

A Comprehensive Updated Review on SARS-CoV-2 and COVID-19

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Yunzhao R. Ren, MD, PhD¹, Amit Golding, MD, PhD², Alfred Sorbello, DO, MPH³, Ping Ji, PhD¹, Jianmeng Chen, MD, PhD¹, Bhawana Saluja, PhD¹, Kimberly Witzmann, MD⁴, Vikram Arya, PhD, FCP⁵, Kellie S. Reynolds, PharmD⁵, Su-Young Choi, PharmD, PhD⁵, Nikolay P. Nikolov, MD², and Chandrahas Sahajwalla, PhD, FCP¹

Abstract

This literature review aims to provide a comprehensive current summary of the pathogenesis, clinical features, disease course, host immune responses, and current investigational antiviral and immunomodulatory pharmacotherapies to facilitate the development of future therapies and measures for prevention and control.

Keywords

COVID-19, clinical characteristics, review, SARS-CoV-2, treatment

The disease name “COVID-19” and the associated virus name “SARS-CoV-2” were coined by the World Health Organization (WHO) and the Coronavirus Study Group of the International Committee on Virus Taxonomy, respectively, on February 11.^{1,2} Currently no specific drug has been approved by the US Food and Drug Administration (FDA) for treating COVID-19, and the current management of patients is mainly supportive. The FDA has issued only emergency use authorizations (EUs) to permit the emergency use of chloroquine phosphate, hydroxychloroquine sulfate, and remdesivir. Therapeutic development for COVID-19 includes repurposing existing medications and developing investigational candidates.

Epidemiology

The first reported confirmed COVID-19 case was presented as atypical pneumonia on December 8, 2019, in Wuhan, China.³ The patient was among a cluster of 41 cases reported to WHO on January 11, 2020.⁴ As of May 19, 2020, SARS-CoV-2 has infected more than 4.6 million people worldwide.⁵ COVID-19 had resulted in a global death toll of more than 319 000.

An epidemiology study noted that of 44 672 confirmed COVID-19 cases in China through February 11, 13.8% and 4.7% of patients were in severe and critical condition, respectively.⁶ The reported median incubation period of COVID-19 is 4 days,⁷ and the median period from symptom onset to hospital ad-

mission ranges from 7 to 10 days.^{8,9} Median time from onset of the first symptom to dyspnea is 4–5 days^{7,8}; to pneumonia, 3 days⁷; to intensive care unit (ICU)

¹Division of Inflammation and Immune Pharmacology, Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, FDA, Silver Spring, Maryland, USA

²Division of Rheumatology and Transplant Medicine, Office of Immunology and Inflammation, Office of New Drugs, Center for Drug Evaluation and Research, FDA, Silver Spring, Maryland, USA

³Office of Translational Sciences, Center for Drug Evaluation and Research, FDA, Silver Spring, Maryland, USA

⁴Office of Bioequivalence, Office of Generic Drugs, Center for Drug Evaluation and Research, FDA, Silver Spring, Maryland, USA

⁵Division of Infectious Disease Pharmacology, Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, FDA, Silver Spring, Maryland, USA

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Corresponding Author:

Chandrahas Sahajwalla, PhD, FCP, 10903 New Hampshire Ave., Building 51, Room 3144, Silver Spring, MD 20993
 Email: chandrahas.sahajwalla@fda.hhs.gov

Chandrahas Sahajwalla is a fellow of the American College of Clinical Pharmacology.

Vikram Arya is a fellow of the American College of Clinical Pharmacology.

Disclaimer: The views expressed are those of the authors and do not necessarily represent the views of the US FDA or the US government. COVID-19 publications are evolving at a rapid pace, and this review is based on articles available at the time of submission to the journal. Therefore, readers are advised to monitor the literature for the most current findings.

admission, 6 days¹⁰; and to acute respiratory distress syndrome (ARDS), 8 days.⁸ The median duration of hospitalization is 12 days⁷ to 22 days¹¹; median length of ICU stay, 18 days¹⁰; median time from admission to invasive mechanical ventilation, 14.5 days¹¹; and median time from admission to death is 18.5 days.¹¹

Modes of Transmission of SARS-CoV-2

The currently estimated reproductive number (R_0) of SARS-CoV-2, the average number of people to which 1 infected individual will pass the virus, ranges from 2.2 to 5.7,¹²⁻¹⁴ whereas the reported R_0 for SARS-CoV is approximately 3.¹⁵

SARS-CoV-2 is an airborne virus that can be transmitted by aerosol.¹⁶ A hospital survey detected the maximum transmission distance of SARS-CoV-2 aerosol might be 4 m from the COVID-19 patients.¹⁷ In the contaminated area of the hospital, the viral nucleic acid-positive rate was 75% for computer mice, 70% for floor swabs, 60% for trashcans, 43% for sickbed handrails, and 8% for doorknobs. In a virus viability test, authors used a nebulizer to generate artificial aerosols with small particle size ($<5 \mu\text{m}$) containing SARS-CoV-2.¹⁸ The results showed that SARS-CoV-2 remained viable in the artificial aerosols for at least 3 hours. The virus is most stable on plastic and stainless steel surfaces, on which viable virus has been detected for up to 72 hours. No viable virus was detected after 4 hours on copper and after 24 hours on cardboard. Therefore, the WHO advises people not to touch their eyes, nose, or mouth with their hands to limit self-contamination.¹⁹ In addition, the Centers for Disease Control and Prevention (CDC) recommends wearing cloth face coverings in public settings where other social distancing measures (6 ft) are difficult to maintain.²⁰ A case study reported that 5 of 39 passengers on a coach bus contracted the virus from an infected patient who did not wear a protective face mask. However, the same patient bought a mask and wore it before transferring to a minibus, and none of the 14 passengers on the minibus contracted the virus.²¹

Live SARS-CoV-2 was isolated from nasal/pharyngeal swabs and sputum, but not from stool, in patients with COVID-19.²² The live viral copies peaked during the early stage of symptom onset (≤ 4 days) and could not be detected after day 8 in samples from 9 mild cases of infection. This discovery suggests that viral transmission occurs primarily through the airborne route rather than the fecal-oral route during early stages of the disease. Indeed, there have been sporadic case reports on human-to-human transmission from asymptomatic or presymptomatic subjects.²³⁻²⁵ One study estimated that the transmissibility of the asymptomatic cases is comparable to that of symptomatic cases.²⁶ An

epidemiology report from China indicated that 1.2% (889) of 72 314 tested/suspected/diagnosed cases were asymptomatic.²⁷ Identification and isolation of asymptomatic subjects has helped to reduce the pandemic in an Italian village.²⁸ These reports suggest that a pan-population screening for viral exposure is an effective way to critically contain the spread of the disease.

Two meteorology models consistently found that higher relative humidity favored SARS-CoV-2 transmission.^{29,30} The 2 models differed regarding the trend of temperature effect on viral transmission, probably because of the different ranges of temperature studied in China (winter) and Brazil (autumn).

Demographics

An epidemiology study from China found that although the proportion of male patients (51.4%) was comparable to that of females (48.6%), male patients (63.8%) comprised almost two-thirds of the total deaths.⁶ Data from the CDC website as of May 14 showed that among approximately 111 000 COVID-19 cases in the United States, 3% were in children (<17 years old), 23% were in the elderly (≥ 65 years old), and most (74%) were in patients between 18 and 64 years old.³¹ Of 580 hospitalized patients in 14 US states from March 1 to March 30, 261 (45%) were non-Hispanic white, 192 (33.1%) were non-Hispanic black, 47 (8.1%) were Hispanic, 32 (5.5%) were Asian, 2 (0.3%) were American Indian/Alaskan Native, and 46 (7.9%) were of other ethnic origins or unknown.³²

Virology and Pathogenesis

Culture of bronchoalveolar lavage fluid collected from early Wuhan cases identified the etiology of the virus.³³ A transmission electron microscope identified corona structure-containing virus particles of about 60 to 140 nm in size. The same research team identified that this ~ 30 -kb virus was a single-stranded RNA virus belonging to the *Betacoronavirus* genus in the *Coronaviridae* family.³³

To date, the virus phylogenetically closest to SARS-CoV-2 by genetic homogeneity is a coronavirus isolated from the horseshoe bat (Bat CoV RaTG13) with an overall genome sequence identity of 96.2%, which is higher than that of SARS-CoV ($<80\%$).³⁴ Angiotensin-converting enzyme 2 (ACE2) was identified as a shared receptor required for cell entry both for SARS-CoV and SARS-CoV-2, with higher binding affinity for SARS-CoV-2.³⁵ Sequence comparison of spike (S) protein, the viral ligand of ACE2, identified 3 short insertions located in the N-terminus region that are conserved in SARS-CoV-2 and Bat CoV RaTG13, but not in SARS-CoV.³⁴ Examination of the receptor-binding

domain (RBD) of S protein surprisingly identified that a Malayan pangolin coronavirus had a higher degree of similarity (97.4%) than Bat CoV RaTG13 (89.2%), indicating that recombination may have occurred during the evolution of SARS-CoV-2.³⁶ Variation analysis based on 95 sequences of SARS-CoV-2 up to February 14 revealed very high homology (>99.9%) among different strains.³⁷ Another group estimated that the evolution rate of SARS-CoV-2 is approximately 1.8×10^{-3} per base per year,³⁸ which indicates that SARS-CoV-2 transmission in humans is a recent event. The SARS-CoV-2 genome sequence can be found at <https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/>.

