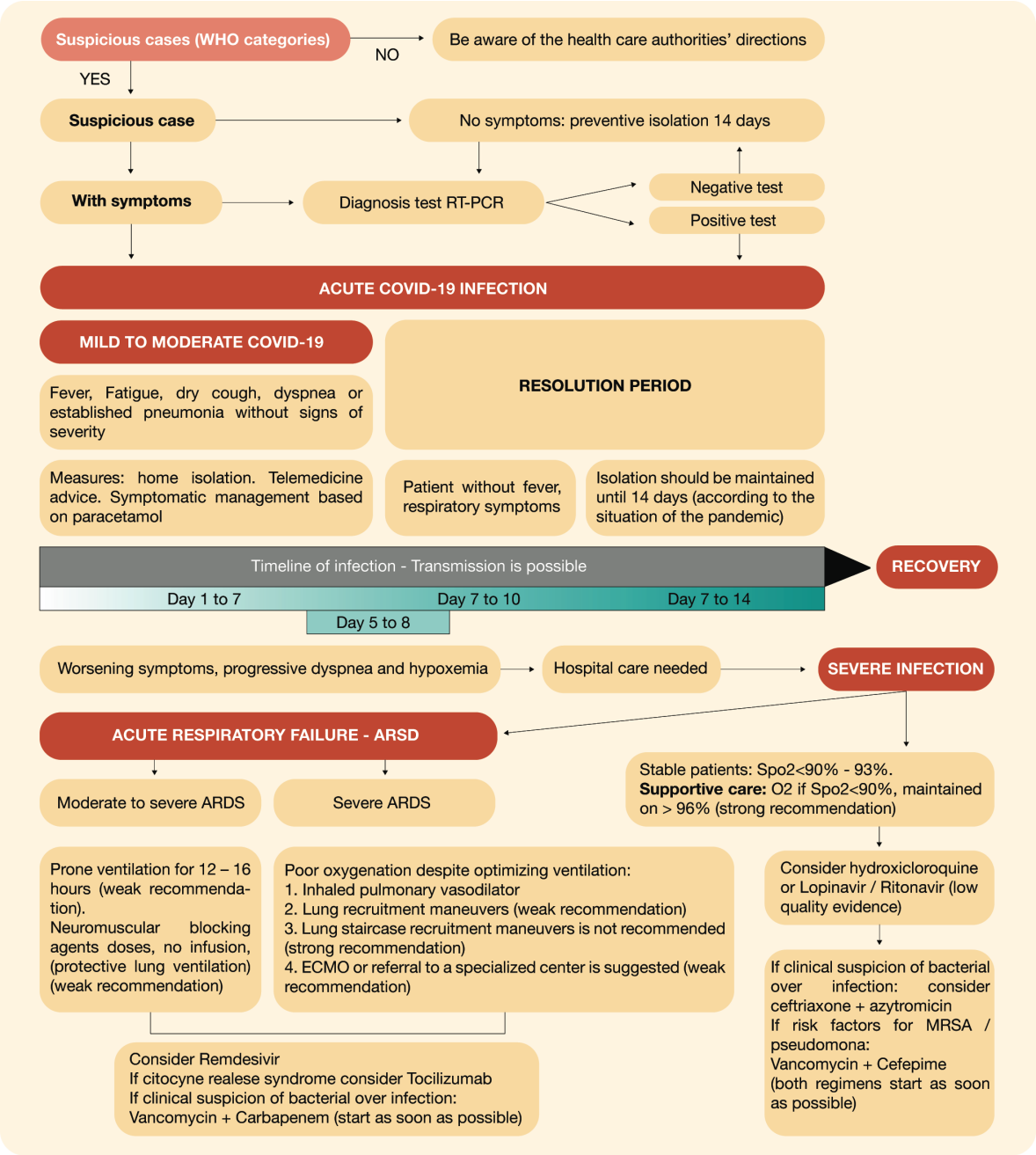

Figure 4 Clinical features of patients with Covid-19

Clinical diagnosis and screening:

The clinical characteristics of symptomatic cases and their severity has been described. In addition to the symptoms reported by the patients, the findings on physical examination may

be absent during mild COIVD-19 infection. Those with moderate to severe COVID-19 infection have various signs on the pulmonary auscultation, however the most common findings include: wet rales; global decrease in respiratory sounds and increased thrill. [146].

Early recognition is essential to classify cases as potential cases and initiate one of the most important measures to contain the pandemic, isolation.

The Center of Disease Control (CDC) and the WHO have established clinical scenarios that should be considered as a high suspicion of COVID 19 infection:

1. Close contact with a confirmed or suspected COVID-19 case, including through work in healthcare settings. Close contact includes being within approximately two meters of a patient for an extended period of time without wearing personal protective equipment or having direct contact with infectious secretions without wearing personal protective equipment.
2. Anyone who has resided or been traveling in areas where widespread community transmission has been reported.
3. Any patient who has had potential exposure through attending events or has spent time in specific settings where cases of COVID-19 have been reported.

The scenarios described respond to the context of a high suspicion of COVID-19 infection. The world health authorities (CDC, WHO) continually update these contexts, that is why they have made several clarifications regarding who to perform the test:

- They have pointed out the importance of fever, cough and dyspnea as sentinel symptoms, since these should form part of the clinical judgment that guides doctors. This allows to expand the group of suspicious patients.
- In cases of severe respiratory distress of undetermined etiology and that do not meet the previously indicated criteria, a screening for COVID-19 would be indicated.
- In areas of limited resources, the suggestion is to prioritize cases that require hospital care, and in this way guide the epidemiological fence to order isolation and protect

the most vulnerable people (chronically ill and over 65 years of age), as well as test those with the greatest possibility of exposure (travelers and health personnel).

Laboratory findings:

At the moment, there is no laboratory data profile that is framed in COVID 19 infection. From a cohort of 43 patients confirmed with COVID 19, these findings were classified as mild, moderate and severe disease [147].

IL-6, D-Dimer, glucose, TTP, fibrinogen and PCR values were associated with the greatest difference in the deviation of their values. Thus, the optimal threshold and area under the ROC curve for IL-6 were 24.3 pg / mL and 0.795 respectively, while for D-Dimer they were 0.28 µg / L and 0.750, respectively. The area under the ROC IL-6 curve (AUC) combined with D-Dimer was 0.840. The specificity of IL-6 and D-Dimer was up to 93.3%, while the sensitivity of IL-6 and D-Dimer in severe COVID was 96.4%, especially in early stages of severe infection.

High levels of D-dimer and more severe lymphopenia have been associated with mortality due to a prothrombotic state that determines multi-organ failure.

In general, leukopenia and / or leukocytosis can be found in the interpretation of blood biometry, however, the most widely described finding is lymphopenia [148]. It should be considered that in the context of viral pneumonia biomarkers such as Procalcitonin and PCR are not useful, in most patients since these biomarkers are in ranges of the normal.

Among other findings, descriptive studies have reported considerable elevations of lactate dehydrogenase and ferritin as well as alteration in aminotransferases; although elevation ranges for these parameters have not been established [149].

Imaging findings:

About the imaging findings, COVID 19 viral pneumonia shows images similar to other viral infections.

Although computed tomography (CT) is the test of choice, it is not useful for a definitive diagnosis due to the wide variety of images that can be found in COVID 19 infection. This statement is derived from a large cohort of more than 1000 Wuhan patients, where RT-PCR confirmation of COVID 19 and chest CT images of these patients were correspondingly analyzed. CT images were determined to have a sensitivity of 98%; however, the specificity was only 25% [150].

In general, the majority of descriptive studies concur that the finding of ground glass opacifications is most common. It is typically basal and bilateral, and rarely associated with underlying consolidation. A multicenter Chinese study that retrospectively reviewed the CT scans of 101 patients found that 87% had typical ground-glass images and up to 53% had this finding along with consolidations. These findings were more frequent in the most severe and older age groups of patients [151].

These findings were compared between 205 viral pneumonia patients with a respiratory panel positive for other viruses versus 219 SARS-CoV-2 positive patients. The most uncommon findings on CT images of patients with COVID 19 were: central distribution of opacifications (14%), air bronchogram (14 %), pleural thickening (15%), pleural effusion (4%), and lymphadenopathy (2.7%) [152].

Diagnosis methods to detect COVID-19

The emergence and outbreak of SARS-CoV-2, the causative agent of COVID-19, has rapidly become a global concern that highlights the need for fast, sensitive, and specific tools to surveil the spread of this infectious agent.

Diagnostic protocols to detect SARS-CoV-2 using real-time quantitative polymerase chain reaction (RT-qPCR) were listed on the World Health Organization (WHO) website as guidance, however, various institutions and governments have chosen to establish their own protocols that might not be publicly available or listed by WHO.

There are important challenges associated with close surveillance of the current SARS-CoV-2 outbreak. Firstly, the rapid increase of cases has overwhelmed diagnostic testing capacity in many countries, underscoring the need for a high-throughput, scalable pipeline for sample processing [153, 154]. Secondly, given that SARS-CoV-2 is closely related to other coronaviruses [87], some of the currently available nucleic acid detection assays can result in false positives [155]. Thirdly, critical concern for molecular detection is the low sensitivity reported for RT-qPCR assays [150] and serological tests [125], particularly in the early stages of infection. Additionally, most of the available RT-qPCR assays require sample processing and equipment only available in diagnostic and/or research laboratories.

The most common tests for COVID-19 involve taking a swab from a patient's nose and throat and checking these swabs for the genetic footprint of the virus. They are called "PCR tests". The first PCR test for COVID-19 was developed within two weeks of the disease being identified[125].

Even though most of the available diagnostics have focused on RT-PCR, additional methods include using microarray or microfluidic technologies, CRISPR to isolate gene segments for diagnostics, serological and full genetic sequencing are available. It is important to note that the FDA has so far granted Emergency Use Authorization (EUA) status only to some PCR-based tests.

Differential diagnosis

COVID-19 pneumonia presents a clinical picture that, as previously stated, may be indistinguishable from other viral pneumonias. Any viruses that causes pneumonia must be in the differential diagnosis of COVID-19 and include influenza, parainfluenza, adenovirus,

respiratory syncytial virus, rhinovirus, human metapneumovirus, SARS-CoV, etc.

It is important to mention that coinfection is a possibility, as some reports from Italy and China had described, the most common pathogen in coinfection was Influenza virus (H1N1, H3N2), Rhinovirus and Respiratory syncytial virus (A/B). In contrast, bacterial coinfection was infrequent [156, 157].

The bacterial etiology that may have clinical and radiographic similarities to COVID-19 is that caused by bacteria such as mycoplasma and chlamydia. Among the pictures that cause non-infectious lung lesions are those autoimmune diseases with lung involvement such as vasculitis, dermatomyositis and other pneumonitis.

COVID-19 in pregnant women

Regarding SARS-nCoV infection in pregnant women, there is currently limited evidences about the effect of the virus on the mother or fetus. However, due to the physiological changes typical of pregnancy, especially on the immune system (immunosuppression) and the cardiopulmonary system, pregnant women are thought to be more susceptible to developing severe symptoms when they acquire viral respiratory disease. In 2009, when Influenza A H1N1 infection occurred, pregnant women were 1% of the infected population, yet accounted for 5% of infection-related deaths [158].

Some of the guidance related to the effects of the coronavirus in pregnant women and the fetus is due to previous studies of various viruses. During the SARS-CoV pandemic in 2002 and 2003, in a very small study of 12 patients, women infected during their first trimester had high a miscarriage rate (57%). During their second and third trimesters they developed intrauterine growth restriction (40%), and preterm delivery (80% [one spontaneous and three induced by maternal condition]), and three women died during pregnancy (25%) [159]. In another study of 11 pregnant patients infected with MERS-CoV, 9 presented adverse results (91%), 6 neonates were admitted to the neonatal intensive care unit (55%) and three of them

died (27%) [160].

With information obtained so far from the Wuhan SARS-CoV-2 outbreak, the infection appears to be less severe for pregnant women, compared to previous SARS-CoV and MERS-CoV outbreaks [158]. However, it is important to take into account that the data obtained are from reviews consist of a small number of patients. Additionally, the majority of pregnancies with confirmed SARS-Cov-2 pneumonia were in the third trimester and there were very few within the first and second trimesters . Therefore, more information should be collected with larger numbers of pregnant women with the infection. Follow-up of positively diagnosed pregnant women during in the first and second trimesters should be encouraged, to understand the impact of the new coronavirus infection on the pregnant mother, the fetus and the course of pregnancy [161, 162]

Mullins et al, carried out a bibliographic review of all the evidence collected until March 10, 2020, relating to any pregnant women with coronavirus diagnosed during her pregnancy or puerperium. 23 studies were included but there is a high probability that reported cases overlap. In total, they found 32 women affected by COVID-19, including one with a twin pregnancy. Delivery of 30 newborns was reported, 27 by Caesarean section 3 by vaginal delivery[163].

The management of pregnant patients with COVID-19, in general, follows the same principles as for the wider population. It is vital to consider that the mother, fetus and, subsequently, the newborn are always considered a high-risk population. Management should include early isolation, oxygen therapy if necessary, avoid fluid overload, empirical antibiotic therapy (due to the risk of bacterial infection), maternal fetal monitoring, Doppler ultrasound is recommended within obstetric surveillance. In patients who are asymptomatic, home management can be done, indicating that they should seek further medical advice if their symptoms develop into more severe disease. All mothers recovering from COVID-19 infection should be monitored with a Doppler ultrasound every two weeks, due to the risk of developing intrauterine growth restriction [164, 165].

