Bernd Sebastian Kamps
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COVID REFERENCE

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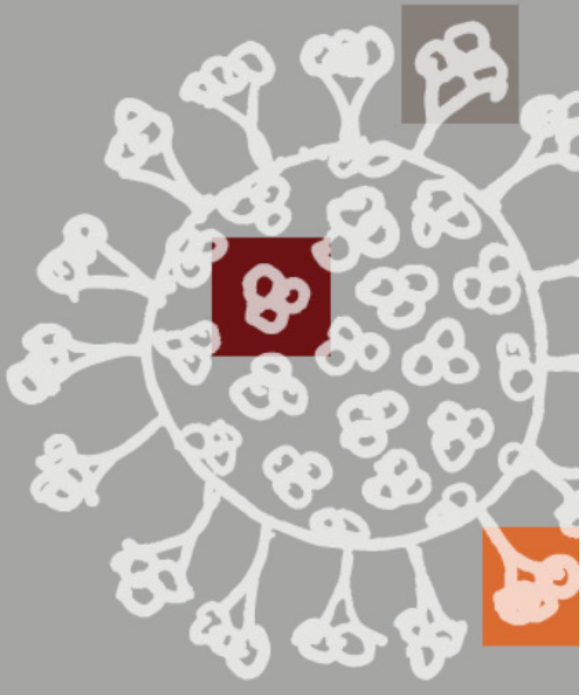

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have received *no support from third parties* to realize this manual.

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Edition 2020~3

Steinhäuser Verlag

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Preface

Seventeen years ago, in the middle of the outbreak, we decided to write a short medical text about the ongoing SARS drama, presenting the scientific data and providing real-time updates. After publishing three editions in 6 months, a scientific magazine concluded that our *SARS Reference* (www.SARSReference.com) was "not fancy", but presented "plenty of information". When we became aware of the new coronavirus epidemic in mid-January 2020, we immediately felt that time had come to repeat our millenium exercise.

While SARS-CoV-2 seems under control in China, the epidemic is moving west briskly. What only weeks ago seemed an impossible feat – imposing and enforcing strict quarantine measures and isolating millions of people – is now a reality in many countries. People all over the world will have to adapt and invent new lifestyles in what is the most disruptive event since World War II.

We believe that the current situation needs a new type of textbook. Humanity is confronting an unknown and threatening disease which is often severe and fatal. Health care systems are overwhelmed. There is no proven treatment and vaccines will not be available soon. Such a situation has not existed since the flu pandemic in 1918.

We believe a clear head is crucial in times of over-information, with dozens of scientific papers published *every day*, news about hundreds of studies being planned or already on the way and social media blending hard data with rumors and fake news. The tedious work of screening the scientific literature and the scientific data has to be done – regularly & constantly, like a Swiss watch.

Over the coming months, COVID Reference will be presenting updates on a weekly basis and narrating the scientific data as coherently as possible.

Remember Science Magazine. It isn't fancy.

Bernd Sebastian Kamps & Christian Hoffmann

29th March 2020

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0. Top 10

Please bookmark www.CovidReference.com/Top10Papers and come back for the **Daily Top 10 Papers** on COVID-19 and SARS-CoV-2 (every day at 19:00 CEST). Each citation comes with a short comment and a link to the full-text article.

1. Timeline

Thursday, 12 December

In **Wuhan**, health officials start investigating patients with viral pneumonia. They eventually find that most patients have visits to the Huanan Seafood Wholesale Market in common. The market is known for being a sales hub for poultry, bats, snakes, and other wildlife animals.

Monday, 30 December 2019

Li Wenliang (en.wikipedia.org/wiki/Li_Wenliang), a 34-year-old ophthalmologist from Wuhan, posts a message on a WeChat group alerting fellow doctors to a new disease at his hospital in late December. He writes that seven patients have symptoms similar to SARS and are in quarantine. Li askes his friends to inform their families and advises his colleagues to wear protective equipment.

Tuesday, 31 December 2019

The Wuhan police announce that they are investigating eight people for spreading rumors about a new infectious diseases outbreak (see 30 December).

The Wuhan Municipal Health Commission reports 27 patients with viral pneumonia and a history of exposure to the Huanan Seafood Wholesale Market. Seven patients are critically ill. The clinical manifestations of the cases were mainly **fever**, a few patients had **difficulty breathing**, and chest radiographs showed **bilateral lung infiltrative lesions**. The report says that the "disease is preventable and controllable". WHO is informed.

Thursday, 1 January

The Huanan Seafood Wholesale Market is shut down.

Friday, 3 January

Li Wenliang is summoned to a local public security office in Wuhan for "spreading false rumours". He is forced to sign a document where he admits having made "false comments" and "disrupted social order." Li signs a statement agreeing not to discuss the disease further.

On the Weibo social network, Wuhan police say they have taken legal action against people who "published and shared rumors online", "causing a negative impact on society". The following day, the information is taken up by CCTV, the state television. CCTV does not specify that the eight people accused of "spreading false rumors" are doctors.

Sunday, 5 January

WHO alerts that 44 patients with pneumonia of unknown etiology have been reported by the national authorities in China. Of the 44 cases reported, 11 are severely ill while the remaining 33 patients are in stable condition. https://www.who.int/csr/don/05-january-2020-pneumonia-of-unkown-cause-china/en/

Tuesday, 7 January

Chinese officials announce that they have identified a **new coronavirus** (CoV) from patients in Wuhan (pre-published 17 days later: https://doi.org/10.1056/NEJMoa2001017). Coronaviruses are a group of viruses that cause diseases in mammals and birds. In humans, the most common coronaviruses (HCoV-229E, -NL63, -OC43, and -HKU1) continuously circulate in

the human population; they cause colds, sometimes associated with fever and sore throat, primarily in the winter and early spring seasons. These viruses are spread by inhaling droplets generated when infected people cough or sneeze, or by touching a surface where these droplets land and then touching one's face.

Sunday, 12 January

The genetic sequence of the new coronavirus has been made available to WHO. Laboratories in different countries start to produce specific **diagnostic PCR tests**. (The Chinese government reports that there is no clear evidence that the virus passes easily from person to person.)

Two days after starting coughing, Li Wenliang (see 30 December) is hospitalized. He will later be diagnosed with COVID.

Monday, 13 January

Thailand reports the first case outside of China, a woman who had arrived from Wuhan. Japan, Nepal, France, Australia, Malaysia, Singapore, South Korea, Vietnam, Taiwan, Thailand and South Korea report cases over the following 10 days.

Saturday, 18 January

The Medical Literature Guide **Amedeo** (www.amedeo.com) draws the attention of 50,000+ subscribers to a study from Imperial College London, *Estimating the potential total number of novel Coronavirus cases in Wuhan City, China*, by Imai et al. The authors estimate that "a total of 1,723 cases of 2019-nCoV in Wuhan City (95% CI: 427 – 4,471) had onset of symptoms by 12th January 2020". Officially, only 41 cases were reported by 16th January.

Monday, 20 January

China reports three deaths and more than 200 infections. Cases are now also diagnosed outside Hubei province (Beijing, Shanghai and Shenzhen). Asian countries begin to introduce mandatory screenings at airports of all arrivals from high-risk areas of China.

Thursday, 23 January

In a bold and unprecedented move, the Chinese government puts tens of millions of people in **quarantine**. Nothing comparable has ever been done in human history. Nobody knows how efficient it will be.

All events for the Lunar New Year (starting on January 25) are cancelled.

WHO declares that the outbreak does not yet constitute a public emergency of international concern as there is "no evidence" of the virus spreading outside of China.

Friday, 24 January

At least 830 cases have been diagnosed in nine countries: China, Japan, Thailand, South Korea, Singapore, Vietnam, Taiwan, Nepal, and the United States.

Zhu et al. publish their comprehensive report about the isolation of a **novel coronavirus** which is different from both MERS-CoV and SARS-CoV (full-text: https://doi.org/10.1056/NEJMoa2001017). They describe sensitive assays to detect viral RNA in clinical specimens.

Wang et al. publish the **clinical features** of 41 patients (full-text: doi.org/10.1016/S0140-6736(20)30185-9).

Chan et al. describe a **familial cluster** of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission (full-text: doi.org/10.1016/S0140-6736(20)30154-9).

Saturday, 25 January

The Chinese government imposes travel restrictions on more cities in Hubei. The number of people affected by the quarantine totals **56 million**.

Hong Kong declares an emergency. New Year celebrations are cancelled and links to mainland China restricted.

Thursday, 30 January

The WHO declares coronavirus a global emergency. In the meantime, China reports 7,711 cases and 170 deaths. The virus has now spread to all Chinese provinces.

Friday, 31 January

Li Wenliang publishes his experience with **Wuhan police station** (see 3 January) with the letter of admonition on social media. His post goes viral.

India, the Philippines, Russia, Spain, Sweden, the United Kingdom, Australia, Canada, Japan, Singapore, the US, the UAE and Vietnam confirm their first cases.

Sunday, 2 February

The first death outside China, of a Chinese man from Wuhan, is reported in the **Philippines**. Two days later a death in Hong Kong is reported.

Thursday, 6 February

Li Wenliang, who was punished for trying to raise the alarm about coronavirus, dies. His death sparks an explosion of anger, grief and demands for freedom of speech: https://www.theguardian.com/global-development/2020/feb/07/coronavirus-chinese-rage-death-whistleblower-doctor-li-wenliang.

Friday, 7 February

Hong Kong introduces **prison sentences** for anyone breaching quarantine rules.

Monday, 10 February

Amedeo launches a weekly Coronavirus literature service which would later be called ***Amedeo COVID-19***.

Tuesday, 11 February

Less than three weeks after introducing mass quarantine measures in China, the number of daily **reported cases starts dropping**.

The WHO announces that the new infectious disease would be called **COVID-19** (**Co**rona**vi**rus **d**isease 20**19**).

Wednesday, 12 February

On board the Diamond Princess **cruise ship** docked in Yokohama, Japan, 175 people are infected with the virus. Over the following days and weeks, almost 700 people will be infected onboard.

Wednesday, 19 February

Iran reports two deaths from the coronavirus.

At the San Siro stadium in Milan, the Atalanta soccer team from Bergamo wins the Champions League match against Valencia 4 to 1 in front of 44,000 fans from Italy (2,000 from Spain). The mass transport from Bergamo to Milan and return, hours of shouting as well as the following festivities in innumerable bars have been considered by some observers as a coronavirus 'biological bomb'.

Thursday, 20 February

A patient in his 30s admitted to the intensive care unit (ICU) in **Codogno** Hospital (Lodi, Lombardy, Italy) tested positive for SARS-CoV-2. Over the next 24 hours, the number of reported cases would increase to 36, without links to the Codogno patient or previously identified positive cases. It is the beginning of the Italian epidemic.
jamanetwork.com/journals/jama/fullarticle/2763188

Sunday, 23 February

Venice Carnival is brought to an early close and sports events are suspended in the most-hit Italian regions.

Monday, 24 February

France, Bahrain, Iraq, Kuwait, Afghanistan and Oman report their first cases.

Tuesday, 25 February

A report of a joint mission of 25 international and Chinese experts is presented to the public. The mission travelled to several different Chinese provinces. The most important findings

are that the Chinese epidemic peaked and plateaued between the 23rd of January and the 2nd of February, and declined steadily thereafter (Table 1).

https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19)

This was the first sign that the **aggressive use of quarantine** ordered by the Chinese government was the **right thing to do**. Unfortunately, European countries which did not experience the SARS epidemic in 2003, would lose precious time before following the Chinese example.

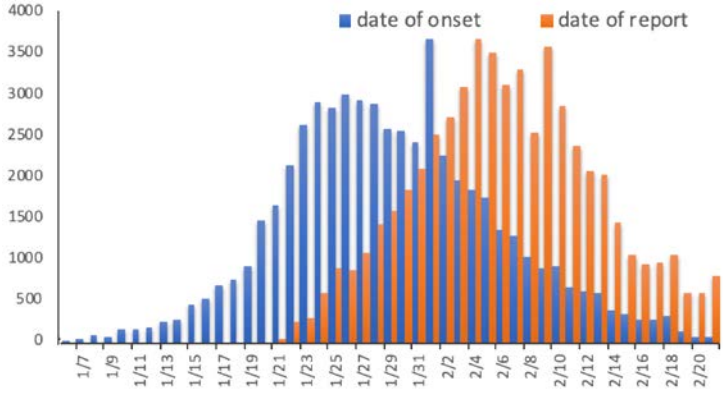

Figure 1. COVID-19 cases in China, January/February 2020. Epidemic curves by symptom onset and date of report on 20 February 2020 for laboratory confirmed COVID-19 cases for all of China. Modified from *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19).* 16-24 February 2020. https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19)

Wednesday, 26 February

A president, fearing for his chances to be re-elected, downplays the threat from the coronavirus pandemic, twittering: "Low Ratings Fake News...are doing everything possible to make the Caronavirus [sic] look as bad as possible, including panicking markets, if possible."

https://www.bmj.com/content/368/bmj.m941

Two days later, the same individual invokes magic: "It's going to disappear. One day, it's like a miracle, it will disappear."

Friday, 28 February

A quick look at European cases diagnosed outside of Italy from February 24-27 reveals that 31 of 54 people (57%) had recently travelled to **Northern Italy**. Epidemiologists immediately realize that an unusual situation is building up and inform the Italian government.

Saturday, 7 March

Official data show that **China's exports** plunged 17.2 percent in the first two months of the year.

Sunday, 8 March

The Italian government led by Prime Minister Giuseppe Conte, deserves credit for instauring the first European lockdown, just two and a half weeks after the first autoctone Italian COVID-19 case was detected. First, strict quarantine measures are imposed on 16 million people in the state of Lombardy and 14 other areas in the north. Two days later, Conte would extend these to the entire country of 60 million people, declaring the Italian territory a "security zone". All people are told to stay at home

unless they need to go out for "valid work or family reasons". Schools are closed.

Monday, 9 March

Iran releases 70,000 prisoners because of the coronavirus outbreak in the country.

Tuesday, 10 March

Xi Jinping tours the city of **Wuhan** and claims a provisional victory in the battle against COVID-19. The last two of 16 temporary hospitals in the city are shut down.

Wednesday, 11 March

WHO declares the coronavirus outbreak a pandemic.

All schools in and around **Madrid**, from kindergartens to universities, are closed for two weeks.

Thursday, 12 March

Italy closes all shops except grocery stores and pharmacies.

In **Spain**, 70,000 people in Igualada (Barcelona region) and three other municipalities are quarantined for at least 14 days. This is the first time Spain adopts measures of isolation for entire municipalities.

Emmanuel Macron, the **French** president, announces the closure of nurseries, schools and universities from Monday, 16 March. He declares: "One principle guides us to define our actions, it guides us from the start to anticipate this crisis and then to manage it for several weeks, and it must continue to do so: it is **confidence in science**. It is to **listen to those who know**." Some of his colleagues should have listened, too.

Friday, 13 March

The prime minister of an **ex-EU country** introduces the notion of 'herd immunity' as a solution to repeated future episodes of coronavirus epidemics. The shock treatment: accepting that 60% of the population will contract the virus, thus developing a collective immunity and avoiding future coronavirus epidemics. The figures are dire. With a little over 66 million inhabitants, some 40 million people would be infected, 4 to 6 million would become seriously ill, and 2 million would require intensive care. Around 400,000 Britons would die. The prime minister projects that "many more families are going to lose loved ones before their time."

Saturday, 14 March

The **Spanish** government puts the whole country into lockdown, telling all people to stay home. Exceptions include buying food or medical supplies, going to hospital, work or other emergencies.

The **French** government announces the closure of all "non-essential" public places (bars, restaurants, cafes, cinemas, nightclubs) after midnight. Only food stores, pharmacies, banks, tobacconists and petrol stations may remain open.

Sunday, 15 March

France calls 47 million voters to the poll. Both government and opposition leaders seem to be in favor of maintaining the municipal elections. Is this a textbook example of unacceptable interference of party politics with the sound management of a deadly epidemic? Future historians will have to investigate.

Monday, 16 March

Ferguson et al. publish a new modelling study on likely UK and US outcomes during the COVID-19 pandemic. In the (unlikely) absence of any control measures or spontaneous changes in individual behaviour, the authors expect a peak in mortality (daily deaths) to occur after approximately 3 months. This would result in 81% of the US population, about 264 million people, contracting the disease. Of those, 2.2 million would die, including 4% to 8% of Americans over age 70. More important, by the second week in April, the demand for critical care beds would be 30 times greater than supply.

The model then analyzes two approaches: mitigation and supression. In the mitigation scenario, SARS-CoV-2 continues to spread at a slow rate so as to avoid a breakdown of hospital systems. In the suppression scenario, extreme social distancing measures and home quarantines would stop the spread of the virus. The study also offers an outlook at the time when strict "Stay at home" measures are lifted. The perspective is grim: the epidemic would bounce back.

France imposes strict confinement measures.

Tuesday, 17 March

Seven million people across the **San Francisco Bay Area** are instructed to "shelter in place" and are prohibited from leaving their homes except for "essential activities" (purchasing food, medicine and other necessities). Most businesses are closed. The exceptions: grocery stores, pharmacies, restaurants (for takeout and delivery only), hospitals, gas stations, banks.

Thursday, 19 March

For the first time since the beginning of the coronavirus outbreak, there have been **no new cases in Wuhan** and in the Hubei province.

Californian Governor Gavin Newsom orders the entire population of **California** (40 million people) to "stay at home". Residents can only leave their homes to meet basic needs like buying food, going to the pharmacy or to the doctor, visiting relatives, exercising.

Friday, 20 March

Italy reports 6,000 new cases and 627 deaths in 24 hours.

In **Spain**, the confinement due to the coronavirus reduces crime by 50%.

China reports no new local coronavirus cases for three consecutive days. Restrictions are eased, **normal life resumes**. The entire world now looks at China. Will the virus spread again?

The state of **New York**, now the center of the U.S. epidemic (population: 20 million), declares a general lockdown. Only essential businesses (grocers, restaurants with takeout or delivery, pharmacies and laundromats) will remain open. Liquor stores? Essential business!

Monday, 23 March

Finally, too late for many observers, the UK puts in place containment measures. They are less strict than those in Italy, Spain and France.

German Chancellor Angela Merkel self-quarantines after coming into contact with a person who tested positive for coronavirus.

Tuesday, 24 March

Off all reported cases in Spain, 12% are among health care workers.

The Tokyo Olympics are postponed until 2021.

India orders a nationwide lockdown. Globally, three billion people are now in lockdown.

Wednesday, 25 March

After weeks of stringent containment measures, Chinese authorities lift travel restrictions in Hubei province. In order to travel, residents will need the "Green Code" provided by a monitoring system that uses the AliPay app.

A 16-year-old girl dies in the south of Paris from COVID-19. The girl had no previous illnesses.

Thursday, 26 March

The US is now the country with most known coronavirus cases in the world.

For fear of reactivating the epidemic, China bans most foreigners from entering the country.

Friday, 27 March

The Prime Minister and the Ministre of Health of an ex-EU country tests positive for coronavirus.

The Lancet publishes *COVID-19 and the NHS—"a national scandal"*.

A paper by McMichael et al. describes a 33% case fatality rate for SARS-CoV-2 infected residents of a long-term care facility in King County, Washington, US.

Sunday, 29 March

The Guardian publishes an article asking if US coronavirus deniers have blood on their hands. The SARS-CoV-2 epidemic is the worst intelligence failure in US history.

Monday, 30 March

Flaxman S et al. from the Imperial College COVID-19 Response Team publish new data on the possibly true number of infected people in **11 European countries**. Their model suggests that as of 28 March, in Italy and Spain, 5.9 million and 7 million people could have been infected, respectively (see Table online). Germany, Austria, Denmark and Norway would have the lowest infection rates (proportion of the population infected). These data suggest that the **mortality of COVID-19 infection** in Italy could be in the range of 0.4% (0.16%-1.2%). Find more details on page 53.

Moscow and **Lagos** (21 million inhabitants) go into lockdown.

The COVID-19 crisis causes some **East European political leaders** to consider legislation giving them extraordinary powers. In one case, a law was passed extending a state of emergency indefinitely.

SARS-CoV-2 is spreading aboard the aircraft carrier USS Theodore Roosevelt. The ship's commanding officer, Captain Brett Crozier, sends an email to three admirals in his chain of command, recommending that he be given permission to evacuate all non-essential sailors, to quarantine known COVID-19 cases, and sanitize the ship. "We are not at war. Sailors do not need to die," writes Crozier in his four-page memo. The letter leaks to the media and generates several headlines. Three days later, 2 April, Captain Crozier is sacked.

Later, testing of 94% of the crew of roughly 4,800 people would reveal around 600 sailors infected, a majority of whom, around 350, were asymptomatic.

Wednesday, 1 April

The United Nations chief warns that the coronavirus pandemic presents the world's "worst crisis" since World War II.

Thursday, 2 April

Worldwide more than one million cases are reported. The true number is probably much higher (see the Flaxman paper on 30 March).

European newspapers run articles about why Germany has so few deaths from COVID-19.

Friday, 3 April

Some economists warn that unemployment could surpass the levels reached during the Great Depression in the 1930s. The good news: almost all governments rate saving tens or hundreds of thousands of lives higher than avoiding a massive economic recession. Has humanity become more human?

Le Monde, the most influential French newspaper, points to a more mundane side effect of the epidemic. As hairdressers are forbidden to work, colors and cuts will degrade. The newspaper predicts that "after two months, 90% of blondes will have disappeared from the face of the Earth".

Saturday, 4 April

In Europe, there are signs of hope. In Italy, the number of people treated in intensive care units decreases for the first time since the beginning of the epidemic.

In France, 6,800 patients are treated in intensive care units. More than 500 of these have been evacuated to hospitals from epidemic hotspots like Alsace and the Greater Paris area to regions with fewer COVID-19 cases. Specially adapted TGV high-speed trains and aircraft have been employed.

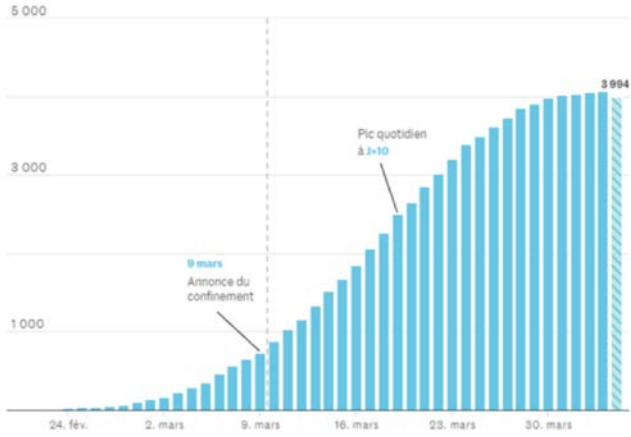

Figure 3. Patients treated in intensive care units in Italy. For the first time since the beginning of the epidemic, the number decreases on 4 April. Souce: Le Monde

Lombardy decides that as of Sunday 5 April, people must wear masks or scarves. Supermarkets must provide gloves and hydroalcoholic gel to their customers.

An Italian politician, less penetrable to scientific reasoning on a par with some of his colleagues in the US and Brazil, asks for churches to be open on Easter (12 April), declaring that "science alone is not enough: the good God is also needed". *Heureux les simples d'esprit*, as the French would say.

Sunday, 5 April

The US surgeon general warns the country that it will face a "Pearl Harbor moment" in the next week.

US is the new epicenter of the COVID-19 epidemic. By the time of this writing (5 April), more than 300,000 cases and almost 10,000 deaths were reported. Almost half were reported from New York and New Jersey.

Tuesday, 7 April

Air quality improves over Italy, the UK and Germany, with falling levels of carbon dioxide and nitrogen dioxide. Will a retrospective analysis of the current lockdown reveal fewer cases of asthma, heart attacks and lung disease?

Wednesday, 8 April

Japan declares a state of emergency, Singapore orders a partial lockdown.

In Wuhan people are allowed to travel for the first time since the city was sealed off 76 days ago.

Thursday, 9 April

EU finance ministers agree to a common emergency plan to limit the impact of the coronavirus pandemic on the European economy. The Eurogroup reaches a deal on a response plan worth more than €500 billion for countries hit hardest by the epidemic.

Passenger air travel has decreased by up to 95%. How many of the 700 airlines will survive the next few months? Will the current interruption of global air travel shape our future travel behaviors?

The epidemic is devastating the US economy. More than 16 million Americans have submitted unemployment claims in the past three weeks.

Friday, 10 April

COVID-19 treatment for one dollar a day? British, American and Australian researchers estimate that it could indeed cost only between 1 and 29 dollars per treatment and per patient. There reasoning (see also our Treatment chapter on page 161): 'Repurposing' existing drugs to treat COVID-19 is vital to reducing mortality and controlling the pandemic. Several promising drugs have been identified and are in various stages of clinical trials globally. Among these are remdesivir, an antiviral tested unsuccessfully against Ebola, the lopinavir/ritonavir treatment for HIV and hydroxychloroquine. If the effectiveness of a treatment is demonstrated, "rapid, mass availability at an affordable cost would be essential to ensuring equity and access especially amongst low- and middle-income economies", write the authors.

The results in detail: Minimum estimated costs of production were US $0.93/day for remdesivir, $1.45/day for favipiravir, $0.08/day for hydroxychloroquine, $0.02/day for chloroquine, $0.10/day for azithromycin, $0.28/day for lopinavir/ritonavir, $0.39/day for sofosbuvir/daclatasvir and $1.09/day for pirfenidone. Costs of production ranged between $0.30 and $31 per treatment course (10–28 days).

Hill A, Wang J, Levi J, Heath K, Fortunatk J. **Minimum costs to manufacture new treatments for COVID-19.** J Virus Erad 2020. Full-text: http://viruseradication.com/journal-details/Minimum_costs_to_manufacture_new_treatments_for_COVID-19/

Message from your mobile phone: “You have been in contact with someone positive for coronavirus.” Google and Apple announce that they are **building a coronavirus tracking system into iOS and Android**. The joint effort would enable the use of Bluetooth technology to establish a voluntary contact-tracing network. Official apps from public health authorities would get extensive access to data kept on phones that have been in close proximity with each other (George Orwell is turning over in his grave). If users report that they’ve been diagnosed with COVID-19, the system would alert people if they were in close contact with the infected person.

Spain discovers *COVID Reference*. Within 24 hours, more than 15,000 people download the PDF of the Spanish edition. The only explanation: a huge media platform displayed the link of our book. Does anyone know who did it?

Figure 4. Google Analytics data for www.CovidReference.com on 10 April. At one moment, more than 500 people, mostly from Spain, were visiting the website simultaneously.

Saturday, 11 April

More than **400 of 700 long-term care facilities** (*EHPAD* in French, ***E**tablissement d'**H**ébergement pour **P**ersonnes **A**gées **D**épendantes*) in the greater Paris region (pop. – 10 million) have COVID-19 cases.

In Italy, 110 doctors and about 30 other hospital workers have died from COVID-19, half of them nurses.

Sunday, 12 April

Easter 2020. Italy reports 361 new deaths, the lowest number in 25 days while Spain reports 603 deaths, down more than 30% from a high 10 days before.

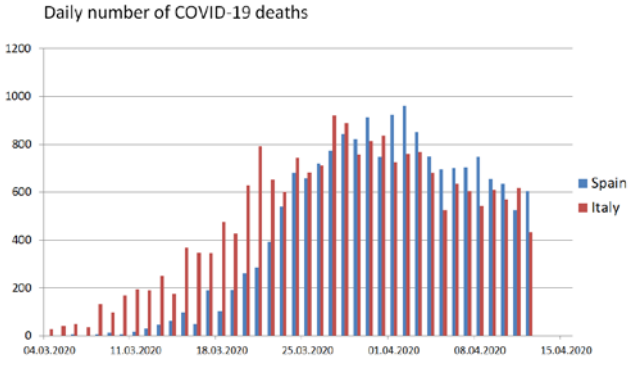

Figure 5. Daily number of COVID-19 deaths in Italy (red) and Spain (blue).

The United Kingdom records its highest daily death toll of almost 1,000. The number of reported COVID-19-linked fatalities now exceeds 10,000. As in many other countries, the true numbers may be slightly higher due to underreporting of people dying in care homes.

The number of COVID-19-related deaths in the United States passes 22,000, while the number of cases tops 500,000. In New York there are signs that the pandemic could be nearing its peak.

Monday, 13 April

The COVID-19 pandemic exposes **bad governance**, not only in Brazil. The French newspaper *Le Monde* reveals the ingredients: denial of reality, search for a scapegoat, omnipresence in the media, eviction of discordant voices, political approach, isolationism and short-term vision in the face of the greatest health challenge in recent decades. The culprit?

Emmanuel Macron announces announces a **month-long extension to France's lockdown**. Only on Monday, May 11, nurseries, primary and high schools would gradually reopen, but not higher education. Cafés, restaurants, hotels, cinemas and other leisure activities would continue to remain closed after May 11.

Tuesday, 14 April

Austria is the first European country to **relax lockdown measures**. It opens up car and bicycle workshops, car washes, shops for building materials, iron and wood, DIY and garden centers (regardless of size) as well as smaller dealers with a customer area under 400 square meters. These shops must ensure that there is only one customer per 20 square meters. In Vienna alone, 4,600 shops are allowed to open today. Opening times are limited to 7.40 a.m. to 7 p.m. The road-map for the coming weeks and months:

- 1 May: All stores, shopping malls and hairdressers reopen (see also the April 3 entry, page 32).
- 15 May: Other services such as restaurants and hotels remain closed at least until mid-May.
- 15 May or later: Possible re-opening of classes in schools.
- July: possible – but improbable – organization of events of all sorts (sport, music, theater, cinema etc.).

There is a general obligation to wear a mask when shopping and on public transport.

The International Monetary Fund (IMF) forecasts a **contraction of 3% of the planet's GDP in 2020**. The possibility of an even more brutal fall in 2021 is not excluded. The possibly worst economic downturn since the Great Depression in 1929 will not spare any continent. In a recession like no other in peacetime for nearly a century, the countries of the euro zone, the United Kingdom and the United States might see a contraction in activity of between 5.9% and 7.5%. China's economy is expected to grow by about 1%.

US: The CDC (Centers for Disease Control and Prevention) reports that more than 9,000 health care workers contracted COVID-19 as and at least 27 died. The median age was 42 years, and 73% were female. Deaths most frequently occurred in HCP aged ≥65 years.

Wednesday, 15 April

Philip Anfinrud and Valentyn Stadnytsky from the National Institutes of Health, Bethesda, report a laser light-scattering experiment in which speech-generated droplets and their trajectories were visualized. They find that when a test person says, "stay healthy," numerous droplets ranging from 20 to 500 µm

are generated. When the same phrase is uttered three times through a slightly damp washcloth over the speaker's mouth, the flash (droplet) count remains close to the background level. The video supports the recommendation of wearing face masks in public. The authors also found that the number of flashes (droplets) increased with the loudness of speech. The new message for billions of people caught in the COVID-19 epidemic: lower your voice!

Anfinrud P, Stadnytskyi V, Bax CE, Bax A. **Visualizing Speech-Generated Oral Fluid Droplets with Laser Light Scattering.** N Engl J Med. 2020 Apr 15. PubMed: https://pubmed.gov/32294341. Full-text: https://doi.org/10.1056/NEJMc2007800

Friday, 17 April

Luiz Inácio Lula da Silva, the former Brazilian president says that the current president is leading Brazil to "the slaughterhouse" with his irresponsible handling of coronavirus. In an interview with The Guardian, Lula says that Brazil's "troglodyte" leader risks repeating the devastating scenes playing out in Ecuador where families have to dump their loved ones' corpses in the streets.

On the **French aircraft carrier Charles-de-Gaulle**, a massive epidemic was confirmed on 17 April. Among the 1760 sailors, 1,046 (59%) were positive for SARS-CoV-2, 500 (28%) presented symptoms, 24 (1.3%) sailors were hospitalized, 8 on oxygen therapy and one in intensive care.

Saturday, 18 April

Care England, Britain's largest representative body for care homes, suggests that up to 7,500 residents may have died of COVID-19. This would be higher than the 1,400 deaths estimated by the government.

In Italy, 131 doctors have died from COVID.

In Catalunya alone, some 6,615 hospital professionals and another 5,934 in old age care homes are also suspected of having or been diagnosed with COVID-19.

Sunday, 19 April

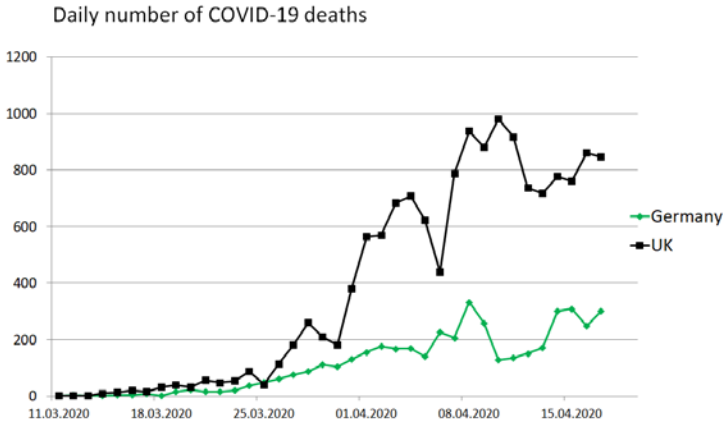

Figure 7. Daily number of COVID-19 deaths in Germany (green) and the United Kingdom (black).

Monday, 20 April

For the first time in history, the West Texas Intermediate (WTI), the benchmark price for US oil, drops below $0. On certain specific contracts, it plunged down to minus 37 dollars (-34 euros). After nearly two months of continuous collapse of the oil market, this paradoxical situation is the result of the COVID-19 pandemic which caused demand to fall by 30%. As oil wells continue to produce, there is no place to store the oil and investors are ready to pay to get rid of it.

Source: Pixabay

Germany's Oktoberfest is cancelled. The iconic beer festival, colloquially known as *Die Wiesn* or "the meadow", attracts around 6 million visitors from around the world. It runs for more than two weeks (September/October) in packed tents with long wooden tables, where people celebrate traditional food, dancing, beer and clothing. The loss for the city of Munich is estimated to be around one billion euros.

2. Epidemiology

Bernd Sebastian Kamps

In December 2019, several patients from Wuhan, People's Republic of China, developed pneumonia and respiratory failure reminiscent of the SARS epidemic in 2003 (WMHC 2019, www.SARSReference.com). In early January 2020, a new virus was grown from bronchoalveolar lavage fluid samples and found to be a betacoronavirus (Zhou 2020). Between then and the time of this writing (19 April), the virus has spread to every corner of the world. More than 2.3 million have been diagnosed and > 160,000 people have died.

In this chapter, we will discuss

- the transmission routes of SARS-CoV-2;
- the natural COVID-19 epidemic and Epidemic 2.0;
- lockdown and measuring its effects;
- the characteristics of the epidemic in selected places;
- lockdown exit;
- 'COVID pass';
- a second epidemic wave.

Transmission

Person-to-person spread

Transmission of coronaviruses is airborne, fecal-oral or through fomites. (A fomite is any inanimate object that, when contaminated with or exposed to infectious agents such as a virus, can transfer a disease to another person, for example

elevator buttons, restroom taps, etc.) (Cai 2020). It is assumed that SARS-CoV-2 is spread mainly through person-to-person contact via respiratory droplets generated by coughing and sneezing. Whether and to what extent other transmission routes are epidemiologically relevant is unclear.