Diagnosis

The direct diagnosis of COVID-19 requires detection of SARS-CoV-2-specific RNA from patients' samples. Reverse-transcription polymerase chain reaction (RT-PCR) is the most widely used technique for diagnosis. A commercial RT-PCR test kit usually uses 2 to 3 pairs of primers detecting the different regions of SARS-CoV-2 genomic RNA to increase the test specificity. The sensitivity of this method is not optimal. One article noted that the sensitivity of RT-PCR (59%), even after 25% of patients had multiple tests, was lower than that of a computed tomography (CT) scan (88%).³⁹ A test report of 4880 Wuhan cases with typical COVID-19 symptoms and history of close patient contact demonstrated that the positive rate was about 40% for nasal and pharyngeal swabs, 50% for sputum samples, and 80% to 100% for bronchoalveolar lavage fluid.⁴⁰ Another study screened 353 subjects in Wuhan and found that the positive rate from nasopharyngeal swabs was 2.5-fold higher than that of oropharyngeal swabs.⁴¹ Interestingly, pharyngeal swab viral nucleic acid screening results of 2510 patients between January 23 and February 25 from a hospital fever clinic in Hunan Province (a neighboring province of Hubei) demonstrated that the positive rate of SARS-CoV-2 (1.3%) was lower than that of influenza A (2.3%) and influenza B (3.3%).⁴² It is unclear whether the lockdown status of Hubei Province or the sensitivity of the detection methods between different viruses contributed to the result.

The disease course also affects viral nucleic acid detection results. One study closely followed throat swab samples or deep nasal cavity swab samples from 56 hospitalized COVID-19 patients and found that the positive rate was the highest (100%) within week 1 since symptom onset.⁴³ However, the positive rate reduced to about one-third in week 3. Similar results were obtained from another study, in which the positive rate of throat swabs from 43 patients was >90% when tested within

1-3 days since symptom onset, but decreased to <80% on day 5, and <50% after day 14.⁴⁴

Other than traditional RT-PCR, viral RNA detecting methods such as loop-mediated isothermal amplification (LAMP) were expeditiously developed and approved by the FDA.⁴⁵ The apparent advantage of LAMP is the much shorter waiting time for the results (<15 minutes) compared with the traditional RT-PCR (~3 hours). CRISPR, the powerful gene-editing technique, premiered in this pandemic and was also approved by FDA, although the commercial kit requires an isothermal amplification step.⁴⁶

Reports on the relationship between viral load in respiratory tracts and disease severity showed conflicting results. One study (n = 12) reported that the high viral load from a patient's respiratory tract is moderately associated with a high Murray score for acute lung injury and low PaO₂/FiO₂.⁴⁷ The same study also reported that the high viral load is associated with high plasma angiotensin II concentration. However, 2 other studies (n = 23 and n = 11) did not find significant differences in viral load between mild and severe cases.^{48,49}

One study demonstrated that the speed of viral clearance differs significantly in mild and severe cases.¹⁰ The average time of viral nucleic acid turning positive to negative was about 10 days in mild cases and 18 days in severe cases. In nonsurvivors, persistent viral RNA was detected until death.¹¹ However, another study with intensive testing was able to detect viral nucleic acid in throat/deep nasal cavity swab samples from 3 of 56 hospitalized patients with mild to moderate confirmed COVID-19 5 weeks after symptom onset.⁴³

SARS-CoV-2 was detected in whole blood and serum.^{50,51} More studies are needed to investigate the correlation between viremia with blood viral load and disease severity.

Clinical and Paraclinical Manifestations

Clinical Signs and Symptoms

The CDC listed 7 common symptoms of COVID-19: cough, dyspnea (shortness of breath or difficulty breathing), fever, chills, muscle pain, sore throat, and new loss of taste or smell.⁵² This review collected 5 early clinical reports with a cutoff of 100 patients published before April 3. The results showed that the most common symptoms were fever (85% to 99%) and cough (43% to 71%); see Table 1.⁷⁻¹¹ The great discrepancy in dyspnea incidence (19% to 71%) between studies may indicate that dyspnea was not the apparent symptom in some patients with hypoxemia ("silent hypoxia"), as 62.4% of severe cases and 46.3% of those who ended up intubated, ventilated, or dead did not

Table 1. Baseline Clinical Symptoms and Signs of Patients With COVID-19 on Hospital Admission

Symptom	Wang et al ⁸	Deng et al ⁹	Zhou et al ¹¹	Wu et al ¹⁰	Guan et al ⁷
Source	Zhongnan Hospital, Wuhan	Tongji Hospital, Wuhan	Jinyintan and Wuhan Pulmonary Hospital, Wuhan	Multiple hospitals in provinces Zhejiang and jiangsu	Multiple hospitals across China
Patients, n	138	225	191	280	1099
Fever	98.6%	87.2%	94%	84.6%	43.8% ^a
Myalgia	34.8%	27.5%	15%	25.4%	14.9% ^b
Fatigue	69.6%		23%	N/A	38.1%
Headache	6.5%	5.5%	N/A	15.4% ^c	13.6%
Cough	59.4%	43.1%	79%	70.4%	67.8%
Sputum	26.8%	32.1%	23%	N/A	33.7%
Sore throat	17.4% ^d	N/A	N/A	11.1%	13.9%
Hemoptysis	N/A	4.6%	N/A	N/A	0.9%
Dyspnea	31.2%	70.6%	N/A	53.6%	18.7%
Diarrhea	10.1%	17.4%	5%	2.5%	3.8%
Nausea	10.1%	N/A	4%	1.1%	5%
Vomiting	3.6%	N/A			
Days from symptom onset to admission ^e	7.0 (4.0-8.0)	7.0 (5.0-10.0) ^{f,g} 10.0 (6.5-12.0)	N/A	N/A	N/A

^a88.7% of patients experienced fever during hospitalization.

^bIncluding arthralgia.

^cIncluding mental disorder.

^dPharyngalgia.

^eMedian (range).

^fRecovered patients.

^gDeceased patients.

present with dyspnea.⁵³ An article hypothesized that SARS-CoV-2 may induce dysfunction of a cortical area of the brain that blunted visceral perception of hypoxemia.⁵⁴ A survey in 417 mild to moderate COVID-19 patients from 12 European hospitals identified that 85.6% and 88.0% of patients reported olfactory and gustatory dysfunction, respectively. The survey also found a significant association between both disorders.⁵⁵ Other less common symptoms associated with COVID-19 include, but are not limited to, lethargy, loss of appetite, diarrhea, nausea, and vomiting (Table 1), conjunctivitis,^{56,57} chilblains or frostbite-like acral skin lesions on hands and/or feet (COVID-19 toes),^{58,59} hypotension,⁶⁰ headache, dizziness, impaired consciousness, and acute cerebrovascular problems.⁶¹ A sudden deterioration in some severe cases was reported around 1 to 2 weeks after the onset of the first symptom.^{62,63}

Age and comorbidities were substantially different between mild/moderate/survived cases and severe/critical/nonsurvived cases (Table 2). Older patients with underlying comorbidities such as hypertension, cardiovascular disease, cerebrovascular disease, or diabetes appear to be at higher risk for developing more severe types of COVID-19. An observational study collected 8910 patients from 169 hospitals across the world and identified that the following comorbidities were significantly associated with an increased risk of in-hospital death: coronary

artery disease, congestive heart failure, cardiac arrhythmia, and chronic obstructive pulmonary disease (COPD).⁶⁴

Laboratory Findings and their Implications

Major peripheral blood biochemical/laboratory findings on admission from the above 5 clinical reports are listed in Table 3. In general, severe cases tend to have low lymphocyte counts, higher plasma D-dimer concentrations, and higher lactate dehydrogenase concentrations. The high D-dimer concentration in severe cases is consistent with the reported higher incidence of disseminated intravascular coagulation in nonsurvivors (71.4%) compared with survivors (0.6%).⁶⁵ A Netherlands study reported that 31% of 184 ICU patients with COVID-19 experienced thrombotic complications.⁶⁶ Other studies measuring more coagulation parameters found that prothrombin time (PT), activated partial thromboplastin time, and fibrinogen degradation products (FDPs) also were higher in patients with severe COVID-19 than patients with mild diseases.^{67,68} Dynamic monitoring of 183 COVID-19 patients with pneumonia observed continuous increases in PT, D-dimer, and FDPs in nonsurvivors.⁶⁵ Zhang et al collected D-dimer concentrations from 343 patients on admission and discovered that the cutoff of 2.0 µg/mL had a sensitivity of 92% and a specificity of 83% in predicting in-hospital mortality.⁶⁹

Table 2. Major Demographic Differences Between Mild/Moderate Cases and Severe Cases