The time of termination of the pregnancy, as well as the method, also depend on several

factors, including gestational age, maternal condition in relation to SARS-CoV-2 infection, presence of maternal comorbidities, and fetal condition. It must be taken within a multidisciplinary team, with individualized management for each patient [166].

Prematurity conditions neonatal morbidity and mortality, so the diagnosis of COVID-19 is not an indication on its own of termination of pregnancy, and the use of corticosteroids is recommended for antenatal fetal lung maturation, with betamethasone or dexamethasone [167]; taking special care in critically nursing patients, as this may worsen their condition, and may delay delivery, which is necessary for the management of these patients [164, 168].

COVID-19 in children

The symptoms presented by children are in themselves similar to adults with an incubation period ranging from 1 to 14 days (mean of 5.2). Cough is the most frequent symptom (65%) followed by fever (60%) with the difference of gastrointestinal symptoms diarrhea (15%), nausea, vomiting (10 %) and abdominal pain, which are usually more flowery than in the adult stage and, sometimes only manifestations along with fever [169, 170].

The clinical manifestations in pediatric patients vary markedly from adults, particularly relating to general progression and severity of the disease. Over 90% of affected children are asymptomatic or have mild to moderate disease [169]. The majority of serious cases in children are related to those with significant comorbidities such as heart disease, immunosuppression, etc. To date of this review, only a few cases of a child without comorbidities died as a result of COVID 19 are reported. This difference of severity of illness between adults and children has not been clarified, however, several theories have been postulated. These include that children express more ACE2 receptors in their lungs which confer some protection to severe injuries such as those caused by RSV and which would decrease dramatically with age [171, 172].

Immunological factors could may also influence outcomes, as in childhood we are most exposed to frequent challenges with recent seasonal viruses such as RSV in the winter months.

Most likely, it is multifactorial and depends on factors from both the host and the virus itself [172].

Abnormal radiological (CT) findings are found in asymptomatic children and consist of bilateral lung lesions (50%). Elevated CRP (C – reactive protein), Procalcitonin PCT (80%), and liver enzymes are present in most affected children, unlike adults in whom PCT is not a reliable marker.

Virus elimination via the stool even after the negativity in the nasopharyngeal mucosa and the disappearance of symptoms makes them a source of contagion through the fecal-oral route [173].

SARS-COV-2 infection and cancer

Patients with cancer are generally more susceptible to infections than healthy people, because they have a state of systemic immunosuppression that is exacerbated during chemotherapy or radiotherapy [174].

In China, according to national surveillance data, coronavirus infection occurs in 1.3% of patients with malignant tumors, a proportion higher than the general incidence of 0.3% of malignant tumors in the country [175]. When comparing non-malignant tumors patients with malignant tumors patients have a higher risk of developing a more serious infection (OR 5.34; 95% CI: 1.80–16.18; p = 0.0026) and health deterioration is accelerated (HR 3.56; 95% CI: 1.65-7.69; p<0.0001) even after adjusting for age [174]. To back up this findings, in a tertiary hospital in Wuhuan - China, it was found that 25% of patients with cancer and SARS-COV-2 infection died, most of them over 60 years of age [176].

Due to these findings, it has been proposed by many international entities that during the pandemic, for prevention it should be developed an individualized plan based on the specific conditions of each patient and treat to minimize the number of visits to health institutions.

- For early-stage patients with need of post-surgical adjuvant chemotherapy, especially those whose clinical, pathologic, and molecular biologic staging suggest a better prognosis, the start time of adjuvant chemotherapy may be delayed up to 90 days after surgery without affecting the overall effect of treatment [177].

- For patients with advanced cancer, the main approach should be to minimize hospitalization in COVID-19 positive installations. Replacing the existing intravenous treatment regimen with oral chemotherapy during this special period may be considered, to ensure that treatment is not interrupted for a long time during the pandemic [178].

However, if there is a suspicion of COVID-19 infection in this population group, it should be followed the same updated diagnostic guidelines and the corresponding management according to their state of severity. Moreover, it should be considered an individualized follow-up due to more likely of complications in this group of population [179].
It should be noted that cancer out-patients have different levels of anxiety, depression and other mental health problems than general population. Studies have shown that approximately 50% of malignant tumor survivors have a moderate to severe fear of tumor recurrence [180]. For this reason, psychologist surveillance of out-patients in quarantine or during hospitalization should be considered.

Complications SARS-CoV-2 infection

Reported complications derived from COVID-19 describe a severe disease that requires management in an intensive care unit (ICU) in approximately 5% of proven infections. Main ones were respiratory failure, cardiovascular dysfunction, cardiomyopathy and acute kidney injury; the average duration between symptom onset and dyspnea and ICU admission has been 7 and 10 days, respectively. Suggesting gradual deterioration in most cases, with older patients (mean> 60 years) the most susceptible. The risk of patient-to-patient transmission in the ICU is currently unknown, therefore adherence to infection control precautions is paramount [181, 182].

Progressive deterioration of respiratory function is undoubtedly the main and worst complication of the infection. The prevalence of hypoxic respiratory failure in COVID-19 patients is 19%, and it can progress to acute respiratory distress syndrome (ARDS), with the need of mechanical ventilation support at 10.5 days on average; between 10 and 32% of hospitalized patients require admission to the ICU due to respiratory deterioration [182]. As the respiratory complication is the main and most severe, its early diagnosis will undoubtedly help in timely support, taking into account risk factors such as advanced age, neutrophilia and organic dysfunction for the development of ARDS. The diagnostic support of pulmonary tomography is undoubtedly a valid tool; images in patients with different clinical types of COVID-19 have characteristic manifestations, but it can become an operational problem due to the difficulty in performing it in critically ill patients; On the contrary, the performance of lung ultrasound at the foot of the bed may replace the performance of radiographs and tomography for its diagnosis [183, 184].

Since more than 70% of hospitalized patients will require supplemental oxygen, it is recommended that it should be started with pulse oximetry values less than 90% with a target of no more than 96%, since higher values have been shown to be harmful [185, 186]. Regarding the use of high-flow nasal cannula (HFNC) oxygen therapy, great variability of results were recorded, because it was not possible to determine whether the progression to orointubation, mortality, or the risk of contamination to health personnel had decreased, but it still should be used instead of non-invasive mechanical ventilation (NIMV). HFNC use should be closely monitored and cared for in an environment where intubation can be facilitated in case of decompensation, due to the failure rate can be high and emergency intubation in an uncontrolled environment increase the risk of nosocomial infection of health providers [187–189].

The recommendation for starting with NIMV is of very low quality, and it is of high risk for both patients and health personnel. In adults with COVID-19 hypoxic respiratory failure, there is no direct evidence to support the use of NIMV; Furthermore, some previous studies suggested that it may be associated with an increased risk of transmission of infections to healthcare workers and may worsen severe forms of lung injury as a result of harmful

transpulmonary pressures and large tidal volumes (TV), in addition to delaying initiation of invasive mechanical ventilation, leading to emerging intubations that may increase the risk of transmission to the healthcare team with increased risk to the patient [190–192].

For the initiation of invasive mechanical ventilation, the recommendation for highly protective ventilation is maintained, with the use of low TV (6 ml / kg of ideal weight), plateau pressure less than 30 cm H2O, conduction pressure between 13-15 cmH2O, respiratory rate can be carried up to 35 per minute, as needed. If hypoxemia progresses to values less than 100–150 mmHg of PaFiO2, there are several therapeutic options, initially increasing positive expiratory pressure (PEEP) by 2–3 cmH2O every 15 to 30 minutes to improve oxygen saturation to 88–90%, maintaining a plateau of less than 30 cm H2O. Recruitment maneuvers are probably of little value, but could be used in selected cases in the presence of a physician to control hemodynamics. If there is considerable asynchrony with positive pressure ventilation, accompanied by an increase in plateau pressure and refractory hypoxemia, deep sedation should be used followed by prompt institution of neuromuscular block. If hypoxemia has been reached refractory to the aforementioned measures, it is recommended to move quickly to ventilation in the prone position and as a final measure venous venous ECMO (VV) should be considered if available or to refer the patient to an ECMO center [193–198]. Routine use of corticosteroids has been discouraged, and restricting it exceptionally for patients who develop ARDS, although without reports of improvement in survival, with discrepancy in results of shorter mechanical ventilation time and ICU stay [199].

Hemodynamic deterioration has a variability of presentation, this depends on the study population and the definition [200], the presence of shock in the intensive care unit may be present between 25 to 35% [181, 201]. Cardiomyopathy related to viral infection is one of the main causes of hemodynamic detriment, occurring in up to 23% of patients with COVID-19 [202]. Hemodynamic failure is one of the main causes of death in these patients, with percentages of up to 40%, inconclusive risk factors are associated to date such as diabetes, hypertension, lymphopenia, and elevation of D-dimer [203]. Acute kidney injury (AKI) is present in up to 12% of critically ill patients, podocytes and proximal tubule cells are

potential host cells for SARS-CoV-2, caused by the virus induced cytopathic effect. The diagnosis is based on markers of early kidney injury and urinary output [187].

Initial management of shock is based on fluid resuscitation, based on the application of dynamic parameters to predict response to fluids, such as variation in stroke volume (SVV), variation in pulse pressure volume (PPV) and change in stroke volume with passive leg elevation or fluid challenge above static parameters [203]. Variables such as skin temperature, capillary refill time and/or serum lactate measurement are currently valid tools. The amount of liquids used in resuscitation should be restricted and administered in relation to dynamic assessment, a liberal water resuscitation strategy is not recommended, preferring balanced crystalloids over colloids as resuscitation liquids, avoiding the use of hydroxyethyl starches, albumin, dextrans or gelatins [204, 205]. Indirect evidence suggests that the target mean arterial pressure (TAM) for patients with septic shock is 65 mmHg using vasoactive support [206]. The recommendation of norepinephrine use as the first agent is maintained. If norepinephrine is unavailable, vasopressin or epinephrine could be used, avoiding the use of dopamine as the initial vasopressor due to the potential development of arrhythmias [207, 208]. In patients with COVID-19 and shock with evidence of cardiac dysfunction and persistent hypoperfusion despite fluid resuscitation and norepinephrine use, dobutamine as inotropic is recommended. Given the development of refractory septic shock, the suggestion of the use of hydrocortisone in continuous infusion is maintained, as indirect evidence, this in favor of reducing the length of stay in the ICU and the resolution time of the shock [207].

Clinical prognosis

According to the investigative mission of the WHO in China, the case-fatality rate ranged from 5.8 percent in Wuhan to 0.7 percent in the rest of China. Of these cases, the deaths were mostly in patients with chronic diseases (cardiovascular disease, diabetes mellitus, chronic lung disease, hypertension and cancer) and the elderly. (WHO, et al. 2020).

Other reports from China have coincided with this clinical risk profile, for example, a study that included 41 confirmed cases, 12 patients who had ARDS had as main underlying

diseases: diabetes and high blood pressure. Of these cases, 6 patients died [138].

Recovery from COVID-19 infection

According to WHO, the recovery time is estimated to be two weeks for mild infections and three to six weeks for serious illnesses. On the other hand, CDC established that people who had symptoms in the mild to moderate spectrum and maintained home isolation have a resolution of 3 days after the fever decrease, and there was a substantial improvement in respiratory symptoms, even without use of medications.

Isolation may be limited to 7 days from resolution of symptoms, however, it must be adapted to the population circumstances of the epidemic [140].

Current treatment strategies

Non - pharmacological measures:

The evolution of epidemiological curve in COVID-19 outbreak makes consider containment strategies in China primarily, and other countries based on non-pharmaceutical interventions (NPIs). According WHO, the most effective measure is hands washing. In general, the recommendations are: “If hands are not visibly dirty, the preferred method is to perform hand hygiene with an alcohol-based hand rub for 20−30 seconds using the appropriate technique. When hands are visibly dirty, they should be washed with soap and water for 40−60 seconds using the appropriate technique” [209].

Five different non-pharmaceutical interventions (NPI) implemented individually and in combination as public health measures reduced contact rates in the population and therefore reduce virus transmission (Table 6) [210].

Table 6 Non - pharmacological measures

Measure	Description
Home isolation	Symptomatic cases stay at home for 7 days, reducing non-household contacts by 75% for this period. Household contacts remain unchanged. Assume 70% of household comply with the policy.

Voluntary home quarantine	Following identification of a symptomatic case in the household, all household members remain at home for 14 days. Household contact rates double during this quarantine period, contacts in the community reduce by 75%. Assume 50% of household comply with the policy.
Social distancing of those over 70 years of age	Reduce contacts by 50% in workplaces, increase household contacts by 25% and reduce other contacts by 75%.
Social distancing of entire population	All households reduce contact outside household, school or workplace by 75%. School contact rates unchanged, workplace contact rates reduced by 25%. Household contact rates assumed to increase by 25%.
Closure of schools and universities	Closure of all schools, 25% of universities remain open. Household contact rates for student families increase by 50% during closure. Contacts in the community increase by 25% during closure.

Increasing the level of hand cleanliness to 60% in places with a high concentration of people, like all airports in the world would have a reduction of 69% in the impact of a potential disease spreading [211].

The specific recommendations from WHO are social distancing and hand washing. About rational use of masks, WHO recommends: “If the person is healthy, only need to wear a mask if he/she is taking care of a person with suspected SARS-CoV-2 infection”. In Japan, the statement in this topic was that effectiveness of wearing a face mask to protect from contracting viruses is thought to be limited. If the use of a face mask in confined, badly ventilated spaces, it might help avoid catching droplets emitted from others but if you are in an open-air environment, the use of face mask is not very efficient. CDC does not recommend that people who are well wear a face mask (including respirators) to protect themselves from respiratory diseases, including COVID-19. Thus, the recommendation is to optimize face mask distribution and priories the needs of frontline health-care workers and the most vulnerable populations in communities who are more susceptible to infection and mortality if infected, including older adults (particularly those older than 65 years) and people with underlying health conditions [212].