Human-to-human transmission of SARS-CoV-2 was proved within weeks (Chan 2020, Rothe 2020). It is unknown if symptom severity is a proxy for infectivity. Even asymptomatic individuals can transmit the virus and a substantial proportion of secondary transmission is believed to occur prior to onset of illness (Nishiura 2020). However, in one case report, there was no evidence of transmission to 16 close contacts, among them 10 high-risk contacts, from a patient with mild illness and positive tests for up to 18 days after diagnosis (Scott 2020).

The SARS-CoV-2 virus is highly contagious, with a basic reproduction number R of around 2.5 (Chan 2020, Tang B 2020, Zhao 2020). [R indicates the average number of infections one case can generate over the course of the infectious period in a naïve, uninfected population.]

The mean incubation is around 5 days (Li 2020, Lauer 2020). The serial interval of COVID-19 – defined as the duration of time between a primary case-patient having symptom onset and a secondary case-patient having symptom onset – has been estimated to be between 5 and 7.5 days (Cereda 2020).

The issue of fomites is still a topic of public anxiety. One study (van Doremalen 2020) showed that the virus can be detectable as an aerosol (in the air) for up to three hours, up to four hours on copper, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel. Hence the imperative advice for regular and thorough handwashing.

Transmissibility of SARS-CoV-2 appears not to be reduced in warm and humid conditions (Luo 2020). However, one study suggests that high temperature and high relative humidity might reduce the transmission of COVID-19 (Wang 2020). It is still unclear if and to which extent the epidemic might temporarily slow down in Europe and North America during the 2020 summer.

Nosocomial spread

Hospitals seem to be a favorable environment for the propagation of the SARS-CoV-2 virus. In some instances, hospitals might be the main COVID-19 carriers, as they are rapidly populated by infected patients, facilitating transmission to uninfected patients (Nacoti 2020). Within the first 6 weeks of the epidemic in China, 1,716 cases among health care workers were confirmed by nucleic acid testing, and at least 5 died (0.3%) (Wu 2020). One study reports that the virus was widely distributed in the air and on object surfaces in both the intensive care units and general wards, implying a potentially high infection risk for medical staff. Contamination was greater in ICUs. Virus was found on floors, computer mice, trash cans, sickbed handrails and was detected in air approximately 4 m from patients (Guo 2020). The virus has also been isolated from toilet bowl and sink samples, suggesting that viral shedding in stool could be a potential route of transmission (Young 2020, Tang 2020). However, most of these studies have evaluated only viral RNA. It remains to be seen whether this translates into infective virus.

Although nosocomial spread of the virus is well documented, appropriate hospital infection control measures can prevent nosocomial transmission of SARS-CoV-2 (Chen 2020). This was nicely demonstrated by the case of a person in her 60s who

travelled to Wuhan on Dec 25, 2019, returned to Illinois on Jan 13, 2020, and transmitted SARS-CoV-2 to her husband. Although both were hospitalized in the same facility and shared hundreds (n=348) of contacts with HCWs, nobody else became infected (Ghinai 2020). However, working in a high-risk department, longer duty hours, and suboptimal hand hygiene after coming into contact with patients, were all associated with an increased risk of infection in health care workers (Ran 2020). At one time during the early epidemic in March 2020, around half of 200 cases in Sardinia were among hospital and other health care workers.

At the end of March, medical personnel represented 12% and 8% of reported Spanish and Italian infections, respectively. Whether there should be universal masking in hospitals is still being debated. The main value could be in giving health care workers the confidence to absorb and implement prevention practices (Klompas 2020).

As of 18 April, 130 doctors had died in Italy (roughly half of them family doctors), 23 in Spain and a yet unknown number in France.

Transfusion

After screening of 2,430 donations in real-time (1,656 platelet and 774 whole blood), authors from Wuhan found plasma samples positive for viral RNA from 4 asymptomatic donors (Chang 2020). It remains unclear whether detectable RNA signifies infectivity.

In a Korean study, seven asymptomatic blood donors were later identified as COVID-19 cases. None of 9 recipients of platelets or red blood cell transfusions tested positive for SARS-CoV-2 RNA

(Kwon 2020). More data are needed before we can conclude that transmission through transfusion is unlikely.

Long-term care facilities

Long-term care facilities are high-risk settings for infectious respiratory diseases. In a skilled nursing facility in King County, Washington, US, 167 cases of COVID-19 were diagnosed within less than three weeks after the identification of the first case: 101 residents, 50 health care personnel and 16 visitors (McMichael 2020) (Table 1).

Among residents (median age: 83 years), the case fatality was 33.7%. Chronic underling conditions included hypertension, cardiac disease, renal disease, diabetes mellitus, obesity and pulmonary disease. The study demonstrates that once introduced in a long-term care facility, SARS-CoV-has the potential to spread rapidly and widely.

Table 1. COVID outbreak in a long-term care facility

	Residents (N = 101)	Healthcare personnel (N = 50)	Visitors (N = 16)
Median age (range)	83 (51-100)	43.5 (21-79)	62.5 (52-88)
Female (%)	68.3	76	31.2
Hospitalized (%)	54.5	6.0	50.0
Died (%)	33.7	0	6.2
Chronic underlying conditions (%)			
Hypertension	67.3	8.0	12.5
Cardiac disease	60.4	8.0	18.8
Renal disease	40.6	0	12.5
Diabetes mellitus	31.7	10.0	6.2
Obesity	30.7	6.0	18.8
Pulmonary disease	31.7	4.0	12.5

Cruise ships and aircraft carriers

Cruise ships carry a large number of people in confined spaces. On 3 February 2020, 10 cases of COVID-19 were reported on the Diamond Princess cruise ship. Within 24 hours, ill passengers were isolated and removed from the ship and the rest of the passengers quarantined on board. Over time, more than 700 of 3,700 passengers and crew tested positive (~20%). One study suggested that without any interventions 2,920 individuals out of the 3,700 (79%) would have been infected (Rocklov 2020). The study also showed that an early evacuation of all passengers on 3 February would have been associated with only 76 infected. Today, all cruise ships are idle in ports around the world and face an uncertain future. Shipping village-loads of people from one place to another may not be a viable business model for years to come.

Large navy vessels seem equally prone to large outbreaks. During an epidemic on the aircraft carrier USS Theodore Roosevelt in late March, around 600 sailors out of a crew of 4,800 were infected with SARS-CoV-2 (see also the March 30 entry of the Timeline); around 60% remained asymptomatic. One active duty sailor has died as of 17 April (USNI News). On the French aircraft carrier Charles-de-Gaulle, a massive epidemic was confirmed on 17 April. Among the 1,760 sailors, 1,046 (59%) were positive for SARS-CoV-2, 500 (28%) presented symptoms, 24 (1.3%) sailors were hospitalized, 8 on oxygen therapy and one in intensive care.

Transmission hotspots during lockdown

It seems that under strict lockdown conditions (with the population confined to their homes and allowed only to go to work and do essential shopping), transmission continues mainly in places where people are crowded and/or working closely together:

- Hospitals
- Long-term care facilities
- Prisons
- Aircraft carriers and other military vessels

The pandemic

Natural Pandemic

The COVID-19 epidemic started in Wuhan, in Hubei province, China, and spread within 30 days from Hubei to the rest of mainland China, to neighboring countries (in particular, South Korea, Hong Kong and Singapore) and west to Iran, Europe and the American continent. The first huge outbreaks occurred in regions with cold winters (Wuhan, Iran, Northern Italy, Alsace region in France).

A hundred or even 50 years ago, the COVID-19 pandemic would have followed its natural course. With a mortality rate of around 0.5%, COVID-19 would have resulted globally in 7.0 billion infections and 40 million deaths during the first year (Patrick 2020). The peak in mortality (daily deaths) would have been observed approximately 3 months after the beginning of local epidemics. One model predicted that 80% of the US population (around 260 million people) would have contracted the disease. Of those, 2.2 million would have died, including 4% to 8% of Americans over age 70 (Ferguson 2020).

Some politicians seriously considered such a Pandemic 1.0 plot, speculating on the advantages of "letting-the-virus-loose":

- The country would avoid the dramatic economic downturn that seems unavoidable in countries and states which opted for strict containment measures (Italy, Spain, France, California, New York, to name a few).
- After three months, 70% of the population would be immunized against further outbreaks (through infection with SARS-CoV-2) and would be able to look ahead to the next winter season with an even temper. (How long would such acquired immunity last? Maybe only a few years. See the *Immunology* chapter, page 89).

In mid-March 2020, the prime minister of an ex-EU country thus introduced the notion of 'herd immunity' as a solution to the epidemic his nation was about to face. The shock treatment: accepting that a large majority of the population would contract the virus, thus developing a collective immunity and avoiding coronavirus epidemics in the immediate future. The figures were dire. With a little over 66 million inhabitants, some 40 million people would have been infected, 4 to 6 million would have become seriously ill, and 2 million would have required intensive care. Around 400,000 Britons would have died. The prime minister forecast: "Many more families are going to lose loved ones before their time."

Pandemic 2.0: Lockdown

Fortunately, for now, the world has been saved from a freely circulating SARS-CoV-2. After all, humanity can change climate, so why shouldn't it be able to change the course of a pandemic? Although economists warned that unemployment could surpass the levels reached during the Great Depression in the 1930s, almost all governments rated saving hundreds of thousands lives higher than avoiding a massive economic recession. First in

China, six weeks later in Italy and still another week later in most Western European countries, an unprecedented experiment of gigantic dimensions was started: ordering entire nations to lockdown. In Italy and Spain, people were ordered to stay home, except for "essential activities" (purchasing food, medicine and other necessities) and going to hospital or work. Italians were told to stay at home even on the popular *Pasquetta* day, Little Easter, where people usually flock to the countryside to enjoy a picnic with family and friends. Italians were not even allowed to move from one village to another.

Lockdown results

The result of lockdown measures can be measured by the number of

- SARS-CoV-2-infected people
- Hospital admissions
- Patients treated in intensive care units (ICU)
- Deaths

Number of infections

The daily communication of newly diagnosed SARS-CoV-2-infected people has become a ritual in most countries. These figures are indeed an indicator for the evolution of a national epidemic and the effects of lockdown measures.

However, these data do *not reflect the true number of infections*. To know the true number, the entire population would need to be tested which is of course impractical. Best estimates can only be made by mathematical modelling. Surprisingly, the first accurate models of the European epidemic revealed that reported COVID-19 cases represent only a fraction of those truly infected. A model based on observed deaths in 11 European countries

suggested that true infections were much higher than reported cases (Flaxman 2020). According to the model, as of 28 March, in Italy and Spain, 5.9 million and 7 million people could have been SARS-CoV-2-infected, respectively (Table 2). Germany, Austria, Denmark and Norway would have the lowest attack rates (proportion of the population infected). If these assumptions are validated, the true number of cases would outnumber the reported cases on March 28 (Italy: 92,472; Spain: 73,235; France: 37,575) by up to two orders of magnitude.

[The data provided by Flaxman et al. immediately invite one to do some *kitchen epidemiology*. First: if on 28 March the number of infected people in Italy was around 6 million (with a credible interval of 2 to 15 million) and if we assume that 18 days later the total number of deaths in Italy was around 30,000 (the official figure reported on 15 April was 21,645 deaths), the mortality of COVID-19 infection in Italy could be in the range of 0.5% (0.19%-1.6%).

Second: if at the end of March, around 60% of all deaths in Italy were reported from Lombardy, 60% of the 6 million projected Italian SARS-CoV-2 infections – 3.6 million – would have occurred in a region with a population of 10 million. Moreover, 20% of all deaths in Italy were reported from the province of Bergamo alone which has a population of one 1.1 million. Seroprevalence studies will sort out these figures soon.]

Table 2. Estimates of total population infected as of 28 March 2020

Country *Deaths on 28 March*	% of population infected*	Population infected*
Austria *68*	1.1% (0.36%-3.1%)	96,800 (31,680-272,800)
Belgium *353*	3.7% (1.3%-9.7%)	425,500 (149,500-1,115,500)
Denmark *65*	1.1% (0.40%-3.1%)	63,800 (23,200-179,800)
France *2,314*	3.0% (1.1%-7.4%)	2,010,000 (737,000-4,958,000)
Germany *433*	0.2% (0.28%-1.8%)	166,000 (232,400-1,494,000)
Italy *10,023*	9.8% (3.2%-26%)	5,919,200 (1,932,800-15,704,000)
Norway *23*	0.41% (0.09%-1.2%)	21,600 (4,860-64,800)
Spain *5,982*	15% (3.7%-41%)	7,035,000 (1,735,300-19,229,000)
Sweden *105*	3.1% (0.85%-8.4%)	316,200 (86,700-856,800)
Switzerland *264*	3.2% (1.3%-7.6%)	275,200 (111,800-653,600)
UK *1,019*	2.7% (1.2%-5.4%)	1,798,200 (799,200-3,596,400)

*mean (95% credible interval)

Data source: Flaxman S et al**.** (Imperial College COVID-19 Response Team). Report 13: Estimating the number of infections and the impact of non-pharmaceutical interventions on COVID-19 in 11 European countries. 30 March 2020. DOI: https://doi.org/10.25561/77731

Admissions into intensive care units

A reliable indicator of the epidemic trend is the number of people treated in intensive care units. In France, the number of new hospital ICU admissions peaked on 1 April (Figure 1), while the daily variation in people treated in ICU (the balance between ICU

entries and exits; Figure 2) started being negative one week later.

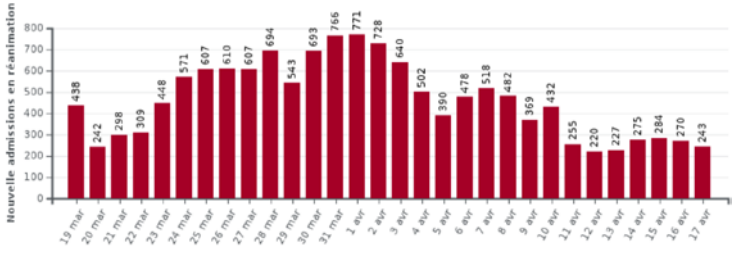

Figure 1. Daily number of new hospital ICU admissions for COVID-19 (y-axis: *Nouvelles admissions en réanimation*).
Source: Pandémie de Covid-19 en France, Wikipedia.

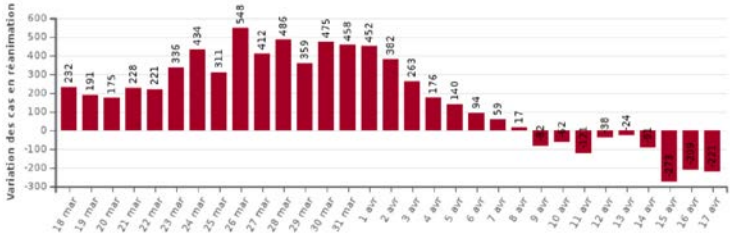

Figure 2. Daily variation in the number of people in ICU for COVID-19 (y-axis: *Variation des cas en réanimation*).
Source: Pandémie de Covid-19 en France, Wikipedia.

Deaths

Asymptomatic infections go unnoticed. Even mild to moderate symptoms may go unnoticed. Deaths do not. Consequently, deaths reflect the reality of the COVID-19 epidemic better than the number of SARS-CoV-2-infected people. Figures 3 and 4 show

the number of deaths in Italy and Spain from 4 March through 19 April.

However, these numbers are incomplete and will soon be corrected upwards. (By 10%, 30%, 50% or more? Nobody knows yet.) In Italy, especially in the most hit Northern regions, a certain number of people died at home and did not appear in the official statistics. In Spain, many municipalities noted an excess mortality not reflected in the national figures. In France, as in other countries, deaths from long-term care facilities were initially not included.

Figure 3 shows that the number of daily deaths decreases about three weeks after the implementation of lockdown measures (Italy: 8/10 March; Spain: 14 March).

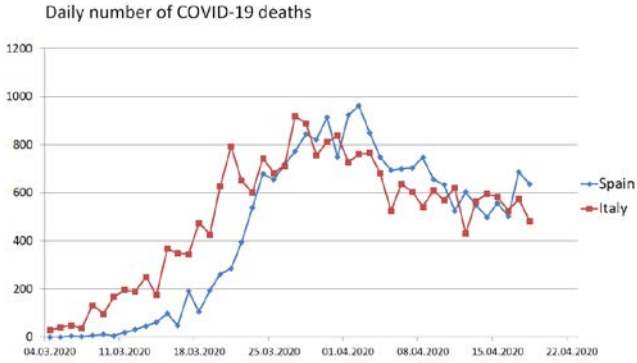

Figure 3. Coronavirus deaths in Italy and Spain from 4 March through 19 April. Source: worldometers.info, Johns Hopkins CSSE

Countries and continents

On 23 January, China ordered the first massive lockdown in history. European countries followed 6 weeks later. Astonishingly, almost no European country was really prepared for the COVID-19 epidemic although everyone could observe the events in China for more than a month. When European countries finally ordered lockdown measures, these were not as strict and swiftly imposed as in China. In some countries, lockdown was powered up over several days (Italy), while in other countries, extended subway systems continued to work and people were joyfully jogging on the streets in large numbers (Paris, France). From the very beginning, it was therefore clear that the European epidemic would need a few days or weeks more than in China to see infection and death figures decline. The following paragraphs summarize distinctive features of some local epidemics.

China

The nationwide spread to all provinces in January 2020 was favored by travelers departing from Wuhan before the Chinese Spring Festival (Zhong 2020).

Starting on 23 January, China imposed a lockdown of the population of Wuhan and later of the entire Hubei province. This astonishing first in human history achieved what even specialists didn't dare dream: curbing an epidemic caused by a highly contagious virus (Lau 2020). This recipe of stringent confinement of people in high-risk areas, is now being re-combined by nations around the world, everyone adding some more or some less efficient ingredients.

Figure 6 proved as early as four weeks after the Wuhan lockdown that strict containment measures are capable of curbing a SARS-

CoV-2 epidemic. The figure presents the Chinese COVID-19 epidemic curves of laboratory-confirmed cases, by symptom onset (blue) and – separately – by date of report (orange). The data were compiled on 20 February 2020, four weeks after the beginning of the containment measures which included a lockdown on nearly 60 million people in Hubei province as well as travel restrictions for hundreds of millions of Chinese citizens. The blue columns show that (1) the epidemic rapidly grew from 10-22 January, (2) reported cases (by date of onset) peaked and plateaued between 23 January and 28 January and (3) steadily declined thereafter (apart from a spike reported on 1 February). Based on these data, we could expect a decline in reported cases around three weeks after the implementation of strict containment measures.

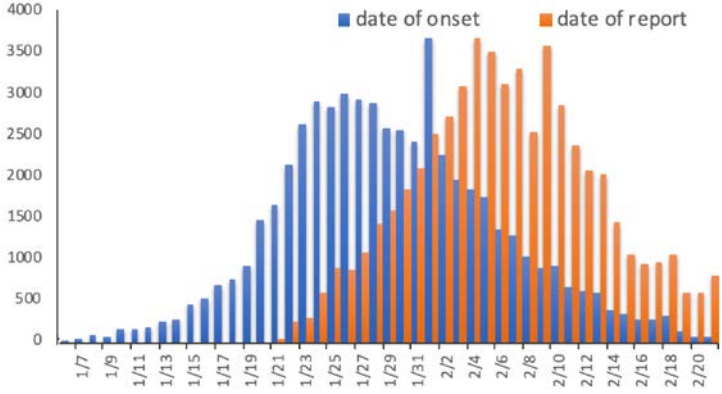

Figure 6. The Chinese outbreak in January/February 2020. Epidemic curves by symptom onset and date of report on 20 February 2020 for laboratory confirmed COVID-19 cases for all of China. Modified from *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19).* 16-24 February 2020. https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19)

Three months after the beginning of the epidemic, Chinese authorities started lifting travel restrictions, slowly restoring life to normal even in the most hard-hit provinces.

In a study on cases reported through 11 February, among 44,672 confirmed cases, most were aged 30-79 years (86.6%), diagnosed in Hubei (74.7%), and considered mild (80.9%) (Wu 2020). A total of 1,023 deaths occurred among confirmed cases for an overall case-fatality rate of 2.3%.

Models have estimated how quarantine and movement restrictions determined the outcome of the first Chinese epidemic. According to one model, without the Wuhan travel ban, there would have been 744,000 cases by February 19, day 50 of the epidemic (Tian 2020). With the Wuhan travel ban alone, the number of cases would have decreased to 202,000.

Lombardy and Italy

Italy was the first European country struck by the pandemic. Complete genome analysis of SARS-CoV-2 isolates suggests that the virus was introduced on multiple occasions (Giovanetti 2020). Although the first local case was diagnosed only on 20 January, the force of the outbreak also suggests that the virus had been circulating for weeks, possibly as early as 1 January (Cereda 2020). People from Milan remember discussing unusual frequent occurrence of pneumonia as early as mid-January (Dario Barone, personal communication).

It is as yet unclear why the epidemic has taken such a dramatic turn in the northern part of Italy, especially in Lombardy, while other areas, especially the southern provinces, are relative spared. One super-spreader event may have been the Champions League soccer match between Atalanta (Bergamo and Valencia) on 19 February at the San Siro stadium in Milan. Forty-four

thousand fans from Italy and Spain witnessed the 4-to-1 win of the Italian team. The mass transport from Bergamo to Milan and back, hours of shouting as well as the following festivities in innumerable bars have been considered by some observers as a coronavirus 'biological bomb'. Support for this assumption comes from a recent study that visualized speech-generated oral fluid droplets with laser light scattering (Anfinrud 2020). The study found that aerosols and droplets increased with the loudness of speech. Loud and persistent shouting as would be usual during a 4-to-1 qualification for the Champions League quarter-final can be assumed to produce the same number of droplets produced by coughing (Chao 2020).

How could the beginning of such an important epidemic be missed? The signs on the wall were there, but deciphering them was not straightforward. During the yearly flu season, COVID-19 deaths in elderly people could easily be interpreted as flu deaths. On the other end of the age spectrum, among the most active social age group – young people crowded in bars, restaurants and discos –, the rapidly SARS-CoV-2 virus would not have caused life-threatening symptoms. Before exploding, the epidemic had time (at least one month) to grow.

Spain

Spain is currently the European country with the highest number of reported and projected cases (Flaxman 2020). The region hardest hit by the epidemic is the Community of Madrid, accumulating 28% of the confirmed cases as of mid-April.

Fortunately, the Mobile World Congress in Barcelona, the world's largest technology congress scheduled for 24-27 February, was canceled two weeks before although health authorities insisted that there was no risk. The decision was made after some of the largest technology companies (among others LG,

Facebook, Sony and Vodafone) suspended their participation for fear of contagion on a large scale from those attending. This was the first blow to the Spanish tourist industry.

On March 14, the Spanish Government decreed a "state of alarm" for fifteen days, extending it later 26 April. It is now extended to May 9, although children under 12 will be able to "circulate" as of 27 April. Free movement of citizens is limited to acquisition of food and medications or going to medical centers or the work-place (as of 20 April, approx. 20% of the workforce is going to work). Masks and gloves are now given to anyone entering the metro, and will be reimbursed by the health authorities from 22 April.

France

The epidemic in France demonstrated the importance of the single most important health care figure in the COVID-19 epidemic: the number of beds available in intensive care units, equipped with respirators and fully operated by specialist staff. The first national outbreak was in the Eastern region of Mulhouse, Alsace, near the Swiss and German border, where a super-spreader event disseminated SARS-CoV-2 among the attendants of a religious meeting from 17 to 24 February. Three weeks later, patients started filling local hospitals, swiftly outstretching the capacities. Patients in serious conditions were flown out across the borders to Germany, Switzerland and Luxembourg. Then, on the weekend of 21 March, virtually from one day to another, patients were pouring into the hospitals of the Greater Paris Region where the number of available intensive care unit beds had been increased from 1,400 to 2,000 during the preceding week. At the height of the epidemic, more than 500 patients were evacuated from epidemic hotspots like Alsace and the Greater Paris area to regions with fewer COVID-19

cases. Specially adapted TGV high-speed trains and aircraft were employed, transporting patients as far away as Brittany and the Bordeaux area in the South-West, 600 km from Paris and 1000 km from Mulhouse. The French management of ICU beds was a huge logistical success.

UK

In the UK (as in some other places like Brazil and the US), clumsy political maneuvering and/or denial of the COVID-19 reality delayed the start of effective lockdown measures by a week or more. With the epidemic doubling in size about every 7 days (Li 2020), around 50% and 75% of all deaths might have been prevented with lockdown or social distancing ordered one or two weeks earlier, respectively. Preliminary data from Ireland and the United Kingdom seem to confirm this assumption. History will remember.

Germany's low fatality rate

German's fatality rate seems to be lower than in other countries. As of 11 April, the country reported 2,736 deaths for 122,171 cases (case fatality ration [CFR]: 1.9%). This is in stark contrast with Italy (18,849 deaths, 147,577 cases; CFR: 12.8%), Spain (13,197 deaths, 124,869 cases; CFR: 10.6) and the UK (8,958 deaths, 73,758 cases; CFR: 12.1%). It is assumed that the main reason for this difference is simply testing. While other countries were conducting a limited number of tests of older patients with severe cases of the virus, Germany was doing many more tests that included milder cases in younger people (Stafford 2020). The more people with no or mild symptoms you test, the lower the fatality rate. Reliable PCR methods have been reported by the end of January (Corman 2020).

Furthermore, in Germany's public health system, SARS-CoV-2 testing is not restricted to a central laboratory as in many other nations but can be conducted at quality-controlled laboratories throughout the country. Within a few weeks, overall capacity reached half a million PCR tests a week. The same low fatality rate is seen in South Korea, another country with high testing rates.

As lockdown measures were less strict in Germany – people were told to stay at home but could move more freely than in Italy and Spain – the coming weeks will show the efficacy of this approach. Another important reason for the low mortality in Germany is the age distribution. During the first weeks of the epidemic, most people became infected during carnival sessions or ski holidays. The majority were younger than 50 years of age. Mortality in this age group is markedly lower than in older people.

North America

Like in Iran, where the regime covered up news of the coronavirus for three days to avoid impacting turnout at parliamentary elections on 21 February, domestic politics (i.e., the fear that economic disruption could harm re-election chances; see the British Medical Journal, 6 March 2020) influenced the epidemic response in the US. As of this writing (19 April), more than 700,000 cases and 40,000 deaths had been reported, almost half being from New York and New Jersey. The total number of deaths of the first COVID-19 wave might reach 60,000, at least half of which could have been prevented (see the UK entry, page 61). Because of an unprecedented vacuum in leadership, the US is now the epicenter of the COVID-19 epidemic.

Africa and South America

New cases are reported from around the world, but the figures are still comparatively low in Africa and South America. One study estimated the risk of transmission of the SARS-CoV-2 through human passenger air flight from four major cities of China (Wuhan, Beijing, Shanghai and Guangzhou) (Haider 2020). From 1-31 January, 388,287 passengers were destined for 1,297 airports in 168 countries or territories across the world. In January, the risk of transmission of the virus to Africa and South America seemed to be low.

On 19 April, Africa, South Africa, Egypt, Algeria and Morocco reported between 2,500 and 3,000 cases each. Algeria had the highest number of deaths (367), many of which can be traced back to citizens living or coming back from France. Such high numbers suggest that the number of infected people in Algeria could be substantially higher than the 2,500 cases officially reported.

In South America, Brazil is on track for a major epidemic fostered by bad governance. Ecuador, a country of 17 million, has the highest death toll relative to the size of its population.

Australia and New Zealand

In Australia, the total number of new cases grew exponentially after the confirmation of the first case on 25 January, levelled out around 22 March, and started falling at the beginning of April. As of 19 April, 6,606 cases had been reported, almost 50% of which from New South Wales.

New Zealand reported the first COVID-19 case on 28 February. On 26 March, the government implemented a nationwide lockdown where citizens could only leave their homes for activities such as accessing essential services. Close contact was only allowed with

persons from the same household. With a population of 5 million, the country had 1,431 cases on 19 April. Twelve people died.

Lockdown exit

Over the coming weeks, countries who ordered lockdown will have to put in place a balanced lockdown exit - normalizing and restoring societal activities - while at the same time minimizing the risk of setting off a second cataclysmic wave of contagion. (Normile 2020). The International Monetary Fund (IMF) forecast a contraction of 3% of the planet's GDP in 2020. In a recession like no other in peacetime for nearly a century, the countries of the euro zone, the United States and the United Kingdom might see a contraction in activity of between 5.9% and 7.5%. Economically, protracted lockdown is unsustainable. What can be done once – the month-long isolation of the entire population – can probably not be repeated.

Countries will have to decide which activities to open in which order, fix a timetable, consider if some regions shall exit lockdown earlier than others and decide which activities would be shut down for 6 months or more, possibly until the general availability of a vaccine:

1. Minimize transmission
    - All mass gatherings will probably have to be banned, including sport events, festivals and the re-opening of cinemas, discos and bars. To be effective, some countries may extend some of these bans until a vaccine is available to all.
    - Postpone partly the opening of universities courses where teaching can be organized as online education.
    - Wearing face masks in public (Anfinrud 2020).

2. Maximize economic activity (while guaranteeing social distancing)
    - Young adults need to be able to return to work, schools need to open as soon as possible to take care of young children.
    - Small shops will open first; other shops will follow.
    - Hotels and restaurants will open at a still later stage.

Austria was the first European country to relax lockdown measures. On 14 April, it opened up car and bicycle workshops, car washes, shops for building materials, iron and wood, DIY and garden centers (regardless of size) as well as smaller dealers with a customer area under 400 square meters. These shops had to ensure that there was only one customer per 20 square meters. In Vienna alone, 4,600 shops were allowed to open. Opening times were limited to 7:40 a.m. to 7 p.m. The roadmap for the following weeks and months considered the following scheme:

- 1 May: All stores, shopping malls and hairdressers reopen.
- 15 May: Possible opening of services such as restaurants and hotels.
- 15 May or later: Possible re-opening of schools.
- July: Possible – but improbable – organization of events of all sorts (sports, music, theater, cinema etc.).

From Monday, 20 April, **Germany** will re-open small shops with a retail space of under 800 square meters, on the condition that hygiene and social distancing measures are in place. Larger car dealerships, bike shops and book shops can also reopen.

Schools will re-open on 4 May, giving priority to students that have to take exams. Mass gatherings will remain banned throughout spring and summer. No decision has been announced as to when and whether lift restrictions on restaurants and bars.

"COVID Pass"

In countries that are currently experiencing huge COVID-19 outbreaks, tens of thousands of people will die. Those who survive severe or less severe illness, with or without hospitalization, will have developed antibodies against the SARS-CoV-2 virus (Zhang 2020, Okba 2020). Even more people, those who were infected but developed no symptoms, will have antibodies, too. All in all, millions of people in Italy, Spain and France will be thus have SARS-CoV-2 antibodies.

In South Korea and elsewhere more than 100 people who had recovered from COVID-19 were retested positive (Ye 2020) and there was concern that patients who recover from COVID-19 may be at risk of reinfection. However, there was no indication that they were contagious. The most likely explanation is that the 'infection had been reactivated' in the patients or that the tests picked up non-infective RNA of the virus. Very preliminary data from an animal study (n=2) suggest that that immunity acquired following primary infection may protect upon subsequent exposure to the virus. Infection of rhesus macaques with SARS-CoV-2 and re-infection after recovery showed that there was no viral replication in nasopharyngeal or anal swabs, nor any other any signs of COVID-19 disease recurrence (Bao 2020).

In mid-April 2020, we still don't know if antibodies protect against a second infection. There is no reason to believe why they would not and most researchers strongly think they do, but further studies are needed to support the inference from our general knowledge of coronavirus infection that neutralizing antibodies are likely to be protective. Once people have recov-

ered from SARS-CoV-2 infection, it is therefore likely that they are not vulnerable to secondary infection.

There has been speculation about the introduction of a SARS-CoV-2 antibody passport, or *COVID Pass*. People with neutralizing antibodies – assumed to be protected from COVID-19 infection, symptomatic and asymptomatic, and therefore unable to transmit the virus – would be allowed to freely move around. However, not only it is too early for issuing such *passe-partout* (see the previous paragraph), but it would also present a huge logistical challenge: Would the pass need to come in the form of a costly national identity card? How would citizens be controlled? After how many months and years would the card be revoked if antibody levels are shown to wane with time (see chapter *Immunology*, page 89)? For the time being, a positive SARS-CoV-2 serological status might be used in health care settings to determine who should be in close contact to confirmed or suspected COVID-19 patients.

The second wave

The dilemma faced by lockdowned countries is to restart and maximize economic activity while, at the same time, minimizing the number of new SARS-CoV-2 infections and the risk of setting off a second cataclysmic wave of contagion.

In the immediate future, there will be no return to "life before COVID-19". The above-mentioned study by Ferguson (Ferguson 2020) predicts that after lifting strict "Stay at home" measures (extreme social distancing measures and home quarantines), the epidemic would simply bounce back (Figure 7)!

So what will our future look like? A pendulum existence of three months "Stay at home" interspersed with a few months of "Go out again"? We have good reason to believe that this is economically not viable. Unless a miraculous drug or vaccine

is/are developed and produced quickly in sufficient quantities, the people of the world will have to invent intermediate measures. Mitigation strategies focusing on shielding the elderly (60% reduction in social contacts) and slowing but not interrupting transmission (40% reduction) will certainly reduce the disease and death burden by half, but would still result in 20 million deaths in 2020 (Patrick 2020). For a long time we might all wear face masks when leaving our homes and rely on intensive contact tracing and isolation of cases once the lockdown is lifted (Hellewell 2020). Fear for the second wave of the epidemic might be with us for years.

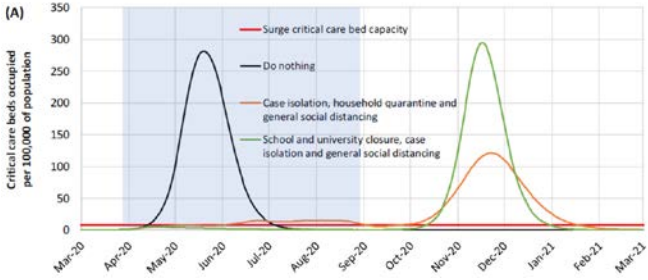

Figure 7. Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand (Source: Ferguson 2020).

Fortunately, people have the ability to learn. In any second wave of the COVID-19 epidemic, there will be no mass gatherings, no 2020 UEFA European Football Championship and no 2020 Summer Olympics in Tokyo. Discos, pubs and all other places which weeks ago brought people into close contact would be closed until further notice. In daily life, everyone would take action when experiencing fever and cough and suggesting action when witnessing it. There will be testing on a massive scale with

extensive contact tracing and ensuing quarantine measures (Nussbaumer-Streit 2020).

Science

Coronaviruses have come a long way (Weiss 2020) and will stay with us for a long time. Questions abound: When will air travel resume? Will we be able to move from one country to another soon? When can we plan our next vacation and return to beaches and nightlife? Will we wear face masks for years? For how long will we live in a closed world?

The French have a precise formula to express unwillingness for living in a world we don't recognize: "Un monde de con!" Fortunately, we will walk out of this *monde de con* thanks to a scientific community which is vaster, stronger and faster than at any time in history. (Should politicians who are skeptical of science be ousted out of office? Yes, please, it might be time now!) Today, we don't know how long, how intense and how deadly the epidemic will be. We are walking on moving ground, and in the coming weeks and months, we will need to be flexible and inventive, finding solutions nobody would have imagined just months ago. Sure enough, though, science will lead the way out. If we leapt three years into the future and read the story of COVID-19, we would be excited.