	Wang et al ⁸	Deng et al ⁹	Zhou et al ¹¹	Wu et al ¹⁰	Guan et al ⁷
Severity definition	Non-ICU versus ICU	Recovered versus death	Survivor versus nonsurvivor	Mild/moderate versus severe/critical ^a	Nonsevere versus severe ^{b,f}
Patients, n	102 versus 36	116 versus 109	137 versus 54	197 versus 83	1099
Median age, y	51 versus 66 ^c	40 versus 69 ^c	52 versus 69 ^c	38 versus 63 ^c	45 versus 52
Sex (male), %	52% versus 61%	44% versus 67% ^c	59% versus 70%	54% versus 54%	58% versus 58%
Comorbidities, %	37% versus 72% ^c	41% versus 73% ^c	40% versus 67% ^c	N/A	21% versus 39%
Hypertension	22% versus 58% ^c	16% versus 37% ^c	23% versus 48%	N/A	13% versus 24%
Cardiovascular disease	11% versus 25%	3% versus 12% ^d	1% versus 24% ^{c,e}	7% versus 52%	1.8% versus 5.8% ^e
Cerebrovascular disease	1% versus 17% ^c	N/A	N/A		1.2% versus 2.3%
Diabetes	6% versus 22% ^{a,c}	8% versus 16%	14% versus 31%	3% versus 34% ^f	6% versus 12%
Pulmonary disease	N/A	3% versus 20% ^c	N/A	1.5% versus 3.6%	N/A
COPD	1% versus 8%	N/A	1% versus 7%	0% versus 1.2%	0.6% versus 3.5%

^aPatients experienced severe acute respiratory syndrome.

^bThe degree of severity at the time of admission using the American Thoracic Society guidelines for community-acquired pneumonia.

^c $P \leq .001$.

^dCounted as heart disease.

^eCounted as coronary heart disease.

^fCounted as endocrine system disease.

Higher levels of hypersensitive troponin I in patients with severe COVID-19 (Table 3) indicates an association of SARS-CoV-2 infection and cardiomyopathy.^{8,11} A review summarized that cardiovascular complications associated with COVID-19 include myocardial injury, myocarditis, acute myocardial infarction, heart failure, dysrhythmias, and thromboembolic events.⁷⁰ Another review attributed COVID-19-associated myocardial injury both to direct viral infection of the myocardium via ACE2 and the accompanying systemic inflammatory response to the virus.⁷¹ The authors suspect that like SRAS-CoV, SARS-CoV-2 also may downregulate ACE expression after the infection, which would increase the angiotensin II/angiotensin 1-7 ratio.⁷² Angiotensin 1-7 is known to have some physiological cardiovascular protective effects.

Interestingly, some clinical reports noted low eosinophil counts in COVID-19 patients.^{42,73,74} One study associated eosinopenia on admission with poor prognosis of the disease.⁷⁴ In addition, several studies closely monitored eosinophil dynamic counts in hospitalized COVID-19 patients and found that faster recovery of eosinophil counts was associated with better outcomes.^{73,75}

Imaging

Between 85%⁷ and 100%⁸ of hospitalized patients with COVID-19 showed pulmonary abnormalities during chest CT scans on hospital admission. A chest radiography study summarized that in patients with identified abnormalities, 96% (73 of 76) demonstrated ground-glass opacity, 66% (50 of 75) demonstrated consolidation, and 62% (47 of 76) demonstrated

interlobular septal thickening.⁷⁶ Dynamic comparison of chest CT images through the disease course found that the lung lesions increased with time since symptom onset. A retrospective study found that 56% of patients in early stage of the disease (0-2 days since symptom onset) had normal CT results.⁷⁷ A similar trend was confirmed from another study, in which there were more consolidated lung lesions in chest CT images in patients 5 days or more from disease onset (61%) versus 4 days or fewer (21%) and more ground-glass opacity lung lesions (79% vs 40%) in patients 5 days or more from disease onset compared with 4 days or fewer.⁷⁸ A follow-up study of 63 hospitalized patients with an interval of 4-14 days since admission demonstrated progression on CT imaging in 86% of the cases (54 of 63).⁷⁹ A study classified the evolution of chest CT presentations in 4 stages, with maximum lung involvement on approximately day 10 since symptom onset.⁸⁰

Presentation of the Disease in Special Populations

COVID-19 in Children. Pediatric COVID-19 cases are relatively infrequent, with better clinical outcomes than in adults. An epidemiology article noted that of 44 672 confirmed COVID-19 cases in China up to February 11, only 965 patients (2.2%) were younger than 20 years of age and 416 patients (0.9%) were younger than 10 years of age. Among these young patients, only 1 died.⁶ Another pediatric epidemiology study summarized the findings for 2135 children (<18 years) tested from January 16 to February 8 and identified 728 (34%) as laboratory-confirmed cases and 1407 (66%) as suspected cases.⁸¹ The median age of all patients, 57% of whom were male, was 7 years. Only 125 patients (6%)

Table 3. Major Laboratory Differences Between Mild/Moderate Cases and Severe Cases on Hospital Admission

	Wang et al ^a	Deng et al ^b	Zhou et al ^c	Wu et al ^d	Guan et al ^e
Severity definition	Non-ICU versus ICU	Recovered versus dead	Survivor versus nonsurvivor	Mild/moderate versus severe/critical	Nonsevere versus severe
Patients, n	102 versus 36	116 versus 109	137 versus 54	197 versus 83	1099
White blood cell count ($\times 10^9/L$)	4.3 versus 6.6	4.5 versus 7.2 ^f	5.2 versus 9.8 ^f	5.0 versus 3.4	4.9 versus 3.7
Neutrophil count ($\times 10^9/L$)	2.7 versus 4.6 ^f	N/A	N/A	3.1 versus 2.2	N/A
Lymphocyte count ($\times 10^9/L$)	0.9 versus 0.8	1.0 versus 0.6 ^f	1.1 versus 0.6 ^f	1.3 versus 0.5 ^f	1.0 versus 0.8
Platelet count ($\times 10^9/L$)	165 versus 142	N/A	220 versus 166 ^f	196 versus 86 ^g	172 versus 138
D-dimer, $\mu g/mL$	0.2 versus 0.4 ^f	N/A	0.6 versus 5.2 ^f	0.2 versus 0.3 ^f	43 versus 60 ^h
Albumin, g/L	N/A	N/A	34 versus 29 ^f	38 versus 42	N/A
Creatine kinase, U/L	87 versus 102	N/A	18 versus 39	67 versus 76	13 versus 19 ^f
Creatine kinase-MB, U/L	13 versus 18 ^f	N/A	N/A	9 versus 13	N/A
Hypersensitive troponin I, pg/mL	5.1 versus 11.0	N/A	3 versus 22 ^f	N/A	N/A
Lactate dehydrogenase, U/L	212 versus 435 ^{a,f}	N/A	254 versus 521 ^{a,f}	184 versus 235	37 versus 58 ^a
ALT, U/L	23 versus 35	19 versus 22 ^{a,f}	27 versus 40	20 versus 24	20 versus 28 ^f
AST, U/L	29 versus 52 ^{a,f}	22 versus 34 ^{a,f}	N/A	26 versus 26	18 versus 39 ^{a,f}
Total bilirubin, $\mu mol/L$	9.3 versus 11.5	N/A	N/A	6.6 versus 6.7	10 versus 13 ^{a,f}
BUN, mmol/L	4.0 versus 5.9 ^{a,f}	N/A	N/A	4.0 versus 4.4	N/A
Serum creatinine, $\mu mol/L$	N/A	65 versus 89 ^{a,f}	2 versus 9 ⁱ	58 versus 63	1 versus 4.3 ⁱ
Procalcitonin, ng/mL	22 versus 75 ^{a,f,j}	N/A	1 versus 25 ^{a,f,k}	1.3 versus 1.5	3.7 versus 13.7 ^k
CRP, mg/L	N/A	3 versus 109 ^{a,f}	N/A	6.9 versus 21.3	56 versus 81 ^c

^aProportion ≥ 250 U/L.

^bProportion ≥ 40 U/L.

^cProportion > 10 mg/L.

^dProportion > 17.1 $\mu mol/L$.

^eProportion ≥ 200 U/L.

^f $P \leq .001$.

^gMean.

^hProportion ≥ 0.5 g/mL.

ⁱProportion > 133 $\mu mol/L$.

^jProportion ≥ 0.05 ng/mL.

^kProportion ≥ 0.1 ng/mL.

were reported to be in severe/critical condition, with 76 cases (61%) under 5 years of age, although the comorbidities of these pediatric patients were not provided. It is unclear if the COVID-19 prevalence in children has been underestimated because of the limited availability of viral nucleic acid test kits for asymptomatic patients and for patients with mild symptoms during the early stages of the pandemic.

There were 2 pediatric reports of 25 and 36 children with COVID-19 from China.^{82,83} The ages of these children ranged from 1 month to 16 years. Among them, only 2 cases were critical, requiring invasive mechanical ventilation. All 51 children recovered.