Pharmacological treatment:

Therapeutic strategies are urgently needed to be applied in the context of COVID-19, as a pandemic. In terms of this public health urgency is important to consider two important definitions: Drug repurposing and compassionate use of drugs. The first one, drug repurposing is an emerging strategy where pre- existing medicines, having already been tested safe in humans, in similar virus or targets in the infection process, are redirected

against unique objective: SARS-Cov 2 (viral structure, infection process). The second one, compassionate use of drugs is the use of a new, unapproved drug to treat a seriously ill patient when no other treatments are available. These concepts have been applied with COVID-19 treatment.

Identifying targets for pharmacological use has been important to develop therapeutically drugs with roles in virus structure and infection process (Figure 2). Some representative existing drugs act on targets in similar RNA viruses like Ebola, hepatitis C, influenza, and others as MERS and SARS viruses. The most important studied targets are 3CLpro and PLpro, the two viral proteases responsible for cleavage viral peptides into functional units for virus replication and packaging within the host cells. Thus as drug repurposing appears Lopinavir and Ritonavir [213]. RdRp is other important target as the RNA polymerase responsible for viral RNA synthesis, blocked by Remdesivir and Favipiravir. About endocytosis process into host cells, viral spike protein and its interaction with ACE2 receptor constitute other important target blocked by arbidol, used also in Influenza. ACE2 is a negative regulator, receptor of renin-angiotensin system, involved in pressure control and inflammatory lung disease. By the knowledge of physiopathology of covid-19 infection, we know that activities of ACE2, AT1 and AT2 receptors are altered, thus some drugs are being studied around these targets, but also in vitro and experimental way. Some homologue target-drug models have been purposed between SARS-CoV and SARS-CoV-2 due to the receptor-binding domain (RBD) in S protein with 76% of sequence similarity. In the same way with PLpro sequences with 83% similar active sites [213].

Other drugs like Chloroquine and analogues (Hydroxicloroquine) acts directly on endosomal pH and interfere with ACE2 glycosylation. In general, the most studied pharmaceutical interventions found for COVID-19 treatment include arbidol, remdesivir, oseltamivir, favipiravir, human immunoglobulin, interferons, chloroquine, hydroxychloroquine, methylprednisolone, ritonavir, darunavir, lopinavir, tocilizumab and convalescent plasma. Drugs listed with their mechanisms of action on COVID 19, and adverse effects can be found on Table 7.

Peer-reviewed version available at Diagnostic Microbiology and Infectious Disease 2020; doi:10.1016/j.diagmicrobio.2020.115094

Table 7 Drugs and targets in SARS-CoV-2

TYPE OF DRUG	TARGET	OTHER DISEASES INDICATION	MECHANISM OF ACTION IN COVID 19 (Drugs Repurposing)	ACTIVITY AGAINST SARS-COV-2	SIDE EFFECTS
ANTIVIRAL DRUGS					
Favipiravir	RdRp, RNA dependent RNA polimerase	Influenza. Ebola, yellow fever, chikungunya, norovirus.	Inhibitor of viral RNA-dependent RNA polymerase. Pyrazinecarboxamide derivative viral RNA polymerase inhibitor.	IN VITRO	ND
Arbidol	S protein, ACE2	Influenza	Entrance. S protein- AC2 receptor	IN VITRO	Gastrointestinal effects
ANTIRETROVIRAL DRUGS					
Lopinavir + Ritonavir	Viral proteases: 3CLpro or PLpro	Combination for HIV infection	HIV reverse transcriptase inhibitors. Rito enhance the action of other drugs by inhibition of CYP3A4 May inhibit the viral proteases: 3CLpro or PLpro	IN VITRO. IN VIVO	Rash, GI upset, abnormal liver tests
Remdesivir	RdRp, RNA dependent RNA polimerase	Ebola and Marburg viruses, SARS-CoV-1 and MERS	Inhibe viral replication	IN VITRO, IN VIVO	Abnormal liver tests, GI
Darunavir	Protease inhibitor	HIV protease inhibitor	In combination with cobicistat, a CYP3A inhibitor,	ND	Rash, GI upset, abnormal liver tests
ANTIMALARIAL DRUGS					
Chloroquine	endosome/ ACE2	Antimalarial actions, chloroquine has some efficacy in HIV-AIDS	Glycosilation Inhibition and elevate endosomal pH and interfere with ACE2 glycosylation	IN VITRO	Retinopathy, QT prologation,
Hidroxichloroquine	endosome/ ACE2	Antimalarial actions, chloroquine has some efficacy in HIV-AIDS	inhibiting virus entry into host cells	IN VITRO	QT prolongation
ANTIBIOTICS					
Azitromicin	Bacterial protein sybthesis, blocking 50S ribosomal	Bacterial infections	For suspected bacterial superinfection	ND	GI effects
ANTIVIRAL DRUGS. NON-SPECIFIC					
Interferon	PKR, Mx protein	Hepatitis B virus and HCV	Inhibite viral replication by inhibition of PKR	ND	Depression, injection site reaction, flu like syndrome
NEUROAMINID ASA INHIBITOR					
Oseltamivir	Neuroaminidasa Inhibitor	Influenza	Not well studied	In vitro	
TYPE OF DRUG	**TARGET**	**OTHER DISEASES INDICATION**	**MECHANISM OF ACTION IN COVID 19 (Drugs Repurposing)**	**ACTIVITY**	**SIDE EFFECTS**
MONOCLONAL ANTIBODY					

Tocilizumab	IL-6 receptors (soluble and membrane-bound)	rheumatoid arthritis, systemic juvenile idiopathic arthritis, juvenile idiopathic polyarthritis, giant - cell arteritis	Inhibit IL-6. Taper immune system in critical patients	ND	Abnormal liver tests, GI perforation
ANTIINFLAMATORY DRUGS					
Corticosteroids	Inflammation cascade	Inflammatory responses	For patients with refractory shock or acute respiratory distress syndrome	ND	Cushing Sd., diabetes, weigh gain.
OTHERS					
Acetylcysteine	Mucolytic	Symptomatic relief;	Syntomatic relief	ND	Nausea, fver, vomiting,
Angiotensin receptor blockers	ACE2 Receptors			ND	Diziness, nausea, diarrea, headache
Thalidomide	Immunosuppressant	Myeloma	Inhibite production of TNF-α, antiangiogenic activity.	ND	Fever, low cell counts, anxiety, weigh gain or loss
Pirfenidone		idiopathic pulmonary fibrosis	reduces fibroblast proliferation, production of fibrosis-associated proteins and cytokines	ND	
Vitamin C	Antioxidant	Sepsis, chronic process,	Module redoz signaling	ND	

*There are several drugs in study to be considering in treatment for Covid-19. This table summarizes the most important in terms of principal outcomes in clinical trials or activity in vitro. ND= Non Data

Actually, there is a great effort to build strong evidence. There are 382 clinical trials in progress. Some antiviral, antimalarial and antibiotic drugs have also been shown to have in vitro activity against SARS CoV 2, but it does not guarantee clinical efficacy. For these there are several completed and in progress clinical studies. Some of them like Darunavir are in phase II, Remdesivir, cloroquine and hydroxycloroquine are in phase III of clinical trials, Lopinavir and Ritonavir (Kaletra) and Umifenovir or Arbidol in phase IV. In order to collect data quickly and get information from many countries on March 20, 2020, the WHO announced a large global trial, called SOLIDARITY. The treatments included in this big trial are: remdesivir, chloroquine and hydroxychloroquine, ritonavir / lopinavir-ritonavir / lopinavir and interferon beta. The completed and clinical trials with evidence that favors them are listed in (Supplementary Table 7) and its relation with clinical features in Table 8 [143]. The clinical trials evaluate some important outcomes. A systematic review of Lopinavir /Ritonavir assess treatment in terms of mortality, mechanical ventilation or extracorporeal membrane oxygenation (ECMO)[214] development of acute respiratory distress syndrome and serious adverse effects [215]. None of the outcomes favors the intervention; nevertheless, in terms of development of the respiratory syndrome, the pharmacological intervention is effective, although the certainty of the evidence was very low. Another systematic review, that included six articles and 23 ongoing clinical trials in China about the use of chloroquine in COVID-19 [216]. Studies indicates chloroquine reduced progression of disease and decreased duration of symptoms, but none of the studies favors the use of cloroquine because of the lack of strong evidence in ramdomized trials. In a recent trial, 100% of patients treated with hydroxychloroquine in combination with azithromycin were “virologically cured” comparing with 57.1% in patients treated with hydroxychloroquine alone, and 12.5% in the control group, but these cannot be completely extrapolated because it requires more quantity and quality studies. The use of chloroquine or hydroxychloroquine in primary health care is not recommended for the management of COVID 19. These drugs are associated with an increased risk of heart damage, especially when administered concurrently with macrolides (QT interval prolongation). Drugs like Tocilizumab has been included in severe or critical patients. Remdesivir is effective against the 2019-nCoV in vitro in Vero E6 cells through mechanism of involving the host cells' post-entry stage. Several randomized trials are underway to evaluate the efficacy of remdesivir for moderate or severe COVID-19.

Oseltamivir inhibits the viral neuraminidase, drug approved for influenza A and B treatment. Its use was reported during the COVID-19 epidemic in China, but it has no effective outcomes. Tocilizumab, an inhibitor of IL-6 is considered in a group of critical patients, in which 75% cursed with improved respiratory function after treatment. The last treatment reported in a 5 patient case series is convalescent plasma. Following plasma transfusion normalized temperature within 3 days in 4 of 5 patients, decreased SOFA score, increase PAO2/FIO2 within 12 days and viral loads also decreased and became negative within 12 days after the transfusion. A promising drug, although the evidence level is low [217]. Bevacizumab is a monoclonal antibody that targets vascular endothelial growth factor (VEGF) that might suppress the edema in patients with COVID-19.

The other drugs studied in pharmaceutical interventions for COVID-19 treatment include arbidol, human immunoglobulin, interferons, chloroquine, methylprednisolone, tocilizumab, vitamin C, pirfenidone, bromhexine, danoprevir, darunavir, cobicistat, convalescent plasma, biological therapies and traditional Chinese medicines (TCM), which are studied in clinical trials in progress and do not appear as strong evidence to recommend them in practice.

Regarding corticosteroids, are beneficial in treating SARS-CoV patients; it prolongs the survival time of clinical cases. Due to the cytokine storm some authors described the use of corticosteroids, but others describe its use in the early stages of SARS infection with increasing values of viral load. Other drugs like vitamin C has been used to prevent but there is no good evidence to support this. All of the evidence including clinical trials, randomized clinical trials, favors the use or not about the therapeutically drugs are detailed in Table 8.

This review summarized some drug repurposing agents currently known to be effective against other RNA viruses including SARS-CoV, MERS-CoV, influenza. Actually, exist some new drugs with high potential impact of biologics targets for Covid-19 treatment. It is important to notice that there is no specific treatment for the coronavirus approach. In context of the scientific evidence and the particular clinical features of each patient, the reader will be able to make clinical and therapeutically decisions.

Vaccines development

When it comes to vaccine design and manufacturing, the main objectives are its safety, its efficacy in activating specific adaptive immune responses and the production of -ideally- long term memory. Thus, eliciting protective immune responses including neutralization antibodies and/or CTL generation is of paramount importance.

Huge challenges need to be tackled in order to minimize the long and cumbersome process of vaccine generation. Among them, candidate antigen targets need to be identified, immunization routes and delivery systems investigated, animal models set, adjuvants optimized, scalability and production facility considered, target population selected, and vaccine safety and long-term efficiency evaluated.

Currently there are no approved vaccines against any human coronavirus, suggesting that their generation is quite trivial. Several candidate vaccines against SARS-CoV had shown promise reaching Phase I or Phase II clinical trials [77, 78], but the rapid containment of SARS-CoV expansion rendered them redundant, did not allow for a test population for Phase III trials and, therefore, put their further assessment to a halt.

However, the accumulated experience from previous coronavirus vaccine designs and the sequence and structural similarity of SARS-CoV and SARS-CoV-2 are significant advantages in the current endeavor. Thorough studies conducted in SARS-CoV-specific T cells of SARS convalescent patients have shown that all memory T cell responses are directed at SARS-CoV structural proteins. T cell epitope mapping showed that CD8+ responses were targeting SARS-CoV membrane (M) and Nucleocapsid (N) proteins and CTL memory could last up to 11 years after infection [218]. These data suggest that vaccine strategies employing viral structural proteins that can elicit effective, long-term memory T cell responses could yield fruitful results.

On the other hand, the S1 spike protein region containing the ACE receptor binding domain (RDB) is the obvious option when neutralizing antibody responses are considered [219–221].

Indeed, a candidate SARS vaccine antigen consisting of the RBD of SARS-CoV Spike protein was created and found it could elicit robust neutralizing antibody responses and long-term protection in vaccinated animals [222].

The fact that COVID-19 convalescent sera shows potential as a therapeutic approach [67] argues that efficient B cell responses are mounted and lead to production of protective antibodies. Two different groups, using an immunoinformatic approach mapped several CTL and B cell epitopes on different proteins of the virus [223, 224]. Moreover, various CTL epitopes were found to be binding MHC class I peptide-binding grooves via multiple contacts, illustrating their probable capacity to elicit immune responses [82]. Consequently, these identified B and T cell epitopes could be potential targets for therapeutic vaccines.