References

Ainslie K et al. (Imperial College COVID-19 Response Team). Report 11: **Evidence of initial success for China exiting COVID-19 social distancing policy after achieving containment.** 24 March 2020. DOI: https://doi.org/10.25561/77646

Anfinrud P, Stadnytskyi V, Bax CE, Bax A. **Visualizing Speech-Generated Oral Fluid Droplets with Laser Light Scattering.** N Engl J Med. 2020 Apr 15. PubMed: https://pubmed.gov/32294341. Full-text: https://doi.org/10.1056/NEJMc2007800

Bae S, Kim MC, Kim JY, et al. **Effectiveness of Surgical and Cotton Masks in Blocking SARS-CoV-2: A Controlled Comparison in 4 Patients**. Ann Intern Med. 2020 Apr 6. pii: 2764367. PubMed: https://pubmed.gov/32251511 . Full-text: https://doi.org/10.7326/M20-1342

Bao L, Deng W, Gao H, et al. **Reinfection could not occur in SARS-CoV-2 infected rhesus macaques.** BioRxiv, 12 March 2020. Full-text: https://www.biorxiv.org/content/10.1101/2020.03.13.990226v1

Cai J, Sun W, Huang J, Gamber M, Wu J, He G. **Indirect Virus Transmission in Cluster of COVID-19 Cases, Wenzhou, China, 2020.** Emerg Infect Dis. 2020 Mar 12;26(6). PubMed: https://pubmed.gov/32163030. Fulltext: https://doi.org/10.3201/eid2606.200412

Cereda D, Tirani M, Rovida F, et al. **The early phase of the COVID-19 outbreak in Lombardy, Italy.** Preprint. Full-text: https://arxiv.org/abs/2003.09320

Chan JF, Yuan S, Kok KH, et al. **A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster.** Lancet. 2020 Feb 15;395(10223):514-523. PubMed: https://pubmed.gov/31986261. Fulltext: https://doi.org/10.1016/S0140-6736(20)30154-9

Chan KH, Yuen KY. **COVID-19 epidemic: disentangling the re-emerging controversy about medical face masks from an epidemiological perspective.** Int J Epidem March 31, 2020. dyaa044, full-text: https://doi.org/10.1093/ije/dyaa044

Chang L, Zhao L, Gong H, Wang L, Wang L. **Severe Acute Respiratory Syndrome Coronavirus 2 RNA Detected in Blood Donations.** Emerg Infect Dis. 2020 Apr 3;26(7). PubMed: https://pubmed.gov/32243255. Full-text: https://doi.org/10.3201/eid2607.200839

Chao CYH, Wan MP, Morawska L, et al. **Characterization of expiration air jets and droplet size distributions immediately at the mouth opening.** J Aerosol Sci. 2009 Feb;40(2):122-133. PubMed: https://pubmed.gov/32287373. Full-text: https://doi.org/10.1016/j.jaerosci.2008.10.003

Chen N, Zhou M, Dong X, et al. **Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study.** Lancet. 2020 Feb 15;395(10223):507-513. PubMed:

https://pubmed.gov/32007143. Fulltext: https://doi.org/10.1016/S0140-6736(20)30211-7

Cheng VCC, Wong SC, Chen JHK, et al. **Escalating infection control response to the rapidly evolving epidemiology of the Coronavirus disease 2019 (COVID-19) due to SARS-CoV-2 in Hong Kong.** Infect Control Hosp Epidemiol 2020;0: PubMed: https://pubmed.gov/32131908. Full-text: https://doi.org/10.1017/ice.2020.58

Corman VM, Landt O, Kaiser M, et al. **Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR.** Euro Surveill. 2020 Jan;25(3). PubMed: https://pubmed.gov/31992387. Full-text: https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

Du Z, Xu X, Wu Y, Wang L, Cowling BJ, Meyers LA. **Serial Interval of COVID-19 among Publicly Reported Confirmed Cases.** Emerg Infect Dis. 2020 Mar 19;26(6). PubMed: https://pubmed.gov/32191173. Fulltext: https://doi.org/10.3201/eid2606.200357

Dudly JP, Lee NT. **Disparities in Age-Specific Morbidity and Mortality from SARS-CoV-2 in China and the Republic of Korea.** Clin Inf Dis 2020, March 31. Full-text: https://doi.org/10.1093/cid/ciaa354

Ferguson et al. (Imperial College COVID-19 Response Team). Report 9: **Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand.** 16 March 2020. DOI: https://doi.org/10.25561/77482

Flaxman S et al. (Imperial College COVID-19 Response Team). Report 13: **Estimating the number of infections and the impact of non-pharmaceutical interventions on COVID-19 in 11 European countries.** 30 March 2020. DOI: https://doi.org/10.25561/77731

Ghinai I, McPherson TD, Hunter JC, et al. **First known person-to-person transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the USA**. Lancet. 2020 Apr 4;395(10230):1137-1144. PubMed: https://pubmed.gov/32178768 . Full-text: https://doi.org/10.1016/S0140-6736(20)30607-3

Giovanetti M, Angeletti S, Benvenuto D, Ciccozzi M. **A doubt of multiple introduction of SARS-CoV-2 in Italy: a preliminary overview.** J Med Virol. 2020 Mar 19. PubMed: https://pubmed.gov/32190908. Fulltext: https://doi.org/10.1002/jmv.25773

Guo ZD, Wang ZY, Zhang SF, et al. **Aerosol and Surface Distribution of Severe Acute Respiratory Syndrome Coronavirus 2 in Hospital Wards, Wuhan, China, 2020**. Emerg Infect Dis. 2020 Apr 10;26(7). PubMed: https://pubmed.gov/32275497. Full-text: https://doi.org/10.3201/eid2607.200885

Haider N, Yavlinsky A, Simons D, et al. **Passengers' destinations from China: low risk of Novel Coronavirus (2019-nCoV) transmission into Africa and South America.** Epidemiol Infect 2020;148: PubMed: https://pubmed.gov/32100667. Full-text: https://doi.org/10.1017/S0950268820000424

Hellewell J, Abbott S, Gimma A, et al. **Feasibility of controlling COVID-19 outbreaks by isolation of cases and contacts.** Lancet Glob Health. 2020 Apr;8(4):e488-e496. PubMed: https://pubmed.gov/32119825. Fulltext: https://doi.org/10.1016/S2214-109X(20)30074-7

Kam KQ, Yung CF, Cui L, et al. **A Well Infant with Coronavirus Disease 2019 (COVID-19) with High Viral Load.** Clin Infect Dis 2020;0: PubMed: https://pubmed.gov/32112082. Full-text: https://doi.org/10.1093/cid/ciaa201

Klompas M, Morris CA, Sinclair J, Pearson M, Shenoy ES. **Universal Masking in Hospitals in the Covid-19 Era**. N Engl J Med. 2020 Apr 1. PubMed: https://pubmed.gov/32237672. Full-text: https://doi.org/10.1056/NEJMp2006372

Kwon SY, Kim EJ, Jung YS, Jang JS, Cho NS. **Post-donation COVID-19 identification in blood donors**. Vox Sang. 2020 Apr 2. PubMed: https://pubmed.gov/32240537. Full-text: https://doi.org/10.1111/vox.12925

Lau H, Khosrawipour V, Kocbach P, et al. **The positive impact of lockdown in Wuhan on containing the COVID-19 outbreak in China.** J Travel Med. 2020 Mar 17. pii: 5808003. PubMed: https://pubmed.gov/32181488. Fulltext: https://doi.org/10.1093/jtm/taaa037

Lauer SA, Grantz KH, Bi Q, et al. **The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application.** Ann Intern Med 2020: PubMed: https://pubmed.gov/32150748. Full-text: https://doi.org/10.7326/M20-0504

Leung NH, Chu Dk, Shiu EY. **Respiratory virus shedding in exhaled breath and efficacy of face masks.** Nature Med 2020, April 3. https://doi.org/10.1038/s41591-020-0843-2

Li Q, Guan X, Wu P, et al. **Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia.** N Engl J Med 2020: PubMed: https://pubmed.gov/31995857. Full-text: https://doi.org/10.1056/NEJMoa2001316

Lu J, Gu J, Li K, et al. COVID-19 **Outbreak Associated with Air Conditioning in Restaurant, Guangzhou, China, 2020**. Emerg Infect Dis. 2020 Apr 2;26(7). PubMed: https://pubmed.gov/32240078. Full-text: https://doi.org/10.3201/eid2607.200764

Luo C, Yao L, Zhang L, et al. **Possible Transmission of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a Public Bath Center in Huai'an,** Jiangsu Province, China. JAMA Netw Open. 2020 Mar 2;3(3):e204583. PubMed: https://pubmed.gov/32227177. Full-text: https://doi.org/10.1001/jamanetworkopen.2020.4583

McMichael TM, Currie DW, Clark S, et al. **Epidemiology of Covid-19 in a Long-Term Care Facility in King County, Washington.** N Engl J Med 28 March 2020. Full-text: https://doi.org/10.1056/NEJMoa2005412.

Nacoti M et al. **At the Epicenter of the Covid-19 Pandemic and Humanitarian Crises in Italy: Changing Perspectives on Preparation and Mitigation.** NEJM Catalyst Innovations in Care Delivery. 21 March 2020. Full-text: https://catalyst.nejm.org/doi/full/10.1056/CAT.20.0080

Nishiura H, Linton NM, Akhmetzhanov AR. **Serial interval of novel coronavirus (COVID-19) infections.** Int J Infect Dis 2020;0: PubMed: https://pubmed.gov/32145466. Full-text: https://doi.org/10.1016/j.ijid.2020.02.060

Normile D. **As normalcy returns, can China keep COVID-19 at bay?** Science. 2020 Apr 3;368(6486):18-19. PubMed: https://pubmed.gov/32241931. Full-text: https://doi.org/10.1126/science.368.6486.18

Nussbaumer-Streit B, Mayr V, Dobrescu AI, et al. **Quarantine alone or in combination with other public health measures to control COVID-19: a rapid review**. Cochrane Database Syst Rev. 2020 Apr 8;4:CD013574. PubMed: https://pubmed.gov/32267544. Full-text: https://doi.org/10.1002/14651858.CD013574

Okba NMA, Muller MA, Li W, et al. **Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients.** Emerg Infect Dis. 2020 Apr 8;26(7). PubMed: https://pubmed.gov/32267220. Full-text: https://doi.org/10.3201/eid2607.200841

Ran L, Chen X, Wang Y, Wu W, Zhang L, Tan X. **Risk Factors of Healthcare Workers with Corona Virus Disease 2019: A Retrospective Cohort Study in a Designated Hospital of Wuhan in China.** Clin Infect Dis. 2020 Mar 17. pii: 5808788. PubMed: https://pubmed.gov/32179890. Fulltext: https://doi.org/10.1093/cid/ciaa287

Rocklov J, Sjodin H, Wilder-Smith A. **COVID-19 outbreak on the Diamond Princess cruise ship: estimating the epidemic potential and effectiveness of public health countermeasures.** J Travel Med 2020;0: PubMed: https://pubmed.gov/32109273. Full-text: https://doi.org/10.1093/jtm/taaa030

Rothe C, Schunk M, Sothmann P, et al. **Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany.** N Engl J Med 2020;382:970-971. https://pubmed.gov/32003551. Full-text: https://doi.org/10.1056/NEJMc2001468

Scott SE, Zabel K, Collins J, et al. **First Mildly Ill, Non-Hospitalized Case of Coronavirus Disease 2019 (COVID-19) Without Viral Transmission in the United States - Maricopa County, Arizona, 2020.** Clin Infect Dis. 2020 Apr 2. PubMed: https://pubmed.gov/32240285. Full-text: https://doi.org/10.1093/cid/ciaa374

Stafford N. Covid-19: **Why Germany's case fatality rate seems so low**. BMJ. 2020 Apr 7;369:m1395. PubMed: https://pubmed.gov/32265194. Full-text: https://doi.org/10.1136/bmj.m1395

Tang A, Tong ZD, Wang HL, et al. **Detection of Novel Coronavirus by RT-PCR in Stool Specimen from Asymptomatic Child, China.** Emerg Infect Dis. 2020

Jun 17;26(6). PubMed: https://pubmed.gov/32150527. Fulltext: https://doi.org/10.3201/eid2606.200301

Tang B, Bragazzi NL, Li Q, Tang S, Xiao Y, Wu J. **An updated estimation of the risk of transmission of the novel coronavirus (2019-nCov).** Infect Dis Model 2020;5:248-255. PubMed: https://pubmed.gov/32099934. Full-text: https://doi.org/10.1016/j.idm.2020.02.001

Tian H, Liu Y, Li Y, et al. **An investigation of transmission control measures during the first 50 days of the COVID-19 epidemic in China**. Science. 2020 Mar 31. pii: science.abb6105. PubMed: https://pubmed.gov/32234804. Full-text: https://doi.org/10.1126/science.abb6105

van Doremalen N, Bushmaker T, Morris DH, et al. **Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1.** N Engl J Med. 2020 Mar 17. PubMed: https://pubmed.gov/32182409. Fulltext: https://doi.org/10.1056/NEJMc2004973

Walker P et al. (Imperial College COVID-19 Response Team). Report 12: **The global impact of COVID-19 and strategies for mitigation and suppression.** 26 March 2020. DOI: https://doi.org/10.25561/77735

Wang J, Tang, K, Feng K, Lv W. **High Temperature and High Humidity Reduce the Transmission of COVID-19** (March 9, 2020). Available at SSRN: https://ssrn.com/PubMed=3551767 or http://dx.doi.org/10.2139/ssrn.3551767

Weiss SR. **Forty years with coronaviruses.** J Exp Med. 2020 May 4;217(5). pii: 151597. PubMed: https://pubmed.gov/32232339. Full-text: https://doi.org/10.1084/jem.20200537

Wells CR, Sah P, Moghadas SM, et al. **Impact of international travel and border control measures on the global spread of the novel 2019 coronavirus outbreak.** Proc Natl Acad Sci U S A. 2020 Mar 13. pii: 2002616117. PubMed: https://pubmed.gov/32170017. Full-text: https://doi.org/10.1073/pnas.2002616117

Wenham C, Smith J, Morgan R. **COVID-19: the gendered impacts of the outbreak.** Lancet. 2020 Mar 14;395(10227):846-848. PubMed: https://pubmed.gov/32151325. Fulltext: https://doi.org/10.1016/S0140-6736(20)30526-2

WHO. **Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19).** https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19)

WMHC. **Wuhan Municipal Health and Health Commission's briefing on the current pneumonia epidemic situation in our city** (31 December 2019). http://wjw.wuhan.gov.cn/front/web/showDetail/2019123108989. Accessed 25 March 2020.

Wolfel R, Corman VM, Guggemos W, et al. **Virological assessment of hospitalized patients with COVID-2019.** Nature. 2020 Apr 1. pii: 10.1038/s41586-020-2196-x. PubMed: https://pubmed.gov/32235945. Full-text: https://doi.org/10.1038/s41586-020-2196-x

Wu Z, McGoogan JM. **Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention.** JAMA. 2020 Feb 24. pii: 2762130. PubMed: https://pubmed.gov/32091533. Fulltext: https://doi.org/10.1001/jama.2020.2648

Ye G, Pan Z, Pan Y, et al. **Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation.** J Infect. 2020 May;80(5):e14-e17. PubMed: https://pubmed.gov/32171867. Full-text: https://doi.org/10.1016/j.jinf.2020.03.001

Young BE, Ong SWX, Kalimuddin S, et al. **Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore.** JAMA. 2020 Mar 3. pii: 2762688. PubMed: https://pubmed.gov/32125362. Fulltext: https://doi.org/10.1001/jama.2020.3204

Zhang W, Du RH, Li B, et al. **Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes.** Emerg Microbes Infect. 2020 Feb 17;9(1):386-389. PubMed: https://pubmed.gov/32065057. Full-text: https://doi.org/10.1080/22221751.2020.1729071

Zhao S, Lin Q, Ran J, et al. **Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from 2019 to 2020: A data-driven analysis in the early phase of the outbreak.** Int J Infect Dis 2020;92:214-217. doi: 10.1016/j.ijid.2020.01.050. Epub 2020 PubMed: https://pubmed.gov/32007643. Full-text: https://doi.org/10.1016/j.ijid.2020.01.050

Zhong P, Guo S, Chen T. **Correlation between travellers departing from Wuhan before the Spring Festival and subsequent spread of COVID-19 to all provinces in China.** J Travel Med. 2020 Mar 17. pii: 5808004. PubMed: https://pubmed.gov/32181483. Fulltext: https://doi.org/10.1093/jtm/taaa036

Zhou P, Yang XL, Wang XG, et al. **A pneumonia outbreak associated with a new coronavirus of probable bat origin.** Nature. 2020 Mar;579(7798):270-273. PubMed: https://pubmed.gov/32015507. Fulltext: https://doi.org/10.1038/s41586-020-2012-7

3. Virology

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Coronaviruses are found in a variety of animals and humans. These enveloped viruses contain a single strand of positive-sense RNA. Virions are mostly spherical, with pronounced spiked glycoprotein (S) embedded in the envelope. Additional structural proteins include envelope (E), matrix (M), and nucleocapsid (N).

The family *Coronaviridae* includes four genera, alpha-, beta-, delta- and gammacoronavirus, as well as several subgenera and species. Phylogenetic analysis on the coronavirus genomes has revealed that SARS-CoV-2 is a new member of the betacoronavirus genus, which includes severe acute respiratory syndrome-related coronavirus (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV), bat SARS-related coronaviruses (SARSr-CoV), as well as others identified in humans and diverse animal species. Intra- and inter-species transmission of CoVs, and genetic recombination events contribute to the emergence of new CoV strains.

SARS-CoV-2 is taxonomically related to the subgenus *Sarbecovirus* together with SARS-CoV and bat SARS-like CoVs. Genomic sequencing showed SARS-CoV-2 to be closely related to betacoronaviruses detected in bats, but distinct from SARS-CoV. The following sections includes some key papers on different topics.

Taxonomy

Please check the comments on these studies.

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. **The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2**. Nat Microbiol. 2020 Apr;5(4):536-544. PubMed: https://pubmed.gov/32123347. Full-text: https://doi.org/10.1038/s41564-020-0695-z

A consensus statement defining the place of SARS-CoV-2 (provisionally named 2019-nCoV) within the Coronaviridae family.

Ceraolo C, Giorgi FM. **Genomic variance of the 2019-nCoV coronavirus**. J Med Virol. 2020 May;92(5):522-528. PubMed: https://pubmed.gov/32027036. Full-text: https://doi.org/10.1002/jmv.25700

Analysis of 56 genomic sequences from distinct patients, showing high sequence similarity (>99%). A few variable genomic regions exist, mainly at the ORF8 locus (coding for accessory proteins).

Zhou P, Yang XL, Wang XG, et al. **A pneumonia outbreak associated with a new coronavirus of probable bat origin**. Nature. 2020 Mar;579(7798):270-273. PubMed: https://pubmed.gov/32015507. Fulltext: https://doi.org/10.1038/s41586-020-2012-7

Full-length genome sequences from five patients at an early stage of the outbreak, showing 79.6% sequence identity to SARS-CoV and 96% to a bat coronavirus.

Origin and hosts

Andersen KG, Rambaut A, Lipkin WA, Holmes EC, Garry RF. **The proximal origin of SARS-CoV-2.** Nature Medicine. Published: 17 March 2020. Fulltext: https://www.nature.com/articles/s41591-020-0820-9

Review on notable genomic features of SARS-CoV-2, compared to alpha- and betacoronaviruses. Insights on the origin, clearly showing that this virus is not a laboratory construct or a purposefully manipulated virus.

Cui J, Li F, Shi ZL. **Origin and evolution of pathogenic coronaviruses**. Nat Rev Microbiol. 2019 Mar;17(3):181-192. PubMed: https://pubmed.gov/30531947. Full-text: https://doi.org/10.1038/s41579-018-0118-9

SARS-CoV and MERS-CoV likely originated in bats, both jumping species to infect humans through different intermediate hosts.

Lam TT, Shum MH, Zhu HC, et al. **Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins**. Nature. 2020 Mar 26. pii: 10.1038/s41586-020-2169-0. PubMed: https://pubmed.gov/32218527. Fulltext: https://doi.org/10.1038/s41586-020-2169-0

Do Malayan pangolins act as intermediate hosts? Metagenomic sequencing identified pangolin-associated coronaviruses, including one with strong similarity to SARS-CoV-2 in the receptor-binding domain.

Zhang T, Wu Q, Zhang Z. **Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak**. Curr Biol. 2020 Mar 13. pii: S0960-9822(20)30360-2. PubMed: https://pubmed.gov/32197085. Fulltext: https://doi.org/10.1016/j.cub.2020.03.022

This study suggests that pangolin species are a natural reservoir of SARS-CoV-2-like CoVs. Pangolin-CoV was 91.0% and 90.6% identical to SARS-CoV-2 and Bat-CoV RaTG13, respectively.

Stability and transmission of the virus

Chin AW, Chu JT, Perera MR, et al. **Stability of SARS-CoV-2 in different environmental conditions**.The Lancet Microbe 2020, April 02. DOI:https://doi.org/10.1016/S2666-5247(20)30003-3. Full-text: https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247(20)30003-3/fulltext

SARS-CoV-2 was highly stable at 4°C (almost no reduction on day 14) but sensitive to heat (70°C: inactivation 5 min, 56°: 30 min, 37°: 2 days). It also depends on the surface: No infectious virus could be recovered from printing and tissue papers after 3-hours, from treated wood and cloth on day 2, from glass and banknotes on day 4, stainless steel and plas-

tic on day 7. Strikingly, a detectable level of infectious virus (<0·1% of the original inoculum) was still present on the outer layer of a surgical mask on day 7.

Kim YI, Kim SG, Kim SM, et al. **Infection and Rapid Transmission of SARS-CoV-2 in Ferrets**. Cell Host Microbe. 2020 Apr 5. pii: S1931-3128(20)30187-6. PubMed: https://pubmed.gov/32259477. Full-text: https://doi.org/10.1016/j.chom.2020.03.023.

Ferrets shed the virus in nasal washes, saliva, urine, and feces up to 8 days post-infection. They may represent an infection and transmission animal model of COVID-19 that may facilitate development of SARS-CoV-2 therapeutics and vaccines.

Leung NH, Chu Dk, Shiu EY. **Respiratory virus shedding in exhaled breath and efficacy of face masks**. Nature Med 2020, April 3. https://doi.org/10.1038/s41591-020-0843-2

This study from Hong Kong (performed 2013-16) quantified virus in respiratory droplets and aerosols in exhaled breath. In total, 111 participants (infected with seasonal coronavirus, influenza or rhinovirus) were randomized to wear or not to wear a simple surgical face mask. Results suggested that masks could be used by ill people to reduce onward transmission. In respiratory droplets, seasonal coronavirus was detected in 3/10 (aerosols: 4/10) samples collected without face masks, but in 0/11 (0/11) from participants wearing face masks. Influenza viruses were detected in 6/23 (8/23) without masks, compared to 1/27 (aerosol 6/27!) with masks. For rhinovirus, there were no significant differences at all. Of note, authors also identified virus in some participants who did not cough at all during the 30 min exhaled breath collection, suggesting droplet and aerosol routes of transmission from individuals with no obvious signs or symptoms.

Shi J, Wen Z, Zhong G, et al. **Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-coronavirus 2**. Science. 2020 Apr 8. pii: science.abb7015. PubMed: https://pubmed.gov/32269068. Full-text: https://doi.org/10.1126/science.abb7015

SARS-CoV-2 replicates poorly in dogs, pigs, chickens, and ducks. However, ferrets and cats are permissive to infection and cats were susceptible to airborne infection. But cat owners can relax. Experiments were done in a small number of cats exposed to high doses of the virus, probably more than found in real-life. It also remains unclear if cats secrete enough coronavirus to pass it on to humans.

van Doremalen N, Bushmaker T, Morris DH, et al. **Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1**. N Engl J Med. 2020 Mar 17. PubMed: https://pubmed.gov/32182409. Fulltext: https://doi.org/10.1056/NEJMc2004973

Stability of SARS-CoV-2 was similar to that of SARS-CoV-1, indicating that differences in the epidemics probably arise from other factors and that aerosol and fomite transmission of SARS-CoV-2 is plausible. The virus can remain viable and infectious in aerosols for hours and on surfaces up to days (depending on the inoculum shed).

Cell tropism

Chu H, Chan JF, Wang Y, et al. **Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19**. Clin Infect Dis. 2020 Apr 9. pii: 5818134. PubMed: https://pubmed.gov/32270184

Cell experiments on replication capacity and immune activation profile of SARS-CoV-2 and SARS-CoV infection in human lung tissues. Both viruses were similar in cell tropism, with both targeting types I and II pneumocytes, and alveolar macrophages. SARS-CoV-2 generated 3.20 times more infectious virus particles than that of SARS-CoV from infected lung tissues.

Wang X, Xu W, Hu G, et al. **SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion**. Cell Mol Immunol. 2020 Apr 7. pii: 10.1038/s41423-020-0424-9. PubMed: https://pubmed.gov/32265513. Full-text: https://doi.org/10.1038/s41423-020-0424-9

It remains unclear whether SARS-CoV-2 can also infect T cells, resulting in lymphocytopenia. Using a model with pseudoviruses, authors showed that SARS-CoV-2 infects (but does not replicate in) T cells through S protein-mediated membrane fusion. T-cell lines were significantly more sensitive to SARS-CoV-2 infection when compared with SARS-CoV. Of note, a very low expression level of hACE2 was found, indicating that a novel receptor might mediate SARS-CoV-2 entry into T cells.

Spike protein and cell entry

Chu H, Chan JF, Wang Y, et al. **Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19**. Clin Infect Dis. 2020 Apr 9. pii: 5818134. PubMed: https://pubmed.gov/32270184.

Cell experiments on replication capacity and immune activation profile of SARS-CoV-2 and SARS-CoV infection in human lung tissues. Both viruses were similar in cell tropism, with both targeting types I and II pneumocytes, and alveolar macrophages. SARS-CoV-2 generated 3.20 folds more infectious virus particles than that of SARS-CoV from infected lung tissues.

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 Apr;176:104742. PubMed: https://pubmed.gov/32057769. Fulltext: https://doi.org/10.1016/j.antiviral.2020.104742

Identification of a peculiar furin-like cleavage site in the Spike protein of SARS-CoV-2, lacking in other SARS-like CoVs. Potential implication for the development of antivirals.

Hoffmann M, Kleine-Weber H, Schroeder S, et al. **SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor**. Cell. 2020 Mar 4. pii: S0092-8674(20)30229-4. PubMed: https://pubmed.gov/32142651. Fulltext: https://doi.org/10.1016/j.cell.2020.02.052

This work shows how viral entry happens. SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming. In addition, sera from convalescent SARS patients cross-neutralized SARS-2-S-driven entry.

Lan J, Ge J, Yu J, et al. **Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor**. Nature. Published: 30 March 2020. Full-text: https://www.nature.com/articles/s41586-020-2180-5

To elucidate the SARS-CoV-2 RBD and ACE2 interaction at a higher resolution/atomic level, authors used X-ray crystallography. Binding mode was very similar to SARS-CoV, arguing for a convergent evolution of both viruses. The epitopes of two SARS-CoV antibodies targeting the RBD were also analysed with the SARS-CoV-2 RBD, providing insights into the future identification of cross-reactive antibodies.

Letko M, Marzi A, Munster V. **Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses**. Nat Microbiol. 2020 Apr;5(4):562-569. PubMed: https://pubmed.gov/32094589. Full-text: https://doi.org/10.1038/s41564-020-0688-y

Important work on viral entry, using a rapid and cost-effective platform which allows to functionally test large groups of viruses for zoonotic potential. Host protease processing during viral entry is a significant barrier for several lineage B viruses. However, bypassing this barrier allows several coronaviruses to enter human cells through an unknown receptor.

Monteil V, Kwon H, Patricia Prado P, et al. **Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2**. Cell 2020. DOI: 10.1016/j.cell.2020.04.004. https://www.cell.com/pb-assets/products/coronavirus/CELL_CELL-D-20-00739.pdf.

This study shows that human recombinant soluble ACE2 (hrsACE2) blocks SARS-CoV-2 infections of different cells, human blood vessel organoids and human kidney organoids. In ARDS patients, hrsACE2 was ineffective but safe at a broad range of doses. Apeiron Biologics plans a randomized study on 200 COVID-19 patients in April.

Ou X, Liu Y, Lei X, et al. **Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV**. Nat Commun. 2020 Mar 27;11(1):1620. PubMed: https://pubmed.gov/32221306. Fulltext: https://doi.org/10.1038/s41467-020-15562-9

More on viral entry and on (the limited) cross-neutralization between SARS-CoV and SARS-CoV-2.

Shang J, Ye G, Shi K. **Structural basis of receptor recognition by SARS-CoV-2**. Nature 2020, March 30. https://doi.org/10.1038/s41586-020-2179-y.

How well does SARS-CoV-2 recognize hACE2? Better than other coronaviruses. Compared to SARS-CoV and RaTG13 (isolated from bats), ACE2-binding affinity is higher. Functionally important epitopes in SARS-CoV-2 RBM are described that can potentially be targeted by neutralizing antibody drugs.

Wang Q, Zhang Y, Wu L, et al. **Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2**. Cell. 2020 Apr 7. pii: S0092-8674(20)30338-X. PubMed: https://pubmed.gov/32275855. Full-text: https://doi.org/10.1016/j.cell.2020.03.045

Atomic details of the crystal structure of the C-terminal domain of SARS-CoV-2 spike protein in complex with human ACE2 are presented. The hACE2 binding mode of SARS-CoV-2 seems to be similar to SARS-CoV, but some key residue substitutions slightly strengthen the interaction and lead to higher affinity for receptor binding. Antibody exper-

iments indicated notable differences in antigenicity between SARS-CoV and SARS-CoV-2.

Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. **Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2**. Science. 2020 Mar 27;367(6485):1444-1448. PubMed: https://pubmed.gov/32132184. Full-text: https://doi.org/10.1126/science.abb2762

Using cryo–electron microscopy, it is shown how SARS-CoV-2 binds to human cells. The first step in viral entry is the binding of the viral trimeric spike protein to the human receptor angiotensin-converting enzyme 2 (ACE2). Authors present the structure of human ACE2 in complex with a membrane protein that it chaperones, B0AT1. The structures provide a basis for the development of therapeutics targeting this crucial interaction.

Yuan M, Wu NC, Zhu X, et al. **A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV**. Science. 2020 Apr 3. pii: science.abb7269. PubMed: https://pubmed.gov/32245784. Full-text: https://doi.org/10.1126/science.abb7269

Insights into antibody recognition and how SARS-CoV-2 can be targeted by the humoral response, revealing a conserved epitope shared between SARS-CoV and SARS-CoV-2. This epitope could be used for vaccines and the development of cross-protective antibodies.

Zhang L, Lin D, Sun X, et al. **Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved alpha-ketoamide inhibitors**. Science. 2020 Mar 20. PubMed: https://pubmed.gov/32198291. Fulltext: https://doi.org/10.1126/science.abb3405

Description of the X-ray structures of the main protease (Mpro, 3CLpro) of SARS-CoV-2 which is essential for processing the polyproteins that are translated from the viral RNA. A complex of Mpro and an optimized protease α ketoamide inhibitor is also described.

RNA-dependent RNA polymerase (RdRp)

Gao Y, Yan L, Huang Y, et al. **Structure of the RNA-dependent RNA polymerase from COVID-19 virus**. Science. 2020 Apr 10. pii: science.abb7498. PubMed: https://pubmed.gov/32277040. Full-text: https://doi.org/10.1126/science.abb7498

Using cryogenic electron microscopy, authors describe the structure of the RNA-dependent RNA polymerase, another central enzyme of the viral replication machinery. It is also shown how remdesivir and sofosbuvir bind to this polymerase.

Other key papers

Chan JF, Zhang AJ, Yuan S, et al. **Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility**. Clin Infect Dis. 2020 Mar 26. PubMed: https://pubmed.gov/32215622. Fulltext: https://doi.org/10.1093/cid/ciaa325

A readily available hamster model as an important tool for studying transmission, pathogenesis, treatment, and vaccination against SARS-CoV-2.

Le TT, Andreadakis Z, Kumar A, et al. **The COVID-19 vaccine development landscape**. Nature reviews drug discovery. 09 April 2020. doi: 10.1038/d41573-020-00073-5. Full-text: https://www.nature.com/articles/d41573-020-00073-5.

Brief data-driven overview by seven experts. The conclusion is that efforts are unprecedented in terms of scale and speed and that there is an indication that vaccine could be available by early 2021. As of 8 April 2020, the global vaccine landscape includes 115 candidates, of which the 5 most advanced candidates have already moved into clinical development, including mRNA-1273 from Moderna, Ad5-nCoV from CanSino Biologics, INO-4800 from Inovio, LV-SMENP-DC and pathogen-specific aAPC from Shenzhen Geno-Immune Medical Institute. The race is on!

Monto AS, DeJonge P, Callear AP, et al. **Coronavirus occurrence and transmission over 8 years in the HIVE cohort of households in Michigan**. J Infect Dis. 2020 Apr 4. pii: 5815743. PubMed: https://pubmed.gov/32246136. Full-text: https://doi.org/10.1093/infdis/jiaa161

It's not clear whether SARS-CoV-2 behaves like other human coronaviruses (hCoVs). A longitudinal surveillance cohort study of children and their households from Michigan found that hCoV infections were sharply seasonal, showing a peak for different HCoV types (229E, HKU1, NL63, OC43) in February. Over 8 years, almost no HCoV infections occurred after March.

4. Immunology

Thomas Kamradt

To date, despairingly little is known about immune responses against SARS-CoV-2. Some of the most important and most urgent questions are:

- Is someone who has overcome COVID-19 protected from a second round of the disease?
- If yes, how long does the immune protection last?
- What are the correlates of protection?
- Why do children and young adults seem to develop only mild, if any, signs and symptoms of COVID-19, and why is the disease so much more severe in the elderly?
- How does the immune response against SARS-CoV-2 contribute to disease development? Are there pathogenic immune responses?
- Can we use immunological parameters to predict an individual patient's risk in developing severe disease?
- Can we develop a vaccine against SARS-CoV-2?

We do not know the answer to any of these questions today.

Protective antibodies

In the absence of robust experimental or clinical data on SARS-CoV-2-induced immune responses we can make some educated guesses based on prior experiences with endemic coronaviruses (e.g. 229E or OC43), the SARS-CoV and the MERS-CoV viruses. Experimental, serological and sero-epidemiological studies strongly suggest that coronaviruses, including SARS-CoV induce

neutralizing and protective antibodies. These studies also seem to indicate that antibody-mediated protection is short-lived.

Cellular immune response

Less is known about cellular immune responses, i.e. T cell responses against coronaviruses. Experimental evidence from studies in mice suggests that T cells residing in the mucosa of the respiratory tract could be an important correlate of protection. However, although mice can be infected with coronaviruses including SARS-CoV, they do not develop the severe pulmonary symptoms that are characteristic of SARS and COVID-19. Therefore, these results have to be interpreted with caution. Human T cells from the respiratory mucosa of ill recovering humans would be necessary to clarify the issue but are difficult to come by.

These questions are not simply of an academic nature. Rational vaccine design is based on solid knowledge about protective immunity. As long as we do not know which protective immune response we need to induce by vaccination, vaccine development remains guesswork.