Two COVID-19-associated Kawasaki disease cases were reported, in a 6-month-old girl and a 5-year-old boy.^{84,85} Both children tested positive on SARS-CoV-2 nucleic acid and presented with persistent fever (38.8°C and 39.4°C), rash, conjunctivitis, leukocytosis, elevated C-reactive protein (CRP), and hypoalbuminemia. The infant girl also presented with prominent tongue papilla and swelling of the hands and lower extremities, which met the diagnostic criteria of Kawasaki disease. The boy presented with hypotension and an

enlarged cardiac silhouette chest x-ray. Both children received intravenous immunoglobulin (IVIg) treatment and recovered.

Another pediatric article reported a cluster of 8 children with hyperinflammatory shock during the COVID-19 pandemic in southeastern England.⁸⁶ Six of the children were of Afro-Caribbean descent, and 5 of the children were boys. Two were SARS-CoV-2-positive, and 4 had known family exposure to SARS-CoV-2. The clinical manifestations were much like those of atypical Kawasaki disease, Kawasaki disease shock syndrome, or toxic shock syndrome. All children received IVIg treatment, and 1 child died. Based on 15 pediatric cases of a multisystem inflammatory syndrome (4 were positive for SARS-CoV-2) with features of Kawasaki disease or toxic shock syndrome, the New York City health department issued a health alert on May 4.⁸⁷

A pediatric study indicated that the immune reaction in young children might be different against SARS-CoV-2 and respiratory syncytial virus (RSV), a common respiratory virus infecting this population.⁸⁸ Compared with RSV-infected children (n = 16; mean

age, 1.4 years), SARS-CoV-2-infected children ($n = 40$; mean age, 5.1 years) had a significantly higher proportion of T cells (CD3⁺) and a lower proportion of B cells (CD19⁺) in total lymphocytes and significantly higher CD8⁺ T-cell counts. Interleukin (IL)-10 was significantly higher in children with RSV pneumonia ($n = 5$) than in children with COVID-19 pneumonia ($n = 30$), whereas no significant differences in IL-2, IL-4, IL-6, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ were found between the 2 groups. Whether the age differences between the 2 groups also contributed to these observed differences is unclear. Two RSV-infected children (12.5%) and 1 SARS-CoV-2-infected child (2.5%) developed severe pneumonia. All children survived.

COVID-19 in Pregnant Women and Neonates. Various case reports, case series, and retrospective and case-control studies of pregnant women with COVID-19 presented clinical and laboratory data on maternal and neonatal manifestations and outcomes.⁸⁹⁻⁹³ Reviews and analyses of published reports provided additional insights.⁹⁴⁻⁹⁷ Most of the pregnant women in these studies and reports were in their third trimester, and many of their babies were delivered by caesarean section. In general, they experienced signs and symptoms of COVID-19 much like those in nonpregnant women. No maternal deaths were reported. Fetal distress, premature births, premature rupture of membranes, respiratory difficulties, and low birth weight were observed among some babies born to mothers with COVID-19.^{89,95,98}

No definitive cases of vertical transmission of the SARS coronavirus 2 (SARS-CoV-2) from mother to fetus have been identified, although 2 highly suspect cases have been reported. Wang et al described a male infant delivered by emergency cesarean section to a 34-year-old woman with COVID-19 confirmed by pharyngeal swab.⁹⁹ The infant had viral nucleic acid detected from a pharyngeal swab approximately 36 hours after birth. Tests of the cord blood, placenta, and the mother's breast milk were negative for SARS-CoV-2. Both mother and infant recovered. Alzamora et al reported on a 41-year-old diabetic woman with COVID-19-induced respiratory failure whose neonate was positive for SARS-CoV-2 nucleic acid from a nasopharyngeal swab 16 hours after a cesarean section delivery.¹⁰⁰ Serologies for SARS-CoV-2 were negative for the mother and the baby at the time of delivery. The mother converted to seropositive status on postpartum day 4, whereas the neonate, who had not been breastfed, remained seronegative at that time.

In relation to neonatal survival, Zhu et al reported 1 neonatal death in which a male baby born to a mother with confirmed COVID-19 developed refractory shock,

gastric bleeding, multiorgan failure, and disseminated intravascular coagulation approximately 8 days after birth.⁹⁸ The neonate died 1 day later. He tested negative for COVID-19 by throat swab.

Careful monitoring and follow-up of pregnant women with COVID-19 and their neonates is imperative. Considering that many of the published case reports and case series involve small numbers of pregnant women, additional information is needed to better characterize and prevent neonatal infection and provide more clinical and laboratory data to investigate the potential for vertical transmission.

COVID-19 Patients with Background Immunosuppressive Therapy. One pediatric center in Bergamo, a COVID-19-endemic area in Italy, showed that among around 200 transplant recipients, including 10 current inpatients, 100 with autoimmune liver disease and 3 under chemotherapy for hepatoblastoma (inpatients), none developed clinical pulmonary disease, despite 3 having tested positive for SARS-CoV-2.¹⁰¹ Another survey of 320 patients with rheumatoid arthritis (57%) and spondyloarthritis (43%) in the Lombardy region of Italy identified 4 confirmed and 4 suspected cases.¹⁰² Of the 320 subjects, 52% were treated with TNF inhibitors, 40% were treated with other biological disease-modifying antirheumatic drugs and 8% with Janus kinase inhibitors. Among 8 confirmed/suspected cases, 6 were on etanercept/abatacept, 2 were on tofacitinib/baricitinib, 3 were on methotrexate, and 3 were on hydroxychloroquine. These results indicated that patients on immunomodulatory therapy, including hydroxychloroquine, can become infected from SARS-CoV-2.

The common practice for patients with organ transplants and for those on maintenance treatment with immunosuppressive drugs is to temporarily discontinue the treatment.¹⁰³⁻¹⁰⁶ Of 2 recovered patients, all received IVIg and IFN- α treatment.^{103,106} Guidance from the American College of Rheumatology recommends that antimalarial therapies (hydroxychloroquine/chloroquine) may be continued, but sulfasalazine, methotrexate, leflunomide, immunosuppressants, non-IL-6 biologics, and JAK inhibitors should be stopped or held (M/H) in rheumatoid patients in the context of documented or presumptive COVID-19 infection, regardless of COVID-19 severity.¹⁰⁷ The European League Against Rheumatism advises patients not to stop or reduce antirheumatic drug treatment.¹⁰⁸

COVID-19 in Patients with Smoking History. Various observational studies, systematic reviews, and meta-analyses have assessed the potential contribution of cigarette smoking to increased risk for COVID-19 severity and mortality. Recent studies have

suggested that smoking may upregulate the angiotensin-converting enzyme receptor, which along with the spike protein, facilitates the entry of SARS-CoV-2 into lung epithelial cells.^{109,110}

Among 7 retrospective or prospective studies of the clinical characteristics and potential risk factors associated with the course, severity, and outcome of COVID-19 infections,^{4,7,64,111-114} 2 studies identified smoking as a significant risk factor.^{64,111}

Three publications reported findings with inconsistent results from systematic reviews or meta-analyses. A systematic review by Vardavas and Nikitara found an association between smoking and COVID-19 illness progression.¹¹⁵ Emami et al in a meta-analysis reported a high prevalence of smoking (7.6%) in hospitalized patients with COVID-19.¹¹⁶ However, a meta-analysis by Lippi and Henry found no association between smoking and COVID-19, although they acknowledged the findings reported by Liu above.¹¹⁷

The inconsistent results on smoking may be attributed to lack of data on smoking quantity and duration, small population size and/or few smokers in certain studies, and the presence of other concurrent comorbid conditions. Future research should consider including documentation of nicotine exposure through vaping and e-cigarettes.

The scientific community recently has debated a possible therapeutic role for nicotine in treating COVID-19. Some epidemiologic data have shown lower numbers of smokers among patients with COVID-19, indicating that nicotine may mediate the viral transmission by lowering ACE2 levels.^{118,119} A randomized clinical study is planned in France to more formally assess if nicotine could reduce the risk of contracting the disease.¹²⁰

Pathology

An analysis of 92 deceased patients with COVID-19 revealed that 91 deaths were from complications directly related to the viral infection.¹²¹ Among them, ARDS was most prevalent (76%), followed by myocardial injury (34%), liver injury (16%), and renal insufficiency (15%). Multiple organ dysfunction syndrome occurred in 15% of cases. Autopsy and biopsy of COVID-19 cases have been sporadically reported. A report of complete autopsy in 12 consecutive COVID-19-positive deaths (8 men and 4 women with a median age of 73 years) in Germany found that half the cases had coronary heart disease and that a quarter of the cases had respiratory diseases (asthma/COPD).¹²² The cause of death was found within the lungs or the pulmonary vascular system in all 12 cases. Deep venous thrombosis was identified in 7 of 12 patients (58%) in whom venous thromboembolism was not

suspected before death. Pulmonary embolism was the direct cause of death in 4 patients. The histopathology examination of lungs found diffuse alveolar damage in 8 cases. The lesions included hyaline membranes, activated pneumocytes, microvascular thromboemboli, capillary congestion, and protein-enriched interstitial edema, which were consistent with ARDS diagnosis.