However, important safety considerations should be taken into account before releasing a new vaccine in the market. Previous studies on macaque models have shown that a vaccine-induced anti-Spike protein antibody at the acute stage of SARS-CoV infection can provoke severe acute lung injury [225]. Similar observations of SARS-CoV vaccine-induced pulmonary injury have also been described in multiple several murine and monkey animal models [226].

Classic vaccine strategies like use of attenuated virus or recombinant protein subunit administration begin to lose support in the scientific community. COVID-19 mainly affects older patients with underlying pathologies that debilitate their immune system. Use of attenuated virus vaccines is contraindicated in these populations as weakened immune systems can permit the reversion of the attenuated pathogen to its wild type state, therefore causing the pathology it was designed to prevent. On the other hand, subunit vaccine design can be challenging when the protein used contains extended glycosylation. Interestingly, nucleic acid-based vaccines showed great promise in response to emerging pathogens like the DNA vaccine designed for Zika virus, entering in Phase I clinical trials [227]. Another nucleic acid-based platform for vaccine development, mRNA vaccines, seems a quite revolutionary strategy. Being designed to possess improved stability and protein translation efficiency these vaccine platforms can act both as adjuvants and antigen sources alike,

inducing potent immune responses [228, 229]. The optimization of the delivery system, such as lipid nanoparticles makes them excellent design candidates [230]. Finally, delivery systems such as recombinant vesicular stomatitis virus particles or the administration of mRNA molecules that codify for virus-like particles have been proven extremely efficient as testified by the recent FDA approved vaccine against Ebola [231].

In an unprecedentedly swift response to develop and manufacture an anti-SARS-CoV-2 vaccine, more than 40 companies and academic institutions are exploring the aforementioned strategies. An example illustrating the rapid reaction of the scientific community to the SARS-CoV-2 outbreak is that of the the biopharmaceutical company Moderna, the first vaccine manufacturer that entered in Phase I clinical trials for one candidate vaccine for COVID-19. On the night of Saturday, January 11, 2020, in the headquarters of the National Institute of Allergies and Infectious Diseases (NIAID) of USA Barney Graham, Deputy Director of the Vaccine Research Center, received the SARS-CoV-2 sequence. During the weekend his group analyzed the data and on Monday, 13 of January he discussed his observations with a group of investigators of the biopharmaceutical company Moderna. On the same day Moderna's infectious disease research team finalized the sequence for mRNA-1273, the company's first vaccine candidate against SARS-CoV-2. On February 7, 2020, the first clinical batch of Moderna was completed. On February 24, 2020, the clinical batch was shipped from Moderna to the NIH to be used in their own Phase I clinical study. On March 4, 2020, the U.S. FDA gave the green light for mRNA-1273 to begin clinical trials. Twelve days later, on March 16, 2020, the NIH announced that the first participant in its Phase I clinical study received the first dose of mRNA-1273. The time between virus sequencing to beginning of Phase I trials was a record total of 63 days.

The pharmaceutical companies that are currently on a race to produce a vaccine for COVID-19 along with the vaccine developing strategies they are using are summarized in Table 9.

Table 8 COVID-19 vaccine development update by manufacturer. Vaccination strategies employed, delivery platforms used, and current development status are presented if official data are provided.

Manufacturer	Vaccine candidate	Vaccination Strategy	Delivery Platform	Current stage of development/Trial Phase ¥	Status ¥	Viruses targeted by candidate vaccines using same strategy
Moderna/ NIAID	mRNA-1273	mRNA codifying for full-length S protein	LNP* encapsulated mRNA	Phase 1 NCT04283461§	Recruiting completed 19 March 2020	SARS-CoV, MERS-CoV
CanSino Biological Inc./ Beijing Institute of Biotechnology	Ad5-nCoV	Recombinant virus/Non-replicating	Adenovirus Type 5 Vector	Phase 1 ChiCTR2000030906§	Currently recruiting	Ebola, MERS-CoV
Inovio Pharmaceuticals	INO-4800	DNA vaccine	Plasmid-Electroporation facilitated entry	Pre-clinical development	Phase 1 clinical trials are expected to begin in April 2020	Lassa, Nipah, HIV, Filovirus, HPV, Zika, Hepatitis B
Takis Biotech & Applied DNA Sciences/ Evvivax	Not announced/4 candidates for COVID-19	DNA vaccine	DNA	Pre-clinical development	Phase 1 clinical trials are expected to begin in fall 2020	Lassa, Nipah, HIV, Filovirus, HPV, Zika, Hepatitis B
Zydus Cadila	Not announced/2 strategies employed	1. DNA vaccine 2. Live attenuated recombinant vaccine	1. Plasmid 2. Recombinant replicating measles virus	Pre-clinical development	Not announced	Lassa, Nipah, HIV, Filovirus, HPV, Zika, Hepatitis B
Sinovac	Not announced	Formalin inactivated & alum adjuvant	Inactivated virus	Pre-clinical development	Not announced	SARS-CoV
Serum Institute of India & Codagenix	Not announced	Live Attenuated Virus	Live Attenuated Virus	Pre-clinical development	*In vivo* testing pending	HAV, InfA, ZIKV, FMD, SIV, RSV, DENV
Geovax/ BravoVax	Not announced	Recombinant viral vector/Non-replicating	Modified vaccinia ankara virus like particles encoded (MVA-CLP)	Pre-clinical development	Narrowing the vaccine candidates down from three to one	LASV, EBOV, MARV, HIV
Janssen Pharmaceutical Companies of Johnson & Johnson/Barda	Not announced	Recombinant viral vector/Non-replicating	Ad26 (alone or with MVA boost) - AdVac and PER.C6 systems	Pre-clinical development	Vaccine candidate is expected end of March 2020/ Clinical testing starting in November 2020	Ebola, HIV, RSV
University of Oxford	ChAdOx1	Recombinant viral vector/Non-replicating	Chimpanzee adenovirus vaccine vector	Pre-clinical development	Not announced	Influenza strains, Mycobacterium tuberculosis, Chikungunya, Zika, MenB, plague
Altimmune	Intranasal COVID-19 vaccine	Recombinant viral vector/Non-replicating	Adenovirus - based NasoVAX expressing SARS2-CoV S protein	Pre-clinical development	Animal testing imminent /Clinical testing is initially scheduled for August 2020	Influenza strains (NasoVAX vaccine)
Greffex	Adenovirus-based vector vaccine for COVID-19	Recombinant viral vector/Non-replicating	Adenovirus-based vector vaccine	Pre-clinical development	Animal testing has begun	MERS-CoV
Vaxart	Not announced	Recombinant viral	Oral Vaccine platform	Pre-clinical development	Not announced	InfA, CHIKV, LASV, NORV;

ExpreS^{2}ion	Not announced	vector/Non-replicating Protein Subunit	Drosophila Schneider 2 insect cell expression system VLPs[#]	Pre-clinical development	Phase 1/2a clinical testing to begin within 12 months	EBOV, RVF, HBV, VEE -
Walter Reed Army Institute of Research/United States Army Medical Research Institute of Infectious Diseases	Not announced	Protein Subunit/S protein	Antigen + adjuvant	Pre-clinical development	Several vaccine candidates developed/ Animal testing has begun	MERS-CoV
Clover Biopharmaceuticals Inc./Glaxo Smith Kline	COVID-19 S-Trimer	Protein Subunits/S-Trimer	Antigen + adjuvant	Pre-clinical development	Pre-clinical trials pending	HIV, REV Influenza
Vaxil Bio	Protein subunit COVID-19 vaccine candidate	Protein Subunit/ signal peptide technology (Patented)	Antigen + adjuvant	Pre-clinical development	Candidate identified/Beginning of trials not announced	
AJ Vaccines	Not announced	Protein Subunit	Antigen + adjuvant	Pre-clinical development	Not announced	
Generex Biotechnology/EpiVax	Ii-Key peptide COVID-19 vaccine	Protein Subunit	li-key/antigenic epitope hybrid peptide vaccine	Pre-clinical development	Intention is to begin human testing within 3 months	Influenza strains, HIV, SARSCoV
EpiVax/University of Georgia	Ii-Key peptide COVID-19 vaccine	Protein Subunit/S protein	li-key/antigenic epitope hybrid peptide vaccine	Pre-clinical development	Not announced	H7N9
Sanofi Pasteur/BARDA	Not announced	Protein Subunit/S protein produced in baculovirus	Antigen + adjuvant	Pre-clinical development	Not announced	Influenza strains, SARS-CoV
Novavax	Not announced	Protein Subunit	Recombinant nanoparticles	Pre-clinical development	Several candidates currently tested in animals/Clinical testing to begin in late spring 2020	RSV; CCHF, HPV, VZV, EBOV
Heat Biologics/University of Miami	gp96-based vaccine	Protein Subunit/gp-96 heat-shock protein backbone	Antigen + adjuvant	Pre-clinical development	Not announced	NSCLC, HIV, malaria, Zika
University of Queensland/CSL	Molecular clamp vaccine for COVID-19	Protein Subunit/ Molecular clamp stabilized Spike protein	Antigen + adjuvant	Pre-clinical development	Further development prior to pre-clinical testing required	Nipah, influenza, Ebola, Lassa
Baylor College of Medicine	Re-purposed SARS vaccine for COVID-19	Protein Subunit/S1 or RBD protein	Antigen + adjuvant	Pre-clinical development	Not announced	SARS-CoV
iBio/CC-Pharming	Plant-based COVID-19 vaccine	Subunit protein/Plant produced	Antigen + adjuvant	Pre-clinical development	Not announced	
VIDO-InterVac/ University of Saskatchewan	Not announced	Protein Subunit	Adjuvanted microsphere peptide	Pre-clinical development	Not announced	

Institute Pasteur/Themis/ Univ. of Pittsburg Center for Vaccine Research	Not announced	Recombinant replicating Viral Vector	Measles Vector	Pre-clinical development	Not announced	West nile, Ebola, Lassa, Zika
Tonix Pharma/Southern Research	Horsepox vaccine with percutaneous administration	Recombinant replicating Viral Vector (used also in TNX-1800 vaccine)	Horsepox vector expressing S-protein	Pre-clinical development	Not announced	Smallpox, monkeypox
Fudan University/ Shanghai Jiao Tong University/RNA Cure Biopharma	mRNA vaccine candidate for COVID-19	mRNA vaccine/2 candidates	1. LNP* encapsulated mRNA cocktail encoding SARS-CoV-2 VLP[#] 2. LNP* encapsulated mRNA encoding RBD of S-protein	Pre-clinical development	Not announced	
China CDC/Tongji University/Sterm ina	Not announced	mRNA vaccine	Not announced	Pre-clinical development	Not announced	
Arcturus/Duke-NUS Medical School	Not announced	mRNA vaccine	Self-replicating RNA and nanoparticle non-viral delivery system	Pre-clinical development	Not announced	Various candidates
BioNTech/Fosun Pharma/Pfizer	BNT162	mRNA vaccine	Not announced	Pre-clinical development	Clinical testing is expected to begin in April 2020	Influenza strains
Curevac	Not announced	mRNA vaccine	Not announced	Pre-clinical development	Clinical trials expected to begin in summer 2020	RABV, LASV, YFV; MERS, InfA, ZIKV, DengV, NIPV
Imperial College London	Self-amplifying (sa) RNA vaccine	saRNA vaccine	Not announced	Pre-clinical development	Animal testing is underway/ Clinical trials expected to begin in summer 2020	EBOV; LASV, MARV, Inf (H7N9), RABV
Medicago Inc.	Plant-based COVID-19 vaccine	Plant-derived VLP[#]	VLP[#]	Pre-clinical development	Human testing expected to begin in July or August 2020	Influenza, Rotavirus, Norovirus, West Nile virus, Cancer

* LNP: Lipid nanoparticle system, [§] Clinical Trial Registry Identifier, [¥] According to manufacturer, [#] VLP: Virus like particle, Table updated until 22/03/2020; Several more companies have announced their intention to manufacture COVID-19 vaccines without disclosing further information.