The quest for a vaccine

The fundamentals:

- Recovery from infections often induces long-term and sometimes life-long immunity against the causative pathogen.
- Immunological memory protects against re-infection and is mediated by specific antibodies and T cells.
- Immunizations confer immunity without exposure to virulent pathogens. Immunization can be passive or active.

- In passive immunization protective antibodies are transferred from a donor to a recipient whereas active immunization induces a protective immune response in the recipient.

Passive immunization against SARS-CoV-2

Passive immunization against COVID-19 can be achieved with convalescent plasma, hyperimmune sera, or with neutralizing monoclonal antibodies.

Convalescent Plasma

Treatment of patients with convalescent plasma is based on the idea that someone who has recovered from an infection will have antibodies against the causative pathogen in their blood. Convalescent plasma is used as post-exposure prophylaxis (e.g. hepatitis) or treatment for some infectious diseases including Argentinian hemorrhagic fever (Casadevall 2004). Prior experience shows antibody transfer is most effective when given prophylactically or early in the disease.

Convalescent plasma has been given to SARS patients. Regrettably, this was not done in the context of controlled clinical studies. A meta-analysis could therefore only conclude that the treatment was probably safe and perhaps helpful (Mair-Jenkins 2015). While drugs or vaccines against COVID-19 are still months or years away, convalescent plasma is available now.

To date, we do not know if all patients who have recovered from COVID-19 will harbor high enough titers of neutralizing antibodies to confer protection upon transfer of plasma. Even the assays to determine the concentration of neutralizing antibodies are not standardized nor widely available.

Currently, convalescent plasma is given to COVID-19 patients (see treatment chapter). Several randomized clinical studies are

underway. The multicenter CONCOR-1 trial in Canada id due to start on April 27th with 1,200 participants planned and the CONCOVID trial in The Netherlands with a target number of more than 400 patients. These and similar studies will show if convalescent plasma is safe and effective.

Given the possibility of antibody-dependent disease enhancement (ADE), safety is an important consideration in these trials. One study on macaques found that passive transfer of anti-SARS-CoV-S protein from immunized monkeys into naïve recipients resulted in acute lung injury after infection. The proposed mechanism was a diversion of macrophage activation from wound healing to pro-inflammatory (Liu 2019).

Enhanced lung-pathology upon antibody-transfer was also observed in a rabbit model of MERS (Houser 2017). Convalescent plasma has been given to MERS patients and one case-report raises the possibility of acute lung Injury following convalescent plasma transfusion (Chun 2016).

Taken together, these data stress the necessity to administer convalescent plasma in controlled trials, which will determine safety and efficacy.

Pooled immunoglobulin preparations

Hyperimmune globulin preparations, e.g. cytomegalovirus immunoglobulin (CMVIG), pooled from many different donors, are currently the most frequently used form of passive antibody transfer. These preparations contain higher concentrations of pathogen-specific antibodies than convalescent plasma. However, they are more difficult to produce and there are currently no SARS-CoV-2 hyperimmune globulin preparations available.

Active immunization against SARS-CoV-2

At the time of this writing, there are more than 100 COVID-19 vaccine candidates in different stages of preclinical development. Five candidate vaccines are in phase I clinical trials (https://www.nature.com/articles/d41573-020-00073-5).

The speed of vaccine development is breathtaking. On 11 January, 2020 Chinese researches published the sequence of the SARS-CoV-2 genome on the internet. Approximately 2 months later, on 16 March, an mRNA-based vaccine entered a phase I clinical trial. This was possible, thanks to knowledge gained in efforts to develop vaccines against SARS and MERS and the availability of innovative technologies.

Earlier work had identified the S protein of SARS-CoV and MERS-CoV as a suitable vaccine target. The S protein binds to its cellular receptor, ACE2, to infect human cells. A high degree of homology between the S proteins of the three viruses was quickly established after the discovery of SARS-CoV-2 and the interaction of SARS-CoV-2 S protein with ACE2 was confirmed. Thus, a vaccine target was identified in record time.

New technologies helped the rapid development of an mRNA-based vaccine. The principle was first used in 2013. The Chinese CDC had discovered H7N9, a novel avian influenza virus strain, and immediately published the sequence of the relevant antigens online. Synthetic biology approaches enabled the generation of a vaccine candidate within 8 days and that vaccine was shown to induce antibodies in mice (https://doi.org/10.1038/emi.2013.54).

Why, then, do we still wait for an effective and safe vaccine against SARS-CoV-2? There are still some obstacles to overcome.

Different strategies to develop a vaccine against SARS-CoV-2

Many fundamentally different strategies are currently used to develop a vaccine against COVID-19 (Amanat and Krammer 2020).

The most traditional way to produce vaccines is the use of **whole viruses**, which are either *attenuated or inactivated*. Currently licensed examples include the vaccines against measles and yellow fever (attenuated virus) and influenza and polio (inactivated viruses). Efforts are ongoing to develop attenuated or inactivated SARS-CoV-2 as a vaccine.

Another approach is to use **recombinant viral proteins** as vaccine; licensed examples include the vaccines against hepatitis B and human papilloma virus. Efforts are ongoing to develop recombinant SARS-CoV-2 S protein as an immunogen.

A more recent approach is to use **recombinant viral vectors** in which a relevant antigen of the pathogenic virus is expressed. The only currently licensed example is the vaccine against Ebola, which is based on a modified vesicular stomatitis virus. An adenovirus-based recombinant vaccine against COVID-19 has entered a clinical phase I trial in March 2020.

DNA vaccines targeting the S protein are also in preclinical development. There are currently no licensed DNA vaccines, which might make the licensing process slower as compared with e.g. protein-based vaccines. A DNA vaccine against COVID-19 entered a clinical phase I trial in April 2020.

An **mRNA vaccine** targeting the S protein has been used in a clinical phase I trial that started on 16 March. There are currently no licensed mRNA vaccines, which might make the licensing process slower as compared with e.g. protein-based vaccines.

A vaccine based on genetically modified dendritic cells expressing a lentivirally encoded SARS-CoV-2 minigene and a study using genetically modified artificial antigen presenting cells entered a clinical phase I trial in March. There are currently no licensed vaccines based on **genetically modified antigen-presenting cells**, which might again make the licensing process slower as compared with e.g. protein-based vaccines.

While it is much too early to make any predictions on the safety, immunogenicity and efficacy of the many vaccines currently under development, it is useful to see what can be learned from prior attempts to develop vaccines against coronaviruses.

Vaccines against coronaviruses can induce pathological immune responses.

Rarely, vaccines can enhance disease rather than protect from disease (Openshaw 2001). Vaccines are administered to healthy people. SARS-CoV-2 causes a mild, if not clinically inapparent disease in at least 80% of those who are infected. Therefore safety considerations are of utmost importance. Unfortunately there is some data hinting at the possibility that the development of a safe vaccine against COVID-19 might be unusually difficult.

Vaccine-induced immune response against FIPV is harmful in kittens

Feline infectious peritonitis (FIP) is a severe and often fatal disease in cats. It is caused by a coronavirus, FIPV. Different attempts at vaccine development have failed. In an early study kittens that were vaccinated with an avirulent FIPV strain were more susceptible to infection with virulent FIPV than the non-vaccinated controls (Pedersen and Black 1983). More worryingly were the results of a later study in which cats were immunized

with a recombinant vaccinia virus that expressed the FIPV S protein. Vaccination induced low titers of neutralizing antibodies. Upon FIPV-challenge the previously immunized animals were not protected but died earlier than the controls (Vennema 1990). It is thought that antibody-mediated infection of macrophages and the deposition of immune complexes cause the more severe disease in immunized animals (Perlman and Dandekar 2005, Weiss and Scott, 1981).

Immunopathology seen in experimental vaccines against SARS

Immunopathological or disease-enhancing effects were reported by many different research groups using different technologies and different animal models in an effort to develop a vaccine against SARS.

Immunization with recombinant modified vaccinia virus Ankara (rMVA) expressing the SARS-CoV spike (S) protein causes severe hepatitis in ferrets.

Ferrets are susceptible to SARS-CoV infection. Weingartl and collegues immunized ferrets with recombinant modified vaccinia virus Ankara (rMVA) expressing the SARS-CoV S protein (Weingartl 2004). Upon challenge with the virus, high titers of neutralizing antibodies were detectable more rapidly in the immunized animals than in the controls. However, the ferrets immunized with rMVA-S developed severe hepatitis which was not the case in the control animals (Weingartl 2004). Ferrets are also highly susceptible to SARS-CoV-2 infection (Kim 2020) and are thus suitable for the evaluation of the safety of future vaccine candidates.

Immunization of mice results in type 2 inflammatory responses in the lungs

A group from North Carolina/USA used inactivated virus with or without adjuvant to immunize mice against SARS-CoV (Bolles 2011). The vaccine protected young and to a lesser extent older animals from morbidity and mortality following high-dose viral challenge. However, challenge with a heterologous virus resulted in inflammatory infiltrates and pulmonary eosinophilia that were more severe in the vaccinated animals. Moreover, in old mice the vaccine did not confer protection but still resulted in inflammatory infiltrates in the lung. The occurrence of lung immunopathology with this vaccine was later confirmed and extended by another group (Tseng 2012). Eosinophilic lung infiltrates were also observed when a recombinant baculovirus expressed S protein or coronavirus-like particles (VLPs) expressing the SARS-CoV S protein were used to immunize mice (Lokugamage 2008; Tseng 2012). It is important to note that these were mainly histopathological findings and the vaccinated mice had reduced viral titers upon challenge. However, these histopathological findings are reminiscent of those that were associated with vaccine-induced pathology in children that had received a vaccine against respiratory syncytial virus (RSV) in the 1960s (Castilow 2007). Moreover, lung pathology and even pneumonia were reported when mice were immunized with recombinant vaccinia virus (VV) expressing SARS-CoV S and nucleocapsid (N) proteins (Yasui 2008). Lung pathology was also observed when Venezuelan equine encephalitis virus replicon particles (VRP) expressing the N protein were used to immunize mice (Deming 2006).

Unfortunately, if perhaps not surprisingly, similar findings were reported for MERS-CoV vaccine candidates. An inactivated MERS-CoV vaccine induced neutralizing antibodies in mice and

also resulted in an enhanced type 2 pathology in the lung, i.e. eosinophilic infiltrates and increased concentrations of IL-5 and IL-13 (Agrawal 2016).

Some studies suggest that this type 2 pathology may be ameliorated or prevented by using toll-like receptor agonists (Iwata-Yoshikawa 2014) or delta inulin (Honda-Okubo 2015) as adjuvants for inactivated whole virus or recombinant spike protein vaccine candidates.

Together, these findings cause concern. Careful histopathological evaluation of the lungs should be part of the pre-clinical development of COVID-19 vaccines.

Immunization of non-human primates results in severe acute lung injury

In a recent study Chinese macaques were vaccinated with a modified vaccinia Ankara (MVA) virus encoding full-length SARS-CoV S glycoprotein (ADS-MVA) and challenged with SARS-CoV 8 weeks later (Liu 2019). Vaccination induced high levels of antibodies and reduced virus loads. However, the vaccinated monkeys had diffuse alveolar damage (DAD) (Liu 2019). An earlier study had used inactivated SARS-CoV to vaccinate four macaques. Three monkeys were protected upon challenge whereas one macaque had lung-pathology consistent with antibody-dependent disease enhancement (ADE) (Wang 2016). These authors further suggested that ADE was mediated by antibodies against certain epitopes of SARS-CoV S but not others (Wang 2016).

Anti-S antibodies enhance infection of human immune cells

Antibodies against SARS-CoV spike protein can enhance virus entry into human cells by interaction with conformational epitopes in the ACE2-binding domain (Yang 2005). Anti-Spike

immune serum was reported to promote the infection of human hematopoietic cell lines by SARS-CoV. Virus entry was not mediated via ACE2 but depended on Fcγ receptor II (Jaume 2011). While the *in vivo* relevance of these findings remains to be determined, they add to the list of concerns that need to be addressed in the development of safe and effective vaccines against COVID-19.

Outlook

Given the massive and diverse ongoing efforts to develop a vaccine against COVID-19, we can be optimistic that a safe and effective vaccine will be available in the not-too-distant future. The development of a vaccine against Ebola took five years and there is reason to believe that the COVID-19 vaccine(s) will be developed even faster than that. We need to keep in mind that vaccine discovery and early development only require 30% of all the work and time required to bring a vaccine to the end user.

One challenge for the developers of COVID-19 vaccine(s) is that the elderly are most susceptible to the infection and carry a particularly high risk for severe or lethal disease. Due to immunosenescence, the elderly are notoriously difficult to immunize, requiring higher doses or particular immunization schemes in order to generate a protective immune response. Studies in mice indicate that older animals are also more likely to develop immunopathology upon vaccination.

A lesson that should have been learned already following the SARS outbreak is that more enzootic viruses will jump from their animal reservoirs to humans. Given the fact that not too many different viruses can cause severe and potentially deadly respiratory infections we should not stop our efforts once a SARS-CoV-2 specific vaccine is available. Instead, efforts should

be made to develop a vaccine platform that can quickly be adapted to newly emerging coronaviruses. We do not know the date of the next outbreak but we can be sure that SARS-CoV-2 is not the last coronavirus humankind has to confront.

References

Agrawal AS, Tao X, Algaissi A, et al. **Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus.** Hum Vaccin Immunother. 2016 Sep;12(9):2351-6. PubMed: https://pubmed.gov/27269431. Full-text: https://doi.org/10.1080/21645515.2016.1177688

Amanat F, Krammer F. **SARS-CoV-2 Vaccines: Status Report.** Immunity. 2020 Apr 14;52(4):583-589. PubMed: https://pubmed.gov/32259480. Full-text: https://doi.org/10.1016/j.immuni.2020.03.007

Bloch EM, Shoham S, Casadevall A, et al. **Deployment of convalescent plasma for the prevention and treatment of COVID-19.** J Clin Invest. 2020 Apr 7. pii: 138745. PubMed: https://pubmed.gov/32254064. Full-text: https://doi.org/138745

Bolles M, Deming D, Long K, 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 Dec;85(23):12201-15. PubMed: https://pubmed.gov/21937658. Full-text: https://doi.org/10.1128/JVI.06048-11

Casadevall A, Dadachova E, Pirofski LA. **Passive antibody therapy for infectious diseases.** Nat Rev Microbiol. 2004 Sep;2(9):695-703. PubMed: https://pubmed.gov/15372080. Full-text: https://doi.org/10.1038/nrmicro974

Castilow EM, Olson MR, Varga SM. **Understanding respiratory syncytial virus (RSV) vaccine-enhanced disease.** Immunol Res. 2007;39(1-3):225-39. PubMed: https://pubmed.gov/17917067. Full-text: https://doi.org/10.1007/s12026-007-0071-6

Chun S, Chung CR, Ha YE, et al. **Possible Transfusion-Related Acute Lung Injury Following Convalescent Plasma Transfusion in a Patient With Middle East Respiratory Syndrome.** Ann Lab Med. 2016 Jul;36(4):393-5. PubMed: https://pubmed.gov/27139619. Full-text: https://doi.org/10.3343/alm.2016.36.4.393

Deming D, Sheahan T, Heise M, et al. **Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants.** PLoS Med. 2006 Dec;3(12):e525. PubMed: https://pubmed.gov/17194199. Full-text: https://doi.org/10.1371/journal.pmed.0030525

Honda-Okubo Y, Barnard D, Ong CH, Peng BH, Tseng CT, Petrovsky N. **Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic immunopathology.** J Virol. 2015 Mar;89(6):2995-3007. PubMed: https://pubmed.gov/25520500. Full-text: https://doi.org/10.1128/JVI.02980-14

Houser KV, Broadbent AJ, Gretebeck L, et al. **Enhanced inflammation in New Zealand white rabbits when MERS-CoV reinfection occurs in the absence of neutralizing antibody.** PLoS Pathog. 2017 Aug 17;13(8):e1006565. PubMed: https://pubmed.gov/28817732. Full-text: https://doi.org/10.1371/journal.ppat.1006565

Iwata-Yoshikawa N, Uda A, Suzuki T, et al. **Effects of Toll-like receptor stimulation on eosinophilic infiltration in lungs of BALB/c mice immunized with UV-inactivated severe acute respiratory syndrome-related coronavirus vaccine.** J Virol. 2014 Aug;88(15):8597-614. PubMed: https://pubmed.gov/24850731. Full-text: https://doi.org/10.1128/JVI.00983-14

Jaume M, Yip MS, Cheung CY, et al. **Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcgammaR pathway.** J Virol. 2011 Oct;85(20):10582-97. PubMed: https://pubmed.gov/21775467. Full-text: https://doi.org/10.1128/JVI.00671-11

Kim YI, Kim SG, Kim SM, et al. **Infection and Rapid Transmission of SARS-CoV-2 in Ferrets.** Cell Host Microbe. 2020 Apr 5. pii: S1931-3128(20)30187-6. PubMed: https://pubmed.gov/32259477. Full-text: https://doi.org/10.1016/j.chom.2020.03.023

Liu L, Wei Q, Lin Q, et al. **Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection.** JCI Insight. 2019 Feb 21;4(4). pii: 123158. PubMed: https://pubmed.gov/30830861. Full-text: https://doi.org/123158

Lokugamage KG, Yoshikawa-Iwata N, Ito N, et al. **Chimeric coronavirus-like particles carrying severe acute respiratory syndrome coronavirus (SCoV) S protein protect mice against challenge with SCoV.** Vaccine. 2008 Feb 6;26(6):797-808. PubMed: https://pubmed.gov/18191004. Full-text: https://doi.org/10.1016/j.vaccine.2007.11.092

Mair-Jenkins J, Saavedra-Campos M, Baillie JK, et al. **The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis.** J Infect Dis. 2015 Jan 1;211(1):80-90. PubMed: https://pubmed.gov/25030060. Full-text: https://doi.org/10.1093/infdis/jiu396

Marston HD, Paules CI, Fauci AS. **Monoclonal Antibodies for Emerging Infectious Diseases - Borrowing from History.** N Engl J Med. 2018 Apr 19;378(16):1469-1472. PubMed: https://pubmed.gov/29513615. Full-text: https://doi.org/10.1056/NEJMp1802256

Openshaw PJ, Culley FJ, Olszewska W. **Immunopathogenesis of vaccine-enhanced RSV disease.** Vaccine. 2001 Oct 15;20 Suppl 1:S27-31. PubMed: https://pubmed.gov/11587806. Full-text: https://doi.org/10.1016/s0264-410x(01)00301-2

Pedersen NC, Black JW. **Attempted immunization of cats against feline infectious peritonitis, using avirulent live virus or sublethal amounts of virulent virus.** Am J Vet Res. 1983 Feb;44(2):229-34 PubMed: https://pubmed.gov/6299143.

Perlman S, Dandekar AA. **Immunopathogenesis of coronavirus infections: implications for SARS.** Nat Rev Immunol. 2005 Dec;5(12):917-27. PubMed: https://pubmed.gov/16322745. Full-text: https://doi.org/10.1038/nri1732

Tseng CT, Sbrana E, Iwata-Yoshikawa N, et al. **Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus.** PLoS One. 2012;7(4):e35421. PubMed: https://pubmed.gov/22536382. Full-text: https://doi.org/10.1371/journal.pone.0035421

Vennema H, de Groot RJ, Harbour DA, et al. **Immunogenicity of recombinant feline infectious peritonitis virus spike protein in mice and kittens.** Adv Exp Med Biol. 1990;276:217-22. PubMed: https://pubmed.gov/1966406. Full-text: https://doi.org/10.1007/978-1-4684-5823-7_30

Wang Q, Zhang L, Kuwahara K, et al. **Immunodominant SARS Coronavirus Epitopes in Humans Elicited both Enhancing and Neutralizing Effects on Infection in Non-human Primates.** ACS Infect Dis. 2016 May 13;2(5):361-76. PubMed: https://pubmed.gov/27627203. Full-text: https://doi.org/10.1021/acsinfecdis.6b00006

Weingartl H, Czub M, Czub S, et al. **Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets.** J Virol. 2004 Nov;78(22):12672-6. PubMed: https://pubmed.gov/15507655. Full-text: https://doi.org/10.1128/JVI.78.22.12672-12676.2004

Weiss RC, Scott FW. **Antibody-mediated enhancement of disease in feline infectious peritonitis: comparisons with dengue hemorrhagic fever.** Comp Immunol Microbiol Infect Dis. 1981;4(2):175-89. PubMed: https://pubmed.gov/6754243. Full-text: https://doi.org/10.1016/0147-9571(81)90003-5

Yang ZY, Werner HC, Kong WP, et al. **Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses.** Proc Natl Acad Sci U S A. 2005 Jan 18;102(3):797-801. PubMed: https://pubmed.gov/15642942. Full-text: https://doi.org/10.1073/pnas.0409065102

Yasui F, Kai C, Kitabatake M, et al. **Prior immunization with severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV.** J Immunol. 2008 Nov 1;181(9):6337-48. PubMed: https://pubmed.gov/18941225. Full-text: https://doi.org/10.4049/jimmunol.181.9.6337

5. Diagnostic Tests and Procedures

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Diagnosis

Rapid identification and isolation of infected individuals is crucial. Diagnosis is made using clinical, laboratory and radiological features. As symptoms and radiological findings of COVID-19 are non-specific, SARS-CoV-2 infection has to be confirmed by nucleic acid-based polymerase chain reaction (PCR), amplifying a specific genetic sequence in the virus. Within a few days after the first cases were published, a validated diagnostic workflow for SARS-CoV-2 was presented (Corman 2020), demonstrating the enormous response capacity achieved through coordination of academic and public laboratories in national and European research networks.

There is an interim guidance for laboratory testing for coronavirus disease (COVID-19) suspected human cases, published by WHO on March 19, 2020 (WHO 2020). Several comprehensive up-to-date reviews of laboratory techniques in diagnosing SARS-CoV-2 have been published recently (Chen 2020, Löffelholz 2020).

In settings with limited resources, no testing capacity should be wasted. Importantly, patients should only be tested if a positive test results in imperative action. This is not the case in the following examples:

- Young people who had contact with an infected person a few days earlier, have mild or moderate symptoms and live alone. They do not need PCR testing, even if they get fever. They'll remain in home quarantine, on sick leave if necessary, at

least 14 days after the onset of symptoms. A test would only be useful to clarify whether they can work in a hospital or other health care facilities after quarantine. Some authorities require at least one negative test (nasopharyngeal) before starting work again (in addition to at least 48 hours of being symptom-free).

- A couple returning from an epidemic hotspot and feel a slight scratch in their throats. As they should remain in quarantine anyway, again, no testing is needed.
- A family of four with typical COVID-19 symptoms. Testing only one (symptomatic) person is sufficient. If the test is positive, it is not necessary to test the other household contacts – as long as they stay at home.

These decisions are not easy to commnicate, particularly to fearful and worried patients.

In other situations, however, a test must be immediately carried out and repeated if necessary, especially for medical professionals with symptoms, but also, for example, in nursing homes, in order to detect an outbreak as quickly as possible.

Even though there are constantly updated recommendations by authorities and institutions of the country's health system about who should be tested by whom and when: they are constantly changing and have to be constantly adapted to the local epidemiological situation. With decreasing infection rates and increasing test capacities, more patients will certainly be able to be tested in the future, and the indication for a test will be expanded.

Specimen collection

SARS-CoV-2 can be detected in different tissues and body fluids. In a study on 1,070 specimens collected from 205 patients with COVID-19, bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15; 93%), followed by sputum (72 of 104; 72%), nasal swabs (5 of 8; 63%), fibrobronchoscopy brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), feces (44 of 153; 29%), and blood (3 of 307; 1%). None of the 72 urine specimens tested positive (Wang 2020). The virus was also not found in the vaginal fluid of 10 women with COVID-19 (Saito 2020). It was also not found in sperm and breast milk (Song 2020, Scorzolini 2020). On rare occasions, however, the virus may be detected in tears and conjunctival secretions (Xia 2020).

Samples can also be taken from sputum (if producible), endotracheal aspirate, or bronchoalveolar lavage. It is likely that lower respiratory samples are more sensitive than nasopharyngeal swabs. Especially in seriously ill patients, there is often more virus in the lower than in the upper respiratory tract (Huang 2020). However, there is always a high risk of "aerosolization" and thus the risk that staff members become infected.

However, viral replication of SARS-CoV-2 is very high in upper respiratory tract tissues which is in contrast to SARS-CoV (Wölfel 2020). According to WHO, respiratory material for PCR should be collected from upper respiratory specimens (nasopharyngeal and oropharyngeal swab or wash) in ambulatory patients (WHO 2020). It is preferred to collect specimens from both nasopharyngeal and oropharyngeal swabs which can be combined in the same tube.

Nasopharyngeal swabs – practical issues

It is important to carry out the swab correctly. Both nasopharynx and oropharyngeal swabs have a number of error options that all can lead to false negative results. In addition, protective measures must be taken in order not to endanger the examiner. Every swab carries a high risk of infection! Respiratory protection, protective glasses, gowns and gloves are required. The correct putting on and taking off of the protective clothing should be practiced! Many mistakes occur even when a protective mask is removed.

For the smear, the patient should sit on a chair and put his head slightly back. The examiner should stand at a slightly offset position in order to avoid a possible cough drop. Tell the patient that it can be uncomfortable for a short time. Swabs should be used that are suitable for virus detection and have the most flexible plastic shaft possible. Wooden sticks can inactivate viruses and pose a high risk of injury. The swab should be held between thumb and forefinger, like a pencil, so the end should not touch anything. The posterior wall of the nasopharynx is often reached after 5-7 cm, indicated by a slight resistance. "Nose popules" is not enough! Touching the teeth and tongue should be avoided when taking a throat swab; the swab should be removed from the back wall, directly next to the uvula. Caution with the gag reflex! There is a wealth of practical videos on the internet for the correct execution of the swabs. After appropriate instruction, many patients can perform the swabs themselves.

We have established swabs for patients who are able to do this (most of them!) at home. A courier with the tubes is sent directly to the patient's home, and the courier places the tubes in front of the door. Direct contact between patient and courier should

be avoided. The swab tubes should not be touched by the courier (either put them directly in a bag or collect them with an inverted bag) and should be brought back directly (no mailing!). This requires prior, precise instruction, but is usually quite feasible.

The swabs can be stored dry or in a small amount of NaCl solution; if necessary, this should be clarified with the laboratory beforehand. Quick PCR examination is important, preferably on the same day if possible. Heat is not favorable. In a small study, samples were inactivated by incubation in a water bath at 56°C for 30 minutes. 7/15 samples with low viral values converted to false negative. Longer storage also led to false negative results (Pan 2020).

Lower respiratory specimens may include sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease. However, a high risk of aerosolization should be considered (adhere strictly to infection prevention and control procedures). Additional clinical specimens may be collected as COVID-19 virus has been detected in blood and stool (see below).

Gathering specimens from nasopharyngeal and throat swabs can cause discomfort for patients and put health-care workers at risk. The virus is present in saliva and several studies have shown that posterior oropharyngeal (deep throat) saliva samples are feasible and more acceptable to patients and healthcare workers (To 2020, Yu 2020). Throat washing may be used for monitoring due to its non-invasiveness and reliability. Throat washing was harvested by asking patients to oscillate over the posterior pharyngeal wall with 20 ml sterile normal saline. After 5-10 seconds, they spit out the normal saline from their throat to a sterile container. In 24 paired throat washings and nasopha-

ryngeal swabs specimens, the positive testing rate of throat washing was much higher than that of swabs (Guo 2020).

Although no cases of transmission via fecal-oral route have yet been reported, there is also increasing evidence that SARS-CoV-2 is actively replicating in the gastrointestinal tract. A larger study from Zhuhai, China showed prolonged presence of SARS-CoV-2 viral RNA in fecal samples. Of the 41 (55%) of 74 patients with fecal samples that were positive for SARS-CoV-2 RNA, respiratory samples remained positive for SARS-CoV-2 RNA for a mean of 16.7 days and fecal samples remained positive for a mean of 27.9 days after first symptom onset (Wu 2020). In 22/133 patients, SARS–CoV-2 was still detected in the sputum or feces (up to 39 and 13 days, respectively) after pharyngeal swabs became negative (Chen 2020). These studies have raised concerns about whether patients with negative pharyngeal swabs are truly virus-free, or sampling of additional body sites is needed. However, the clinical relevance of these finding remains unclear and there is one study that did not detect infectious virus from stool samples, despite having high virus RNA concentrations (Wölfel 2020).

SARS-CoV-2 is rarely detected in blood (Wang 2020, Wölfel 2020). What about transmission risk associated with transfusions? In a screening study of 2,430 blood donations in Wuhan, plasma samples were found positive for viral RNA from 4 asymptomatic donors (Kwon 2020). Another study from Korea found seven asymptomatic blood donors who were later identified as COVID-19 confirmed cases. None of 9 recipients of platelets or red blood cell transfusions tested positive for SARS-CoV-2 RNA. Transfusion transmission of SARS-CoV-2 was considered to be unlikely (Chang 2020). As with feces, it remains unclear whether detectable RNA in the blood signifies infectivity.

PCR

Several different qPCR-based detection kits are available as labs worldwide have customized their PCR tests for SARS-CoV-2, using different primers targeting different sections of the virus's genetic sequence. A review of different assays and diagnostic devices was recently published (Löffelholz 2020). A protocol for real-time (RT)-PCR assays for the detection of SARS-CoV-2 for two RdRp targets (IP2 and IP4) is described at 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

Novel real-time RT-PCR assays targeting the RNA-dependent RNA polymerase (RdRp)/helicase, spike and nucleocapsid genes of SARS-CoV-2 may help to improve the laboratory diagnosis of COVID-19. Compared to the reported RdRp-P2 assay which is used in most European laboratories, these assays do not cross-react with SARS-CoV in cell culture and may be more sensitive and specific (Chan 2020).

If not, the limits of detection of six commercial kits differ substantially (up to 16-fold difference), with the poorest limits likely leading to false-negative results when RT–PCR were used to detect SARS-CoV-2 infection (Wang 2020). According to the authors, manufacturers should analyze the existing problems according to the clinical application and further improve their products.

Qualitative PCR

A qualitative PCR ("positive or negative") is usually sufficient in routine diagnostics. Quantification of viral RNA is currently (still) only of academic interest. False positive results are rare. The main problem of any qualitative PCR is above all the false

negative results. They have many causes. Incorrect smears are particularly common, but laboratory errors also occur.

Several studies have shown that asymptomatic patients also have positive PCR results and can transmit the virus (Bai 2020, Cereda 2020, Rothe 2020). Viral shedding may begin 2 to 3 days before the appearance of the first symptoms. Analyzing a total of 414 throat swabs in 94 patients, the highest viral load in throat swabs was found at the time of symptom onset. Infectiousness started from 2.3 days (95% CI, 0.8–3.0 days) before symptom onset and peaked at 0.7 days before symptom onset (He 2020). Infectiousness was estimated to decline quickly within 7 days.

In a cohort of 113 symptomatic patients, the median duration of detection of SARS-CoV-2 RNA was 17 days (interquartiles 13-22 days), measured from the onset of the disease. In some patients, the PCR was positive even longer: male gender and a severe course (invasive mechanical ventilation) were independent risk factors for prolonged shedding (Xu 2020).

Recent reports from patients have repeatedly gained much media attraction, showing positive results after repeated negative PCR and clinical recovery (Lan 2020, Xiao 2020, Yuan 2020). These studies raise the question of re-activation or re-infection of COVID-19 (see below, clinical chapter). Currently, the results are much more likely due to methodological problems (Li 2020). At low virus levels, especially during the last days of an infection, the viral load can fluctuate and sometimes be detectable, sometimes not (Wölfel 2020). Reactivation, and also a rapid reinfection would be very unusual for coronaviruses.

Quantification of viral load

Several studies have evaluated the SARS-CoV-2 viral load in different specimens. In a small prospective study, the viral load in nasal and throat swabs obtained from 17 symptomatic

patients was analyzed in relation to day-of-onset of any symptoms (Zou 2020). Of note, the viral load detected in the asymptomatic patients was similar to that in the symptomatic patients, which suggests the transmission potential of asymptomatic or minimally symptomatic patients.

In another study on 82 infected individuals, the viral loads in throat swab and sputum samples peaked at around 5–6 days after symptom onset, ranging from around 79,900 copies/ml in the throat to 752,000 copies per mL in sputum (Pan 2020). In a study on oropharyngeal saliva samples, unlike SARS, patients with COVID-19 had the highest viral load near presentation, which could account for the fast-spreading nature of this epidemic (To 2020). The median viral load in posterior oropharyngeal saliva or other respiratory specimens at presentation was 5.2 $\log_{10}$ copies per mL (IQR 4.1-7.0) in this study. In a total of 323 samples from 76 patients, the average viral load in sputum (17,429 copies/test) was significantly higher than in throat swabs (2,552 copies) and nasal swabs (651 copies). Viral load was higher in the early and progressive stages than in the recovery stage (Yu 2020). According to a recently published study, viral shedding may already begin 2-3 days before the appearance of the first symptoms and the infectiousness profile may more closely resemble that of influenza than of SARS (He 2020).

Higher viral loads might be associated with severe clinical outcomes. In a study evaluating serial samples from 21 mild and 10 severe cases (Liu 2020), mild cases were found to have an early viral clearance, with 90% of these patients repeatedly testing negative on RT-PCR by day 10 post-onset. By contrast, all severe cases still tested positive at or beyond day 10 post-onset. However, large and prospective trials are needed to evaluate the

role of SARS-CoV-2 viral load as a marker for assessing disease severity and prognosis.

Diagnosis in the setting of shortage of PCR test kits

There is no doubt that the overall goal must be to detect as many infections as possible. However, in many countries, a shortage of supply test kits does not meet the need of a growing infected population. Thus, pooled samples are often used to save material. Several samples are examined together. Only when such a pooled sample is positive, will the samples be examined individually.

Some studies have also investigated whether the diagnosis in high prevalence periods and countries cannot be made without PCR detection if necessary. A large retrospective case-control study from Singapore has evaluated predictors for SARS-CoV-2 infection, using exposure risk factors, demographic variables, clinical findings and clinical test results (Sun 2020). Even in the absence of exposure risk factors and/or radiologic evidence of pneumonia, clinical findings and tests can identify subjects at high risk of COVID-19. Low leukocytes, low lymphocytes, higher body temperature, higher respiratory rate, gastrointestinal symptoms and decreased sputum production were strongly associated with a positive SARS-CoV-2 test. However, those preliminary prediction models are sensitive to the local epidemiological context and phase of the global outbreak. They make only sense during times of high incidence. In other words: if I see a patient during the peak of an epidemic, presenting with fever, cough, shortness of breath and lymphopenia, I can be almost sure that this patient suffers from COVID-19. During phases, when the incidence is lower, these models do not make sense. There is no doubt that the nucleic acid test or genetic sequencing serves as the gold standard method for confirmation of infection. Whenever PCR is available, PCR should be performed.

Serology

Detection of past viral infections by looking for antibodies an infected person has produced will be among the most important goals in the fight against the COVID-19 pandemic (Brief review: Petherick 2020). Antibody testing is multipurpose: these serological assays are of critical importance to determine seroprevalence, previous exposure and identify highly reactive human donors for the generation of convalescent serum as therapeutic. They will also support contact tracing and screening of health care workers to identify those who are already immune. How many people really got infected, in how many did the virus escape the PCR diagnosis, and for what reasons, how many patients are asymptomatic, and what is the real mortality rate in a defined population? Only with comprehensive serology testing (and well-planned epidemiological studies) will we be able to answer these questions and reduce the ubiquitous undisclosed number in the current calculations. Several investigations are already underway in a wide variety of locations worldwide.