However, another immunohistology investigation of lung tissues from 2 COVID-19 patients who died with respiratory failure found that the pattern of COVID-19 pneumonitis was predominantly a pauci-inflammatory septal capillary injury with significant septal capillary mural and luminal fibrin deposition and permeation of the interalveolar septa by neutrophils without hallmarks of classic ARDS.¹²³ In addition, there were prominent deposits of C5b-9, C4d, and mannose-binding lectin-associated serine protease 2 in the microvasculature, indicating activation of the complement pathway. The same study also examined skin tissues from 3 severe COVID-19 patients with respiratory failure and purpuric rash. The purpuric skin lesions similarly showed a pauci-inflammatory thrombogenic vasculopathy, with deposition of C5b-9 and C4d in both grossly involved and normal-appearing skin. The authors concluded that severe COVID-19 condition was accompanied by catastrophic microvascular injury syndrome mediated by activation of the complement pathways associated with procoagulant status.

Host Immune Response to SARS-CoV-2

Details of the immune response to SARS-CoV-2 are still under investigation. A study summarized the hallmarks of differences in immune profiles between COVID-19 and other infectious diseases.⁶³ Compared with H1N1 virus infection, COVID-19 patients had lower counts of CD3⁺CD4⁺CD45⁺ lymphocytes, but higher counts of CD3⁺CD16⁺CD45⁺ and CD19⁺CD45⁺ lymphocytes. The study identified that all investigated COVID-19 patients (n = 28) who developed severe respiratory failure were associated either with macrophage activation syndrome (MAS), characterized by high ferritin levels, or immune dysregulation (characterized by low expression of human leukocyte antigen-DR isotype on CD14 monocytes without elevated ferritin).

Innate Immune Response

Innate immune response is the host's first wave of response to a pathogen without previous exposure to it. It usually dominates during the initial hours to days of viral infection, which is characterized by activation of phagocytes and natural killer (NK) cells. Highlighted cytokines during this stage usually include interferons, TNF- α , IL-1, IL-6, IL-8, and IL-12, most of which

are produced by macrophages and monocytes. Three clinical studies recorded the baseline cytokine plasma concentrations in 21,¹²⁴ 43,¹²⁵ and 274¹²⁶ patients with COVID-19 on admission or from initial tests. The median time from symptom onset to admission in these 3 articles was 7-8 days,¹²⁴ 6 days,¹²⁵ and 9-10 days,¹²⁶ respectively. However, the cytokine examination date from a fourth study may be even later,⁴ because the median time for blood sample collection in this article was 4 days since subjects were transferred to a designated hospital.

One study reported that IFN- γ , TNF- α , IL-1 β , and IL-8 plasma concentrations in COVID-19 patients were significantly higher compared with results from 4 healthy subjects.⁴ When comparing the cytokine concentrations by disease severity, most studies found higher TNF- α , IL-6, and IL-8 in severe cases versus mild cases. However, these results should be interpreted with caution, as they are from a single point, and most samples may reflect a sum of both innate and adaptive immune responses to the virus.

Flow cytometry results^{124,127,128} showed abnormalities in NK cells from COVID-19 patients. NK cell number was significantly lower in severe/critical cases compared with those in healthy subjects and/or mild cases.^{127,128} The expression of NKG2A, the checkpoint receptor of NK cells,¹²⁹ was significantly higher in COVID-19 patients than in healthy subjects. This indicates the exhaustion of NK cell function¹³⁰ during SARS-CoV-2 infection. Indeed, the proportion of activated NK cells collected from patients, detected by the expression of intracellular cytokines such as CD107a, INF- γ , IL-2, TNF- α , and granzyme B, were all significantly lower in COVID-19 patients than in healthy subjects.¹²⁷ Whether the reduction of activated NK cells in COVID-19 patients is because of overwhelming viral load or of certain inhibiting mechanisms of SARS-CoV-2 is unclear. NK cell function appears to be associated with prognosis; in 5 available convalescent patients, the percentage of NKG2A⁺ NK cells in the blood decreased during the convalescent period.¹²⁷

T Cells

CD4⁺ T-helper cells play a pivotal role in activating the adaptive immune response to viral infection. CD8⁺ cytotoxic T cells are responsible for killing certain virus-infected cells by detecting viral antigen presented with major histocompatibility complex class I complex on the surface of infected cells.

In addition to the consistent trend of lower lymphocyte counts observed in severe cases of COVID-19 (Table 3) from different studies, total T cells, CD4⁺ T cells, and CD8⁺ T cells also were significantly lower in severe/critical COVID-19 cases than in nonsevere cases.^{124,127,128} Subgroups of CD4⁺ T cells did not show

significant proportion changes of CD45RA⁺-naive T cells and CD45RO⁺ memory T cells between severe cases and moderate cases.¹²⁴ However, the proportion of CD45RA⁺ regulatory T cells in severe cases (0.5%) was only half the value of that in moderate cases (1.1%). One study noted a slight improvement in mean T-cell counts (including CD4⁺ or CD8⁺ subpopulations) compared with baseline values after 5-14 days of in-hospital treatment.¹²⁸ Count improvement appears to be baseline-proportional, regardless of disease severity.

Several published studies have observed features of cellular exhaustion in T cells analogous to that described for NK cells.¹²⁹ In 2 related studies, the authors showed that healthy individuals could be distinguished from mild and severe COVID-19 patients based on levels of cell-surface markers associated with exhaustion on CD8 T cells; specifically, the severe group had much lower levels of nonexhausted (PD-1⁻CTLA-4⁻TIGIT⁻) CD8⁺ T cells.^{127,131} They also found that CD8⁺ T cells in COVID-19 patients exhibit many aspects of exhaustion and reduced function, such as diminished expression of cell-surface CD107a, reduced expression of activation cytokines IFN- γ and IL-2, and reduced mean fluorescence intensity of granzyme B. In addition, the proportion of IFN- γ -expressing CD4⁺ T cells was about 40% lower in severe cases (14.1%) than in moderate cases (22.8%).¹²⁴ Interestingly, in COVID-19 patients who recovered after treatment with antiviral medications (including lopinavir/ritonavir), both NK and T cells showed reduced signs of exhaustion, including a reduced percentage of NKG2A⁺ NK cells. In a separate patient cohort, exhaustion markers PD-1 and TIM-3 on CD4 and CD8 T cells were seen as COVID-19 patients progressed from early to later symptomatic stages of disease.¹³² Whether the reduction in T-cell counts and its impaired function in circulation represented local immune reactions in the lung is unclear.

Antibody Production

The antibody production temporal profile after SARS-CoV-2 infection follows a typical naive humoral immune response toward the virus. Antinucleocapsid protein N (anti-N) antibodies can be detected in most patients around days 5-10 after symptom onset,⁴⁸ and the anti-N IgG titer peaked starting in weeks 2 and 3.⁴⁴ Anti-N IgA titer peaked in week 3. The study showed that anti-N IgG from patients with COVID-19 did not have cross-reactivity with some common coronaviruses such as NL63, 229E, OC43, and HKU1, but could recognize protein N of SARS-CoV. The anti-spike protein S (anti-S) RBD antibodies generally appeared slightly later than did anti-N antibodies.⁴⁸ In patients with serum specimens available for at least 14 days since symptom onset, seropositivity was 94% (15 of 16) for anti-N IgG and 100% (16 of 16) for anti-S RBD IgG.

Both anti-N and anti-S RBD IgG and IgM demonstrated neutralization capability *in vitro*, and the antibody titer was strongly associated with neutralization capability.⁴⁸

One study noted that the titers of both anti-N and anti-S IgG and IgM in some severe cases started to decrease after days 18-20 since symptom onset.⁴⁸ Meanwhile, most mild cases demonstrated a gradual, steadier increase in titer, with the trend extending beyond days 15-20 since symptom onset.

The antibody production temporal profile also coincided with reduction of viral load in samples from oropharyngeal saliva or the respiratory tract,^{48,133} indicating seroconversion. In a study that closely followed both viral load (from throat swabs) and serum antibody profiles, the anti-N IgM-positive rate was 76% in nucleic acid-positive patients and 93% in nucleic acid-negative patients.⁴⁴ Combined nucleic acid and anti-N IgM tests resulted in 99% diagnostic sensitivity in recently infected patients.

Cytokine Storm Syndrome

Severe cases of COVID-19 are distinguished by a prominent increase in T-cell-related cytokines IL-6, IL-10, IL-2R (alpha)/CD25, and macrophage-secreted IL-8 (Table 4), indicating ongoing cytokine storm syndrome (CSS). Compared with survivors, the time profile of IL-6 in nonsurvivors showed an accelerated increase pattern starting on day 13 from illness onset.¹¹ The IL-6 pattern and impaired function of NK cells support the “second wave” hypothesis of proinflammatory cytokine-induced MAS-like pathology, which eventually leads to ARDS and multiorgan damage.^{63,134} Henderson et al pointed out in a review that the COVID-19-associated CSS resembles the classical MAS observed in patients with familial hemophagocytic lymphohistiocytosis (HLH), also seen in a subset of patients with systemic juvenile idiopathic arthritis, who carry heterozygous variants of the same genes (with biallelic mutations) as those in familial HLH patients.¹³⁵ The mutations, particularly in genes mediating cytotoxic granule release from NK cells and CD8⁺ T cells, result in impaired clearance of infected cells and activated macrophages, leaving the latter to release massive amounts of inflammatory cytokines.