As can be easily deduced from Table 9, optimistic predictions dictate that a vaccine for COVID-19 will not be ready in the next 12-18 months. An indirect course of action that could help to mitigate the impact of COVID-19 pandemic would be a plan of vaccination against influenza strains and *Strepococcus pneumoniae*. Influenza is a major universal health problem accounting for 3 to 5 million cases of severe illness and about 350 000 to 650 000 respiratory deaths yearly. For the time period from 17 February 2020 to 01 March 2020 alone the WHO laboratories tested positive for influenza viruses 62423 samples [232]. On the other

hand, *Streptococcus pneumoniae* is the most common cause of community acquired pneumonia. In the present context of COVID-19 global outbreak vaccination against the most prevalent strains of influenza and *Streptococcus pneumoniae* would have a multifaceted effect. Firstly, it would lower the risk of severe disease, reduce hospitalization and admission to already heavily charged ICUs due to these pathologies that could prove critical for weaker health systems that would struggle to carry the burden of combined outbreaks. Moreover, vaccinating health care workers is crucial for reducing the risk of absence due to disease, thereby strengthening the healthcare workforce and minimizing the risk to infect COVID-19 hospitalized patients with additional pneumonia-causing pathogens. Lastly, COVID-19 patients vaccinated for influenza and *Streptococcus pneumoniae* allow their immune system to focus on one pathogen and, therefore, give it a better fighting chance against SARS-CoV-2 infection [233]. High risk groups prioritized for vaccination for these two pathogens include pregnant women, persons with immunocompromised immune systems (either due to congenital or acquired immunodeficiencies), children, adults ≥65 years and health care professionals.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 16 April 2020 doi:10.20944/preprints202004.0283.v1

Peer-reviewed version available at *Diagnostic Microbiology and Infectious Disease* **2020**; doi:10.1016/j.diagmicrobio.2020.115094

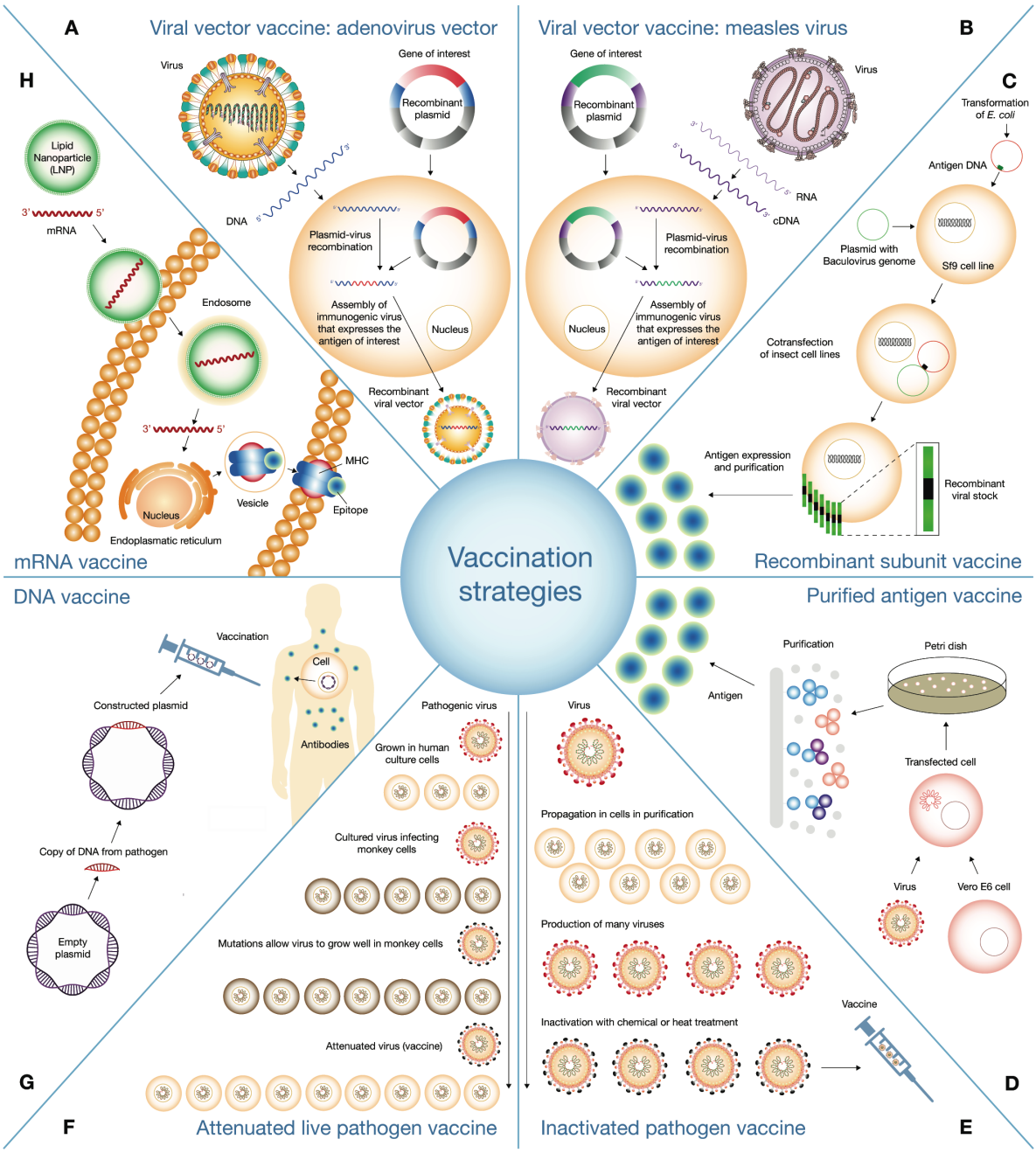

Figure 4. *Strategies used or proposed for COVID-19 vaccine development and delivery. A) and B) Adenoviral and measles recombinant viral vectors can be manipulated to express and therefore elicit robust immune responses against the Spike (S) protein of SARS-CoV-2. C. Recombinant subunit vaccine strategies use the Sf9-baculovirus insect cell expression system resulting in the production of high-quality antigen that can be used to elicit immune responses. D) Purified antigen vaccine strategies implicate the replication of large numbers of virus in cell cultures and the subsequent purification of viral antigens to be used for vaccination. E) Attenuated vaccines contain whole pathogen that has been submitted to heat or chemical treatment inactivation. F) Attenuated live pathogen vaccine strategies consist in administering a live pathogen that due to cell culture passaging has lost its virulence. They usually elicit robust and long-term memory immune responses without the need to administer an adjuvant. G) In DNA vaccines the DNA codifying a highly immunogenic antigen is administered and captured by professional antigen presenting cells (APCs) leading to antigen production and presentation by these cells. H. Moderna's vaccine candidate already in Phase I clinical trials uses an mRNA vaccine approach whereby the genetic information codifying for the S protein of SARS-CoV-2 is delivered in LNPs to enhance absorption by APCs. Once*

uptaken by APCs the mRNA induces the expression of S antigen that is subsequently mounted on and presented by MHC molecules to elicit adaptive immune response.

Climate and SARS-CoV-2

Numerous studies confirm that climate has an impact on virus (i.e., influenza, coronavirus, etc.) spread through manipulating the conditions of i) its diffusion, ii) the virus survival outside the host, and iii) the immunity of host population [234]. Meteorological conditions, such as temperature, humidity, wind speed and direction, atmospheric pressure, solar radiation (including ultraviolet (UV) spectrum) and precipitation amount and intensity depend on the latitude and the elevation of the location, thus creating distinct climatic zones in the planet. While in some regions, such as temperate climate zones, human influenza peaks have clear seasonal cycles, in others it is not as predictable [234–238].

An array of studies, investigating the relationship between climatic factors and the activity of influenza all over the world, concluded that at the high latitudes of the world the peaks of influenza correlate with cold and dry weather conditions (i.e., winter season), while around the equatorial zone, it is more common during the months of high humidity and precipitation [239–245]. Essentially, it depends on explicit threshold conditions based on monthly averages of specific humidity and temperature. When specific humidity drops below 11-12 g/kg and temperature drops below 18-21°C, the peak of influenza is stimulated during the cold-dry season, however, for tropical and subtropical (always humid and warm) regions, it is likely to prevail during the high precipitation (≥150 mm) months [239]. The "cold-dry" set of climatic conditions endorses a greater survival of the virus outside human body, and, thus, results in better transmission [237, 246]. Similar temperature dependency was concluded for SARS (strain CoV-P9) coronavirus. Laboratory experiments testing virus stability, demonstrated a decreasing infectivity with increasing ambient temperatures, where at 4°C, 56°C and 75°C the survival rates outside host decreased from at least 96 to 1.5 and to 0.5 hours, respectively [247]. In addition, cold air cools nasal epithelium which, in turn, decreases mechanical defenses of the respiratory and immune systems [248].

Duan et. al., (2003) concluded that, even a relatively short exposure (1 hour) to UV radiation destroys viral infectivity of SARS (strain CoV-P9) coronavirus. Other studies also correlate vitamin D secretion and influenza immunity, due to the UV role in vitamin D production [249, 250]. The latter, and the reduced immune system due to melatonin oscillations during the dark (lack of sunlight hours) winter seasons could further explain winter outbreaks of influenza at high latitude regions [251].

Finally, wind speed may contribute to the spread of influenza nanoparticles. While low winds might improve its transmission from one host to another, strong winds contribute to its dispersion and ventilation [252], which could be a positive effect depending on wind direction.

Conclusions

The authors of this study examined the most important literature available in terms of the genetic, virologic, clinical and therapeutic evidence on the SARS-CoV-2 virus and the novel coronavirus diseases 2019 (COVID-19).

This extensive and comprehensive literature review tries to offer a good insight of the most recent information available. This review was designed to offer a good insight of the virus and the diseases to the entire medical community. This document although summarized, tries to bring well-supported information on this new disease. A disease that has been keeping us on a partial or total lockdown all over the planet.

List of Abbreviations:

ACE2: Angiotensin Converting Enzyme-2

ARDS: acute respiratory distress syndrome

CDCC: The Center for Disease Control in China

COVID-19: Coronavirus disease 2019

ELISA: The enzyme-linked immunosorbent assay

GISAID: Global Initiative on Sharing All Influenza Data

INF: Interferon

ISGs: IFN-stimulated genes

MERS: Middle East Respiratory Syndrome

MHC-I: Major Histocompatibility complex I

ORF: Open reading frames

PDB: Protein Data Bank archive

pDCs: plasmacytoid dendritic cells

RCSB: Research Collaboratory for Structural Bioinformatics

RNA: Ribonucleic acid

RdRp: RNA-dependent RNA polymerase

RBD: receptor-binding domain

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

SARS: Severe acute respiratory syndrome

TCGA: The Cancer Genome Atlas

TLRs: endosomal Toll like receptors

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

According to the local and international regulation, this project did no required ethical approval.

Consent to publish

Not Applicable.

Availability of data and materials

All the information used for this analysis can be found

Competing interests

The authors declare that they have no competing interests.

Funding

This work did not receive financial support of any kind.

Authors' Contributions

EOP was responsible for the full conceptualization and he was in charge of drafting the document in all of the stages. KSR and LGB contributed with the Cancer and Covid-19 section. MRN completed the section about covid-19 in children. LG and CBO completed the diagnosis section of the review. NK completed the immunological response to SARS CoV2. CM completed the virologic aspects of the manuscript. AMG, DC, HSS and LU completed the clinical section of the manuscript, the therapeutic strategies and the gynecological and complication section of the manuscript. RZ completed the environmental effects and Covid-19. NG critically review the manuscript and ALC was partially responsible for the conceptualization of the study, the genetic aspects of the virus and he completed all the figures for this work.

References

1. Shanker A. The Possible Origins of the New Coronavirus SARS-CoV-2. 2020.

2. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020;395:565–574.

3. Giri R, Bhardwaj T, Shegane M, Gehi BR, Kumar P, Gadhave K, et al. When Darkness Becomes a Ray of Light in the Dark Times: Understanding the COVID-19 via the Comparative Analysis of the Dark Proteomes of SARS-CoV-2, Human SARS and Bat SARS-Like Coronaviruses. bioRxiv. 2020.

4. Lorusso A, Calistri P, Petrini A, Savini G, Decaro N. Novel coronavirus (SARS-CoV-2) epidemic: a veterinary perspective. Vet Ital. 2020.

5. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;:102433.

6. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis. 2020.

7. Peiris JSM, Guan Y, Yuen KY. Severe acute respiratory syndrome. Nature Medicine. 2004.

8. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:727–33.

9. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020.

10. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. Science. 2020.

11. Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science. 2020;:eabb3405.

12. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020.

13. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020.

14. Gralinski LE, Menachery VD. Return of the coronavirus: 2019-nCoV. Viruses. 2020.

15. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, et al. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. Cell Host Microbe. 2020.

16. Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm Sin B. 2020.

17. Elbe S, Buckland-Merrett G. Data, disease and diplomacy: GISAID's innovative contribution to global health. Glob Chall. 2017.

18. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data – from vision to reality. Eurosurveillance. 2017.

19. Kirchdoerfer RN, Cottrell CA, Wang N, Pallesen J, Yassine HM, Turner HL, et al. Pre-fusion structure of a human coronavirus spike protein. Nature. 2016;531:118–21.

20. Yuan Y, Cao D, Zhang Y, Ma J, Qi J, Wang Q, et al. Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. Nat Commun. 2017;8:15092.

21. Li W, Moore MJ, Vasllieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426:450–4.

22. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181:1–10.

23. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020.

24. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J Virol. 2020.

25. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The Proximal Origin of SARS-CoV-2. Virological. 2020.

26. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res. 2000;87.

27. Liu L, Wei Q, Alvarez X, Wang H, Du Y, Zhu H, et al. Epithelial cells lining salivary gland ducts are early target cells of severe acute respiratory syndrome coronavirus infection in the upper respiratory tracts of rhesus macaques. J Virol. 2011;85:4025–30.

28. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med. 2005;11:875–9.

29. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020.

30. Forrest ARR, Kawaji H, Rehli M, Baillie JK, De Hoon MJL, Haberle V, et al. A promoter-level mammalian expression atlas. Nature. 2014.

31. Hoffmann M, Kleine-Weber H, Schroeder S, Mü MA, Drosten C, Pö S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor Article SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181:1–10.

32. Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res. 2020.

33. de Wilde AH, Snijder EJ, Kikkert M, van Hemert MJ. Host factors in coronavirus replication. In: Current Topics in Microbiology and Immunology. Springer Verlag; 2018. p. 1–42.

34. Lim Y, Ng Y, Tam J, Liu D. Human Coronaviruses: A Review of Virus–Host Interactions. Diseases. 2016;4:26.

35. Knoops K, Kikkert M, Van Den Worm SHE, Zevenhoven-Dobbe JC, Van Der Meer Y, Koster AJ, et al. SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. PLoS Biol. 2008;6:1957–74.

36. Weiss SR, Navas-Martin S. Coronavirus Pathogenesis and the Emerging Pathogen Severe Acute Respiratory Syndrome Coronavirus. Microbiol Mol Biol Rev. 2005;69:635–64.

37. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, et al. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol. 2011;174:11–22.

38. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 2020;395:507–13.

39. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395:497–506.

40. Yang Y, Lu Q, Liu M, Wang Y, Zhang A, Jalali N, et al. Epidemiological and clinical features of the 2019 novel coronavirus outbreak in China. medRxiv. 2020.

41. Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. Curr Opin Virol. 2011;1:519–25.

42. Kell AM, Gale Jr M. RIG-I in RNA virus recognition. Virology. 2015;479:110–21.

43. Yoneyama M, Fujita T. RNA recognition and signal transduction by RIG-I-like receptors. Immunol Rev. 2009;227:54–65.

44. Diebold SS. Recognition of viral single-stranded RNA by Toll-like receptors. Adv Drug Deliv Rev. 2008;60:813–23.

45. Ma DY, Suthar MS. Mechanisms of innate immune evasion in re-emerging RNA viruses. Curr Opin Virol. 2015;12:26–37.

46. Nelemans T, Kikkert M. Viral Innate Immune Evasion and the Pathogenesis of Emerging RNA Virus Infections. Viruses. 2019;11:961.

47. Dandekar AA, Perlman S. Immunopathogenesis of coronavirus infections: implications for SARS. Nat Rev Immunol. 2005;5:917–27.

48. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Springer; 2017. p. 529–39.

49. Zumla A, Hui DS, Perlman S. Middle East respiratory syndrome. The Lancet. 2015;386:995–1007.

50. Kindler E, Thiel V, Weber F. Interaction of SARS and MERS coronaviruses with the antiviral interferon response. In: Advances in virus research. Elsevier; 2016. p. 219–43.

51. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol. 2016;14:523.

52. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. China Febr 17 2020. 2020.

53. Wong C, Lam C, Wu A, Ip W, Lee N, Chan I, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol. 2004;136:95–103.

54. Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. Cytokine. 2018;104:8–13.

55. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA, et al. Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of the macrophage activation syndrome: Re-analysis of a prior Phase III trial. Crit Care Med. 2016;44:275.

56. A multicenter, randomized controlled trial for the efficacy and safety of tocilizumab in the treatment of new coronavirus pneumonia (COVID-19). Chinese Clinical Trial Registry. 2020. http://www.chictr.org.cn/showprojen.aspx?proj=49409. Accessed 26 Mar 2020.

57. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases. Lancet Infect Dis. 2003;3:722–7.

58. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005;2:69.

59. Yao X, Ye F, Zhang M, Cui C, Huang B, Niu P, et al. 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). Clin Infect Dis. 2020.

60. Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, et al. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. The Lancet. 2020;395:e30–1.

61. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. The Lancet. 2020.

62. Liu J, Wu P, Gao F, Qi J, Kawana-Tachikawa A, Xie J, et al. Novel immunodominant peptide presentation strategy: a featured HLA-A* 2402-restricted cytotoxic T-lymphocyte epitope stabilized by intrachain hydrogen bonds from severe acute respiratory syndrome coronavirus nucleocapsid protein. J Virol. 2010;84:11849–57.

63. Keicho N, Itoyama S, Kashiwase K, Phi NC, Long HT, Van Ban V, et al. Association of human leukocyte antigen class II alleles with severe acute respiratory syndrome in the Vietnamese population. Hum Immunol. 2009;70:527–31.

64. Chen Y-MA, Liang S-Y, Shih Y-P, Chen C-Y, Lee Y-M, Chang L, et al. Epidemiological and genetic correlates of severe acute respiratory syndrome coronavirus infection in the hospital with the highest nosocomial infection rate in Taiwan in 2003. J Clin Microbiol. 2006;44:359–65.

65. Wang S-F, Chen K-H, Chen M, Li W-Y, Chen Y-J, Tsao C-H, et al. Human-leukocyte antigen class I Cw 1502 and class II DR 0301 genotypes are associated with resistance to severe acute respiratory syndrome (SARS) infection. Viral Immunol. 2011;24:421–6.

66. Hajeer AH, Balkhy H, Johani S, Yousef MZ, Arabi Y. Association of human leukocyte antigen class II alleles with severe Middle East respiratory syndrome-coronavirus infection. Ann Thorac Med. 2016;11:211.

67. Chan JF-W, Yuan S, Kok K-H, To KK-W, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The Lancet. 2020;395:514–23.

68. Tang F, Quan Y, Xin Z-T, Wrammert J, Ma M-J, Lv H, et al. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. J Immunol. 2011;186:7264–8.

69. Liu W, Fontanet A, Zhang P-H, Zhan L, Xin Z-T, Baril L, et al. Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. J Infect Dis. 2006;193:792–5.

70. Li G, Chen X, Xu A. Profile of specific antibodies to the SARS-associated coronavirus. N Engl J Med. 2003;349:508–9.

71. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. 2020.

72. Amanat F, Nguyen T, Chromikova V, Strohmeier S, Stadlbauer D, Javier A, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. medRxiv. 2020.

73. Bao L, Deng W, Gao H, Xiao C, Liu J, Xue J, et al. Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. bioRxiv. 2020.

74. Casadevall A, Pirofski L. The convalescent sera option for containing COVID-19. J Clin Invest. 2020;130.

75. Hopkins JS. Drugmaker Takeda Is Working on Coronavirus Drug. Wall Street Journal. 2020. https://www.wsj.com/articles/drugmaker-takeda-is-working-on-coronavirus-drug-11583301660. Accessed 26 Mar 2020.

76. Thevarajan I, Nguyen THO, Koutsakos M, Druce J, Caly L, van de Sandt CE, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nat Med. 2020;:1–3. doi:10.1038/s41591-020-0819-2.

77. Sariol CA, Nogueira ML, Vasilakis N. A Tale of Two Viruses: Does Heterologous Flavivirus Immunity Enhance Zika Disease? Trends Microbiol. 2018;26:186–90. doi:10.1016/j.tim.2017.10.004.

78. Yang Z, Werner HC, Kong W, Leung K, Traggiai E, Lanzavecchia A, et al. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. Proc Natl Acad Sci. 2005;102:797–801. doi:10.1073/pnas.0409065102.

79. Yip MS, Leung NHL, Cheung CY, Li PH, Lee HHY, Daëron M, et al. Antibody-dependent infection of human macrophages by severe acute respiratory syndrome coronavirus. Virol J. 2014;11:82. doi:10.1186/1743-422X-11-82.

80. Ho M-S, Chen W-J, Chen H-Y, Lin S-F, Wang M-C, Di J, et al. Neutralizing Antibody Response and SARS Severity. Emerg Infect Dis. 2005;11:1730–7. doi:10.3201/eid1111.040659.

81. Jaume M, Yip MS, Cheung CY, Leung HL, Li PH, Kien F, et al. Anti-Severe Acute Respiratory Syndrome Coronavirus Spike Antibodies Trigger Infection of Human Immune Cells via a pH- and Cysteine Protease-Independent FcγR Pathway. J Virol. 2011;85:10582–97. doi:10.1128/JVI.00671-11.

82. Yoshikawa T, Hill T, Li K, Peters CJ, Tseng C-TK. Severe Acute Respiratory Syndrome (SARS) Coronavirus-Induced Lung Epithelial Cytokines Exacerbate SARS Pathogenesis by Modulating Intrinsic Functions of Monocyte-Derived Macrophages and Dendritic Cells. J Virol. 2009;83:3039–48. doi:10.1128/JVI.01792-08.

83. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, et al. Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. Cell Host Microbe. 2016;19:181–93. doi:10.1016/j.chom.2016.01.007.

84. Kelly-Cirino C, Mazzola LT, Chua A, Oxenford CJ, Kerkhove MDV. An updated roadmap for MERS-CoV research and product development: focus on diagnostics. BMJ Glob Health. 2019;4 Suppl 2:e001105. doi:10.1136/bmjgh-2018-001105.

85. Drosten C, Günther S, Preiser W, van der Werf S, Brodt H-R, Becker S, et al. Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome. N Engl J Med. 2003;348:1967–76. doi:10.1056/NEJMoa030747.

86. Corman V, Müller M, Costabel U, Timm J, Binger T, Meyer B, et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Eurosurveillance. 2012;17.

87. Chan JF-W, Kok K-H, Zhu Z, Chu H, To KK-W, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect. 2020;9:221–36.

88. WHO. Technical guidance Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. 2020. https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117. Accessed 30 Mar 2020.

89. CDC China. Specific primers and probes for detection 2019 novel coronavirus. Novel primers and probes for detection of novel coronavirus in coronavirus. 2020. http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html. Accessed 30 Mar 2020.

90. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25. doi:10.2807/1560-7917.ES.2020.25.3.2000045.

91. Hong Kong University. Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR. 2020. https://www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf?sfvrsn=af1aac73_4.

92. Institut Pasteur, Paris. Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2. 2020. https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2.

93. National Institute of Infectious Disease Japan. Detection of second case of 2019-nCoV infection in Japan. 2020. https://www.niid.go.jp/niid/en/2019-ncov-e/9334-ncov-vir3-2.html. Accessed 30 Mar 2020.

94. Department of Medical Sciences, Ministry of Public Health, Thailand. RT-PCR protocol for the detection of 2019-nCoV. 2020. https://www.who.int/docs/default-source/coronaviruse/conventional-rt-pcr-followed-by-sequencing-for-detection-of-ncov-rirl-nat-inst-health-t.pdf?sfvrsn=42271c6d_4.

95. Centers for Disease Control and Prevention. Real-Time RT-PCR Panel for Detection2019-Novel Coronavirus. 2020. https://www.who.int/docs/default-source/coronaviruse/uscdcrt-pcr-panel-for-detection-instructions.pdf?sfvrsn=3aa07934_2.

96. Coronavirus in California | COVID-19 Testing. Carbon Health. 2020. https://carbonhealth.com/coronavirus#learn. Accessed 30 Mar 2020.

97. IDbyDNA | Explify Platform. 2020. https://www.idbydna.com/explify-platform/. Accessed 30 Mar 2020.

98. Xpert® Xpress SARS-CoV-2 has received FDA Emergency Use Authorization. Cepheid. 2020. https://www.cepheid.com/. Accessed 30 Mar 2020.

99. Roche. cobas® SARS-CoV-2 Test. Diagnostics. 2020. https://diagnostics.roche.com/global/en/products/params/cobas-sars-cov-2-test.html. Accessed 30 Mar 2020.

100. Abbott. Abbott launches Novel Coronavirus Test. Abbott. 2020. https://www.abbott.com/corpnewsroom/product-and-innovation/abbott-launches-novel-coronavirus-test.html. Accessed 30 Mar 2020.

101. CDC. Coronavirus Disease 2019 (COVID-19): Information for Laboratories: 2019-nCoV. Centers for Disease Control and Prevention. 2020. https://www.cdc.gov/coronavirus/2019-ncov/lab/index.html. Accessed 30 Mar 2020.

102. COVID-19 Test DiaSorin Molecular. DiaSorin Molecular. 2020. http://molecular.diasorin.com/us/. Accessed 30 Mar 2020.

103. Thermo Fisher Scientific. TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, Inc.). 2020. https://www.thermofisher.com/es/es/home/life-science/oligonucleotides-primers-probes-genes/applied-biosystems-custom-primers-probes.html. Accessed 30 Mar 2020.

104. Coronavirus Update | Hologic Molecular Test Receives FDA Emergency Use Authorization. Hologic. 2020. https://www.hologic.com/coronavirus-test. Accessed 30 Mar 2020.

105. Quidel. Lyra SARS-CoV-2 Assay. 2020. https://www.quidel.com/molecular-diagnostics/lyra-sars-cov-2-assay. Accessed 30 Mar 2020.

106. GenMark Receives FDA Emergency Use Authorization for its ePlex® SARS-CoV-2 Test. GenMark Diagnostics, Inc. http://ir.genmarkdx.com/news-releases/news-release-details/genmark-receives-fda-emergency-use-authorization-its-eplexr-sars. Accessed 30 Mar 2020.

107. IDT. 2019-nCoV coronavirus reagents - qPCR assays and genes. 2020. https://www.idtdna.com/pages/landing/coronavirus-research-reagents#media. Accessed 30 Mar 2020.

108. 2019-nCoV CDC-qualified Probe and Primer Kits for SARS-CoV-2. LGC Biosearch Technologies. https://www.biosearchtech.com/products/pcr-kits-and-reagents/pathogen-detection/2019-ncov-cdc-probe-and-primer-kit-for-sars-cov-2. Accessed 30 Mar 2020.

109. Wadsworth Center. New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel. 2020. https://health.ny.gov/health_care/medicaid/program/update/2020/2020-03-10_covid-19.htm. Accessed 30 Mar 2020.

110. Coronavirus Disease (COVID-19) | LabCorp. https://www.labcorp.com/coronavirus-disease-covid-19. Accessed 3 Apr 2020.

111. COVID-19 : genesig. https://www.genesig.com/products/10039-coronavirus-covid-19-ce-ivd. Accessed 3 Apr 2020.

112. New Coronavirus (2019-nCoV) Nucleic Acid Detection Kit. PerkinElmer Applied Genomics. https://perkinelmer-appliedgenomics.com/home/products/new-coronavirus-2019-ncov-nucleic-acid-detection-kit/. Accessed 3 Apr 2020.

113. FDA OKs Cellex's antibody-based test for Covid-19 - MedCity News. https://medcitynews.com/2020/04/fda-oks-cellexs-antibody-based-test-for-covid-19/. Accessed 3 Apr 2020.