But, outside clinical studies: who should be tested now? Testing actually makes no sense for patients with a previous, proven COVID-19 disease. However, it can still be done if, for example, you want to validate a test. In addition to those involved in health care or working in other professions with a high risk of transmission, such testing can also be useful in order to identify possible contact persons retrospectively.

Several groups are working towards these tests (Amanat 2020), some of them are already commercially available.

Antibody testing usually focuses on antigens (proteins). In the case of SARS-CoV-2, different Enzyme-Linked Immunosorbent Assay (ELISA) kits based on recombinant nucleocapsid protein and spike protein are used (Löffelholz 2020). The SARS-CoV-2

spike protein seems to be the best target. However, which part of the spike protein to use is less obvious and there is a lot hanging on the uniqueness of the spike protein. The more unique it is, the lower the odds of cross-reactivity with other coronaviruses—false positives resulting from immunity to other coronaviruses. Cross reactivity to other coronaviruses can be challenging. So called confirmation tests (usually neutralization tests) can be used to reduce false positive testings.

Even with a very high specificity of 99% and above, especially in low-prevalence areas, the informative value is limited and a high rate of false positive tests can be assumed. An example: With a specificity of 99%, it is expected that one test out of 100 is positive. In a high prevalence setting, this is less relevant. However, if a person is tested in a low prevalence setting, the likelihood that a positive test is really positive (the positive predictive value, i.e. the number of really positive tests divided by the number of all positive tests) is low. In a population with a given prevalence of 1%, the predictive value would only be 50%! Current estimates from Iceland, a well-defined but unselected population, still have shown a relatively constant rate of around 0.8% in March 2020 (Gudbjartsson 2020). Even in apparently more severely affected countries, the infection rates are only slightly higher. If we assume an infection number of 133,800 (April 17) for Germany, a country with one of the world's largest number of infections, and assume that the number of undetected infections is about 5 times as high, then the prevalence in Germany is overall still below 1%. Almost every hundredth is infected, every second positive test would be false positive, even with a specificity of 99%. General antibody screening in the population will therefore produce a fairly high rate of false positive tests.

Of note, serologic responses to coronaviruses are only transient. Antibodies to other human, seasonal coronaviruses may disappear even after a few months. Preliminary data suggest that the profile of antibodies to SARS-CoV-2 is similar to SARS-CoV (Xiao 2020). For SARS-CoV, antibodies were not detected within the first 7 days of illness, but IgG titre increased dramatically on day 15, reaching a peak on day 60, and remained high until day 180 from when it declined gradually until day 720. IgM was detected on day 15 and rapidly reached a peak, then declined gradually until it was undetectable on day 180 (Mo 2006). As with other viruses, IgM antibodies occur somewhat earlier than IgG antibodies which are more specific. IgA antibodies are relatively sensitive but less specific (Okba 2020).

The first larger study on the host humoral response against SARS-CoV-2 has shown that humoral response to SARS-CoV-2 can aid to the diagnosis of COVID-19, including subclinical cases (Guo 2020). In this study, IgA, IgM and IgG response using an ELISA-based assay on the recombinant viral nucleocapsid protein was analyzed in 208 plasma samples from 82 confirmed and 58 probable cases (Guo 2020). The median duration of IgM and IgA antibody detection were 5 days (IQR 3-6), while IgG was detected on day 14 (IQR 10-18) after symptom onset, with a positive rate of 85.4%, 92.7% and 77.9% respectively. The detection efficiency by IgM ELISA was higher than that of PCR after 5.5 days of onset of symptoms. In another study of 173 patients, the seroconversion rates (median time) for IgM and IgG were 82.7% (12 days) and 64.7% (14 days), respectively. A higher titer of antibodies was independently associated with severe diseases (Zhao 2020). It will be seen in the coming months how the human antibody response to SARS-CoV-2 evolves over time and how this response and titres correlate with immunity. It is also conceivable that in some patients (e.g. those with immunodeficiency), the antibody response remains reduced.

Radiology

Computed tomography

Computed tomography (CT) can play a role in both diagnosing and assessment of disease extent and follow-up. Chest CT has a relatively high sensitivity for diagnosis of COVID-19 (Ai 2020, Fang 2020). However, around half of patients may have a normal CT during the first 1-2 days after symptom onset (Bernheim 2020). On the other hand, it became clear very early in the current pandemic that a considerable proportion of subclinical patients (scans done before symptom onset) may already have pathological CT findings (Chan 2020, Shi 2020). In some of these patients showing pathological CT findings evident for pneumonia PCR in nasopharyngeal swabs was still negative (Xu 2020). On the other hand, half of the patients who later develop CT morphologically visible pneumonia can still have a normal CT in the first 1-2 days after the symptoms appear (Bernheim 2020).

However, one should not overestimate the value of chest CT. The recommendation by some Chinese researchers to include CT as an integral part in the diagnosis of COVID-19 has led to harsh criticism, especially from experts in Western countries. The Chinese studies have been exposed to significant errors and shortcomings. In view of the high effort and also because of the risk of infection for the staff, many experts strictly reject the general CT screening in SARS-CoV-2 infected patients or in those with suspicion (Hope 2020, Raptis 2020). According to the recommendation of the British Radiology Society, which made attempts to incorporate CT into diagnostic algorithms for COVID-19 diagnostics, the value of CT remains unclear – even if a PCR is negative or not available (Nair 2020, Rodrigues 2020). A chest CT should only be performed if complications or differential diagnoses are considered (Raptis 2020).

In blinded studies, radiologists from China and the United States have attempted to differentiate COVID-19 pneumonia from other viral pneumonia. The specificity was quite high, the sensitivity nuch lower (Bai 2020). A recent metaanalysis found a high sensitivity but low specificity (Kim 2020). The sensitivity of CT was affected by the distribution of disease severity, the proportion of patients with comorbidities, and the proportion of asymptomatic patients. In areas with low prevalence, chest CT had a low positive predictive value (1.5-30.7%).

If pathological, images usually show bilateral involvement, with multiple patchy or ground-glass opacities (GGO) with subpleural distribution in multiple bilateral lobes. Lesions may display significant overlap with those of SARS and MERS (Hosseiny 2020).

A systematic review of imaging findings in 919 patients found bilateral multilobar GGO with a peripheral or posterior distribution, mainly in the lower lobes and less frequently within the right middle lobe as the most common feature (Salehi 2020). In this review, atypical initial imaging presentation of consolidative opacities superimposed on GGO were found in a smaller number of cases, mainly in the elderly population. Septal thickening, bronchiectasis, pleural thickening, and subpleural involvement were less common, mainly in the later stages of the disease. Pleural effusion, pericardial effusion, lymphadenopathy, cavitation, CT halo sign, and pneumothorax were uncommon (Salehi 2020).

The evolution of the disease on CT is not well understood. However, with a longer time after the onset of symptoms, CT findings are more frequent, including consolidation, bilateral and peripheral disease, greater total lung involvement, linear opacities, “crazy-paving” pattern and the “reverse halo” sign (Bernheim 2020). Some experts have proposed that imaging can be sorted into four different phases (Li 2020). In the early phase,

multiple small patchy shadows and interstitial changes emerge. In the progressive phase, the lesions increase and enlarge, developing into multiple GGOs as well as infiltrating consolidation in both lungs. In the severe phase, massive pulmonary consolidations and "white lungs" are seen, but pleural effusion is rare. In the dissipative phase, the GGOs and pulmonary consolidations were completely absorbed, and the lesions began to change into fibrosis.

In a longitudinal study analyzing 366 serial CT scans in 90 patients with COVID-19 pneumonia, the extent of lung abnormalities progressed rapidly and peaked during illness days 6-11 (Wang 2020). The predominant pattern of abnormalities after symptom onset in this study was ground-glass opacity (45-62%). As pneumonia progresses, areas of lesions enlarge and developed into diffuse consolidations in both lungs within a few days (Guan 2020).

Most patients discharged had residual disease on final CT scans (Wang 2020). Studies with longer follow-up are needed to evaluate long-term or permanent lung damage including fibrosis, as is seen with SARS and MERS infections. Pulmonary fibrosis is expected to be the main factor leading to pulmonary dysfunction and decline of quality of life in COVID-19 survivors after recovery. More research is needed into the correlation of CT findings with clinical severity and progression, the predictive value of baseline CT or temporal changes for disease outcome, and the sequelae of acute lung injury induced by COVID-19 (Lee 2020).

Of note, chest CT is not recommended in all COVID-19 patients, especially in those who are well enough to be sent home or those with only short symptomatic times (< 2 days). In case of COVID-19, a large number of patients with infection or suspected infection swarm into the hospital. Consequently, the examination workload of the radiology department increases sharply. Because the transmission route of SARS-CoV-2 is through respira-

tory droplets and close contact transmission, unnecessary CT scan should be avoided. An overview of the prevention and control of the COVID-19 epidemic in the radiology department is given by An et al.

Ultrasound and PET

Some experts have postulated that lung ultrasound (LUS) may be helpful, since it can allow the concomitant execution of clinical examination and lung imaging at the bedside by the same doctor (Buonsenso 2020, Soldati 2020). Potential advantages of LUS include portability, bedside evaluation, safety and possibility of repeating the examination during follow-up. Experience especially from Italy with lung ultrasound as a bedside tool has improved evaluation of lung involvement, and may also reduce the use of chest x-rays and CT. A point scoring system is employed by region and ultrasound pattern (Vetrugno 2020). However, the diagnostic and prognostic role of LUS in COVID-19 is uncertain.

Whether there is any potential clinical utility of other imaging techniques such as 18F-FDG PET/CT imaging in the differential diagnosis of complex cases also remains unclear (Deng 2020, Qui 2020).

References

Ai T, Yang Z, Hou H, 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 Feb 26:200642. PubMed: https://pubmed.gov/32101510. Full-text: https://doi.org/10.1148/radiol.2020200642

Amanat F, Nguyen T, Chromikova V, et al. **Serological assay to detect SARS-CoV-2 seroconversion in humans.** Full-text: https://doi.org/10.1101/2020.03.17.20037713

An P, Ye Y, Chen M, Chen Y, Fan W, Wang Y. **Management strategy of novel coronavirus (COVID-19) pneumonia in the radiology department: a Chinese experience.** Diagn Interv Radiol. 2020 Mar 25. PubMed:

https://pubmed.gov/32209526. Full-text: https://doi.org/10.5152/dir.2020.20167

Bai HX, Hsieh B, Xiong Z, et al. **Performance of radiologists in differentiating COVID-19 from viral pneumonia on chest CT**. Radiology. 2020:200823. [PMID: 32155105] doi:10.1148/radiol.2020200823

Bai HX, Hsieh B, Xiong Z, et al. **Performance of radiologists in differentiating COVID-19 from viral pneumonia on chest CT.** Radiology. 2020 Mar 10:200823. Full-text: https://doi.org/10.1148/radiol.2020200823

Bai Y, Yao L, Wei T, et al. **Presumed Asymptomatic Carrier Transmission of COVID-19.** JAMA. 2020 Feb 21. PubMed: https://pubmed.gov/32083643. Full-text: https://doi.org/10.1001/jama.2020.2565

Bernheim A, Mei X, Huang M, Yang Y, et al. **Chest CT Findings in Coronavirus Disease-19 (COVID-19): Relationship to Duration of Infection.** Radiology. 2020 Feb 20:200463. https://doi.org/10.1148/radiol.2020200463.

Buonsenso D, Pata D, Chiaretti A. **COVID-19 outbreak: less stethoscope, more ultrasound.** Lancet Respir Med. 2020 Mar 20. PubMed: https://pubmed.gov/32203708. Full-text: https://doi.org/10.1016/S2213-2600(20)30120-X

Cereda D, Tirani M, Rovida F, et al. **The early phase of the COVID-19 outbreak in Lombardy, Italy.** https://arxiv.org/ftp/arxiv/papers/2003/2003.09320.pdf. Accessed 27 March 2020.

Chan JF, Yip CC, To KK, et al. **Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-polymerase chain reaction assay validated in vitro and with clinical specimens.** J Clin Microbiol. 2020 Mar 4. PubMed: https://pubmed.gov/32132196. Full-text: https://doi.org/10.1128/JCM.00310-20

Chan JF, Yuan S, Kok KH, et al. **A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster.** Lancet. 2020 Feb 15;395(10223):514-523. PubMed: https://pubmed.gov/31986261. Full-text: https://doi.org/10.1016/S0140-6736(20)30154-9

Chang L, Zhao L, Gong H, Wang L, Wang L. **Severe Acute Respiratory Syndrome Coronavirus 2 RNA Detected in Blood Donations.** Emerg Infect Dis. 2020 Apr 3;26(7). PubMed: https://pubmed.gov/32243255. Full-text: https://doi.org/10.3201/eid2607.200839

Chen C, Gao G, Xu Y, et al. **SARS-CoV-2–Positive Sputum and Feces After Conversion of Pharyngeal Samples in Patients With COVID-19.** Ann Intern Med. 2020, March 30. Full-text: https://annals.org/aim/fullarticle/2764036/sars-cov-2-positive-sputum-feces-after-conversion-pharyngeal-samples

Cheng MP, Papenburg J, Desjardins M, et al. **Diagnostic Testing for Severe Acute Respiratory Syndrome-Related Coronavirus-2: A Narrative Review.** Ann Intern Med. 2020 Apr 13. pii: 2764737. PubMed:

https://pubmed.gov/32282894. Full-text: https://doi.org/10.7326/M20-1301.

Corman VM, Landt O, Kaiser M, et al. **Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR**. Euro Surveill. 2020 Jan;25(3). PubMed: https://pubmed.gov/31992387. Full-text: https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

Deng Y, Lei L, Chen Y, Zhang W. **The potential added value of FDG PET/CT for COVID-19 pneumonia.** Eur J Nucl Med Mol Imaging. 2020 Mar 21. PubMed: https://pubmed.gov/32198615. Full-text: https://doi.org/10.1007/s00259-020-04767-1

Fang Y, Zhang H, Xie J, et al. **Sensitivity of Chest CT for COVID-19: Comparison to RT-PCR**. Radiology. 2020 Feb 19:200432. PubMed: https://pubmed.gov/32073353. Full-text: https://doi.org/10.1148/radiol.2020200432

Guan W, Liu J, Yu C. **CT Findings of Coronavirus Disease (COVID-19) Severe Pneumonia.** AJR Am J Roentgenol. 2020 Mar 24:W1-W2. PubMed: https://pubmed.gov/32208010. Full-text: https://doi.org/10.2214/AJR.20.23035

Gudbjartsson DF, Helgason A, Jonsson H, et al. **Spread of SARS-CoV-2 in the Icelandic Population**. N Engl J Med. 2020 Apr 14. PubMed: https://pubmed.gov/32289214. Full-text: https://doi.org/10.1056/NEJMoa2006100.

Guo L, Ren L, Yang S, et al. **Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19).** Clin Infect Dis. 2020 Mar 21. PubMed: https://pubmed.gov/32198501. Full-text: https://doi.org/10.1093/cid/ciaa310

Guo WL, Jiang Q, Ye F, et al. **Effect of throat washings on detection of 2019 novel coronavirus**. Clin Infect Dis. 2020 Apr 9. pii: 5818370. PubMed: https://pubmed.gov/32271374. Full-text: https://doi.org/10.1093/cid/ciaa416.

Hao W. **Clinical Features of Atypical 2019 Novel Coronavirus Pneumonia with an initially Negative RT-PCR Assay.** J Infect. 2020 Feb 21. PubMed: https://pubmed.gov/32092387. Full-text: https://doi.org/10.1016/j.jinf.2020.02.008

He X, Lau EHY, Wu P, et al. **Temporal dynamics in viral shedding and transmissibility of COVID-19**. Nat Med. 2020 Apr 15. pii: 10.1038/s41591-020-0869-5. PubMed: https://pubmed.gov/32296168. Full-text: https://doi.org/10.1038/s41591-020-0869-5.

Hope MD, Raptis CA, Henry TS. **Chest Computed Tomography for Detection of Coronavirus Disease 2019 (COVID-19): Don't Rush the Science**. Ann Intern Med. 2020 Apr 8. pii: 2764546. PubMed: https://pubmed.gov/32267912. Full-text: https://doi.org/10.7326/M20-1382.

Hosseiny M, Kooraki S, Gholamrezanezhad A, Reddy S, Myers L. **Radiology Perspective of Coronavirus Disease 2019 (COVID-19): Lessons From Severe Acute Respiratory Syndrome and Middle East Respiratory Syndrome.**

AJR Am J Roentgenol. 2020 Feb 28:1-5. PubMed: https://pubmed.gov/32108495. Full-text: https://doi.org/10.2214/AJR.20.22969

Huang Y, Chen S, Yang Z, et al. **SARS-CoV-2 Viral Load in Clinical Samples of Critically Ill Patients**. Am J Respir Crit Care Med. 2020 Apr 15. PubMed: https://pubmed.gov/32293905. Full-text: https://doi.org/10.1164/rccm.202003-0572LE

Kwon SY, Kim EJ, Jung YS, Jang JS, Cho NS. **Post-donation COVID-19 identification in blood donors.** Vox Sang. 2020 Apr 2. PubMed: https://pubmed.gov/32240537. Full-text: https://doi.org/10.1111/vox.12925

Lan L, Xu D, Ye G, et al. Positive RT-PCR Test Results in Patients Recovered From COVID-19. JAMA. 2020 Feb 27. PubMed: https://pubmed.gov/32105304. Full-text: https://doi.org/10.1001/jama.2020.2783

Lee EYP, Ng MY, Khong PL. **COVID-19 pneumonia: what has CT taught us?** Lancet Infect Dis. 2020 Apr;20(4):384-385. PubMed: https://pubmed.gov/32105641. Full-text: https://doi.org/10.1016/S1473-3099(20)30134-1

Li M, Lei P, Zeng B, et al. **Coronavirus Disease (COVID-19): Spectrum of CT Findings and Temporal Progression of the Disease.** Acad Radiol. 2020 Mar 20. pii: S1076-6332(20)30144-6. PubMed: https://pubmed.gov/32204987. Full-text: https://doi.org/10.1016/j.acra.2020.03.003

Li Y, Xia L. **Coronavirus Disease 2019 (COVID-19): Role of Chest CT in Diagnosis and Management.** AJR Am J Roentgenol. 2020 Mar 4:1-7. PubMed: https://pubmed.gov/32130038. Full-text: https://doi.org/10.2214/AJR.20.22954

Li Y, Yao L, Li J, et al. **Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19**. J Med Virol. 2020 Mar 26. PubMed: https://pubmed.gov/32219885. Full-text: https://doi.org/10.1002/jmv.25786

Liu Y, Yan LM, Wan L, et al. **Viral dynamics in mild and severe cases of COVID-19.** Lancet Infect Dis. 2020 Mar 19. PubMed: https://pubmed.gov/32199493. Full-text: https://doi.org/10.1016/S1473-3099(20)30232-2

Loeffelholz MJ, Tang YW. **Laboratory diagnosis of emerging human coronavirus infections - the state of the art.** Emerg Microbes Infect. 2020 Dec;9(1):747-756. PubMed: https://pubmed.gov/32196430. Full-text: https://doi.org/10.1080/22221751.2020.1745095

Mo H, Zeng G, Ren X, et al. **Longitudinal profile of antibodies against SARS-coronavirus in SARS patients and their clinical significance.** Respirology. 2006 Jan;11(1):49-53. PubMed: https://pubmed.gov/16423201. Full-text: https://doi.org/10.1111/j.1440-1843.2006.00783.x

Nair A, Rodrigues JCL, Hare S, et al. **A British Society of Thoracic Imaging statement: considerations in designing local imaging diagnostic algorithms for the COVID-19 pandemic**. Clin Radiol. 2020 May;75(5):329-334.

PubMed: https://pubmed.gov/32265036. Full-text: https://doi.org/10.1016/j.crad.2020.03.008.

Okba NMA, Muller MA, Li W, et al. **Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease 2019 Patients**. Emerg Infect Dis. 2020 Apr 8;26(7). PubMed: https://pubmed.gov/32267220.

Pan Y, Long L, Zhang D, et al. **Potential false-negative nucleic acid testing results for Severe Acute Respiratory Syndrome Coronavirus 2 from thermal inactivation of samples with low viral loads**. Clin Chem. 2020 Apr 4. pii: 5815979. PubMed: https://pubmed.gov/32246822. Full-text: https://doi.org/10.1093/clinchem/hvaa091

Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. **Viral load of SARS-CoV-2 in clinical samples.** Lancet Infect Dis. 2020 Feb 24. PubMed: https://pubmed.gov/32105638. Full-text: https://doi.org/10.1016/S1473-3099(20)30113-4

Petherick A. **Developing antibody tests for SARS-CoV-2.** Lancet. 2020 Apr 4;395(10230):1101-1102. PubMed: https://pubmed.gov/32247384. Full-text: https://doi.org/10.1016/S0140-6736(20)30788-1

Qin C, Liu F, Yen TC, Lan X. **(18)F-FDG PET/CT findings of COVID-19: a series of four highly suspected cases.** Eur J Nucl Med Mol Imaging. 2020 Feb 22. PubMed: https://pubmed.gov/32088847. Full-text: https://doi.org/10.1007/s00259-020-04734-w

Qiu L, Liu X, Xiao M, et al. **SARS-CoV-2 is not detectable in the vaginal fluid of women with severe COVID-19 infection.** Clin Infect Dis 2020, April 2. Full-text: https://doi.org/10.1093/cid/ciaa375

Raptis CA, Hammer MM, Short RG, et al. **Chest CT and Coronavirus Disease (COVID-19): A Critical Review of the Literature to Date**. AJR Am J Roentgenol. 2020 Apr 16:1-4. PubMed: https://pubmed.gov/32298149. Full-text: https://doi.org/10.2214/AJR.20.23202

Rodrigues JCL, Hare SS, Edey A, et al. **An update on COVID-19 for the radiologist - A British society of Thoracic Imaging statement**. Clin Radiol. 2020 May;75(5):323-325. PubMed: https://pubmed.gov/32216962. Full-text: https://doi.org/10.1016/j.crad.2020.03.003

Rothe C, Schunk M, Sothmann P, et al. **Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany.** N Engl J Med. 2020 Mar 5;382(10):970-971. PubMed: https://pubmed.gov/32003551. Full-text: https://doi.org/10.1056/NEJMc2001468

Saito M, Adachi E, Yamayoshi S, et al. **Gargle lavage as a safe and sensitive alternative to swab samples to diagnose COVID-19: a case report in Japan.** Clin Infect Dis. 2020 Apr 2. pii: 5815296. PubMed: https://pubmed.gov/32241023. Full-text: https://doi.org/10.1093/cid/ciaa377

Salehi S, Abedi A, Balakrishnan S, Gholamrezanezhad A. **Coronavirus Disease 2019 (COVID-19): A Systematic Review of Imaging Findings in 919 Patients.** AJR Am J Roentgenol. 2020 Mar 14:1-7. PubMed:

https://pubmed.gov/32174129. Full-text: https://doi.org/10.2214/AJR.20.23034

Scorzolini L, Corpolongo A, Castilletti C, Lalle E, Mariano A, Nicastri E. **Comment of the potential risks of sexual and vertical transmission of Covid-19 infection**. Clin Infect Dis. 2020 Apr 16. pii: 5820874. PubMed: https://pubmed.gov/32297915. Full-text: https://doi.org/10.1093/cid/ciaa445

Shi H, Han X, Jiang N, et al. **Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study.** Lancet Infect Dis. 2020 Apr;20(4):425-434. PubMed: https://pubmed.gov/32105637. Full-text: https://doi.org/10.1016/S1473-3099(20)30086-4

Soldati G, Smargiassi A, Inchingolo R, et al. **Is there a role for lung ultrasound during the COVID-19 pandemic?** J Ultrasound Med. 2020 Mar 20. PubMed: https://pubmed.gov/32198775. Full-text: https://doi.org/10.1002/jum.15284

Song C, Wang Y, Li W, et al. **Absence of 2019 Novel Coronavirus in Semen and Testes of COVID-19 Patients**. Biol Reprod. 2020 Apr 16. pii: 5820830. PubMed: https://pubmed.gov/32297920. Full-text: https://doi.org/10.1093/biolre/ioaa050

Sun Y, Koh V, Marimuthu K, et al. **Epidemiological and Clinical Predictors of COVID-19.** Clin Infect Dis. 2020 Mar 25. pii: 5811426. PubMed: https://pubmed.gov/32211755. Full-text: https://doi.org/10.1093/cid/ciaa322

To KK, Tsang OT, Leung WS, et al. **Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study.** Lancet Infect Dis. 2020 Mar 23. pii: S1473-3099(20)30196-1. PubMed: https://pubmed.gov/32213337. Full-text: https://doi.org/10.1016/S1473-3099(20)30196-1

Vetrugno L, Bove T, Orso D, et al. **Our Italian Experience Using Lung Ultrasound for Identification, Grading and Serial Follow-up of Severity of Lung Involvement for Management of Patients with COVID-19.** Echocardiography. 2020 Apr 1. PubMed: https://pubmed.gov/32239532. Full-text: https://doi.org/10.1111/echo.14664

Wang W, Xu Y, Gao R, et al. **Detection of SARS-CoV-2 in Different Types of Clinical Specimens.** JAMA. 2020 Mar 11. pii: 2762997. PubMed: https://pubmed.gov/32159775. Full-text: https://doi.org/10.1001/jama.2020.3786

Wang X, Yao H, Xu X, et al. **Limits of Detection of Six Approved RT-PCR Kits for the Novel SARS-coronavirus-2 (SARS-CoV-2)**. Clin Chem. 2020 Apr 13. pii: 5819547. PubMed: https://pubmed.gov/32282874.

Wang Y, Dong C, Hu Y, et al. **Temporal Changes of CT Findings in 90 Patients with COVID-19 Pneumonia: A Longitudinal Study.** Radiology. 2020 Mar 19:200843. PubMed: https://pubmed.gov/32191587. Full-text: https://doi.org/10.1148/radiol.2020200843

Wolfel R, Corman VM, Guggemos W, et al. **Virological assessment of hospitalized patients with COVID-2019.** Nature. 2020 Apr 1. pii: 10.1038/s41586-020-2196-x. PubMed: https://pubmed.gov/32235945. Full-text: https://doi.org/10.1038/s41586-020-2196-x

Wu Y, Guo C, Tang L, et al. **Prolonged presence of SARS-CoV-2 viral RNA in faecal samples.** Lancet Gastroenterol Hepatol. 2020 Mar 19. pii: S2468-1253(20)30083-2. PubMed: https://pubmed.gov/32199469. Full-text: https://doi.org/10.1016/S2468-1253(20)30083-2

Xia J, Tong J, Liu M, Shen Y, Guo D. **Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection**. J Med Virol. 2020;1-6. https://doi.org/10.1002/jmv.25725.

Xiao AT, Tong YX, Zhang S. **False-negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: Rather than recurrence**. J Med Virol. 2020 Apr 9. PubMed: https://pubmed.gov/32270882. Full-text: https://doi.org/10.1002/jmv.25855

Xiao DAT, Gao DC, Zhang DS. **Profile of Specific Antibodies to SARS-CoV-2: The First Report.** J Infect. 2020 Mar 21. pii: S0163-4453(20)30138-9. PubMed: https://pubmed.gov/32209385. Full-text: https://doi.org/10.1016/j.jinf.2020.03.012

Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. **Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing.** Radiology. 2020 Feb 12:200343. PubMed: https://pubmed.gov/32049601. Full-text: https://doi.org/10.1148/radiol.2020200343

Xu J, Wu R, Huang H, et al. **Computed Tomographic Imaging of 3 Patients With Coronavirus Disease 2019 Pneumonia With Negative Virus Real-time Reverse-Transcription Polymerase Chain Reaction Test.** Clin Infect Dis. 2020 Mar 31. pii: 5814104. PubMed: https://pubmed.gov/32232429. Full-text: https://doi.org/10.1093/cid/ciaa207

Xu K, Chen Y, Yuan J, et al. **Factors associated with prolonged viral RNA shedding in patients with COVID-19**. Clin Infect Dis. 2020 Apr 9. pii: 5818308. PubMed: https://pubmed.gov/32271376. Full-text: https://doi.org/10.1093/cid/ciaa351

Yu F, Yan L, Wang N, et al. **Quantitative Detection and Viral Load Analysis of SARS-CoV-2 in Infected Patients.** Clin Infect Dis. 2020 Mar 28. PubMed: https://pubmed.gov/32221523. Full-text: https://doi.org/10.1093/cid/ciaa345

Yuan J, Kou S, Liang Y, Zeng J, Pan Y, Liu L. **PCR Assays Turned Positive in 25 Discharged COVID-19 Patients**. Clin Infect Dis. 2020 Apr 8. pii: 5817588. PubMed: https://pubmed.gov/32266381. Full-text: https://doi.org/10.1093/cid/ciaa398

Zhao J, Yuan Q, Wang H, et al. **Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019.** Clin Infect Dis. 2020 Mar 28. PubMed: https://pubmed.gov/32221519. Full-text: https://doi.org/10.1093/cid/ciaa344

Zou L, Ruan F, Huang M, et al. **SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients.** N Engl J Med. 2020 Mar 19;382(12):1177-1179. PubMed: https://pubmed.gov/32074444. Full-text: https://doi.org/10.1056/NEJMc2001737

6. Clinical Presentation

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After an average incubation time of around 5 days (range: 2-14 days), a typical COVID-19 infection begins with dry cough and low-grade fever (38.1–39°C or 100.5–102.1°F), often accompanied by smell and taste diminishment. In a more advanced stage, patients may experience shortness of breath and require mechanical ventilation.

Laboratory findings include lymphocytopenia. In patients with a fatal outcome, levels of d-dimer, serum ferritin, serum lactate dehydrogenase and IL-6 were elevated compared to survivors.

The outcome of COVID-19 is often unpredictable, especially in older patients with comorbidities. The clinical picture ranges from completely asymptomatic to rapidly devastating courses. Much of the clinical data to date is still based on the experiences in China (Table 1 provides an overview of the most important studies). With the massive spread of the infection in Europe and the USA, it will become clear whether these experiences can be transferred to more local conditions.

Incubation period

A pooled analysis of 181 confirmed COVID-19 cases with identifiable exposure and symptom onset windows estimated the median incubation period to be 5.1 days with a 95% CI of 4.5 to 5.8 days (Lauer 2020). The authors estimated that 97.5% of those who develop symptoms will do so within 11.5 days (8.2 to 15.6 days) of infection. Fewer than 2.5% of infected persons will show symptoms within 2.2 days, whereas symptom onset will occur within 11.5 days in 97.5%. However, these estimates imply that,

under conservative assumptions, 101 out of every 10,000 cases will develop symptoms after 14 days of active monitoring or quarantine. Another analysis of 158 confirmed cases outside Wuhan estimated a very similar median incubation period of 5.0 days (95 % CI, 4.4 to 5.6 days), with a range of 2 to 14 days (Linton 2020). In a detailed analysis of 36 cases linked to the first three clusters of circumscribed local transmission in Singapore, the median incubation period was 4 days with a range of 1-11 days (Pung 2020). Taken together, the incubation period of around 4-6 days is in line with that of other coronaviruses causing SARS or MERS (Virlogeux 2016).

Of note, the time from exposure to onset of infectiousness (latent period) may be shorter. There is little doubt that transmission of SARS-CoV-2 during the late incubation period is possible (Li 2020). In a longitudinal study, the viral load was high 2-3 days before the onset of symptoms, and the peak was even reached 0.7 days before the onset of symptoms. The authors of this *Nature Medicine* paper estimated that approximately 44% (95% CI 25-69%) of all secondary infections are caused by such presymptomatic patients (He 2020).

Symptoms

Fever, cough, shortness of breath

Symptoms occur in the majority of cases (for symptomatic, see below). In the largest study published to date (Guan 2020, see Table 1 and 2), fever was the most common symptom in 88.7%, with a median maximum of 38.3 C; only 12.3% had a temperature of > 39 C. The absence of fever seems to be somewhat more frequent than in SARS or MERS; fever alone may therefore not be sufficient to detect cases in public surveillance. The second most common symptom is cough, occurring in about two thirds of all patients.

In the study from Wuhan on 191 patients hospitalized with severe COVID-19 (Zhou 2020), among survivors, median duration of fever was 12.0 days (8-13 days) and cough persisted for 19 days (IQR 12-23 days). Shortness of breath is also common, especially in severe cases (Table 2). Myalgia, chills and headache also may occur.

Table 1. Outstanding clinical studies, main characteristics

	Guan 2020	Wu 2020	Mizumoto 2020	Zhou 2020
n	1,099	73,314	634	191
	China	China	Japan	Wuhan (China)
Median age	47 (IQR 35-58)	NA	58	56 (IQR 46-67)
"Older" age	15.1% (> 65 yrs)	11.9% (> 70 yrs)	75.1% (> 60 yrs)	NA
Female	41.9%	NA	49.4%	37.7%
Severe Dis.	15.7% (CAP definition)	18.6% (more than mild pneumonia)	NA	NA
Death	1.4% (15)*	2.3% (1,023)	1.1% (7**)	28.3%

*short FU, outcomes unknown at time of data cut-off. **longer FU expected

The study by Guan (N Engl J Med) is the largest clinical cohort to date with 1,099 relatively well documented patients from 552 hospitals in 30 Chinese provinces, admitted as of January 29 (Guan 2020).

The second (Wu 2020) is a report from the Chinese CDC, summarizing what happened in during the first weeks.

The third study describes an outbreak onboard the Diamond Princess cruise ship (Mizumoto 2020).

The fourth study reports from hospitalized patients in Wuhan with severe COVID-19 who have a definite outcome (Zhou 2020).

In a meta-analysis of COVID-19 in papers published until February 23, fever (88.7%), cough (57.6%) and dyspnea (45.6%) were the most prevalent clinical manifestations (Rodriguez-Morales 2020). In another review, the corresponding percentages were 88.5%, 68.6% and 21.9%, respectively (Li 2020). As shown in Table 1, some differences between severe and non-severe cases are evident. In the Wuhan study on patients with severe COVID-19, multivariate analysis revealed that a respiratory rate of >24 breaths per minute at admission was higher in non-survivors (63% versus 16%). Others found higher rates of shortness of breath, and high temperature of >39.0 in older patients compared with younger patients (Lian 2020).

A plethora of symptoms have been described in the past few weeks, clearly indicating that COVID-19 is a complex disease, which in no way consists only of a respiratory infection. Although the symptoms are unspecific so that the differential diagnosis encompasses a wide range of infections, respiratory and other diseases, a close look at the patient should nevertheless be taken. The symptoms are briefly discussed below.

Otolaryngeal symptoms (including anosmia)

Although upper respiratory tract symptoms such as rhinorrhea, nasal congestion, sneezing and sore throat are relatively unusual, several groups have recently reported on anosmia and hyposmia as an early sign (Luers 2020, Gane 2020). Interestingly, these otolaryngological symptoms appear to be much more common in Europe than in Asia. However, it is still unclear whether this is a real difference or whether these complaints in the initial phase in China were not recorded well enough. There is now very good data from Europe:

Among 417 mild-to-moderate COVID-19 patients (from 12 European hospitals), 86% and 88% reported olfactory and gustatory dysfunctions, respectively (Lechien 2020). The vast majority was

anosmic (hyposmia, parosmia, phantosmia did also occur) and the early olfactory recovery rate was 44%. Females were more affected than males. Olfactory dysfunction appeared before (12%), at the same time (23%) or after (65%) the appearance of other symptoms. There is no doubt that sudden anosmia or ageusia need to be recognized as important symptoms of COVID-19.