An extremely high level of ferritin is a common feature of MAS/HLH not seen in COVID-19. However, COVID-19-associated CSS does share many features with MAS/HLH, including fever, cytopenias, chemistry abnormalities, relatively high ferritin, and elevated cytokines, including IL-1 β , IL-6, and IFN- γ . In particular, substantial elevation of soluble CD25, 1 of the listed diagnostic criteria of HLH,¹³⁶ was observed in severe COVID-19 cases and in nonsurvivors.^{124,126} Whether soluble CD25 is simply a marker of CD8⁺

T-cell hyperactivation in MAS/HLH¹³⁷ and COVID-19-associated CSS or whether it contributes to the pathophysiology of these conditions needs to be further elucidated.

IL-6 can be produced by many cell types, including monocytes/macrophages, T cells, B cells, fibroblasts, endothelial cells, and smooth muscle cells.¹³⁸ Its production can be triggered by infection and tissue damage. Th1-type cytokines such as IL-1, TNF, and IFN- β can enhance IL-6 production, whereas IL-6 suppresses endotoxin-induced IL-1 and TNF production. IL-6 rapidly induces production of a plethora of acute-phase proteins from the liver, including CRP, serum amyloid protein A, antitrypsin, and hepcidin.¹³⁹ It promotes a procoagulation status by facilitating maturation of megakaryocytes, increasing synthesis of thrombopoietin and fibrinogen and inducing tissue factor on the cell surface of monocytes. In addition, IL-6 breaks immune balance by inducing differentiation of Th17 cells with Transforming growth factor beta (TGF- β), while inhibiting TGF- β -induced Treg differentiation.

Elevation of IL-10 is commonly observed in viral infections.¹⁴⁰ It functions synergistically with IL-6 to stimulate B-cell differentiation, proliferation, and antibody production¹⁴¹ and is traditionally considered a Th1-inhibiting cytokine produced by monocytes and Th2 cells. The abnormally high levels of IL-10 in patients with COVID-19 may contribute to the imbalanced immune response to SARS-CoV-2. However, IL-10 may not be a good target for treating COVID-19-associated CSS because of its immunomodulatory effect.

IL-8 primarily functions as a neutrophil chemotactic factor and belongs to the CXC chemokine family. It also induces respiratory burst and release of lysosomal enzymes from neutrophils.¹⁴² Whether IL-8 is highly produced by macrophage residing in the lung in patients with COVID-19, which overrecruits and overactivates neutrophils that cause excessive local damage, is unclear.

Several other circulating inflammatory markers in COVID-19 patients have been studied. One study involved examining 48 cytokine profiles from 50 COVID-19 patients and identified 5—IFN- γ -induced protein 10 (IP-10), monocyte chemoattractant protein-3 (MCP-3), hepatocyte growth factor, monokine-induced gamma interferon, and macrophage inflammatory protein 1 alpha—which were observed to be highly associated with disease severity.¹⁴³ A combination of IP-10 and MCP-3 showed good potential to predict subjects' progression from mild to severe disease.

Antibody-Dependent Enhancement

Antibody-dependent enhancement (ADE) describes a phenomenon in which the virus-bound antibody

Table 4. Comparison of Immunologic Parameters in Patients With COVID-19

Parameter	Huang et al ⁴	Chen et al ²⁴	Wang et al ²⁵	Chen et al ²⁶	Xu et al ²⁸	Zheng et al ²⁷
Source	Jinyintan Hospital, Wuhan	Tongji Hospital, Wuhan	Union Hospital, Wuhan	Tongji Hospital, Wuhan	Hubei Provincial Hospital	Multiple hospitals in province Anhui
Patients, n	13 ICU cases versus 28 non-ICU cases ^a	11 severe cases versus 10 moderate cases ^a	7 SpO ₂ < 90% versus 36 SpO ₂ ≥ 90% ^a	113 nonsurvivors versus 161 recovered ^a	107 severe/critical cases versus 80 mild cases	13 severe cases versus 55 mild cases ^a
Cytokines						
TNF- α	Higher in ICU cases ^b	Higher in severe cases	Comparable	~50% higher in nonsurvivors	N/A	N/A
IL-1 β	Comparable	Comparable	N/A	N/A	Comparable	N/A
IFN- γ	Comparable to slightly higher in ICU cases	N/A	N/A	N/A	N/A	N/A ^a
IL-2	Higher in ICU cases ^b	Higher in severe cases ^b	Comparable	~2-fold higher in nonsurvivors	N/A	N/A
IL-2R	N/A	Higher in severe cases ^b	N/A	N/A	N/A	N/A
IL-4	Comparable	N/A	Comparable	N/A	N/A	N/A
IL-6	Comparable to slightly higher in ICU cases	Higher in severe cases ^b	~7.7-fold higher in cases SpO ₂ < 90%	~5.5-fold higher in nonsurvivors	Higher in critical cases	N/A
IL-8	Higher in ICU cases	Higher in severe cases	N/A	~2.5-fold higher in nonsurvivors	N/A	N/A
IL-10	Comparable to slightly higher in ICU cases	Higher in severe cases ^b	~65% higher in cases SpO ₂ < 90%	~2.5-fold higher in nonsurvivors	Higher in severe/critical cases ^b	N/A
Immune cells						
Total T cells ^c	N/A	Lower in severe cases ^b	N/A	N/A	Lower in severe/critical cases ^b	Lower in severe cases ^b
CD4 ⁺ T cells ^c	N/A	Lower in severe cases ^b	Comparable	N/A	Lower in severe/critical cases ^b	N/A
CD8 ⁺ T cells ^c	N/A	Lower in severe cases ^b	Lower in cases SpO ₂ < 90%	N/A	Lower in severe/critical cases ^b	Lower in severe cases ^b
Total B cells ^c	N/A	Comparable	Comparable	N/A	Lower in severe/critical cases ^b	N/A
NK cells ^c	N/A	Lower in severe cases	N/A	N/A	Lower in severe/critical cases ^b	Lower in severe cases ^b
NKG2A+ NK cells	N/A	N/A	N/A	N/A	N/A	Higher in COVID-19 patients compared with healthy subjects ^b

^aBaseline (on admission) results.^bStatistically significant ($P < .05$).^cCount ($\times 10^6/L$).^dInpatient results.

facilitates cellular uptake of virus-antibody complex via FcR expressed on the surface of target cells. Once in the cell, the virus is not killed or neutralized; instead, it may continue to replicate, and/or stimulate or kill the target cells, causing more inflammation and damage. Of note is that ADE is not necessarily associated with past humoral response to a related pathogen, such as dengue virus,¹⁴⁴ and can occur during the primary humoral response, when the neutralizing antibody is at a suboptimal level. This was documented in some severe cases following SARS-CoV infection.^{145,146}

In a study reporting 75 patients with SARS, the lung radiographic worsening of some severe cases was correlated with the time of IgG seroconversion.¹⁴⁵ The pattern is consistent with that of COVID-19, in which the disease in some severe cases suddenly worsened after around 1 to 2 weeks,^{62,63} when seroconversion of anti-SARS-CoV-2 occurred (around 5 to 10 days after the onset of the first symptom).⁴⁸ In addition, the recovered SARS patients had higher and sustainable or steadily increasing levels of both anti-N antibody and anti-S neutralizing antibody since the seroconversion.¹⁴⁶ However, the titer of anti-N antibody in the SRAS nonsurvivors was low, and the titer of anti-S antibody decreased rapidly approximately 5 days after the peak, an observation much like that in some patients with severe cases of COVID-19.⁴⁸

In vitro studies demonstrated that human anti-S serum enhanced SARS-CoV infection in human monocyte-derived macrophages (MDMs).¹⁴⁷ The infection mechanism is very different from that of the ACE2-mediated, endosomal/lysosomal-dependent pathway and can be blocked by anti-Fc γ R II antibody.¹⁴⁸ A study challenged rhesus monkeys with SARS virus and found acute diffuse alveolar damage in monkeys that were vaccinated with S protein and had high titers of anti-S antibody before the challenge. However, most challenged monkeys who were vaccinated with the control vehicle showed minor to moderate inflammation.¹⁴⁹ Combining anti-S IgG sera with SARS-CoV causes dose-dependent production of IL-6, IL-8, and MCP-1 from human MDMs in vitro. In addition, elevated serum IL-8 concentration is strongly associated with anti-S antibody titer ($r = 0.94$) in S protein-vaccinated monkeys; however, the IL-8 production in MDMs stimulated by SARS-CoV pseudovirus and sera from deceased SARS patients could be blocked by the anti-Fc γ R antibody. Subtyping of macrophages in the lungs of S protein-vaccinated monkeys showed a skewed wound-healing response to uncontrolled inflammation and tissue damage.

Therefore, based on the similarity of SARS-CoV and SARS-CoV-2, the disease course and the cytokine pattern, some severe COVID-19 cases likely experi-

enced ADE. The mechanism underlying why only a small proportion of patients experienced ADE needs to be investigated further.