114. Ipsum Diagnostics Coronavirus Test Gets FDA Emergency Use Authorization | GenomeWeb. https://www.genomeweb.com/molecular-diagnostics/ipsum-diagnostics-coronavirus-test-gets-fda-emergency-use-authorization. Accessed 3 Apr 2020.

115. Corporation L. Flexible Testing Options for SARS-CoV-2 Coronavirus. http://info.luminexcorp.com/covid19. Accessed 3 Apr 2020.

116. Mesa Biotech COVID-19 Test Receives FDA Emergency Use Authorization | GenomeWeb. https://www.genomeweb.com/regulatory-news-fda-approvals/mesa-biotech-covid-19-test-receives-fda-emergency-use-authorization. Accessed 3 Apr 2020.

117. NeuMoDx Secures FDA Emergency Use Authorization for Coronavirus Test. Hospimedica.com. 2020. https://www.hospimedica.com/covid-19/articles/294781507/neumodx-secures-fda-emergency-use-authorization-for-coronavirus-test.html. Accessed 3 Apr 2020.

118. QIAGEN launches QIAstat-Dx test kit for detection of SARS-CoV-2 coronavirus in Europe following CE marking. https://corporate.qiagen.com/newsroom/press-releases/2020/20200318_qiastat_covid19_ce-ivd. Accessed 3 Apr 2020.

119. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000;28:e63–e63.

120. Perera RS, Ding XC, Tully F, Oliver J, Bright N, Bell D, et al. Development and clinical performance of high throughput loop-mediated isothermal amplification for detection of malaria. PloS One. 2017;12.

121. Park G-S, Ku K, Baek S-H, Kim SJ, Kim SI, Kim B-T, et al. Development of Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) Assays Targeting SARS-CoV-2. bioRxiv. 2020.

122. Shen M, Zhou Y, Ye J, AL-maskri AAA, Kang Y, Zeng S, et al. Recent advances and perspectives of nucleic acid detection for coronavirus. J Pharm Anal. 2020.

123. Pang J, Wang MX, Ang IYH, Tan SHX, Lewis RF, Chen JI-P, et al. Potential rapid diagnostics, vaccine and therapeutics for 2019 novel Coronavirus (2019-ncoV): a systematic review. J Clin Med. 2020;9:623.

124. FIND. SARS-CoV-2 diagnostic pipeline. https://www.finddx.org/covid-19/pipeline/. Accessed 30 Mar 2020.

125. Li Z, Yi Y, Luo X, Xiong N, Liu Y, Li S, et al. Development and Clinical Application of A Rapid IgM-IgG Combined Antibody Test for SARS-CoV-2 Infection Diagnosis. J Med Virol. 2020. doi:10.1002/jmv.25727.

126. Cohen J, KupferschmidtFeb. 11 K, 2020, Pm 5:15. Labs scramble to spot hidden coronavirus infections. Science | AAAS. 2020. https://www.sciencemag.org/news/2020/02/labs-scramble-spot-hidden-coronavirus-infections. Accessed 30 Mar 2020.

127. Al Johani S, Hajeer AH. MERS-CoV diagnosis: An update. J Infect Public Health. 2016;9:216–9. doi:10.1016/j.jiph.2016.04.005.

128. SureScreen Diagnostics. COVID-19 Coronavirus Rapid Test Cassette. 2020. https://www.surescreen.com/products/covid-19-coronavirus-rapid-test-cassette. Accessed 30 Mar 2020.

129. Shenzhen Bioeasy Biotechnology. Bioeasy Novel Coronavirus (2019-nCoV) Test Kits. Bioeasy. http://en.bioeasy.com.tr/bioeasy-novel-coronavirus-2019-ncov-test-kits/. Accessed 30 Mar 2020.

130. Broughton JP, Deng X, Yu G, Fasching CL, Singh J, Streithorst J, et al. Rapid Detection of 2019 Novel Coronavirus SARS-CoV-2 Using a CRISPR-based DETECTR Lateral Flow Assay. medRxiv. 2020;:2020.03.06.20032334. doi:10.1101/2020.03.06.20032334.

131. Cawte AD, Unrau PJ, Rueda DS. Live cell imaging of single RNA molecules with fluorogenic Mango II arrays. Nat Commun. 2020;11:1–11. doi:10.1038/s41467-020-14932-7.

132. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA - J Am Med Assoc. 2020;:1–9.

133. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: Retrospective case series. The BMJ. 2020;368 January:1–7.

134. Shen P, Fillatreau S, Thase ME, Mahableshwarkar AR, Dragheim M, Loft H, et al. cr ip t ce pt e d us cr ip t Ac ce pt us. Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol. 2015;26:1–15.

135. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med. 2020;2019.

136. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 2020;395:507–13.

137. Medical Association A. Covid-19 Jama. 2019;2019.

138. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395:497–506.

139. Who W. WHO-2019-nCoV-clinical-2020.4-eng. 2020;:1–21.

140. Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;:1–13.

141. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med. 2020;:1–10.

142. Remuzzi A, Remuzzi G. Imported from https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30627-9/fulltext?utm_campaign=tlcoronavirus20&utm_source=twitter&utm_medium=social. The Lancet. 2020;2:10–3.

143. WHO. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: interim guidance, 13 March 2020. 2020. https://apps.who.int/iris/handle/10665/331446. Accessed 29 Mar 2020.

144. Poston JT, Patel BK, Davis AM. Management of Critically Ill Adults With COVID-19. 2020;:2–4.

145. Ginsberg LE. "If clinically indicated:" Is it? Radiology. 2010;254:324–5.

146. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res. 2020;7:1–23.

147. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic Utility of Clinical Laboratory Data Determinations for Patients with the Severe COVID-19. J Med Virol. 2020;:0–1.

148. Gorbalenya AE. Severe acute respiratory syndrome-related coronavirus – The species and its viruses, a statement of the Coronavirus Study Group. bioRxiv. 2020;:2020.02.07.937862.

149. Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;19:1–2.

150. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology. 2020;2019:200642.

151. Zhao W, Zhong Z, Xie X, Yu Q, Liu J. Relation Between Chest CT Findings and Clinical Conditions of Coronavirus Disease (COVID-19) Pneumonia: A Multicenter Study. AJR Am J Roentgenol. 2020; May:1–6.

152. Bai HX, Hsieh B, Xiong Z, Halsey K, Choi JW, Tran TML, et al. Performance of radiologists in differentiating COVID-19 from viral pneumonia on chest CT. Radiology. 2020;:200823.

153. Wee S-L. As Deaths Mount, China Tries to Speed Up Coronavirus Testing. The New York Times. 2020. https://www.nytimes.com/2020/02/09/world/asia/china-coronavirus-tests.html. Accessed 29 Mar 2020.

154. Myhrvold C, Freije CA, Gootenberg JS, Abudayyeh OO, Metsky HC, Durbin AF, et al. Field-deployable viral diagnostics using CRISPR-Cas13. Science. 2018;360:444–8. doi:10.1126/science.aas8836.

155. Wang M, Wu Q, Xu W, Qiao B, Wang J, Zheng H, et al. Clinical diagnosis of 8274 samples with 2019-novel coronavirus in Wuhan. medRxiv. 2020.

156. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull. 2020;25:1–8.

157. Bordi L, Nicastri E, Scorzolini L, Di Caro A, Capobianchi MR, Castilletti C, et al. Differential diagnosis of illness in patients under investigation for the novel coronavirus (SARS-CoV-2), Italy, February 2020. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull. 2020;25:2–5.

158. Yang H, Wang C, Poon L. Novel coronavirus infection and pregnancy. Ultrasound Obstet Gynecol. 2020.

159. Wong SF, Chow KM, Leung TN, Ng WF, Ng TK, Shek CC, et al. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. Am J Obstet Gynecol. 2004;191:292–7.

160. Alfaraj SH, Al-Tawfiq JA, Memish ZA. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection during pregnancy: Report of two cases & review of the literature. J Microbiol Immunol Infect Wei Mian Yu Gan Ran Za Zhi. 2019;52:501–3.

161. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. The Lancet. 2020;395:809–15. doi:10.1016/S0140-6736(20)30360-3.

162. Liu Y, Chen H, Tang K, Guo Y. Clinical manifestations and outcome of SARS-CoV-2 infection during pregnancy. J Infect. 2020.

163. Mullins E, Evans D, Viner RM, O'Brien P, Morris E. Coronavirus in pregnancy and delivery: rapid review. Ultrasound Obstet Gynecol. n/a n/a. doi:10.1002/uog.22014.

164. Rasmussen SA, Smulian JC, Lednicky JA, Wen TS, Jamieson DJ. Coronavirus Disease 2019 (COVID-19) and Pregnancy: What obstetricians need to know. Am J Obstet Gynecol. 2020. doi:10.1016/j.ajog.2020.02.017.

165. Favre G, Pomar L, Qi X, Nielsen-Saines K, Musso D, Baud D. Guidelines for pregnant women with suspected SARS-CoV-2 infection. Lancet Infect Dis. 2020;0. doi:10.1016/S1473-3099(20)30157-2.

166. Zhang L, Jiang Y, Wei M, Cheng B, Zhou X, Li J, et al. Analysis of the pregnancy outcomes in pregnant women with COVID-19 in Hubei Province. Zhonghua Fu Chan Ke Za Zhi. 2020;55:E009–E009.

167. Liang H, Acharya G. Novel corona virus disease (COVID-19) in pregnancy: What clinical recommendations to follow? Acta Obstet Gynecol Scand. 2020;99:439–42. doi:10.1111/aogs.13836.

168. Poon LC, Yang H, Lee JC, Copel JA, Leung TY, Zhang Y, et al. ISUOG Interim Guidance on 2019 novel coronavirus infection during pregnancy and puerperium: information for healthcare professionals. Ultrasound Obstet Gynecol. 2020.

169. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, et al. Epidemiological Characteristics of 2143 Pediatric Patients With 2019 Coronavirus Disease in China. Pediatrics. 2020.

170. Xu Y, Li X, Zhu B, Liang H, Fang C, Gong Y, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nat Med. 2020;:1–4. doi:10.1038/s41591-020-0817-4.

171. Gu H, Xie Z, Li T, Zhang S, Lai C, Zhu P, et al. Angiotensin-converting enzyme 2 inhibits lung injury induced by respiratory syncytial virus. Sci Rep. 2016;6:1–10. doi:10.1038/srep19840.

172. Xia W, Shao J, Guo Y, Peng X, Li Z, Hu D. Clinical and CT features in pediatric patients with COVID-19 infection: Different points from adults. Pediatr Pulmonol. 2020.

173. Lee P-I, Hu Y-L, Chen P-Y, Huang Y-C, Hsueh P-R. Are children less susceptible to COVID-19? J Microbiol Immunol Infect Wei Mian Yu Gan Ran Za Zhi. 2020.

174. Liang W, Guan W, Chen R, Wang W, Li J, Xu K, et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. Lancet Oncol. 2020;21:335–7. doi:10.1016/S1470-2045(20)30096-6.

175. Fuhai M, Haitao H, Yantao T. Surgical treatment strategy for digestive system malignancies during the outbreak of novel coronavirus pneumonia. Chin J Oncol. 2020;42:E001–E001. doi:10.3760/cma.j.cn112152-20200223-00117.

176. Yu J, Ouyang W, Chua MLK, Xie C. SARS-CoV-2 Transmission in Patients With Cancer at a Tertiary Care Hospital in Wuhan, China. JAMA Oncol. 2020. doi:10.1001/jamaoncol.2020.0980.

177. Chavez-MacGregor M, Clarke CA, Lichtensztajn DY, Giordano SH. Delayed Initiation of Adjuvant Chemotherapy Among Patients With Breast Cancer. JAMA Oncol. 2016;2:322–9. doi:10.1001/jamaoncol.2015.3856.

178. Binliang L, Fei M, Jiani W, Ying F, Hongnan M, Binghe X. Health management of breast cancer patients outside the hospital during the outbreak of 2019 novel coronavirus disease. Chin J Oncol. 2020;42:E002–E002. doi:10.3760/cma.j.cn112152-20200221-00110.

179. Jazieh A-R, Al Hadab A, Al Olayan A, AlHejazi A, Al Safi F, Al Qarni A, et al. Managing Oncology Services During a Major Coronavirus Outbreak: Lessons From the Saudi Arabia Experience. JCO Glob Oncol. 2020;:518–24. doi:10.1200/GO.20.00063.

180. Reiche EMV, Nunes SOV, Morimoto HK. Stress, depression, the immune system, and cancer. Lancet Oncol. 2004;5:617–25. doi:10.1016/S1470-2045(04)01597-9.

181. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med. 2020;0. doi:10.1016/S2213-2600(20)30079-5.

182. Murthy S, Gomersall CD, Fowler RA. Care for Critically Ill Patients With COVID-19. JAMA. 2020. doi:10.1001/jama.2020.3633.

183. Soldati G, Demi M, Smargiassi A, Inchingolo R, Demi L. The role of ultrasound lung artifacts in the diagnosis of respiratory diseases. Expert Rev Respir Med. 2019;13:163–72.

184. Soldati G, Smargiassi A, Inchingolo R, Buonsenso D, Perrone T, Briganti DF, et al. Is there a role for lung ultrasound during the COVID-19 pandemic? J Ultrasound Med Off J Am Inst Ultrasound Med. 2020.

185. Chu DK, Kim LH-Y, Young PJ, Zamiri N, Almenawer SA, Jaeschke R, et al. Mortality and morbidity in acutely ill adults treated with liberal versus conservative oxygen therapy (IOTA): a systematic review and meta-analysis. The Lancet. 2018;391:1693–705.