"Flu plus 'loss of smell' means COVID-19". Among 263 patients presenting in March (at a single center in San Diego) with flu-like symptoms, loss of smell was found in 68% of COVID-19 patients (n=59), compared to only 16% in negative patients (n=203). Smell and taste impairment were independently and strongly associated with positivity (anosmia: adjusted odds ratio 11, 95%CI: 5-24). Conversely, sore throat was independently associated with negativity (Yan 2020).

Cardiovascular symptoms and issues

There is growing evidence of direct and indirect effects of SARS-CoV-2 on the heart, especially in patients with pre-existing heart diseases (Bonow 2020). SARS-CoV-2 has the potential to infect cardiomyocytes, pericytes and fibroblasts via the ACE2 pathway leading to direct myocardial injury, but that pathophysiological sequence remains unproven (Hendren 2020). A second hypothesis to explain COVID-19-related myocardial injury centers on cytokine excess and/or antibody mediated mechanisms. Clinically, COVID-19 can manifest with an acute cardiovascular syndrome (termed "ACovCS"). Numerous cases with ACovCS have been described, not only with typical thoracic complaints, but also with very diverse cardiovascular manifestations. Troponin is an important parameter (see below). In a case series of 18 COVID-19 patients who had ST segment elevation, there was variability in presentation, a high prevalence of nonobstructive disease, and a poor prognosis. 6/9 patients undergoing coronary

angiography had obstructive disease. Of note, all 18 patients had elevated D-dimer levels (Bangalore 2020).

In patients with a seemingly typical coronary heart syndrome, COVID-19 should also be considered in the differential diagnosis, even in the absence of fever or cough (Fried 2020, Inciardi 2020).

Gastrointestinal symptoms

In the Chinese studies, gastrointestinal symptoms were rarely seen. In a meta-analysis of 60 studies comprising 4,243 patients, the pooled prevalence of gastrointestinal symptoms was 18% (95% CI, 12%-25%); prevalence was lower in studies in China than other countries. Among the first 393 consecutive patients who were admitted to two hospitals in New York City, diarrhea (24%), and nausea and vomiting (19%) were more frequent than in the reports from China (Goyal 2020). Stool viral RNA was detected at higher frequency among those with diarrhea (Cheung 2020). As with otolaryngeal symptoms, it remains unclear whether this difference reflects geographic variation or differential reporting).

Neurologic symptoms

Neuroinvasive propensity has been demonstrated as a common feature of human coronaviruses. These viruses can invade the brainstem via a synapse-connected route from the lung and airways. With regard to SARS-CoV-2, early occurrences such as olfactory symptoms (see above) should be further evaluated for CNS involvement. Potential late neurological complications in cured COVID-19 patients are possible (Baig 2020). A retrospective, observational case series found 78/214 patients (36%) with neurologic manifestations, ranging from fairly specific symptoms (loss of sense of smell or taste, myopathy, and stroke) to more non-specific symptoms (headache, low consciousness, dizziness, or seizure). Whether these more non-specific symptoms

are manifestations of the disease itself remains to be seen (Mao 2020).

Especially in patients with severe COVID-19, neurological symptoms are common. In an observational series of 58 patients, ARDS due to SARS-CoV-2 infection was associated with encephalopathy, prominent agitation and confusion, and corticospinal tract signs. It remains unclear which of these features were due to critical illness–related encephalopathy, cytokines, or the effect or withdrawal of medication, and which features were specific to SARS-CoV-2 infection (Helms 2020).

Other and atypical symptoms and manifestations

In a case series from China, 12/38 patients (32%, more common in severe cases) had ocular manifestations consistent with conjunctivitis, including conjunctival hyperemia, chemosis, epiphora, or increased secretions. Two patients had positive PCR results from conjunctival swabs (Wu 2020).

Other new and sometimes puzzling clinical presentations have emerged in the current pandemic. There are case reports of non-specific symptoms, especially in the elderly population, underlining the need for extensive testing in the current pandemic (Nickel 2020).

Other signs of infection such as throat congestion, tonsil swelling, enlargement of lymph nodes or rash were almost inexistent. All symptoms are non-specific so that the differential diagnosis includes a wide range of infections, respiratory disorders that may not be distinguished clinically.

Laboratory findings

The most evident laboratory findings in the large cohort study from China (Guan 2020) are shown in Table 2. On admission, lymphocytopenia was present in 83.2% of the patients,

thrombocytopenia in 36.2%, and leukopenia in 33.7%. In most patients, C-reactive protein was elevated to moderate levels; less common were elevated levels of alanine aminotransferase, and D-dimer. Most patients have normal procalcitonin on admission.

Patients with severe disease had more prominent laboratory abnormalities (including lymphocytopenia) than those with non-severe disease. This was also seen in a large retrospective study of hospitalized patients in Wuhan where lymphocyte and leukocyte count was significantly lower in non-survivors. In these, also levels of D-dimer, serum ferritin, high-sensitivity cardiac troponin I, serum lactate dehydrogenase and IL-6 were clearly elevated compared to survivors (Zhou 2020). In particular, D-dimer seemed to be of prognostic value. In the Wuhan study, all patients surviving had low D-dimer during hospitalization, whereas levels in non-survivors tended to increase sharply at day 10. In a multivariate analysis, D-dimer of > 1 µg/mL remained the only lab finding which was significantly associated with in-hospital death, with an odds ratio of 18.4 (2.6-129, p=0.003). However, D-dimer has a reported association with mortality in patients with sepsis. Many of these died from sepsis in the Wuhan study.

Table 2. Percentage of symptoms in the largest cohort to date (Guan 2020). Disease severity was classified according to American Thoracic Society (Metlay 2019) guidelines

Clinical symptoms	All	Severe Disease	Non-Severe
Fever,%	88.7	91.9	88.1
Cough,%	67.8	70.5	67.3
Fatigue,%	38.1	39.9	37.8
Sputum production,%	33.7	35.3	33.4
Shortness of breath,%	18.7	37.6	15.1
Myalgia or arthralgia,%	14.9	17.3	14.5
Sore throat,%	13.9	13.3	14.0
Headache,%	13.6	15.0	13.4
Chills,%	11.5	15.0	10.8
Nausea or vomiting,%	5.0	6.9	4.6
Nasal congestion,%	4.8	3.5	5.1
Diarrhea,%	3.8	5.8	3.5
Radiological findings			
Abnormalities on X-ray,%	59.1	76.7	54.2
Abnormalities on CT,%	86.2	94.6	84.4
Laboratory findings			
WBC <4,000 per mm^3,%	33.7	61.1	28.1
Lymphocytes <1,500 per mm^3,%	83.2	96.1	80.4
Platelets <150,000 per mm^3,%	36.2	57.7	31.6
C-reactive protein ≥10 mg/L,%	60.7	81.5	56.4
Lactate dehydrogenase ≥250 U/L,%	41.0	58.1	37.1
AST >40 U/L,%	22.2	39.4	18.2
D-dimer ≥0.5 mg/L,%	46.6	59.6	43.2

Low lymphocytes and high LDH are also used in (not yet validated) risk scores to predict the risk of progression (Ji 2020). Low platelets have different causes (Review: Xu 2020).

In addition to low lymphocytes, LDH and d-dimer, a meta-analysis of 341 patients found that cardiac troponin I levels are significantly increased only in patients with severe COVID-19 (Lippi 2020). It remains to be seen whether troponin levels can

be used as a prognostic factor. A comprehensive review on the interpretation of elevated troponin levels in COVID-19 was recently published (Chapman 2020).

In another retrospective observational study of 69 patients with severe COVID-19, the decrease of interleukin-6 (IL-6) levels was closely related to treatment effectiveness, while the increase of IL-6 indicated disease exacerbation. The authors concluded that the dynamic change of IL-6 levels can be used as a marker in disease monitoring in patients with severe COVID-19 (Liu 2020).

There is some data on immunological consequences of COVID-19 from two retrospective studies of 21 and 44 HIV-negative patients with COVID-19, showing significant decreases of CD4+ T-cells in almost all patients, with a more pronounced decline to even less than 200 CD4+ T-cells/µl in severe cases (Chen 2020, Quin 2020). There is also evidence from a larger study on SARS-CoV, showing a prolonged lymphopenia before returning towards normal after five weeks, with the lowest mean CD4+ T-cell count of 317 cells/µl (He 2005). Up to now, however, it remains unclear whether this is of clinical value.

Radiological findings

The primary findings on chest x-ray and CT are those of atypical pneumonia. The predominant CT abnormalities are bilateral, peripheral and basal predominant ground-glass opacity, consolidation, or both (Pan 2020). Patterns of radiological findings are described in a more detail in the chapter *Diagnosis*.

Asymptomatic cases

When considering asymptomatic patients, it is important to distinguish those in which infection is still too early to cause any symptoms and those who will remain asymptomatic during the whole time of infection. Asymptomatic patients may transmit the virus (Bai 2020, Rothe 2020). In a study from Northern Italy viral loads in nasal swabs between asymptomatic and symptomatic subjects did not differ significantly, suggesting the same potential for transmitting the virus (Cereda 2020). In an outbreak in a long-term care facility, 13/23 residents who tested positive were asymptomatic or presymptomatic on the day of testing (Kimball 2020).

While physicians need to be aware of asymptomatic cases, the true percentage of those who remain asymptomatic during the course of infection is difficult to assess. The probably best data come from 3,600 people on board the cruise ship Diamond Princess (Mizumoto 2020) who became involuntary actors in a "well-controlled experiment" where passengers and crew comprised an environmentally homogeneous cohort. Due to insufficient hygienic conditions, >700 people became infected while the ship was quarantined in the port of Yokohama, Japan. After systematic testing, 328 (51.7%) of the first 634 confirmed cases were found to be asymptomatic. Considering the varying of the incubation period between 5.5 and 9.5 days, the authors calculated the true asymptomatic proportion at 17.9% (Mizumoto 2020).

From a total of 565 Japanese citizens evacuated from Wuhan, the asymptomatic ratio was estimated to be 41.6% (Nishiura 2020). In another study on 55 asymptomatic patents with confirmed SARS-CoV-2, the majority was of middle age and had close contact with infected family members (Wang 2020). In a

screening study conducted in Iceland, the number of patients testing positive for SARS-CoV-2 but without symptoms was 44%, although some of these may have been pre-symptomatic (Gudbjartsson 2020).

Taken together, these preliminary studies indicate that around 20-40% of all COVID-19 infected subjects may remain asymptomatic during their infection. But it may well be that we are still quite wrong. Only large-scale field studies on seroprevalence will be able to clarify the exact proportion.

Clinical classification

There is no broadly accepted or valid clinical classification for COVID-19. The largest clinical study distinguished between severe and non-severe cases (Guan 2020), according to the Diagnosis and Treatment Guidelines for Adults with Community-acquired Pneumonia, published by the American Thoracic Society and Infectious Diseases Society of America (Metlay 2019). In these validated definitions, severe cases include either one major criterion or three or more minor criteria. Minor criteria are a respiratory rate > 30 breaths/min, PaO2/FIO2 ratio <250, multilobar infiltrates, confusion/disorientation, uremia, leukopenia, low platelet count, hypothermia, hypotension requiring aggressive fluid resuscitation. Major criteria comprise septic shock with need for vasopressors or respiratory failure requiring mechanical ventilation.

Some authors (Wang 2020) have used the following classification including four categories:

1. Mild cases: clinical symptoms were mild without pneumonia manifestation through image results
2. Ordinary cases: having fever and other respiratory symptoms with pneumonia manifestation through image results

3. Severe cases: meeting any one of the following: respiratory distress, hypoxia (SpO2 ≤93%), abnormal blood gas analysis: (PaO2 <60mmHg, PaCO2 >50mmHg)
4. Critical cases: meeting any one of the following: Respiratory failure which requires mechanical ventilation, shock, accompanied by other organ failure that needs ICU monitoring and treatment.

In the report of the Chinese CDC, estimation of disease severity used almost the same categories (Wu 2020) although numbers 1 and 2 were combined. According to the report, there were 81% mild and moderate cases, 14% severe cases and 5% critical cases. There are preliminary reports from the Italian National Institute of Health, reporting on 24.9% severe and 5.0% critical cases (Livingston 2020). However, these numbers are believed to strongly overestimate the disease burden, given the very low number of diagnosed cases in Italy at the time. Among 7,483 US heath care workers with COVID-19, a total of 184 (2.1–4.9%) had to be admitted to ICUs. Rate was markedly higher in HCWs older 65 of age, reaching 6.9–16.0% (CDC 2020).

Outcome

We are facing rapidly increasing numbers of severe and fatal cases in the current pandemic. The two most difficult but most frequently asked clinical questions are 1. How many patients end up with severe or even fatal courses of COVID-19? 2. What is the true proportion of asymptomatic infections? We will learn more about this shortly through serological testing studies. However, it will be important that these studies are carefully designed and carried out, especially to avoid bias and confounding.

Case fatality rates

The case fatality rates (CFR) or infection fatality rates (IFR) are difficult to assess in such a dynamic pandemic. CFR can be biased upwards by underreporting of cases and downwards by insufficient follow up or unknown outcome. A downward trend may also indicate improvements in epidemiological surveillance. COVID-19 fatality is likely overestimated and especially early estimates are susceptible to uncertainty about asymptomatic or subclinical infections and several biases, including biases in detection, selection or reporting (Niforatos 2020).

Dividing the number of deaths by the number of total confirmed cases (April 14 for Italy: 13.2%, Sweden 10.6%, Spain 10.4%, South Korea 2.2%, Germany 3.0%) is not appropriate.

The picture is much more complex and these simple calculations probably do not reflect the true mortality in each country without taking into factor three other issues:

1. The testing policies (and capacities) in a country. This is the most important factor. The fewer people you test (all people, only symptomatic patients, only those with severe symptoms) the higher the mortality. In Germany, testing systems and high lab capacities were established rapidly (Stafford 2020).

2. Age of the total population and especially of the population which is affected first. For example, in Italy, higher percentages of older people became infected during the first weeks, compared to Germany (where many people acquired SARS-CoV during ski holidays or carnival sessions). Especially if high-risk sites (such as retirement homes) are affected, death cases in the country will increase considerably. For example, a single outbreak in Washington has led to 34 deaths among 101 residents of a long-term care facility (McMichael 2020) – this is exactly the same number of death cases which Australia has

reported as whole country on April 4, among a total of 5,635 confirmed COVID-19 cases.

3. Stage of the epidemic. Some countries have experienced their epidemic grow early, some are still a few days or weeks behind. Death rates only reflect the infection rate of 2-3 weeks previously. In the large retrospective study from Wuhan, the time from illness onset to death was 18.5 days (IQR 15-22 days).

The "death rates" for some selected countries, based on the number of deaths and tests, is shown in Figure 1. These curves reflect test readiness and test capacities. A country such as Sweden, which initially relied on "herd immunity", differs significantly from countries in which a lot has been tested from the beginning of the epidemic, such as Germany. The USA is still at the beginning, in Korea the outbreak was stopped relatively quickly by intensive tracking measures.

The summarizing report from the Chinese CDC found a death rate of 2.3%, representing 1,023 among 44,672 confirmed cases (Wu 2020). Mortality increased markedly in older people. In the cases aged 70 to 79 years, CFR was 8.0% and cases in those aged 80 years older had a 14.8% CFR. CFR was also elevated among those with cardiovascular diseases (10.5%), chronic respiratory diseases (6.3%) for hypertension (6.0%) and cancer (5.6%). Among 1,716 health care workers (HCW), 14.8% of confirmed cases were classified as severe or critical and 5 deaths were observed. In an updated study, 23/3,387 HCWs in China have died, which corresponds to a mortality of 0.68%. The median age was 55 years (range, 29 to 72), and 11 of the 23 deceased HCWs had been reactivated from retirement (Zhang 2020). Current studies in the USA have found similar rates, mortality estimates were 0.3-0.6% (CDC 2020). Of the 27 HCW who have died from COVID-19 until mid-April, 18 were over 54 years of age. The overall low mortality rates were probably due to the fact that HCWs were younger and

healthier, but also that they had been tested earlier and more frequently. However, these rates may better reflect true CFRs.

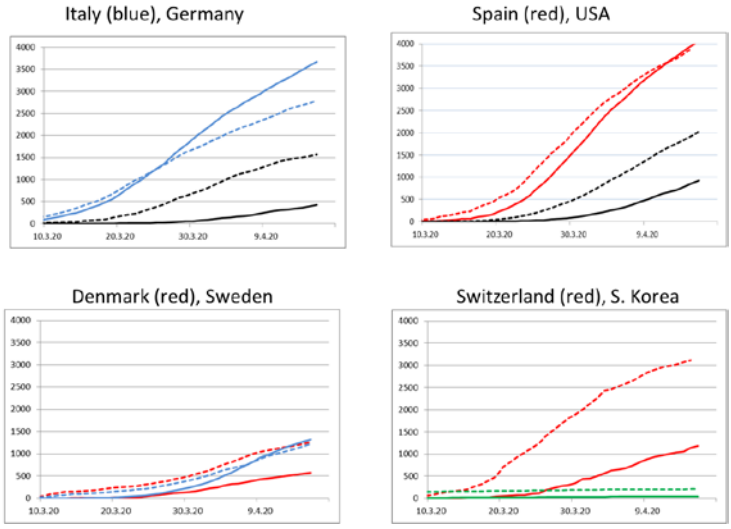

Figure 1. People who tested positive (among 1 million inhabitants, dashed) and deaths (among 10 million inhabitants). "Mortality" reaches 10% at the point where the curves intersect. This has happened for countries such as Spain, Italy or Sweden, but is unlikely for others like Germany, Switzerland or Denmark.

An in-depth analysis of 48,557 cases and 2,169 deaths from the epicenter, Wuhan, found lower rates (Wu 2020). The authors estimated an overall symptomatic case fatality risk (SCFR, the probability of dying after developing symptoms) of only 1.4% (0.9–2.1%). Compared to those aged 30–59 years, those aged below 30 and above 59 years were 0.6 (0.3–1.1) and 5.1 (4.2–6.1) times more likely to die after developing symptoms (Wu 2020). Other groups have confirmed these lower rates (Verity 2020).

Again, the most valid data seem to come from the Diamond Princess. As of April 17, the total number of infected reached 712, and 13 patients have died from the disease leading to a CFR of 1.8%. However, this rate may yet increase, as at least 7 patients were in serious condition (Moriarty 2020). If all patients seriously ill at the last follow up (April 14) die, this would result in a CFR of 2.8%. On the other hand, around 75% of the patients on the Diamond Princess were of 60 years or older, many of them in their eighties. Projecting the Diamond Princess mortality rate onto the age structure of the general population, it is obvious that the mortality rate may be much lower in other broader populations. Mortality would be in a range of 0.2-0.4 %.

The mortality rates from the probably well-monitored HCWs also come relatively close to these rates (CDC 2020, Zhang 2020). Again, we will learn more from limited outbreaks affecting homogeneous populations, such as cruise ships and aircraft carriers. Two large "involuntary field studies" are currently taking place: more than 600 seafarers are infected on the US aircraft carrier Theodore Roosevelt (one soldier has already died), and more than 1,000 COVID-19 patients on the French aircraft carrier Charles de Gaulle. These populations are probably young, healthy and correspond more to the general population.

Risk factors for severe disease

From the beginning of the epidemic, older age has been identified as an important risk factor for disease severity (Huang 2020, Guan 2020). In Wuhan, there was a clear and considerable age dependency in symptomatic infections (susceptibility) and outcome (fatality) risks, by multiple folds in each case (Wu 2020). According to the Italian National Institute of Health, an analysis of the first 2,003 death cases, median age was 80.5 years (IQR 74.3-85.9). Only 17 (0.8%) were 49 years or younger, and 87.7% were older than 70 years (Livingston 2020). More recently,

another important study had highlighted the severity of COVID-19 in older people (McMichael 2020). In an outbreak reported from King County/Washington, a total of 167 confirmed cases were observed in 101 residents (median age 83 years) of a long-term care facility, in 50 health care workers (HCW, median age 43 years), and 16 visitors. The case fatality rate for residents was 33.7% (34 of 101) and 0% among HCW.

Beside older age, several risk factors have been evaluated in the current pandemic. In the largest clinical study to date, some comorbidities such as hypertension have been identified as the main risk factors for severe disease and death (Table 3).

Others have confirmed a higher rate for patients with comorbidities such as hypertension or diabetes. In multivariate analysis of hospitalized patients with severe COVID-19, however, no comorbidity remained significantly associated with outcome (Wang 2020, Zhou 2020).

In another retrospective cohort of 487 COVID-19 patients in Zhejiang Province of China with detailed clinical data, severe cases were also older and more male. Severe cases had a higher incidence of hypertension, diabetes, cardiovascular diseases, and malignancy, and less exposure to epidemic area, but more infected family members. In a multivariate analysis, beside older age, male gender (OR 3.68, 95% CI 1.75–7.75, p=0.001) and presence of hypertension (OR 2.71, 95% CI 1.32–5.59, p=0.007) were independently associated with severe disease at admission, irrespective of adjustment of time to admission (Shi 2020). Among 1,590 hospitalised patients from mainland China, after adjusting for age and smoking status, COPD (hazard ratio 2.7, 95%CI 1.4-5.0), diabetes (HR 1.6, 95%CI 1.03-2.5), hypertension (HR 1.6, 95%CI 1.1-2.3) and malignancy (HR 3.5, 95%CI 1.6-7.7) were risk factors of reaching endpoints (Guan 2020). Among the first 393 consecutive patients who were admitted to two

hospitals in New York City, obese patients were more likely to require mechanical ventilation (Goyal 2020).

As shown in Table 3, there was a slightly higher rate of current smokers in patients with severe disease. A meta-analysis of 5 studies comprising 1,399 patients observed only a trend but no significant association between active smoking and severity of COVID-19 (Lippi 2020). However, other authors have emphasized that current data do not allow to draw firm conclusions about the association of severity of COVID-19 with smoking status (Berlin 2020).

Table 3. Age and comorbidities in the NEJM paper (Guan 2020)

	All	Severe Disease	Non-Severe
Age > 65	15.1	27.0	12.9
Age < 50	56.0	41.7	58.7
Never smoker	85.4	77.9	86.9
Former or current smoker	14.5	22.1	13.1
COPD,%	1.1	3.5	0.6
Diabetes,%	7.4	16.2	5.7
Hypertension,%	15.0	23.7	13.4
Coronary heart disease,%	2.5	5.8	1.8
Cerebrovascular disease,%	1.4	2.3	1.2
Hepatitis B infection,%	2.1	0.6	2.4
Cancer,%	0.9	1.7	0.8
Chronic renal disease,%	0.7	1.7	0.5
Immune deficiency,%	0.2	0	0.2

So far there are no reliable, validated risk scores. The CURB-65 used in community-acquired pneumonia does not seem to be

very meaningful. In a study of 208 patients, a new score was developed to predict progression. It is based on age, comorbidities, lymphocytes and LDH and seems to work quite well, but it must still be validated by larger studies (Ji 2020). This also applies to other, sometimes even more complicated scores (Gong 2020).

More research is needed on the deleterious effect of comorbidities, especially with regard to the renin-angiotensin-aldosterone system (RAAS). Hypertension, cardiovascular disease and diabetes share underlying RAAS pathophysiology that may be clinically insightful. In particular, activity of the angiotensin-converting enzyme 2 (ACE2) is dysregulated (increased) in cardiovascular disease (Hanff 2020). As SARS-CoV-2 cell entry depends on ACE2 (Hoffmann 2020), increased ACE2 levels may increase the virulence of SARS-CoV-2 within the lung and heart.

In the largest study to date of 1,099 patients with COVID-19, hypertension was associated with an increased risk (24% versus 13%) of severe course of disease (Guan 2020). However, comedication was not recorded in this study, and several medical societies and reviews explicitly advise against discontinuing ACE inhibitors (Bavishi 2020, ESH 2020, Vaduganathan 2020).

Furthermore, the binding of SARS-CoV-2 to ACE2 appears to lead to an imbalance in the RAS (RAAS) system. Animal studies have shown that this imbalance could even be influenced favourably by ACE inhibitors or sartans in the course of pneumonia (Gurwitz 2020, Sun 2020). The biological plausibility of the salutary effects of RAAS inhibitors is intriguing and several trials of starting losartan in patients with COVID-19 are currently planned.

More recently, the first clinical study has indicated no deleterious effect of RAAS inhibitors in COVID-19. Among 42 of 417 patients admitted to Shenzhen Hospital while on antihypertensive therapy, patients receiving these drugs had a

lower rate of severe diseases than those without (5/17 compared to 12/25), and a trend toward a lower level of IL-6 in peripheral blood (Meng 2020). In another study, patients with ACE inhibitors also had no increased risk of severe courses (Wang 2020).

Predisposition

COVID-19 shows an extremely variable course, from completely asymptomatic to fulminantly fatal. In some cases it affects young and apparently healthy people, for whom the severity of the disease is neither caused by age nor by any comorbidities – just think of the Chinese doctor Li Wenliang, who died at the age of 34 from COVID-19 (see chapter Timeline). So far, only assumptions can be made. Is there a genetic predisposition for severe courses? Some preliminary reports suggest that this is the case. For example, a report from Iran describes three brothers aged 54 to 66 who all died of COVID-19 after less than two weeks of fulminating progress. All three had previously been healthy and there were no underlying illnesses (Yousefzadegan 2020).

In addition to the genetic predisposition, other potential reasons for a severe course need to be considered: the amount of viral exposure (probably high for Li Wenliang?), the route by which the virus enters the body, ultimately also the virulence of the pathogen and a possible (partial) immunity from previous viral diseases. All of this will have to be investigated in the coming months.

Overburdened health care systems

Mortality may be also higher in situations where hospitals are unable to provide intensive care to all the patients who need it, in particular ventilator support. Mortality would thus also be correlated with health-care burden. Preliminary data show clear disparities in mortality rates between Wuhan (>3%), different

regions of Hubei (about 2.9% on average), and across the other provinces of China (about 0.7% on average). The authors have postulated that this is likely to be related to the rapid escalation in the number of infections around the epicenter of the outbreak, which has resulted in an insufficiency of health-care resources, thereby negatively affecting patient outcomes in Hubei, while this has not yet been the situation in other parts of China (Ji 2020). Another study estimated the risk of death in Wuhan as high as 12% in the epicentre and around 1% in other more mildly affected areas (Mizumoto 2020).

The nightmare of insufficient ressources is currently the reality in Northern Italy. In Italy, on March 15, the cumulative death numbers exceeded for the first time those of admissions to intensive care units – a clear sign for a collapsing health care system. Other countries or regions will face the same situation soon.

Reactivations, reinfections

There are several reports of patients who become positive again after negative PCR tests (Lan 2020, Xiao 2020, Yuan 2020). These reports have gained much attention, because this could indicate both reactivations as well as reinfections. After closer inspection of these reports, however, there is no good evidence for reactivations or reinfections, and other reasons are much more likely. Methodological problems of PCR always have to be considered; the results can considerably fluctuate (Li 2020). Insufficient material collection or storage are just two examples of many problems with PCR. Even if everything is done correctly, it can be expected that a PCR could fluctuate between positive and negative at times when the values are low and the viral load drops at the end of an infection (Wölfel 2020). It also depends on the assay used, the detection limit is between a few hundred and several thousand virus copies/mL (Wang 2020).

The largest study to date found a total of 25 (14.5%) of 172 discharged COVID-19 patients who had a positive test at home after two negative PCR results at hospital (Yuan 2020). On average, the time between the last negative and the first positive test was 7.3 (standard deviation 3.9) days. There were no differences to patients who remained negative. This and the short period of time suggest that in these patients, no reactivations are to be expected.

Reactivations as well as rapid new infections would be very unusual, especially for coronaviruses. If a lot of testing is done, you will find a number of such patients who become positive again after repeated negative PCR and clinical convalescence. The phenomenon is likely to be overrated. Most patients get well anyway; moreover, it is unclear whether renewed positivity in PCR is synonymous with infectiousness.

Outlook

Over the coming months, serological studies will give a clearer picture of the true number of asymptomatic patients and those with unusual symptoms. More importantly, we have to learn more about risk factors for severe disease, in order to adapt prevention strategies. Older age is not the only risk factor. Recently, a 106-year-old COVID-19 patient recently recovered in the UK. The precise mechanisms how comorbidities (and comedications) may contribute to an increased risk for a severe disease course have to be elucidated. Genetic and immunological studies have to reveal susceptibility and predisposition for both severe and mild courses. Who is really at risk, who is not? Quarantining only the old is too easy.

References

Bai Y, Yao L, Wei T, et al. **Presumed Asymptomatic Carrier Transmission of COVID-19.** JAMA. 2020 Feb 21. pii: 2762028. PubMed: https://pubmed.gov/32083643. Full-text: https://doi.org/10.1001/jama.2020.2565

Baig AM. **Neurological manifestations in COVID-19 caused by SARS-CoV-2.** CNS Neurosci Ther. 2020 Apr 7. PubMed: https://pubmed.gov/32266761. Full-text: https://doi.org/10.1111/cns.13372

Bangalore S, Sharma A, Slotwiner A, et al. **ST-Segment Elevation in Patients with Covid-19 - A Case Series.** N Engl J Med. 2020 Apr 17. PubMed: https://pubmed.gov/32302081. Full-text: https://doi.org/10.1056/NEJMc2009020

Bavishi C, Maddox TM, Messerli FH. **Coronavirus Disease 2019 (COVID-19) Infection and Renin Angiotensin System Blockers.** JAMA Cardiol. 2020 Apr 3. pii: 2764299. PubMed: https://pubmed.gov/32242890. Full-text: https://doi.org/10.1001/jamacardio.2020.1282

Berlin I, Thomas D, Le Faou AL, Cornuz J. **COVID-19 and smoking.** Nicotine Tob Res. 2020 Apr 3. pii: 5815378. PubMed: https://pubmed.gov/32242236. Full-text: https://doi.org/10.1093/ntr/ntaa059

Bonow RO, Fonarow GC, O´Gara PT, Yancy CW. **Association of Coronavirus Disease 2019 (COVID-19) With Myocardial Injury and Mortality.** JAMA Cardiol. 2020 Mar 27. pii: 2763844. PubMed: https://pubmed.gov/32219362. Full-text: https://doi.org/10.1001/jamacardio.2020.1105

CDC Covid Response Team. **Characteristics of Health Care Personnel with COVID-19 - United States, February 12-April 9, 2020.** MMWR Morb Mortal Wkly Rep. 2020 Apr 17;69(15):477-481. PubMed: https://pubmed.gov/32298247. Full-text: https://doi.org/10.15585/mmwr.mm6915e6

Cereda D, Tirani M, Rovida F, et al. **The early phase of the COVID-19 outbreak in Lombardy, Italy.** https://arxiv.org/ftp/arxiv/papers/2003/2003.09320.pdf. Accessed 27 March 2020.