Current Antiviral and Immunomodulatory Pharmacotherapies

On April 21, the National Institutes of Health (NIH) issued general treatment guidelines for COVID-19,¹⁵⁰ with the recommendations based on scientific evidence and expert opinion. The guidelines acknowledge a lack of definitive clinical trial data for identifying optimal treatments for the disease. Although no specific pharmacotherapy had been approved by the FDA for COVID-19, the biomedical research field and clinical studies were unprecedentedly enthusiastic and rigorous on drug development in this pandemic. By May 19, 1590 COVID-19-related clinical studies were registered at ClinicalTrials.gov, and using keyword "COVID-19" identified more than 14 300 publications on PubMed. Other than the experimental therapeutics directly targeting the virus and the disease, intensive discussions continued even on some common concomitant medications such as nonsteroidal anti-inflammatory drugs^{151,152} and angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers.^{153,154} Some thorough reviews summarized current experimental pharmacotherapy for COVID-19.¹⁵⁵⁻¹⁵⁸ We do not intend to repeat the previously well-documented work in our review, but prefer to focus on 2 important topics: antiviral and immunomodulatory pharmacotherapies. Because results from clinical trials currently are being generated at such a blazing pace, this review was up to date at the time it was written.

Hasan and Siddiqi¹⁵⁹ proposed the use of a 3-stage clinical phenotyping scale with each stage corresponding to increased disease severity. The first stage is associated with an incubation period, during which SARS-CoV-2 replicates; hence, the use of antiviral drugs during this stage may alleviate symptoms and attenuate disease progression. The second stage typically is marked by viral pneumonia, and patients may require mechanical ventilation depending on the extent of disease progression. The third stage is marked by hyperinflammation and elevation in inflammatory cytokines; therefore, use of immunomodulatory agents to reduce systemic inflammation is being proposed.

Antiviral Therapeutics

As with other viral infections, antiviral therapeutics for treating COVID-19 are recommended and suggested to be prioritized, especially during the early stages of viral infection.¹⁵⁹ This is supported by the observation that the viral load from both upper respiratory tract samples

(swab) and relatively lower respiratory tract samples (sputum) were highest during the first several days since symptom onset.²²

Many viral cellular adherence/endocytosis-blocking reagents were proposed. The cellular infection mechanism of SARS-CoV-2 is believed to be the same as SARS-CoV, which is an ACE2-mediated, endosomal-dependent pathway. Chloroquine¹⁶⁰ and hydroxychloroquine¹⁶¹ were first identified through *in vitro* drug screening to reduce viral titers from the supernatant of infected cell cultures. The mechanism probably is through interference with viral entry/endocytosis by increasing the pH of the endosome.¹⁶²

Two series of open-label, nonrandomized studies in France reported only 1 death in 80 patients with relatively mild disease treated with hydroxychloroquine sulfate (200 mg 3 times a day for 14 days) and azithromycin (500 mg on day 1 followed by 250 mg once daily).^{163,164} The authors justified the use of azithromycin because it had been shown to be effective against Zika and Ebola viruses *in vitro*. Of note is that azithromycin also prolongs the QT interval.

Based on limited scientific information, it is reasonable to believe that hydroxychloroquine may be an effective treatment. The FDA issued an EUA on March 28 to permit the emergency use of chloroquine phosphate and hydroxychloroquine sulfate supplied from the Strategic National Stockpile to treat adults and adolescents who weigh 50 kg or more and are hospitalized with COVID-19 for whom a clinical trial is not available or participation is not feasible.¹⁶⁵ It should be noted that the FDA's typical process for an EUA is to review its circumstances and appropriateness periodically. The review would include regular assessments based on additional information from the sponsor regarding progress on the unapproved product's—or unapproved use of an approved product's—approval, licensure, or clearance.

An observational study in 1376 patients from New York City did not find a significant difference in the rate of intubation or mortality between patients who received hydroxychloroquine and those who did not.¹⁶⁶ A randomized, double-masked, parallel-group Brazilian study in hospitalized patients with severe respiratory syndrome secondary to COVID-19 demonstrated that more patients (16 of 41, 39%) died in a high-dose chloroquine group (600 mg twice daily for 10 days) than did patients (6 of 40, 15%) in a low-dose chloroquine group (450 mg twice daily on day 0 followed by 450 mg once daily for 4 days) through day 13 of the treatment.¹⁶⁷

Multiple studies monitored QT prolongation events in COVID-19 patients following chloroquine/hydroxychloroquine/azithromycin treatment. The

Brazilian study reported that a greater proportion of patients in the high-dose group (19%) had QTcF greater than 500 milliseconds versus patients in the low-dose group (11%).¹⁶⁷ Authors of a French study treating 40 COVID-19 patients with hydroxychloroquine and/or azithromycin showed that 37 patients (93%) observed an increase in QTc and prolonged QTc (QTc \geq 500 milliseconds) in 7 patients (18%) after the treatment.¹⁶⁸ Another study in 90 COVID-19 patients receiving hydroxychloroquine found that patients on concomitant azithromycin had a greater median change in QT interval (23 milliseconds) than patients only on hydroxychloroquine treatment,¹⁶⁹ which confirmed that the 2-drug treatment had an additive effect on QTc prolongation. To remind health care professionals and patients of the known risks of QT interval prolongation and serious and potentially fatal arrhythmias associated with both hydroxychloroquine and chloroquine, the FDA issued a drug safety communication on April 24. The American Heart Association, the American College of Cardiology, and the Heart Rhythm Society recommended caution on COVID-19 treatment with hydroxychloroquine and azithromycin for patients with cardiovascular disease.¹⁷⁰ NIH treatment guidelines recommended monitoring QTc intervals in patients on chloroquine or hydroxychloroquine treatment and against the combined use of hydroxychloroquine and azithromycin because of potential toxicities.¹⁵⁰

Although viremia could occur in both SARS-CoV¹⁷¹ and SARS-CoV-2⁵⁰-infected patients, its prevalence and relationship with disease severity needs to be further investigated. Systemically available small molecules are expected to distribute both systemically and in the airways. However, systemic administration of biological molecules such as soluble ACE2, soluble RBD of ACE2, anti-ACE2 antibody,¹⁷² and EK1 pan-coronavirus fusion inhibiting peptide¹⁷³ may have limited distribution in the airways, where the viral load may be highest.

With the appearance of COVID-19, some antiviral reagents were repurposed immediately, a strategy borrowed from the SARS treatment experience.⁴ To date, the best clinical evidence has come from a randomized, controlled open-label study using lopinavir and ritonavir combination therapy to treat patients with severe COVID-19.¹⁷⁴ Lopinavir and ritonavir are 2 protease inhibitors approved for combination treatment of AIDS and have been used experimentally as treatment for SARS and Middle East respiratory syndrome (MERS).¹⁷⁵ The study failed to demonstrate statistical significance on the primary end point, the time to clinical improvement in patients on lopinavir/ritonavir treatment (400 mg/100 mg twice daily for 14 days) compared

with a standard-of-care (SOC) group (hazard ratio, 1.24; 95%CI, 0.90 to 1.72), with a mortality rate (19.2% vs 25.0%; difference, -5.8%; 95% CI, -17.3 to 5.7) as the secondary end point. A post hoc subgroup analysis demonstrated accelerated clinical recovery (16.0 vs 17.0 days) and reduced mortality rate (19.0% vs 27.1%) in patients treated within 12 days from symptom onset, indicating that further clinical studies on early treatment of COVID-19 are needed. Mechanistically, it is unclear how aspartyl protease inhibitors could also inhibit the protease of coronavirus, which is a cysteine protease.¹⁷⁶ The time profile of viral load from oropharyngeal swab samples recorded from the study demonstrated no apparent postbaseline change in viral load difference between the lopinavir/ritonavir-treated and SOC groups.

Remdesivir, an RNA-dependent RNA polymerase inhibitor, has shown activity against SARS-CoV-2 in vitro.¹⁶⁰ A randomized, double-blind, placebo-controlled study in China¹⁷⁷ enrolled 237 RT-PCR-confirmed severe COVID-19 patients with pneumonia and hypoxemia. The study was terminated before attaining the prespecified sample size because the outbreak of COVID-19 was brought under control in China, as stated by the authors. Patients were randomized (2:1) to receive either remdesivir (200 mg on day 1, followed by 100 mg on days 2-10 in single daily infusions) or placebo treatment. The study did not reveal a statistically significant difference in the primary end point, time to clinical improvement up to day 28 between remdesivir and placebo groups (hazard ratio, 1.23; 95%CI, 0.87-1.75). The 28-day mortality was 14.7% (22 of 150) in the remdesivir group and 13.0% in the placebo group. The viral load time profiles of nasopharyngeal/oropharyngeal swabs were similar in the 2 treatment groups.