186. van den Boom W, Hoy M, Sankaran J, Liu M, Chahed H, Feng M, et al. The Search for Optimal Oxygen Saturation Targets in Critically Ill Patients: Observational Data From Large ICU Databases. Chest. 2020;157:566–73.

187. Wax RS, Christian MD. Practical recommendations for critical care and anesthesiology teams caring for novel coronavirus (2019-nCoV) patients. Can J Anaesth J Can Anesth. 2020.

188. Loh N-HW, Tan Y, Taculod J, Gorospe B, Teope AS, Somani J, et al. The impact of high-flow nasal cannula (HFNC) on coughing distance: implications on its use during the novel coronavirus disease outbreak. Can J Anesth Can Anesth. 2020. doi:10.1007/s12630-020-01634-3.

189. Hui DS, Chow BK, Lo T, Tsang OTY, Ko FW, Ng SS, et al. Exhaled air dispersion during high-flow nasal cannula therapy versus CPAP via different masks. Eur Respir J. 2019;53.

190. Cheung JC-H, Ho LT, Cheng JV, Cham EYK, Lam KN. Staff safety during emergency airway management for COVID-19 in Hong Kong. Lancet Respir Med. 2020.

191. Alraddadi BM, Qushmaq I, Al-Hameed FM, Mandourah Y, Almekhlafi GA, Jose J, et al. Noninvasive ventilation in critically ill patients with the Middle East respiratory syndrome. Influenza Other Respir Viruses. 2019;13:382–90.

192. Brochard L, Lefebvre J-C, Cordioli RL, Akoumianaki E, Richard J-CM. Noninvasive ventilation for patients with hypoxemic acute respiratory failure. Semin Respir Crit Care Med. 2014;35:492–500.

193. Petrucci N, Iacovelli W. Ventilation with lower tidal volumes versus traditional tidal volumes in adults for acute lung injury and acute respiratory distress syndrome. Cochrane Database Syst Rev. 2004. doi:10.1002/14651858.CD003844.pub2.

194. Matthay MA, Aldrich JM, Gotts JE. Treatment for severe acute respiratory distress syndrome from COVID-19. Lancet Respir Med. 2020.

195. Fielding-Singh V, Matthay MA, Calfee CS. Beyond Low Tidal Volume Ventilation: Treatment Adjuncts for Severe Respiratory Failure in Acute Respiratory Distress Syndrome. Crit Care Med. 2018;46:1820–31.

196. Yasuda H, Nishimura T, Kamo T, Sanui M, Nango E, Abe T, et al. Optimal plateau pressure for patients with acute respiratory distress syndrome: a protocol for a systematic review and meta-analysis with meta-regression. BMJ Open. 2017;7:e015091.

197. Munshi L, Del Sorbo L, Adhikari NKJ, Hodgson CL, Wunsch H, Meade MO, et al. Prone Position for Acute Respiratory Distress Syndrome. A Systematic Review and Meta-Analysis. Ann Am Thorac Soc. 2017;14 Supplement_4:S280–8.

198. Papazian L, Aubron C, Brochard L, Chiche J-D, Combes A, Dreyfuss D, et al. Formal guidelines: management of acute respiratory distress syndrome. Ann Intensive Care. 2019;9:69.

199. Wang Y, Jiang W, He Q, Wang C, Wang B, Zhou P, et al. Early, low-dose and short-term application of corticosteroid treatment in patients with severe COVID-19 pneumonia: single-center experience from Wuhan, China. medRxiv. 2020;:2020.03.06.20032342.

200. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020. doi:10.1001/jama.2020.2648.

201. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020.

202. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet. 2020;395:1054–62.

203. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020.

204. Silversides JA, Major E, Ferguson AJ, Mann EE, McAuley DF, Marshall JC, et al. Conservative fluid management or deresuscitation for patients with sepsis or acute respiratory distress syndrome following the resuscitation phase of critical illness: a systematic review and meta-analysis. Intensive Care Med. 2017;43:155–70.

205. Lewis SR, Pritchard MW, Evans DJ, Butler AR, Alderson P, Smith AF, et al. Colloids versus crystalloids for fluid resuscitation in critically ill people. Cochrane Database Syst Rev. 2018;8:CD000567.

206. Lamontagne F, Richards-Belle A, Thomas K, Harrison DA, Sadique MZ, Grieve RD, et al. Effect of Reduced Exposure to Vasopressors on 90-Day Mortality in Older Critically Ill Patients With Vasodilatory Hypotension: A Randomized Clinical Trial. JAMA. 2020.

207. Møller MH, Granholm A, Junttila E, Haney M, Oscarsson-Tibblin A, Haavind A, et al. Scandinavian SSAI clinical practice guideline on choice of inotropic agent for patients with acute circulatory failure. Acta Anaesthesiol Scand. 2018;62:420–50.

208. Gamper G, Havel C, Arrich J, Losert H, Pace NL, Müllner M, et al. Vasopressors for hypotensive shock. Cochrane Database Syst Rev. 2016;2:CD003709.

209. WHO. Water, sanitation, hygiene and waste management for COVID-19. 2020. https://www.who.int/publications-detail/water-sanitation-hygiene-and-waste-management-for-covid-19. Accessed 30 Mar 2020.

210. Ferguson NM, Laydon D, Nedjati-Gilani G, Imai N, Ainslie K, Baguelin M, et al. Impact of non-pharmaceutical interventions (NPIs) to reduce COVID- 19 mortality and healthcare demand. 2020;:20.

211. Nicolaides C, Avraam D, Cueto-Felgueroso L, González MC, Juanes R. Hand-Hygiene Mitigation Strategies Against Global Disease Spreading through the Air Transportation Network. Risk Anal. 2019;:risa.13438. doi:10.1111/risa.13438.

212. Feng S, Shen C, Xia N, Song W, Fan M, Cowling BJ. Rational use of face masks in the COVID-19 pandemic. Lancet Respir Med. 2020;0. doi:10.1016/S2213-2600(20)30134-X.

213. Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, Carter LJ, et al. Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases. ACS Cent Sci. 2020;6:315–31. doi:10.1021/acscentsci.0c00272.

214. Bin Cao. Severe 2019-nCoV Remdesivir RCT -. 2020. https://clinicaltrials.gov/ct2/show/NCT04257656. Accessed 26 Mar 2020.

215. Epistemonikos Foundation O. SYSTEMATIC REVIEW - PRELIMINARY REPORT Lopinavir/ritonavir for the treatment of COVID-19 -. 2020;:8.

216. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care. 2020;:S0883944120303907. doi:10.1016/j.jcrc.2020.03.005.

217. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. JAMA. 2020. doi:10.1001/jama.2020.4783.

218. Ng O-W, Chia A, Tan AT, Jadi RS, Leong HN, Bertoletti A, et al. Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection. Vaccine. 2016;34:2008–14. doi:10.1016/j.vaccine.2016.02.063.

219. Al-amri SS, Abbas AT, Siddiq LA, Alghamdi A, Sanki MA, Al-Muhanna MK, et al. Immunogenicity of Candidate MERS-CoV DNA Vaccines Based on the Spike Protein. Sci Rep. 2017;7:1–8. doi:10.1038/srep44875.

220. Du L, He Y, Zhou Y, Liu S, Zheng B-J, Jiang S. The spike protein of SARS-CoV — a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009;7:226–36. doi:10.1038/nrmicro2090.

221. Du L, Zhao G, He Y, Guo Y, Zheng B-J, Jiang S, et al. Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. Vaccine. 2007;25:2832–8. doi:10.1016/j.vaccine.2006.10.031.

222. Chen W-H, Du L, Chag SM, Ma C, Tricoche N, Tao X, et al. Yeast-expressed recombinant protein of the receptor-binding domain in SARS-CoV spike protein with deglycosylated forms as a SARS vaccine candidate. Hum Vaccines Immunother. 2014;10:648–58. doi:10.4161/hv.27464.

223. Baruah V, Bose S. Immunoinformatics-aided identification of T cell and B cell epitopes in the surface glycoprotein of 2019-nCoV. J Med Virol. 2020;92:495–500. doi:10.1002/jmv.25698.

224. Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. A Sequence Homology and Bioinformatic Approach Can Predict Candidate Targets for Immune Responses to SARS-CoV-2. Cell Host Microbe. 2020. doi:10.1016/j.chom.2020.03.002.

225. Liu L, Wei Q, Lin Q, Fang J, Wang H, Kwok H, et al. Anti–spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. JCI Insight. 4. doi:10.1172/jci.insight.123158.

226. M B, D D, K L, S A, A W, M F, et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. J Virol. 2011;85:12201–15. doi:10.1128/JVI.06048-11.

227. Tebas P, Roberts CC, Muthumani K, Reuschel EL, Kudchodkar SB, Zaidi FI, et al. Safety and Immunogenicity of an Anti-Zika Virus DNA Vaccine - Preliminary Report. N Engl J Med. 2017.

228. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines — a new era in vaccinology. Nat Rev Drug Discov. 2018;17:261–79. doi:10.1038/nrd.2017.243.

229. Maruggi G, Zhang C, Li J, Ulmer JB, Yu D. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. Mol Ther. 2019;27:757–72. doi:10.1016/j.ymthe.2019.01.020.

230. Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. Ther Deliv. 2016;7:319–34. doi:10.4155/tde-2016-0006.

231. Commissioner O of the. First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response. FDA. 2020. http://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health. Accessed 26 Mar 2020.

232. WHO. Influenza update - 363. http://www.who.int/influenza/surveillance_monitoring/updates/latest_update_GIP_surveillance/en/. Accessed 26 Mar 2020.

233. Mendelson M. Could enhanced influenza and pneumococcal vaccination programs help limit the potential damage from SARS-CoV-2 to fragile health systems of southern hemisphere countries this winter? Int J Infect Dis IJID Off Publ Int Soc Infect Dis. 2020.

234. Tamerius J, Nelson M, Zhou S, Viboud C, Miller M, Alonso W. Global influenza seasonality: reconciling patterns across temperate and tropical regions. Environ Health Perspect. 2011;119:439–45.

235. Caini S, El-Guerche Seblain C, Ciblak M, Paget J. Epidemiology of seasonal influenza in the Middle East and North Africa regions, 2010-2016: Circulating influenza A and B viruses and spatial timing of epidemics. Influenza Other Respir Viruses. 2018;12:344–52.

236. Cox N. Influenza seasonality: timing and formulation of vaccines. Bull World Health Organ. 2014;92:311.

237. Lowen A, Steel J. Roles of humidity and temperature in shaping influenza seasonality. J Virol. 2014;88:7692–5.

238. Nisar N, Badar N, Aamir U, Yaqoob A, Tripathy J, Laxmeshwar C, et al. Seasonality of influenza and its association with meteorological parameters in two cities of Pakistan: A time series analysis. PLOS ONE. 2019;14:e0219376.

239. Tamerius J, Shaman J, Alonso W, Bloom-Feshbach K, Uejio C, Comrie A, et al. Environmental Predictors of Seasonal Influenza Epidemics across Temperate and Tropical Climates. PLOS Pathog. 2013;9:e1003194.

240. Viboud C, Boëlle P-Y, Pakdaman K, Carrat F, Valleron A-J, Flahault A. Influenza epidemics in the United States, France, and Australia, 1972-1997. Emerg Infect Dis. 2004;10:32–9.

241. Finkelman BS, Viboud C, Koelle K, Ferrari M, Bharti N, Grenfell B. Global Patterns in Seasonal Activity of Influenza A/H3N2, A/H1N1, and B from 1997 to 2005: Viral Coexistence and Latitudinal Gradients. PLOS ONE. 2007;2:e1296.

242. Moura F, Perdigao A, Siqueira M. Seasonality of influenza in the tropics: a distinct pattern in northeastern Brazil. Am J Trop Med Hyg. 2009;81:180–3.

243. Rao B, Banerjee K. Influenza surveillance in Pune, India, 1978-90. Bull World Health Organ. 1993;71:177–81.

244. Dosseh A, Ndiaye K, Spiegel A, Sagna M, Mathiot C. Epidemiological and virological influenza survey in Dakar, Senegal: 1996-1998. Am J Trop Med Hyg. 2000;62:639–43.

245. Roussel M, Pontier D, Cohen J-M, Lina B, Fouchet D. Quantifying the role of weather on seasonal influenza. BMC Public Health. 2016;16:441.

246. Lofgren E, Fefferman NH, Naumov YN, Gorski J, Naumova EN. Influenza Seasonality: Underlying Causes and Modeling Theories. J Virol. 2007;81:5429 LP – 5436.

247. Duan S-M, Zhao X-S, Wen R-F, Huang J-J, Pi G-H, Zhang S-X, et al. Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. Biomed Environ Sci BES. 2003;16:246–55.

248. Eccles R. An explanation for the seasonality of acute upper respiratory tract viral infections. Acta Otolaryngol (Stockh). 2002;122:183–91.

249. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, et al. Epidemic influenza and vitamin D. Epidemiol Infect. 2006;134:1129–40.

250. Helming L, Böse J, Ehrchen J, Schiebe S, Frahm T, Geffers R, et al. 1α,25-dihydroxyvitamin D3 is a potent suppressor of interferon γ–mediated macrophage activation. Blood. 2005;106:4351–8.

251. Dowell SF. Seasonal variation in host susceptibility and cycles of certain infectious diseases. Emerg Infect Dis. 2001;7:369–74.

252. Xiao H, Tian H, Lin X, Gao L, Dai X, Zhang X, et al. Influence of extreme weather and meteorological anomalies on outbreaks of influenza A (H1N1). Chin Sci Bull. 2013;58:741–9.