Chapman AR, Bularga A, Mills NL. **High-Sensitivity Cardiac Troponin Can Be An Ally in the Fight Against COVID-19.** Circulation. 2020 Apr 6. PubMed: https://pubmed.gov/32251612. Full-text: https://doi.org/10.1161/CIRCULATIONAHA.120.047008

Chen G, Wu D, Guo W, et al. **Clinical and immunologic features in severe and moderate Coronavirus Disease 2019.** J Clin Invest. 2020 Mar 27. pii: 137244. PubMed: https://pubmed.gov/32217835. Full-text: https://doi.org/10.1172/JCI137244

Cheung KS, Hung IF, Chan PP, et al. **Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples from the Hong Kong Cohort and Systematic Review and Meta-analysis.** Gastroenterology. 2020 Apr 3. pii: S0016-5085(20)30448-0. PubMed:

https://pubmed.gov/32251668. Full-text: https://doi.org/10.1053/j.gastro.2020.03.065

ESH. European Society of Hypertension: www.eshonline.org/spotlights/esh-statement-on-covid-19/

Fried JA, Ramasubbu K, Bhatt R, et al. **The Variety of Cardiovascular Presentations of COVID-19.** Circulation. 2020 Apr 3. PubMed: https://pubmed.gov/32243205. Full-text: https://doi.org/10.1161/CIRCULATIONAHA.120.047164

Gane SB, Kelly C, Hopkins C. **Isolated sudden onset anosmia in COVID-19 infection. A novel syndrome?** Rhinology. 2020 Apr 2. pii: 2449. PubMed: https://pubmed.gov/32240279. Full-text: https://doi.org/10.4193/Rhin20.114

Gong J, Ou J, Qiu X, et al. **A Tool to Early Predict Severe Corona Virus Disease 2019 (COVID-19) : A Multicenter Study using the Risk Nomogram in Wuhan and Guangdong, China**. Clin Infect Dis. 2020 Apr 16. pii: 5820684. PubMed: https://pubmed.gov/32296824. Full-text: https://doi.org/10.1093/cid/ciaa443

Goyal P, Choi JJ, Pinheiro LC, et al. **Clinical Characteristics of Covid-19 in New York City**. N Engl J Med. 2020 Apr 17. PubMed: https://pubmed.gov/32302078. Full-text: https://doi.org/10.1056/NEJMc2010419

Guan WJ, Liang WH, Zhao Y, et al. **Comorbidity and its impact on 1590 patients with Covid-19 in China: A Nationwide Analysis.** Eur Respir J. 2020 Mar 26. pii: 13993003.00547-2020. PubMed: https://pubmed.gov/32217650. Full-text: https://doi.org/10.1183/13993003.00547-2020

Guan WJ, Ni ZY, Hu Y, et al. **Clinical Characteristics of Coronavirus Disease 2019 in China.** N Engl J Med. 2020 Feb 28. PubMed: https://pubmed.gov/32109013. Full-text: https://doi.org/10.1056/NEJMoa2002032

Gudbjartsson DF, Helgason A, Jonsson H, et al. **Spread of SARS-CoV-2 in the Icelandic Population**. N Engl J Med. 2020 Apr 14. PubMed: https://pubmed.gov/32289214. Full-text: https://doi.org/10.1056/NEJMoa2006100

Gurwitz D. **Angiotensin receptor blockers as tentative SARS-CoV-2 therapeutics**. Drug Dev Res. 2020 Mar 4. PubMed: https://pubmed.gov/32129518. Full-text: https://doi.org/10.1002/ddr.21656

Hanff TC, Harhay MO, Brown TS, Cohen JB, Mohareb AM. **Is There an Association Between COVID-19 Mortality and the Renin-Angiotensin System-a Call for Epidemiologic Investigations.** Clin Infect Dis. 2020 Mar 26. pii: 5811880. PubMed: https://pubmed.gov/32215613. Full-text: https://doi.org/10.1093/cid/ciaa329

He X, Lau EHY, Wu P, et al. **Temporal dynamics in viral shedding and transmissibility of COVID-19**. Nat Med. 2020 Apr 15. pii: 10.1038/s41591-020-0869-5. PubMed: https://pubmed.gov/32296168. Full-text: https://doi.org/10.1038/s41591-020-0869-5

He Z, Zhao C, Dong Q, et al. **Effects of severe acute respiratory syndrome (SARS) coronavirus infection on peripheral blood lymphocytes and their subsets.** Int J Infect Dis. 2005 Nov;9(6):323-30. PubMed: https://pubmed.gov/16095942. Full-text: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7110876/

Helms J, Kremer S, Merdji H, et al. **Neurologic Features in Severe SARS-CoV-2 Infection**. N Engl J Med. 2020 Apr 15. PubMed: https://pubmed.gov/32294339. Full-text: https://doi.org/10.1056/NEJMc2008597

Hendren NS, Drazner MH, Bozkurt B, Cooper LT Jr. **Description and Proposed Management of the Acute COVID-19 Cardiovascular Syndrome**. Circulation. 2020 Apr 16. PubMed: https://pubmed.gov/32297796. Full-text: https://doi.org/10.1161/CIRCULATIONAHA.120.047349

Hoffmann M, Kleine-Weber H, Schroeder S, et al. **SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.** Cell. 2020 Mar 4. pii: S0092-8674(20)30229-4. PubMed: https://pubmed.gov/32142651. Full-text: https://doi.org/10.1016/j.cell.2020.02.052

Huang C, Wang Y, Li X, et al. **Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China.** Lancet. 2020 Feb 15;395(10223):497-506. PubMed: https://pubmed.gov/31986264. Full-text: https://doi.org/10.1016/S0140-6736(20)30183-5

Inciardi RM, Lupi L, Zaccone G, et al. **Cardiac Involvement in a Patient With Coronavirus Disease 2019 (COVID-19).** JAMA Cardiol. 2020 Mar 27. pii: 2763843. PubMed: https://pubmed.gov/32219357. Full-text: https://doi.org/10.1001/jamacardio.2020.1096

Ji D, Zhang D, Xu J, et al. **Prediction for Progression Risk in Patients with COVID-19 Pneumonia: the CALL Score**. Clin Infect Dis. 2020 Apr 9. pii: 5818317. PubMed: https://pubmed.gov/32271369. Full-text: https://doi.org/10.1093/cid/ciaa414

Ji Y, Ma Z, Peppelenbosch MP, Pan Q. **Potential association between COVID-19 mortality and health-care resource availability.** Lancet Glob Health. 2020 Apr;8(4):e480. PubMed: https://pubmed.gov/32109372. Full-text: https://doi.org/10.1016/S2214-109X(20)30068-1

Kim H, Hong H, Yoon SH. **Diagnostic Performance of CT and Reverse Transcriptase-Polymerase Chain Reaction for Coronavirus Disease 2019: A Meta-Analysis**. Radiology. 2020 Apr 17:201343. PubMed: https://pubmed.gov/32301646. Full-text: https://doi.org/10.1148/radiol.2020201343

Kimball A, Hatfield KM, Arons M, et al. **Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020.** MMWR Morb Mortal Wkly Rep. 2020 Apr 3;69(13):377-381. PubMed: https://pubmed.gov/32240128. Full-text: https://doi.org/10.15585/mmwr.mm6913e1

Lan L, Xu D, Ye G, et al. **Positive RT-PCR Test Results in Patients Recovered From COVID-19**. JAMA. 2020 Feb 27. pii: 2762452. Abstract: https://pubmed.gov/32105304. Fulltext: https://doi.org/10.1001/jama.2020.2783

Lauer SA, Grantz KH, Bi Q, et al. **The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application.** Ann Intern Med. 2020 Mar 10. pii: 2762808. PubMed: https://pubmed.gov/32150748. Full-text: https://doi.org/10.7326/M20-0504

Lechien JR, Chiesa-Estomba CM, De Siati DR, et al. **Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study**. Eur Arch Otorhinolaryngol. 2020 Apr 6. pii: 10.1007/s00405-020-05965-1. PubMed: https://pubmed.gov/32253535. Full-text: https://doi.org/10.1007/s00405-020-05965-1

Li P, Fu JB, Li KF, et al. **Transmission of COVID-19 in the terminal stage of incubation period: a familial cluster.** Int J Infect Dis. 2020 Mar 16. pii: S1201-9712(20)30146-6. PubMed: https://pubmed.gov/32194239. Full-text: https://doi.org/10.1016/j.ijid.2020.03.027

Li Y, Yao L, Li J, et al. **Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19**. J Med Virol. 2020 Mar 26. PubMed: https://pubmed.gov/32219885. Full-text: https://doi.org/10.1002/jmv.25786

Lian J, Jin X, Hao S, et al. **Analysis of Epidemiological and Clinical features in older patients with Corona Virus Disease 2019 (COVID-19) out of Wuhan.** Clin Infect Dis. 2020 Mar 25. pii: 5811557. PubMed: https://pubmed.gov/32211844. Full-text: https://doi.org/10.1093/cid/ciaa242

Linton NM, Kobayashi T, Yang Y, et al. **Incubation Period and Other Epidemiological Characteristics of 2019 Novel Coronavirus Infections with Right Truncation: A Statistical Analysis of Publicly Available Case Data.** J Clin Med. 2020 Feb 17;9(2). pii: jcm9020538. PubMed: https://pubmed.gov/32079150. Full-text: https://doi.org/10.3390/jcm9020538

Lippi G, Henry BM. **Active smoking is not associated with severity of coronavirus disease 2019 (COVID-19).** Eur J Intern Med. 2020 Mar 16. pii: S0953-6205(20)30110-2. PubMed: https://pubmed.gov/32192856. Full-text: https://doi.org/10.1016/j.ejim.2020.03.014

Lippi G, Lavie CJ, Sanchis-Gomar F. **Cardiac troponin I in patients with coronavirus disease 2019 (COVID-19): Evidence from a meta-analysis.** Prog Cardiovasc Dis. 2020 Mar 10. pii: S0033-0620(20)30055-4. PubMed: https://pubmed.gov/32169400. Full-text: https://doi.org/10.1016/j.pcad.2020.03.001

Liu T, Zhang J, Yang Y, et al. **The potential role of IL-6 in monitoring severe case of coronavirus disease 2019.** MedRxiv 2020, https://doi.org/10.1101/2020.03.01.20029769

Livingston E, Bucher K. **Coronavirus Disease 2019 (COVID-19) in Italy.** JAMA. 2020 Mar 17. pii: 2763401. PubMed: https://pubmed.gov/32181795. Full-text: https://doi.org/10.1001/jama.2020.4344

Luers JC, Klussmann JP, Guntinas-Lichius O. [**The Covid-19 pandemic and otolaryngology: What it comes down to?**] Laryngorhinootologie. 2020 Mar 26. PubMed: https://pubmed.gov/32215896. Full-text: https://doi.org/10.1055/a-1095-2344

Mao L, Jin H, Wang M, et al. **Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China**. JAMA Neurol. 2020 Apr 10. pii: 2764549. PubMed: https://pubmed.gov/32275288. Full-text: https://doi.org/10.1001/jamaneurol.2020.1127

McMichael TM, Currie DW, Clark S, et al. **Epidemiology of Covid-19 in a Long-Term Care Facility in King County, Washington.** N Engl J Med. 2020 Mar 27. PubMed: https://pubmed.gov/32220208. Full-text: https://doi.org/10.1056/NEJMoa2005412

Meng J, Xiao G, Zhang J, et al. **Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension.** Emerg Microbes Infect. 2020 Dec;9(1):757-760. PubMed: https://pubmed.gov/32228222. Full-text: https://doi.org/10.1080/22221751.2020.1746200

Metlay JP, Waterer GW, Long AC, et al. **Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America.** Am J Respir Crit Care Med. 2019 Oct 1;200(7):e45-e67. PubMed: https://pubmed.gov/31573350. Full-text: https://doi.org/10.1164/rccm.201908-1581ST

Mizumoto K, Chowell G. **Estimating Risk for Death from 2019 Novel Coronavirus Disease, China, January-February 2020.** Emerg Infect Dis. 2020 Mar 13;26(6). PubMed: https://pubmed.gov/32168464. Full-text: https://doi.org/10.3201/eid2606.200233

Mizumoto K, Kagaya K, Zarebski A, Chowell G. **Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020.** Euro Surveill. 2020 Mar;25(10). PubMed: https://pubmed.gov/32183930. Full-text: https://doi.org/10.2807/1560-7917.ES.2020.25.10.2000180

Moriarty LF, Plucinski MM, Marston BJ, et al. **Public Health Responses to COVID-19 Outbreaks on Cruise Ships — Worldwide, February–March 2020.** MMWR Morb Mortal Wkly Rep. ePub: 23 March 2020. https://doi.org/10.15585/mmwr.mm6912e3.

Nickel CH, Bingisser R. **Mimics and chameleons of COVID-19.** Swiss Med Wkly. 2020 Mar 23;150:w20231. PubMed: https://pubmed.gov/32202647. Full-text: https://doi.org/Swiss Med Wkly. 2020;150:w20231

Niforatos JD, Melnick ER, Faust JS. **Covid-19 fatality is likely overestimated.** BMJ. 2020 Mar 20;368:m1113. PubMed: https://pubmed.gov/32198267. Full-text: https://doi.org/10.1136/bmj.m1113

Nishiura H, Kobayashi T, Suzuki A, et al. **Estimation of the asymptomatic ratio of novel coronavirus infections (COVID-19).** Int J Infect Dis. 2020 Mar 13. pii: S1201-9712(20)30139-9. PubMed: https://pubmed.gov/32179137. Full-text: https://doi.org/10.1016/j.ijid.2020.03.020

Pan F, Ye T, Sun P, et al. **Time Course of Lung Changes On Chest CT During Recovery From 2019 Novel Coronavirus (COVID-19) Pneumonia.** Radiology. 2020 Feb 13:200370. PubMed: https://pubmed.gov/32053470. Full-text: https://doi.org/10.1148/radiol.2020200370

Pung R, Chiew CJ, Young BE, et al. **Investigation of three clusters of COVID-19 in Singapore: implications for surveillance and response measures.** Lancet. 2020 Mar 16. pii: S0140-6736(20)30528-6. PubMed: https://pubmed.gov/32192580. Full-text: https://doi.org/10.1016/S0140-6736(20)30528-6

Qin C, Zhou L, Hu Z, et al. **Dysregulation of immune response in patients with COVID-19 in Wuhan, China.** Clin Infect Dis. 2020 Mar 12. pii: 5803306. PubMed: https://pubmed.gov/32161940. Full-text: https://doi.org/10.1093/cid/ciaa248

Rodriguez-Morales AJ, Cardona-Ospina JA, Gutierrez-Ocampo E, et al. **Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis.** Travel Med Infect Dis. 2020 Mar 13:101623. PubMed: https://pubmed.gov/32179124. Full-text: https://doi.org/10.1016/j.tmaid.2020.101623

Rothe C, Schunk M, Sothmann P, et al. **Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany.** N Engl J Med. 2020 Mar 5;382(10):970-971. PubMed: https://pubmed.gov/32003551. Full-text: https://doi.org/10.1056/NEJMc2001468

Shi H, Han X, Jiang N, et al. **Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study.** Lancet Infect Dis. 2020 Apr;20(4):425-434. PubMed: https://pubmed.gov/32105637. Full-text: https://doi.org/10.1016/S1473-3099(20)30086-4

Shi Y, Yu X, Zhao H, Wang H, Zhao R, Sheng J. **Host susceptibility to severe COVID-19 and establishment of a host risk score: findings of 487 cases outside Wuhan.** Crit Care. 2020 Mar 18;24(1):108. PubMed: https://pubmed.gov/32188484. Full-text: https://doi.org/10.1186/s13054-020-2833-7

Stafford N. **Covid-19: Why Germany's case fatality rate seems so low**. BMJ. 2020 Apr 7;369:m1395. PubMed: https://pubmed.gov/32265194. Full-text: https://doi.org/10.1136/bmj.m1395

Sun ML, Yang JM, Sun YP, Su GH. **Inhibitors of RAS Might Be a Good Choice for the Therapy of COVID-19 Pneumonia**. Zhonghua Jie He He Hu Xi Za Zhi. 2020 Mar 12;43(3):219-222. PubMed: https://pubmed.gov/32164092. Full-text: https://doi.org/10.3760/cma.j.issn.1001-0939.2020.03.016

Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. **Renin-Angiotensin-Aldosterone System Inhibitors in Patients with Covid-19.** N Engl J Med. 2020 Mar 30. PubMed: https://pubmed.gov/32227760. Full-text: https://doi.org/10.1056/NEJMsr2005760

Verity R, Okell LC, Dorigatti I, et al. **Estimates of the severity of coronavirus disease 2019: a model-based analysis.** Lancet Infect Dis. 2020 Mar 30. pii: S1473-3099(20)30243-7. PubMed: https://pubmed.gov/32240634. Full-text: https://doi.org/10.1016/S1473-3099(20)30243-7

Virlogeux V, Fang VJ, Park M, Wu JT, Cowling BJ. **Comparison of incubation period distribution of human infections with MERS-CoV in South Korea and Saudi Arabia.** Sci Rep. 2016 Oct 24;6:35839. PubMed: https://pubmed.gov/27775012. Full-text: https://doi.org/10.1038/srep35839

Wang X, Yao H, Xu X, et al. **Limits of Detection of Six Approved RT-PCR Kits for the Novel SARS-coronavirus-2 (SARS-CoV-2)**. Clin Chem. 2020 Apr 13. pii: 5819547. PubMed: https://pubmed.gov/32282874.

Wang Y, Liu Y, Liu L, Wang X, Luo N, Ling L. **Clinical outcome of 55 asymptomatic cases at the time of hospital admission infected with SARS-Coronavirus-2 in Shenzhen, China.** J Infect Dis. 2020 Mar 17. pii: 5807958. PubMed: https://pubmed.gov/32179910. Full-text: https://doi.org/10.1093/infdis/jiaa119

Wang Y, Lu X, Chen H, et al. **Clinical Course and Outcomes of 344 Intensive Care Patients with COVID-19**. Am J Respir Crit Care Med. 2020 Apr 8. PubMed: https://pubmed.gov/32267160. Full-text: https://doi.org/10.1164/rccm.202003-0736LE

Wölfel R, Corman VM, Guggemos W. et al. **Virological assessment of hospitalized patients with COVID-2019**. Nature 2020, April 1. Full-text: https://doi.org/10.1038/s41586-020-2196-x

Wu JT, Leung K, Bushman M. **Estimating clinical severity of COVID-19 from the transmission dynamics in Wuhan, China**. Nature Medicine. 2020. https://www.nature.com/articles/s41591-020-0822-7

Wu P, Duan F, Luo C, et al. **Characteristics of Ocular Findings of Patients With Coronavirus Disease 2019 (COVID-19) in Hubei Province, China.** JAMA Ophthalmol. 2020 Mar 31. pii: 2764083. PubMed: https://pubmed.gov/32232433. Full-text: https://doi.org/10.1001/jamaophthalmol.2020.1291

Wu Z, McGoogan JM. **Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention.** JAMA. 2020 Feb 24. pii: 2762130. PubMed: https://pubmed.gov/32091533. Full-text: https://doi.org/10.1001/jama.2020.2648

Xiao AT, Tong YX, Zhang S. **False-negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: Rather than recurrence**. J Med Virol. 2020

Apr 9. PubMed: https://pubmed.gov/32270882. Full-text: https://doi.org/10.1002/jmv.25855

Xu P, Zhou Q, Xu J. **Mechanism of thrombocytopenia in COVID-19 patients**. Ann Hematol. 2020 Apr 15. pii: 10.1007/s00277-020-04019-0. PubMed: https://pubmed.gov/32296910. Full-text: https://doi.org/10.1007/s00277-020-04019-0

Yan CH, Faraji F, Prajapati DP, Boone CE, DeConde AS. **Association of chemosensory dysfunction and Covid-19 in patients presenting with influenza-like symptoms**. Int Forum Allergy Rhinol. 2020 Apr 12. PubMed: https://pubmed.gov/32279441. Full-text: https://doi.org/10.1002/alr.22579

Yousefzadegan S, Rezaei N. **Case Report: Death Due to Novel Coronavirus Disease (COVID-19) in Three Brothers**. Am J Trop Med Hyg. 2020 Apr 10. PubMed: https://pubmed.gov/32277694. Full-text: https://doi.org/10.4269/ajtmh.20-0240

Yuan J, Kou S, Liang Y, Zeng J, Pan Y, Liu L. **PCR Assays Turned Positive in 25 Discharged COVID-19 Patients**. Clin Infect Dis. 2020 Apr 8. pii: 5817588. PubMed: https://pubmed.gov/32266381. Full-text: https://doi.org/10.1093/cid/ciaa398

Zhou F, Yu T, Du R, et al. **Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study.** Lancet. 2020 Mar 11. pii: S0140-6736(20)30566-3. PubMed: https://pubmed.gov/32171076. Full-text: https://doi.org/10.1016/S0140-6736(20)30566-3

7. Treatment

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The number of people infected with SARS-CoV-2 is increasing rapidly. Because up to 5-10% can have a severe, potentially life-threatening course, there is an urgent need for effective drugs. No proven effective therapy for this virus currently exists. The time in this pandemic is too short for the development of new, specific agents; a vaccine will also be a long time coming. Thus, existing antivirals or immune modulators with known safety profiles will gain traction as the fastest route to fight COVID-19. Those compounds that have already been tested in other indications now have priority, in particular those that have been shown to be effective in other beta-coronaviruses such as SARS and MERS.

Many current suggestions have emerged from animal models, cell lines or even virtual screening models. While some approaches have at least some evidence for clinical benefit, for others this remains highly speculative. A brief look at ClinicalTrials.gov may illustrate the intensive research efforts that are underway: on April 18, the platform listed 657 studies, with 284 recruiting, among them 121 in Phase III randomized clinical trials (RCTs, assessed on April 19).

Several very different therapeutic approaches are in the treatment pipeline for COVID-19: antiviral compounds that inhibit enzyme systems, those inhibiting the entry of SARS-CoV-2 into the cell and, finally, immunomodulators that are supposed to reduce the cytokine storm and associated pulmonary damage that is seen in severe case. In an interim guidance, the WHO stated on March 13, that "there is no current evidence to recommend any specific anti-COVID-19 treatment" and that use

of investigational therapeutics "should be done under ethically approved, randomized, controlled trials" (WHO 2020).

However, performing clinical trials remains challenging during a public health crisis (Rome 2020) and enrolling patients in clinical trials will not be possible everywhere. For these, this chapter may support in decision-making. The following agents will be discussed here:

1.	**Inhibitors of viral RNA synthesis**	
	RdRp Inhibitors	Remdesivir, Favipiravir (and Ribavirin, Sofosbuvir)
	Protease Inhibitors	Lopinavir/r (and Darunavir)
2.	**Antiviral Entry Inhibitors**	
	TMPRSS2 Inhibitors	Camostat
	Fusion Inhibitors	Umifenovir
	Others	Hydroxy/chloroquine, Oseltamivir, Baricitinib
3.	**Immunomodulators and other immune therapies**	
	Corticosteroids	
	IL-6 targeting therapies	Tocilizumab, Siltuximab
	Passive immunization	Convalescent plasma

Inhibitors of the viral RNA synthesis

SARS-CoV-2 is a single-stranded RNA beta-coronavirus. Potential targets are some non-structural proteins such as protease, RNA-dependent RNA polymerase (RdRp) and helicase, but also accessory proteins. Coronaviruses do not use reverse transcriptase. There is only a total of 82% genetic identity between SARS-CoV and SARS-CoV-2. However, the strikingly

high genetic homology for one of the key enzymes, the RdRp which reaches around 96% (Morse 2020), suggests that substances effective for SARS may also be effective for COVID-19.

RdRp inhibitors

Remdesivir

Remdesivir (RDV) is a nucleotide analogue and the prodrug of an adenosine C nucleoside which incorporates into nascent viral RNA chains, resulting in premature termination. From WHO, remdesivir has been ranked as the most promising candidate for the treatment of COVID-19. *In vitro* experiments have shown that remdesivir has a broad anti-CoV activity by inhibiting RdRp in airway epithelial cell cultures, even at submicromolar concentrations (Sheahan 2017). This RdRp inhibition also applies to SARS-CoV-2 (Wang 2020). The substance is very similar to tenofovir alafenamide, another nucleotide analogue used in HIV therapy. Remdesivir was originally developed by Gilead Sciences for the treatment of the Ebola virus but was subsequently abandoned, after disappointing results in a large randomized clinical trial (Mulangu 2019). Experimental data from mouse models showed better prophylactic and therapeutic efficacy in MERS than a combination of lopinavir/ritonavir (see below) and interferon beta. Remdesivir improved lung function and reduced viral load and pulmonary damage (Sheahan 2020). Resistance to remdesivir in SARS was generated in cell cultures, but was difficult to select and seemingly impaired viral fitness and virulence (Agostini 2018). The same is seen with MERS viruses (Cockrell 2016). Animal models suggest that a once-daily infusion of 10 mg/kg remdesivir may be sufficient for treatment; pharmacokinetic data for humans are still lacking. Gilead is currently "in the process" of opening expanded access programs

in Europe (refer to gilead.com). In the US, this program is already in place.

Clinical data: Safety has been shown in the Ebola trial. Remdesivir is currently being tested in several randomized phase III RCTs in >1,000 patients with both mild-to-moderate and with severe COVID-19 disease. These studies recruiting patients in China and several European countries are planned to be completed by the end of April 2020. Remdesivir is among four treatment options which are tested in the large WHO SOLIDARITY RCT (see below). In the phase III studies on COVID-19, an initial dose of 200 mg is started on day 1, similar to the Ebola studies, followed by 100 mg for another 9 days.

There are some case reports on critically ill patients, improving rapidly after intravenous treatment with remdesivir (Holshue 2020, Hillaker 2020). On April 10, the New England Journal of Medicine published data on the first 53 patients who were treated with 10 days of remdesivir on a compassionate use basis (Grein 2020). These results gained a lot of media attraction as the authors offered an optimistic view on remdesivir. Although viral data were not available, they concluded with a clinical "improvement in 68%" (36/53) and a "noteworthy" low mortality of 13%, seemingly lower than seen in a RCT on lopinavir/r (Cao 2020). The authors also emphasize repeatedly the severity of disease in their patients, as many required ventilation – more than in the lopinavir/r trial. However, for several reasons we feel that this report is a cautionary tale for rushing science. In the absence of primary end points and viral data, this fragmentary report may arouse false expectations. For more details, see www.CovidReference.com/remdesivir.

Favipiravir

Favipiravir is another broad antiviral RdRp inhibitor that has been approved for influenza A and B in Japan and other countries (Shiraki 2020). Favipiravir is converted into an active form intracellularly and recognized as a substrate by the viral RNA polymerase, acting like a chain terminator and thus inhibiting RNA polymerase activity (Delang 2018). In an *in vitro* study, this compound showed no strong activity against a clinical isolate of SARS-CoV-2 (Wang 2020). On February 14, however, a press release with promising results was published in Shenzhen (PR Favipiravir 2020). In the absence of scientific data, favipiravir has been granted five-year approval in China under the trade name Favilavir® (in Europe: Avigan®). A loading dose of 2400 mg BID is recommended, following a maintenance dose of 1200-1800 mg QD. Potential drug-drug interactions (DDIs) have to be considered. As the parent drug undergoes metabolism in the liver mainly by aldehyde oxidase (AO), potent AO inhibitors such as cimetidine, amlodipine, or amitriptyline are expected to cause relevant DDIs (review: Du 2020).

Clinical data: Preliminary results (press release) on encouraging results in 340 COVID-19 patients were reported from Wuhan and Shenzhen. With favipiravir, patients showed shorter periods of fever (2.5 versus 4.2 days), faster viral clearance (4 versus 11 days) and improvement in radiological findings (Bryner 2020). A first open-label randomized trial (RCT) was posted on March 26 (Chen 2020). This RCT was conducted in 3 hospitals from China, comparing arbidol and favipiravir in 236 patients with COVID-19 pneumonia. Primary outcome was the 7-day clinical recovery rate (recovery of fever, respiratory rate, oxygen saturation and cough relief). In "ordinary" COVID-19 patients (not critical), recovery rates were 56% with arbidol (n=111) and 71% (n=98) with favipiravir (p=0.02), which was well tolerated, except for

some elevated serum uric acid levels. However, it remains unclear whether these striking results are credible. In the whole study population, no difference was evident. Many cases were not confirmed by PCR. There were also imbalances between subgroups of "ordinary" patients.

Other RdRp inhibitors

Some other compounds inhibiting RdRp have been discussed. Ribavirin is a guanosine analogue and RNA synthesis inhibitor that was used for many years for hepatitis C infection and is also thought to inhibit RdRp (Elfiky 2020). In SARS and MERS, ribavirin was mostly combined with lopinavir/ritonavir or interferon; however, a clinical effect has never been shown (Arabi 2017). Ribavirin is now available generically. Its use is limited by considerable side effects, especially anemia. Sofosbuvir is a polymerase inhibitor which is also used as a direct-acting agent in hepatitis C. It is usually very well tolerated. Modelling studies have shown that sofosbuvir could also inhibit RdRp by competing with physiological nucleotides for RdRp active site (Elfiky 2020). Sofosbuvir could be combined with HCV PIs. Among these, the fixed antiviral combinations with ledipasvir or velpatasvir could be particularly attractive as they may inhibit the both RdRp and protrease of SARS-CoV-2 (Chen 2020). Studies are planned but not yet officially registered (assessed April 17).

Protease inhibitors

Lopinavir

Some HIV protease inhibitors (PI) such as lopinavir and darunavir are thought to inhibit the 3-chymotrypsin-like protease of coronaviruses. Both are administered orally. To achieve appropriate plasma levels, these PIs have to be boosted

with another HIV PI called ritonavir (usually indicated by "/r": lopinavir/r and darunavir/r). For lopinavir/r, at least two case-control studies on SARS (Chan 2003, Chu 2004) and one prophylactic study on MERS (Park 2019) have indicated a beneficial effect, but the evidence remains poor. A small substudy indicated that SARS-CoV viral load seems to decrease more quickly with lopinavir than without (Chu 2004). However, all studies were small and non-randomized. It therefore remained unclear, whether all prognostic factors were matched appropriately. As with all HIV PIs, one should be always aware of drug-drug interactions. Ritonavir is a strong pharmacoenhancer. For example, tacrolimus has to be reduced by 10-100 fold to maintain concentration within the therapeutical range. In a case report, a woman with kidney transplantation was treated with lopinavir/r for COVID-19 while receiving full dose tacrolimus. Levels went incredibly high and were still above the therapeutical range 9 days after stopping both lopinavir/r and tacrolimus (Bartiromo 2020).

Clinical data: Lopinavir/r was used in many patients in China at the beginning of the outbreak (Chen 2020). A sharp decline has been seen in individual cases (Lim 2020, Liu 2020, Wang 2020). However, in a small study from Singapore study, lopinavir/r showed no effect on SARS-CoV-2 clearance in nasal swabs (Young 2020). In addition, the first randomized open-lable trial in 199 adults hospitalized with severe COVID-19 did not find any clinical benefit with lopinavir/r treatment beyond standard care (Cao 2020) in patients receiving the drug 10 to 17 days after onset of illness. The percentages of patients with detectable viral RNA at various time points were similar, suggesting no discernible effect on viral shedding. Although PK data is lacking, it seems to be possible that concentrations of protein-unbound lopinavir achieved by current HIV dosing is too low for

inhibiting viral replication. It remains to be seen whether levels will be sufficient for (earlier) treatment of mild cases or as post-exposure prophylaxis. There is one retrospective study on 280 cases in which early initiation of lopinavir/r and/or ribavirin showed some benefits (Wu 2020). Lopinavir/r will be tested in WHO's huge SOLIDARITY trial.

Darunavir

For the other HIV PI, darunavir, there are also press releases on antiviral effects in cell cultures (PR 2020). In HIV infection, darunavir is more effective than lopinavir which led to speculations about an effect in COVID-19. However, the manufacturer Janssen-Cilag published a letter to the European Medical Agency on March 13, pointing out that "based on preliminary, unpublished results from a previously reported *in vitro* experiment, it is not likely darunavir will have significant activity against SARS-CoV-2 when administered at the approved safe and efficacious dose for the treatment of HIV-1 infection." *In vitro* there was no antiviral activity against a clinical isolate at clinically relevant concentrations (EC50 >100 µM).

Clinical data: None. However, we have seen at least 4 HIV-infected patients developing COVID-19 while on darunavir. Nevertheless, a large study (CQ4COV19) with 3,040 participants was started on March 18 in Spain for darunavir and is still ongoing (assessed April 14). Patients with mild symptoms are treated with darunavir/ritonavir and chloroquine immediately after a positive SARS-CoV-2 test.

Other PIs

It is hoped that the recently published pharmacokinetic characterization of crystal structure of the main protease SARS-

CoV-2 may lead to the design of optimized protease inhibitors (Zhang 2020). Virtual drug screening to identify new drug leads that target protease which plays a pivotal role in mediating viral replication and transcription, have already identified several compounds. Six compounds inhibited M(pro) with IC50 values ranging from 0.67 to 21.4 muM, among them with disulfiram and carmofur (a pyrimidine analogue used as an antineoplastic agent) two approved drugs (Jin 2020).

Antiviral entry inhibitors

Most coronaviruses attach to cellular receptors by their spike (S) protein. Within a few weeks, several groups have elucidated the entry of SARS-CoV-2 into the target cell (Hoffmann 2020, Zhou 2020). Similar to SARS-CoV, SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as a key receptor, a surface protein that is found in various organs and on lung AT2 alveolar epithelial cells. The affinity for this ACE-2 receptor appears to be higher with SARS-CoV-2 than with other coronaviruses. The hypothesis that ACE inhibitors promote severe COVID-19 courses through increased expression of the ACE2 receptor remains unproven (see clinical chapter).

Camostat

In addition to binding to the ACE2 receptor, priming or cleavage of the spike protein is also necessary for viral entry, enabling the fusion of viral and cellular membranes. SARS-CoV-2 uses the cellular protease transmembrane protease serine 2 (TMPRSS2). Compounds inhibiting this protease may therefore inhibit viral entry (Kawase 2012). The TMPRSS2 inhibitor camostat, which was approved in Japan for the treatment of chronic pancreatitis (trade name: Foipan®), may block the cellular entry of the SARS-CoV-2 virus (Hoffmann 2020).

Clinical data: pending. Phase II studies are underway (Denmark). Another study (CLOCC trial) is planned for June in Germany, comparing camostat and hydroxychloroquine.

Umifenovir

Umifenovir (Arbidol®) is a broad-spectrum antiviral drug which is approved as a membrane fusion inhibitor in Russia and China for the prophylaxis and treatment of influenza. Chinese guidelines recommend it for COVID-19, according to a Chinese press release it is able to inhibit the replication of SARS-CoV-2 in low concentrations of 10-30 μM (PR 2020).

Clinical data: In a small retrospective and uncontrolled study in mild to moderate COVID-19 cases, 16 patients who were treated with oral umifenovir 200 mg TID and lopinavir/r were compared with 17 patients who had received lopinavir/r as monotherapy for 5–21 days (Deng 2020). At day 7 (day 14), in the combination group, SARS-CoV-2 nasopharyngeal specimens became negative in 75% (94%), compared to 35% (53%) with lopinavir/r monotherapy. Chest CT scans were improving for 69% versus 29%, respectively. Similar results were seen in another retrospective analysis (Zhu 2020). However, a clear explanation for this remarkable benefit was not provided. There is a preliminary report of a randomized study indicating a weaker effect of umifenovir compared to favipiravir (Chen 2020).

Hydroxychloroquine (HCQ) and Chloroquine (CQ)

Chloroquine is used for prevention and treatment of malaria and is effective (but not approved) as an anti-inflammatory agent for rheumatoid arthritis and lupus erythematosus. The potential broadly antiviral effect is due to an increase in the endosomal pH value, which disrupts the virus-cell fusion. The glycosylation of cellular receptors of SARS-CoV is also disturbed (Savarino 2003,

Vincent 2005, Yan 2013). In SARS-CoV-2 infection, chloroquine may possibly also inhibit post-entry steps (Wang 2020). In addition to the antiviral effect, anti-inflammatory effects could also be beneficial in COVID-19 pneumonia. A Chinese consensus paper dated March 12 recommended chloroquine for patients with both mild and severe pneumonia (EC 2020). Hydroxychloroquine may be more effective than chloroquine (Yao 2020); it is approved for malaria and certain autoimmune diseases and is also better tolerated. According to *in vitro* data, hydrochloroquine is recommended in a loading dose of 400 mg twice daily, followed by maintenance therapy of 200 mg twice daily (Yao 2020).

An early mini-review stated that "results from more than 100 patients" showed that chloroquine phosphate would be able to alleviate and shorten the course of the disease (Gao 2020). Other experts have raised considerable doubts (Touret 2020). A benefit of chloroquine would be the first positive signal, after decades and hundreds of unsuccessfully studies conducted in a huge number of acute viral diseases. There are also experts arguing that CQ/HCQ could not only be useless but even harmful, as it was seen for Chikungunya virus infection which may be explained by a delay in immune adaptive response (Guastalegname 2020). In cell and animal studies, the effects on other viruses such as avian influenza, Epstein-Barr, or Zika have been variable (Ferner 2020). Precautions for HCQ also include QTc >500 msec and several diseases such as myasthenia gravis, epilepsy etc. Wide use of these drugs will expose patients to rare but potentially fatal harms, including serious cutaneous adverse reactions, fulminant hepatic failure, and ventricular arrhythmias (especially when prescribed with azithromycin).

Clinical data: On March 17, a preliminary report from Marseille, France (Gautret 2020) appeared to show some benefit in a small non-randomized trial on 36 patients. Patients who refused treatment or had an exclusion criteria, served as controls. At day 6, 70% were virologically cured (100% when azithromycin was added) as assessed by nasopharyngeal swabs, compared to 13% in the control group. After reviewing these data, several methodological issues have raised doubts on validity of the data. It became evident that essential standards of data generation and interpretation were seen to be lacking (Kim 2020). However, someone's swanky tweet claiming that the combination of HCQ and azithromycin has "a real chance to be one of the biggest game changers in the history of medicine" (March 21), has attracted world-wide attention. On March 31, a careful review of the risks of HCQ was published, showing how pretentious dissemination of overpromised data may cause severe harm (Yazdany 2020). A small randomized trial from China on 30 patients failed to show any clinical or virological benefit (Chen 2020). However, hydroxychloroquine is currently tested in several trials, including WHO's SOLIDARITY trial. Optimal dosing still remains unclear. Ongoing clinical trials use different dosing regimens. In a PK study on 13 critically ill patients with COVID-19, a dosing regimen of 200 mg three times daily dosing was inappropriate to reach a supposed target blood level of 1-2 mg/L. Authors proposed 800 mg once daily on day 1, followed by 200 mg twice daily for 7 days (Perinel 2020). However, further PK studies are needed.

Others

Baricitinib (Olumiant®) is a Janus-associated kinase (JAK) inhibitor approved for rheumatoid arthritis. Using virtual screening algorithms, baricitinib was identified as a substance

that could inhibit ACE2-mediated endocytosis (Stebbing 2020). Like other JAK inhibitors such as fedratinib or ruxolitinib, signaling inhibition may also reduce the effects of the increased cytokine levels that are frequently seen in patients with COVID-19. There is some evidence that baricitinib could be the optimal agent in this group (Richardson 2020). Other experts have argued that the drug would be not an ideal option due the fact that baricitinib causes lymphocytopenia, neutropenia and viral reactivation (Praveen 2020). However, several studies are underway in Italy and the US.