An interim analysis from a randomized, controlled clinical trial sponsored by the National Institute of Allergy and Infectious Diseases, however, demonstrated a faster time to recovery in patients in the remdesivir group than in the placebo group.¹⁷⁸ On May 1, the FDA granted EUA for the emergency use of remdesivir for the treatment of hospitalized COVID-19 patients.¹⁷⁹

An open-label, randomized study that combined lopinavir/ritonavir treatment with 2 drugs repurposed from treating viral hepatitis—interferon beta-1b (8 million IU every other day) and ribavirin (400 mg twice daily)—showed promise for reducing symptoms of COVID-19, shortening hospital stays, and reducing the duration of viral shedding in the combination group compared with the lopinavir/ritonavir group.¹⁸⁰

Targeted Immune Therapies

Systemic corticosteroid was administered in 18.6% to 44.9% of patients with COVID-19,¹⁸¹ with no

clear effective outcome reported. Use of corticosteroids in treating patients with SARS also did not show clear beneficial effects.⁶² A meta-analysis pooling 11 reports of SARS-CoV-2-, SARS-CoV-, and MERS-CoV-infected patients found that corticosteroid treatment was associated with delayed virus clearance, prolonged hospitalization, increased mechanical ventilation rate, and no significant reduction in mortality.¹⁸² Therefore, NIH treatment guidelines for COVID-19 only recommend using low-dose corticosteroid therapy for adults with COVID-19 and refractory shock.¹⁵⁰

Target-mediated immunomodulatory therapeutics for COVID-19-associated CSS have been proposed and discussed¹⁸³⁻¹⁸⁵; however, a discussion of all targeted therapies being actively studied in COVID-19 is beyond the scope of this article. In addition to the specific approaches described below, some additional examples of active studies (<https://clinicaltrials.gov/>) targeting inflammatory cytokines or cytokine receptor signaling using repurposed drugs approved for treatment of autoimmune/rheumatologic/autoinflammatory conditions include the IL-1 inhibitors anakinra and canakinumab, the IL-6 inhibitors sarilumab, clazakizumab and tocilizumab, and the JAK inhibitors tofacitinib, baricitinib, and ruxolitinib. In addition, an online database (<https://cdc.org/corona/>) includes other immunosuppressive drugs that may show promise in treating COVID-19, for example, the TNF inhibitors infliximab and adalimumab and the IL-12/23 inhibitor ustekinumab.

- Tocilizumab, an anti-IL-6 receptor monoclonal antibody, was the only drug approved for treating CSS induced by chimeric antigen receptor T-cell therapy for hematological malignancies. Sporadic cases of successful tocilizumab treatment in COVID-19 patients with malignant comorbidities have been reported.^{186,187} One patient with multiple myeloma was treated with a single dose of intravenous 8 mg/kg tocilizumab on hospital day 9, after experiencing no improvement in chest CT imaging for 6 days and exhibiting a high concentration of serum IL-6 on day 7 (122 pg/mL). After the treatment, the patient's serum IL-6 decreased steadily to 21 pg/mL on day 18, accompanied by improved results of chest CT imaging and clinical symptoms.¹⁸⁶ The IL-6 concentration in another patient with metastatic renal cell carcinoma was unknown; however, he was given 2 doses of intravenous 8 mg/kg tocilizumab 8 hours apart on day 8 of hospitalization, at the peak of his symptoms.¹⁸⁷ The patient experienced clinical improvement thereafter and fully recovered. No major changes in the

- proportion of T-cell subpopulations were observed before and after this patient's tocilizumab treatment.
- Intravenous immunoglobulin (IVIg) has been used to treat SARS patients.¹⁸⁸⁻¹⁹⁰ Although the exact mechanism is unclear, high doses of IVIg can block FcR-mediated phagocytosis activity, superoxide anion release, and chemotaxis.¹⁹¹ Authors of a study reported using high-dose IVIg (25 g/d for 5 days) successfully to treat 3 severe COVID-19 cases (all discharged).¹⁹² However, other study authors found that 23 of 58 patients with severe or critical COVID-19 received IVIg treatment died over a 28-day period.¹⁹⁰ The detailed IVIg treatment plan was not available.
 - IL-1 pathway inhibitors, including anakinra (IL-1Ra antagonist) and canakinumab (anti-IL-1 β antibody), also have been proposed to treat COVID-19-associated CSS because of their effectiveness in patients with secondary HLH/MAS.¹⁹³ However, unlike IL-6, IL-1 β plasma concentration is comparable in mild and severe COVID-19 cases.^{4,124} In a retrospective cohort study, 29 patients with COVID-19 from Milan received high-dose intravenous anakinra (5 mg/kg twice daily) and were compared retrospectively with 16 patients treated with the SOC.¹⁹⁴ The findings were encouraging, with no major safety signals in the anakinra group and a higher percentage of patients showing clinical improvement (72% versus 50% in the SOC group). At 21 days, survival was 90% in the anakinra group and 56% in the SOC group.
 - Although elevated IFN- γ plays a pivotal role in HLH¹³⁷ and emapalumab, an approved anti-IFN- γ monoclonal antibody, can effectively treat HLH,¹⁹⁵ more studies are needed to evaluate the circulating IFN- γ levels in COVID-19 patients. The authors of this review only identified 1 study showing a slight increase of IFN- γ in ICU cases compared with non-ICU-cases⁴; this is probably because IFN- γ cannot be easily measured in the peripheral blood.¹³⁵ Therefore, CXCL9, a more stable chemokine, has been proposed as a potential useful surrogate for IFN- γ in MAS and potentially may be used as an indicator of IFN- γ activity in COVID-19 patients.¹³⁵ We note that IFN- γ is one of the most important antiviral cytokines that modulate both innate and adaptive immune networks. For different viruses, it has pleiotropic functions such as inhibiting viral cellular entry and release, viral replication, and viral gene expression.¹⁹⁶ As with other targeted cytokine therapies, a balance between suppression of harmful cytokine storm and the potential to impair viral clearance should be considered when targeting IFN- γ in COVID-19 patients.

Based on immunology characteristics of COVID-19, Ferro et al proposed a "window of opportunity" using the target-mediated immunomodulatory drugs to treat patients with COVID-19.¹⁸⁴ The proposed "window of opportunity" is for balancing the risk of infections and treatment efficacy. For example, tocilizumab should be avoided when the disease is still in a mild and stable stage (≤ 7 days) and when IL-6-dependent immune response for viral clearance and antibody production is critical; but it could be used when the disease is rapidly progressive, with or without lung involvement. Because of its low availability and high cost, IVIg has limited application in an epidemic viral disease; the author did not recommend it as the first choice of treatment.

Plasma Therapy

Plasma from convalescent subjects has been used experimentally during the past century to treat actively infected patients during epidemics. The procedure can be traced back as early as the 1917-1919 H1N1 Spanish flu, and to recent SARS and Ebola outbreaks.¹⁹⁷ A meta-analysis described the effectiveness of this passive immunity in treating SARS and influenza.¹⁹⁸ One study reported using convalescent plasma to effectively treat 5 COVID-19 patients with ARDS.¹⁹⁹ Each patient received a direct transfusion of 400 mL of convalescent plasma from a donor who had been well for at least 10 days and tested negative for SARS-CoV-2 and other relevant viruses. The titer of anti-SARS-CoV-2 antibody and neutralizing antibody in the plasma was at least 1:1000 and 1:40, respectively, for each donor. The viral load in nasopharyngeal specimens from 5 patients became negative between day 1 and day 12 posttransfusion. The Sequential Organ Failure Assessment score and chest CT imaging all showed improvement posttransfusion. IL-6 concentration in 4 of 5 patients showed a transient peak between days 1 and 5 posttransfusion, followed by a steady reduction. Three patients eventually were discharged, and 2 patients on mechanical ventilation were in stable condition.

Convalescent plasma transfusion cannot be widely implemented. It is limited by qualified donor, sophisticated plasma collection device, laboratory capability to exclude blood-borne pathogens, and appropriate antibody titer/function-detecting methods. To regulate investigational convalescent plasma therapy, the FDA issued a Guidance in April 2020.²⁰⁰

Summary

The COVID-19 pandemic is still ongoing, and a long time may pass before we can fully grasp the complete picture of the pathogen's characteristics, including its

vulnerabilities, which can be used to inform development of effective and efficient treatments. Development of antiviral therapeutics, led by DNA/RNA polymerase and protease inhibitors, has been streamlined since their invention in combating HIV. Given worldwide extensive efforts, we are hopeful that anti-SARS-CoV-2 replication drugs will be discovered. It is unclear whether overreactive immune response and/or CSS play important roles in patients with severe COVID-19. However, some case reports suggest the efficacy of immunomodulatory agents in treating patients with severe COVID-19, which could pave the way for large-scale randomized, blinded, and controlled clinical trials. Last, but not least, the antibody profiles and timelines in recovered COVID-19 patients are encouraging. These should inform and guide the development of the ultimate antiviral weapon, the vaccine, for preventing COVID-19 in the 21st century.

Information Update After Acceptance

On June 15, 2020, the FDA revoked* the EUA of chloroquine phosphate and hydroxychloroquine sulfate to be used to treat certain hospitalized patients with COVID-19. Based on its ongoing analysis of the EUA and emerging scientific data, the FDA determined that chloroquine and hydroxychloroquine are unlikely to be effective in treating COVID-19 for the authorized uses in the EUA. Additionally, in light of ongoing serious cardiac adverse events and other potential serious side effects, the known and potential benefits of chloroquine and hydroxychloroquine no longer outweigh the known and potential risks for the authorized use. *(<https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-revokes-emergency-use-authorization-chloroquine-and>)

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Conflicts of Interest

The authors have declared no conflicts of interest for this article.

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