Oseltamivir (Tamiflu®) is a neuraminidase inhibitor that is also approved for the treatment and prophylaxis of influenza in many countries. Like lopinavir, oseltamivir has been widely used for the current outbreak in China (Guan 2020). Initiation may be crucial immediately after the onset of symptoms. Oseltamivir is best indicated for accompanying influenza coinfection, which has been seen as quite common in MERS patients at around 30% (Bleibtreu 2018). There is no valid data for COVID-19. It is more than questionable whether there is a direct effect in influenza-negative patients with COVID-19 pneumonia. SARS-CoV-2 does not require neuramidases to enter target cells.

Immunomodulators and other immune therapies

While antiviral drugs are most likely to prevent mild COVID-19 cases from becoming severe, adjuvant strategies will be particularly necessary in severe cases. Coronavirus infections may induce excessive and aberrant, ultimately ineffective host immune responses that are associated with severe lung damage (Channappanavar 2017). Similar to SARS and MERS, some patients with COVID-19 develop acute respiratory distress

syndrome (ARDS), often associated with a cytokine storm (Mehta 2020). This is characterized by increased plasma concentrations of various interleukins, chemokines and inflammatory proteins.

Various host-specific therapies aim to limit the immense damage caused by the dysregulation of pro-inflammatory cytokine and chemokine reactions (Zumla 2020). Immunosuppressants, interleukin-1 blocking agents such as anakinra or JAK-2 inhibitors are also an option (Mehta 2020). These therapies may potentially act synergistically when combined with antivirals. Several marketed drugs are discussed, including those for lowering cholesterol, for diabetes, arthritis, epilepsy and cancer, but also antibiotics. They are said to modulate autophagy, promote other immune effector mechanisms and the production of antimicrobial peptides. However, clinical data is pending for most strategies.

Corticosteroids

Corticosteroids are often used, especially in severe cases. In the largest uncontrolled cohort study to date of 1,099 patients with COVID-19, a total of 19% were treated with corticosteroids, in severe cases almost half of all patients (Guan 2020). However, according to current WHO guidelines, steroids are not recommended outside clinical trials.

A systematic review of several observational SARS studies (Stockman 2006) yielded no benefit and various side effects (avascular necrosis, psychosis, diabetes). However, the use of corticosteroids COVID-19 is still very controversial (Russell 2020, Shang 2020). In a retrospective study of 401 patients with SARS, it was found that low doses reduce mortality and are able to shorten the length of hospital stay for critically ill patients, without causing secondary infection and/or other complications (Chen 2006).

In another retrospective study involving a total of 201 COVID-19 patients, methylprednisolone reduced mortality in patients with ARDS (Wu 2020). On the other hand, there is strong evidence of a delayed viral clearance (Ling 2020), which has also been observed with SARS (Stockman 2006). In a consensus statement by the Chinese Thoracic Society on February 8, corticosteroids should only be used with caution, after careful consideration, at low doses (≤ 0.5–1 mg/kg methylprednisolone or equivalent per day) and, last but not least, as short as possible (≤ 7 Days) (Zhao 2020).

Tocilizumab

Tocilizumab is a monoclonal antibody that targets the interleukin-6 receptor. Tocilizumab (RoActemra® or Actemra®) is used for rheumatic arthritis and has a good safety profile. There is no doubt that tocilizumab should be reserved for patients with severe disease who have failed other therapies. However, some case reports have suggested that IL-6-blocking treatment given for chronic autoimmune diseases may even prevent the development of severe COVID-19 (Mihai 2020).

Clinical data: Some case reports exist. Three patients showed rapid relief of respiratory symptoms, resolution of fever and reduction in CRP following tocilizumab administration (Di Giambenedetto 2020). One uncontrolled, retrospective study has been published (not yet peer reviewed), showing encouraging results in 91% of 21 patients with severe COVID-19 and elevated IL-6 levels, as measured by improved respiratory function, rapid defervescence and successful discharge (Xu 2020). The initial dose should be 4-8 mg/kg, with the recommended dosage being 400 mg (infusion over more than 1 hour). Controlled trials are underway as well as for sarilumab (Kevzara®), another IL-6 receptor antagonist.

Siltuximab

Siltuximab (Sylvant®) is another anti-IL-6-blocking agent. However, this chimeric monoclonal antibody targets interleukin-6 directly and not the receptor. Siltuximab has been approved for idiopathic multicentric Castleman's disease (iMCD). In these patients it is well tolerated.

Clinical data: First results of a pilot trial in Italy ("SISCO trial") have shown encouraging results. According to interim interim data, presented on April 2 from the first 21 patients treated with siltuximab and followed for up to seven days, one-third (33%) of patients experienced a clinical improvement with a reduced need for oxygen support and 43% of patients saw their condition stabilise, indicated by no clinically relevant changes (McKee 2020).

Passive immunization (antibodies)

A meta-analysis of observational studies on passive immunotherapy for SARS and severe influenza indicates a decrease in mortality, but the studies were commonly of low or very low quality and lacked control groups (Mair-Jenkins 2015). In MERS, fresh frozen convalescent plasma or immunoglobulin from recovered patients have been discussed (Zumla 2015, Arabi 2017). Recovered SARS patients develop a neutralizing antibody response against the viral spike protein (Liu 2006). Preliminary data indicate that this response also extends to SARS-CoV-2 (Hoffmann 2020), but the effect on SARS-CoV-2 was somewhat weaker. Others have argued that human convalescent serum could be an option for prevention and treatment of COVID-19 disease to be rapidly available when there are sufficient numbers of people who have recovered and can donate immunoglobulin-containing serum (Casadevall 2020). Recently, an overview on

current evidence of benefit, regulatory considerations, logistical work flow (recruitment of donors etc) and proposed clinical trials has been published (Bloch 2020). Passive immune therapy appears to be safe. However, an unintended consequence of receiving convalescent plasma or globulins may be that recipients won't develop their own immunity, putting them at risk for reinfection.

Clinical data: In a preliminary uncontrolled case series of 5 critically ill patients with COVID-19 and ARDS, administration of convalescent plasma containing neutralizing antibody was followed by improvement in their clinical status (Shen 2020). All 5 patients were receiving mechanical ventilation at the time of treatment and all had received antiviral agents and methylprednisolone. In another pilot study, a single dose (200 mL) of convalescent plasma was given to 10 patients (9 treated with umifenovir, 6 with methylprednisolone, 1 with remdesivir). In all 7 patients with viremia, serum SARS-CoV-2 RNA decreased to an undetectable level within 2-6 days (Duan 2020). Meanwhile, clinical symptoms and paraclinical criteria rapidly improved - within three days. On March 26, the FDA has approved the use of plasma from recovered patients to treat people who are critically ill with COVID-19 (Tanne 2020). It's now time for larger studies.

Others

Interferons: In patients with MERS, interferon studies were disappointing. Despite impressive antiviral effects in cell cultures (Falzarano 2013), no convincing benefit was shown in clinical studies in combination with ribavirin (Omrani 2014, Shalhoub 2015, Arabi 2019). Nevertheless, inhalation of interferon is still recommended as an option in Chinese treatment guidelines.

Other immunomodulatory and other approaches in clinical testing include bevacizumab, brilacidin, cyclosporin, fedratinib (Wu 2020), fingolimod, lenadilomide and thalidomide, sildenafil, teicoplanin (Baron 2020), monoclonal antibodies (Shanmugaraj 2020) and many more. Cellular therapy approaches are also being discussed. However, there is no doubt that these strategies are still far away from broad clinical use.

Outlook

It is hoped that local health systems can withstand the current outbreak and that at least some of the options given in this overview will show positive results over time. It is also important that in this difficult situation, despite the immense pressure, the basic principles of drug development and research including repurposing are not abandoned.

Four different options, namely lopinavir/r, alone and in combination with interferon, remdesivir and (hydroxy) chloroquine will be tested in the SOLIDARITY study launched on March 18 by the WHO. Results of this large-scale, pragmatic trial will generate the robust data we need, to show which treatments are the most effective (Sayburn 2020).

So in the present dark times, which are the best options to offer patients? There is currently no evidence from controlled clinical trials to recommend a specific treatment for SARS-CoV-2 coronavirus infection. A task force of diverse groups of Belgian clinicians has developed "Interim Guidelines for patients suspected of/confirmed with COVID-19 in Belgium" that were published on March 24. They also refer to other Interim Guidelines, as shown in Table 1.

Table 1. Preliminary guidelines for COVID-19 in different countries, according to disease severity (https://epidemio.wiv-isp.be)

Disease severity	Italy (Lombardia protocol)	France	Netherlands	Belgium
Mild to moderate, no risk factors	No	No	No	No
Mild to moderate, risk factors	LPV/r + (H)CQ for 5-7 days	Consider LPV/r, duration depending on viral shedding	Consider CQ for 5 days	Consider HCQ 400 BID, then 200 mg BID for 4 days
Severe	RDV + (H)CQ for 5-20 days	RDV, duration depending on viral shedding	CQ (600 mg, then 300 mg) for 5 days	HCQ 400 BID, then 200 mg BID for 4 days
Severe, 2nd Choice	LPV/r with CQ	No	LPV/r for 10-14 days	LPV/r for 14 days
Critical	RDV + (H)CQ for 5-20 days	RDV, duration depending on viral shedding	RDV for 10 days + CQ for 5 days	RDV
Critical, 2nd Choice	LPV/r with CQ	LPV/r		HCQ (TOC within RCTs)

RDV Remdesivir, LPV/r Lopinavir/ritonavir, (H)CQ (Hydroxy) Chloroquine, TOC Tocilizumab. Risk factors: age > 65 years and/or underlying end organ dysfunction (lung, heart, liver), diabetes, CVD, COPD, hypertension

We predict that within months, we will shake our heads in disbelief at these recommendations but this is no reason to remain inactive today. The task of medicine is to offer the best known treatment at a given moment. At present, the best treatment is supportive care for respiratory failure and hope that some of the above mentioned drugs have a marginal benefit. Even a marginal

benefit might help patients to surpass *in extremis* the divide between life and death.

References

Agostini ML, Andres EL, Sims AC, et al. **Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease.** mBio. 2018 Mar 6;9(2). pii: mBio.00221-18. PubMed: https://pubmed.gov/29511076. Full-text: https://doi.org/10.1128/mBio.00221-18

Arabi YM, Balkhy HH, Hayden FG, et al. **Middle East Respiratory Syndrome.** N Engl J Med. 2017 Feb 9;376(6):584-594. PubMed: https://pubmed.gov/28177862. Full-text: https://doi.org/10.1056/NEJMsr1408795

Arabi YM, Shalhoub S, Mandourah Y, et al. **Ribavirin and Interferon Therapy for Critically Ill Patients With Middle East Respiratory Syndrome: A Multicenter Observational Study.** Clin Infect Dis. 2019 Jun 25. pii: 5523209. PubMed: https://pubmed.gov/31925415. Full-text: https://doi.org/10.1093/cid/ciz544

Baron SA, Devaux C, Colson P, Raoult D, Rolain JM. **Teicoplanin: an alternative drug for the treatment of coronavirus COVID-19?** Int J Antimicrob Agents. 2020 Mar 13:105944. PubMed: https://pubmed.gov/32179150. Full-text: https://doi.org/10.1016/j.ijantimicag.2020.105944

Bartiromo M, Borchi B, Botta A, et al. **Threatening drug-drug interaction in a kidney transplant patient with Coronavirus Disease 2019 (COVID-19).** Transpl Infect Dis. 2020 Apr 12. PubMed: https://pubmed.gov/32279418. Full-text: https://doi.org/10.1111/tid.13286

Bleibtreu A, Jaureguiberry S, Houhou N, et al. **Clinical management of respiratory syndrome in patients hospitalized for suspected Middle East respiratory syndrome coronavirus infection in the Paris area from 2013 to 2016.** BMC Infect Dis. 2018 Jul 16;18(1):331. PubMed: https://pubmed.gov/30012113. Full-text: https://doi.org/10.1186/s12879-018-3223-5

Bloch EM, Shoham S, Casadevall A, et al. **Deployment of convalescent plasma for the prevention and treatment of COVID-19.** J Clin Invest. 2020 Apr 7. pii: 138745. PubMed: https://pubmed.gov/32254064. Full-text: https://doi.org/1387454

Bryner J. **Flu drug used in Japan shows promise in treating COVID-19.** www.Livescience.com

Cao B, Wang Y, Wen D, et al. **A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19.** N Engl J Med. 2020 Mar 18. PubMed: https://pubmed.gov/32187464. Full-text: https://doi.org/10.1056/NEJMoa2001282

Casadevall A, Pirofski LA. **The convalescent sera option for containing COVID-19. J Clin Invest.** 2020 Mar 13. pii: 138003. PubMed: https://pubmed.gov/32167489. Full-text: https://doi.org/10.1172/JCI138003

Chan KS, Lai ST, Chu CM, et al. **Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study.** Hong Kong Med J. 2003 Dec;9(6):399-406 PubMed: https://pubmed.gov/14660806. Full-text: https://www.hkmj.org/PubMeds/v9n6/399.htm

Channappanavar R, Perlman S. **Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology.** Semin Immunopathol. 2017 Jul;39(5):529-539. PubMed: https://pubmed.gov/28466096. Full-text: https://doi.org/10.1007/s00281-017-0629-x

Chen C, Huang J, Cheng Z, et al. **Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial.** Posted March 27, medRxiv 2020.03.17.20037432; https://doi.org/10.1101/2020.03.17.20037432

Chen J, Danping L, Liu L, et al. **A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19.** J Zhejiang University. March 2020

Chen N, Zhou M, Dong X, et al. **Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study.** Lancet. 2020 Feb 15;395(10223):507-513. PubMed: https://pubmed.gov/32007143. Full-text: https://doi.org/10.1016/S0140-6736(20)30211-7

Chen RC, Tang XP, Tan SY, et al. **Treatment of severe acute respiratory syndrome with glucosteroids: the Guangzhou experience.** Chest. 2006 Jun;129(6):1441-52. PubMed: https://pubmed.gov/16778260. Full-text: https://doi.org/10.1378/chest.129.6.1441

Chen YW, Yiu CB, Wong KY. **Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CL (pro)) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates.** F1000Res. 2020 Feb 21;9:129. PubMed: https://pubmed.gov/32194944. Full-text: https://doi.org/10.12688/f1000research.22457.1

Chu CM, Cheng VC, Hung IF, et al. **Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings.** Thorax. 2004 Mar;59(3):252-6. PubMed: https://pubmed.gov/14985565. Full-text: https://doi.org/10.1136/thorax.2003.012658

Cockrell AS, Yount BL, Scobey T, et al. **A mouse model for MERS coronavirus-induced acute respiratory distress syndrome.** Nat Microbiol. 2016 Nov 28;2:16226. PubMed: https://pubmed.gov/27892925. Full-text: https://doi.org/10.1038/nmicrobiol.2016.226

Delang L, Abdelnabi R, Neyts J. **Favipiravir as a potential countermeasure against neglected and emerging RNA viruses.** Antiviral Res. 2018

May;153:85-94. PubMed: https://pubmed.gov/29524445. Full-text: https://doi.org/10.1016/j.antiviral.2018.03.003

Deng L, Li C, Zeng Q, et al. **Arbidol combined with LPV/r versus LPV/r alone against Corona Virus Disease 2019: A retrospective cohort study.** J Infect. 2020 Mar 11. pii: S0163-4453(20)30113-4. PubMed: https://pubmed.gov/32171872. Full-text: https://doi.org/10.1016/j.jinf.2020.03.002

Di Giambenedetto S, Ciccullo A, Borghetti A, et al. **Off-label Use of Tocilizumab in Patients with SARS-CoV-2 Infection**. J Med Virol. 2020 Apr 16. PubMed: https://pubmed.gov/32297987. Full-text: https://doi.org/10.1002/jmv.25897

Du YX, Chen XP. **Favipiravir: pharmacokinetics and concerns about clinical trials for 2019-nCoV infection**. Clin Pharmacol Ther. 2020 Apr 4. PubMed: https://pubmed.gov/32246834. Full-text: https://doi.org/10.1002/cpt.1844

Duan K, Liu B, Li C, et al. **Effectiveness of convalescent plasma therapy in severe COVID-19 patients**. PNAS 2020, April 6. https://doi.org/10.1073/pnas.2004168117

EC. **Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia.** Zhonghua Jie He He Hu Xi Za Zhi 2020 Mar 12;43(3):185-188.

Elfiky AA. **Anti-HCV, nucleotide inhibitors, repurposing against COVID-19.** Life Sci. 2020 May 1;248:117477. PubMed: https://pubmed.gov/32119961. Full-text: https://doi.org/10.1016/j.lfs.2020.117477

Falzarano D, de Wit E, Rasmussen AL, et al. **Treatment with interferon-alpha2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques.** Nat Med. 2013 Oct;19(10):1313-7. PubMed: https://pubmed.gov/24013700. Full-text: https://doi.org/10.1038/nm.3362

Ferner RE, Aronson JK. **Chloroquine and hydroxychloroquine in covid-19**. BMJ. 2020 Apr 8;369:m1432. PubMed: https://pubmed.gov/32269046. Full-text: https://doi.org/10.1136/bmj.m1432

Gao J, Tian Z, Yang X. **Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies.** Biosci Trends. 2020 Mar 16;14(1):72-73. PubMed: https://pubmed.gov/32074550. Full-text: https://doi.org/10.5582/bst.2020.01047

Gautret P, Lagier JC, Parola P, et al. **Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial.** Int J Antimicrob Agents. 2020 Mar 20:105949. PubMed: https://pubmed.gov/32205204. Full-text: https://doi.org/10.1016/j.ijantimicag.2020.105949

Grein J, Ohmagari N, Shin D, et al. **Compassionate Use of Remdesivir for Patients with Severe Covid-19**. N Engl J Med. 2020 Apr 10. PubMed: https://pubmed.gov/32275812. Full-text: https://doi.org/10.1056/NEJMoa2007016

Guastalegname M, Vallone A. **Could chloroquine /hydroxychloroquine be harmful in Coronavirus Disease 2019 (COVID-19) treatment?** Clin Infect Dis. 2020 Mar 24. pii: 5811416. PubMed: https://pubmed.gov/32211771. Full-text: https://doi.org/10.1093/cid/ciaa321

Hillaker E, Belfer JJ, Bondici A, Murad H, Dumkow LE. **Delayed Initiation of Remdesivir in a COVID-19 Positive Patient**. Pharmacotherapy. 2020 Apr 13. PubMed: https://pubmed.gov/32281114. Full-text: https://doi.org/10.1002/phar.2403

Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S. **The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells.** 2020. https://www.biorxiv.org/content/10.1101/2020.01.31.929042v1

Hoffmann M, Kleine-Weber H, Schroeder S, et al. **SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.** Cell. 2020 Mar 4. pii: S0092-8674(20)30229-4. PubMed: https://pubmed.gov/32142651. Full-text: https://doi.org/10.1016/j.cell.2020.02.052

Holshue ML, DeBolt C, Lindquist S, et al. **First Case of 2019 Novel Coronavirus in the United States.** N Engl J Med. 2020 Mar 5;382(10):929-936. PubMed: https://pubmed.gov/32004427. Full-text: https://doi.org/10.1056/NEJMoa2001191

Ji Y, Ma Z, Peppelenbosch MP, Pan Q. **Potential association between COVID-19 mortality and health-care resource availability.** Lancet Glob Health. 2020 Apr;8(4):e480. PubMed: https://pubmed.gov/32109372. Full-text: https://doi.org/10.1016/S2214-109X(20)30068-1

Jin Z, Du X, Xu Y, et al. **Structure of M(pro) from COVID-19 virus and discovery of its inhibitors**. Nature. 2020 Apr 9. pii: 10.1038/s41586-020-2223-y. PubMed: https://pubmed.gov/32272481. Full-text: https://doi.org/10.1038/s41586-020-2223-y

Kawase M, Shirato K, van der Hoek L, Taguchi F, Matsuyama S. **Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry.** J Virol. 2012 Jun;86(12):6537-45. PubMed: https://pubmed.gov/22496216. Full-text: https://doi.org/10.1128/JVI.00094-12

Kim AH, Sparks JA, Liew JW. **A Rush to Judgment? Rapid Reporting and Dissemination of Results and Its Consequences Regarding the Use of Hydroxychloroquine for COVID-19.** Ann Intern Med 2020. DOI: https://doi.org/10.7326/M20-1223

Lim J, Jeon S, Shin HY, et al. **Case of the Index Patient Who Caused Tertiary Transmission of COVID-19 Infection in Korea: the Application of Lopinavir/Ritonavir for the Treatment of COVID-19 Infected Pneumonia Monitored by Quantitative RT-PCR.** J Korean Med Sci. 2020 Feb

17;35(6):e79. PubMed: https://pubmed.gov/32056407. Full-text: https://doi.org/10.3346/jkms.2020.35.e79

Ling Y, Xu SB, Lin YX, et al. **Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients.** Chin Med J (Engl). 2020 Feb 28. PubMed: https://pubmed.gov/32118639. Full-text: https://doi.org/10.1097/CM9.0000000000000774

Liu F, Xu A, Zhang Y, et al. **Patients of COVID-19 may benefit from sustained lopinavir-combined regimen and the increase of eosinophil may predict the outcome of COVID-19 progression.** Int J Infect Dis. 2020 Mar 12. pii: S1201-9712(20)30132-6. PubMed: https://pubmed.gov/32173576. Full-text: https://doi.org/10.1016/j.ijid.2020.03.013

Mair-Jenkins J, Saavedra-Campos M, Baillie JK, et al. **The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis.** J Infect Dis. 2015 Jan 1;211(1):80-90. PubMed: https://pubmed.gov/25030060. Full-text: https://doi.org/10.1093/infdis/jiu396

McKee S. **Positive early data from siltuximab COVID-19 trial.** 2nd April 2020. http://www.pharmatimes.com/news/positive_early_data_from_siltuximab_covid-19_trial_1334145

Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. **COVID-19: consider cytokine storm syndromes and immunosuppression.** Lancet. 2020 Mar 16. pii: S0140-6736(20)30628-0. PubMed: https://pubmed.gov/32192578. Full-text: https://doi.org/10.1016/S0140-6736(20)30628-0

Mihai C, Dobrota R, Schroder M, et al. **COVID-19 in a patient with systemic sclerosis treated with tocilizumab for SSc-ILD.** Ann Rheum Dis. 2020 Apr 2. pii: annrheumdis-2020-217442. PubMed: https://pubmed.gov/32241792. Full-text: https://doi.org/10.1136/annrheumdis-2020-217442

Morse JS, Lalonde T, Xu S, Liu WR. **Learning from the Past: Possible Urgent Prevention and Treatment Options for Severe Acute Respiratory Infections Caused by 2019-nCoV.** Chembiochem. 2020 Mar 2;21(5):730-738. PubMed: https://pubmed.gov/32022370. Full-text: https://doi.org/10.1002/cbic.202000047

Mulangu S, Dodd LE, Davey RT Jr, et al. **A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics.** N Engl J Med. 2019 Dec 12;381(24):2293-2303. PubMed: https://pubmed.gov/31774950. Full-text: https://doi.org/10.1056/NEJMoa1910993

Omrani AS, Saad MM, Baig K, et al. **Ribavirin and interferon alfa-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort study.** Lancet Infect Dis. 2014 Nov;14(11):1090-1095. PubMed: https://pubmed.gov/25278221. Full-text: https://doi.org/10.1016/S1473-3099(14)70920-X

Park SY, Lee JS, Son JS, et al. **Post-exposure prophylaxis for Middle East respiratory syndrome in healthcare workers.** J Hosp Infect. 2019

Jan;101(1):42-46. PubMed: https://pubmed.gov/30240813. Full-text: https://doi.org/10.1016/j.jhin.2018.09.005

Perinel S, Launay M, Botelho-Nevers E, et al. **Towards Optimization of Hydroxychloroquine Dosing in Intensive Care Unit COVID-19 Patients**. Clin Infect Dis. 2020 Apr 7. pii: 5816960. PubMed: https://pubmed.gov/32255489. Full-text: https://doi.org/10.1093/cid/ciaa394

PR Press release. **Favipiravir**. https://www.chinadaily.com.cn/a/202002/17/WS5e49efc2a310128217277fa3.html

PR. Press release. **Abidol and darunavir can effectively inhibit coronavirus.** www.sd.chinanews.com/2/2020/0205/70145.html (accessed February 21, 2020).

Praveen D, Chowdary PR, Aanandhi MV. **Baricitinib - a januase kinase inhibitor - not an ideal option for management of COVID-19**. Int J Antimicrob Agents. 2020 Apr 4:105967. PubMed: https://pubmed.gov/32259575. Full-text: https://doi.org/10.1016/j.ijantimicag.2020.105967

Richardson P, Griffin I, Tucker C, et al. **Baricitinib as potential treatment for 2019-nCoV acute respiratory disease.** Lancet. 2020 Feb 15;395(10223):e30-e31. PubMed: https://pubmed.gov/32032529. Full-text: https://doi.org/10.1016/S0140-6736(20)30304-4

Rome BN, Avorn J. **Drug Evaluation during the Covid-19 Pandemic**. N Engl J Med. 2020 Apr 14. PubMed: https://pubmed.gov/32289216. Full-text: https://doi.org/10.1056/NEJMp2009457

Russell CD, Millar JE, Baillie JK. **Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury.** Lancet. 2020 Feb 15;395(10223):473-475. PubMed: https://pubmed.gov/32043983. Full-text: https://doi.org/10.1016/S0140-6736(20)30317-2

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 Nov;3(11):722-7. PubMed: https://pubmed.gov/14592603. Full-text: https://doi.org/10.1016/s1473-3099(03)00806-5

Sayburn A. **Covid-19: trials of four potential treatments to generate "robust data" of what works.** BMJ. 2020 Mar 24;368:m1206. PubMed: https://pubmed.gov/32209549. Full-text: https://doi.org/10.1136/bmj.m1206

Shalhoub S, Farahat F, Al-Jiffri A, et al. **IFN-alpha2a or IFN-beta1a in combination with ribavirin to treat Middle East respiratory syndrome coronavirus pneumonia: a retrospective study.** J Antimicrob Chemother. 2015 Jul;70(7):2129-32. PubMed: https://pubmed.gov/25900158. Full-text: https://doi.org/10.1093/jac/dkv085

Shang L, Zhao J, Hu Y, Du R, Cao B. **On the use of corticosteroids for 2019-nCoV pneumonia.** Lancet. 2020 Feb 29;395(10225):683-684. PubMed:

https://pubmed.gov/32122468. Full-text: https://doi.org/10.1016/S0140-6736(20)30361-5

Shanmugaraj B, Siriwattananon K, Wangkanont K, Phoolcharoen W. **Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19).** Asian Pac J Allergy Immunol. 2020 Mar;38(1):10-18. PubMed: https://pubmed.gov/32134278. Full-text: https://doi.org/10.12932/AP-200220-0773

Sheahan TP, Sims AC, Graham RL, et al. **Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses.** Sci Transl Med. 2017 Jun 28;9(396). pii: 9/396/eaal3653. PubMed: https://pubmed.gov/28659436. Full-text: https://doi.org/10.1126/scitranslmed.aal3653

Sheahan TP, Sims AC, Leist SR, et al. **Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV.** Nat Commun. 2020 Jan 10;11(1):222. PubMed: https://pubmed.gov/31924756. Full-text: https://doi.org/10.1038/s41467-019-13940-6

Shen C, Wang Z, Zhao F, et al. **Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma.** JAMA. 2020 Mar 27. pii: 2763983. PubMed: https://pubmed.gov/32219428. Full-text: https://doi.org/10.1001/jama.2020.4783

Shiraki K, Daikoku T. **Favipiravir, an anti-influenza drug against life-threatening RNA virus infections.** Pharmacol Ther. 2020 Feb 22:107512. PubMed: https://pubmed.gov/32097670. Full-text: https://doi.org/10.1016/j.pharmthera.2020.107512

Stebbing J, Phelan A, Griffin I, et al. **COVID-19: combining antiviral and anti-inflammatory treatments.** Lancet Infect Dis. 2020 Feb 27. pii: S1473-3099(20)30132-8. PubMed: https://pubmed.gov/32113509. Full-text: https://doi.org/10.1016/S1473-3099(20)30132-8

Stockman LJ, Bellamy R, Garner P. **SARS: systematic review of treatment effects.** PLoS Med. 2006 Sep;3(9):e343. PubMed: https://pubmed.gov/16968120. Full-text: https://doi.org/10.1371/journal.pmed.0030343

Tanne JH. **Covid-19: FDA approves use of convalescent plasma to treat critically ill patients.** BMJ. 2020 Mar 26;368:m1256. PubMed: https://pubmed.gov/32217555. Full-text: https://doi.org/10.1136/bmj.m1256

Touret F, de Lamballerie X. **Of chloroquine and COVID-19.** Antiviral Res. 2020 Mar 5;177:104762. PubMed: https://pubmed.gov/32147496. Full-text: https://doi.org/10.1016/j.antiviral.2020.104762

Vincent MJ, Bergeron E, Benjannet S, et al. **Chloroquine is a potent inhibitor of SARS coronavirus infection and spread.** Virol J. 2005 Aug 22;2:69. PubMed: https://pubmed.gov/16115318. Full-text: https://doi.org/10.1186/1743-422X-2-69

Wang M, Cao R, Zhang L, et al. **Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro.** Cell Res. 2020 Mar;30(3):269-271. PubMed: https://pubmed.gov/32020029. Full-text: https://doi.org/10.1038/s41422-020-0282-0

Wang Z, Chen X, Lu Y, Chen F, Zhang W. **Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment.** Biosci Trends. 2020 Mar 16;14(1):64-68. PubMed: https://pubmed.gov/32037389. Full-text: https://doi.org/10.5582/bst.2020.01030

WHO. **Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected.** March 13 https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected

Wu C, Chen X, Cai Y, 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 Mar 13. pii: 2763184. PubMed: https://pubmed.gov/32167524. Full-text: https://doi.org/10.1001/jamainternmed.2020.0994

Wu D, Yang XO. **TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib.** J Microbiol Immunol Infect. 2020 Mar 11. pii: S1684-1182(20)30065-7. PubMed: https://pubmed.gov/32205092. Full-text: https://doi.org/10.1016/j.jmii.2020.03.005

Wu J, Li W, Shi X, et al. **Early antiviral treatment contributes to alleviate the severity and improve the prognosis of patients with novel coronavirus disease (COVID-19).** J Intern Med. 2020 Mar 27. PubMed: https://pubmed.gov/32220033. Full-text: https://doi.org/10.1111/joim.13063

Xu X, Han M, Li T. **Effective treatment of severe COVID-19 patients with Tocilizumab.** chinaXiv:202003.00026v1

Yan Y, Zou Z, Sun Y, et al. **Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model.** Cell Res. 2013 Feb;23(2):300-2. PubMed: https://pubmed.gov/23208422. Full-text: https://doi.org/10.1038/cr.2012.165

Yao X, Ye F, Zhang M, 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 Mar 9. pii: 5801998. PubMed: https://pubmed.gov/32150618. Full-text: https://doi.org/10.1093/cid/ciaa237

Yazdany J, Kim AH. **Use of Hydroxychloroquine and Chloroquine During the COVID-19 Pandemic: What Every Clinician Should Know.** Ann Intern Med. 2020. Full-text: https://annals.org/aim/fullarticle/2764199/use-hydroxychloroquine-chloroquine-during-covid-19-pandemic-what-every-clinician

Young BE, Ong SWX, Kalimuddin S, et al. **Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore.** JAMA. 2020 Mar 3. pii: 2762688. PubMed: https://pubmed.gov/32125362. Full-text: https://doi.org/10.1001/jama.2020.3204

Zhang L, Lin D, Sun X, et al. **Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved alpha-ketoamide inhibitors.** Science. 2020 Mar 20. pii: science.abb3405. PubMed: https://pubmed.gov/32198291. Full-text: https://doi.org/10.1126/science.abb3405

Zhao JP, Hu Y, Du RH, et al. **Expert consensus on the use of corticosteroid in patients with 2019-nCoV pneumonia.** Zhonghua Jie He He Hu Xi Za Zhi 2020 Mar 12;43(3):183-184. Full-text: https://doi.org/10.3760/cma.j.issn.1001-0939.2020.03.008

Zhou P, Yang XL, Wang XG, et al. **A pneumonia outbreak associated with a new coronavirus of probable bat origin.** Nature. 2020 Mar;579(7798):270-273. PubMed: https://pubmed.gov/32015507. Full-text: https://doi.org/10.1038/s41586-020-2012-7

Zhu Z, Lu Z, Xu T, et al. **Arbidol Monotherapy is Superior to Lopinavir/ritonavir in Treating COVID-19**. J Infect. 2020 Apr 10. pii: S0163-4453(20)30188-2. PubMed: https://pubmed.gov/32283143. Full-text: https://doi.org/10.1016/j.jinf.2020.03.060

Zumla A, Azhar EI, Arabi Y, et al. **Host-directed therapies for improving poor treatment outcomes associated with the middle east respiratory syndrome coronavirus infections.** Int J Infect Dis. 2015 Nov;40:71-4. PubMed: https://pubmed.gov/26365771. Full-text: https://doi.org/S1201-9712(15)00215-5

Zumla A, Hui DS, Azhar EI, Memish ZA, Maeurer M. **Reducing mortality from 2019-nCoV: host-directed therapies should be an option.** Lancet. 2020 Feb 22;395(10224):e35-e36. PubMed: https://pubmed.gov/32035018. Full-text: https://doi.org/10.1016/S0140-6736(20)30305-6

8. Severe COVID-19

This chapter about severe COVID-19 in the hospital/ICU is still under construction. In the meantime, please find the following recommendations.

Checklists for hospitals

European Centre for Disease Prevention and Control. **Checklist for hospitals preparing for the reception and care of coronavirus 2019 (COVID-19) patients.** ECDC: Stockholm; 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-checklist-hospitals-preparing-reception-care-coronavirus-patients.pdf

Patient admission to ICUs

Swiss Society Of Intensive Care Medicine. **Recommendations for the admission of patients with COVID-19 to intensive care and intermediate care units (ICUs and IMCUs).** Swiss Med Wkly. 2020 Mar 24;150:w20227. Fulltext: https://doi.org/10.4414/smw.2020.20227

Management of critically ill patients

Thomas-Ruddel D, Winning J, Dickmann P, et al. **Coronavirus disease 2019 (COVID-19): update for anesthesiologists and intensivists March 2020.** Anaesthesist. 2020 Mar 24. Fulltext: https://doi.org/10.1007/s00101-020-00760-3

Excellent detailed update for anesthesiologists and those working in intensive care.

Sorbello M, El-Boghdadly K, Di Giacinto I, et al. **The Italian COVID-19 outbreak: experiences and recommendations from clinical practice**. Anaesthesia. 2020 Mar 27. PubMed: https://pubmed.gov/32221973. Fulltext: https://doi.org/10.1111/anae.15049

Detailed practical recommendations, based on experiences in Italy. Key elements of clinical management, airway management, personal protective equipment and non-technical aspects.

Matthay MA, Aldrich JM, Gotts JE. **Treatment for severe acute respiratory distress syndrome from COVID-19.** Lancet Respir Med. 2020 Mar 20. pii:

S2213-2600(20)30127-2. PubMed: https://pubmed.gov/32203709. Fulltext: https://doi.org/10.1016/S2213-2600(20)30127-2

Brief overview of therapeutic options for severe acute respiratory distress syndrome.

Poston JT, Patel BK, Davis AM. **Management of Critically Ill Adults With COVID-19.** JAMA. 2020 Mar 26. Fulltext: https://doi.org/10.1001/jama.2020.4914

Short recommendations, made by the Surviving Sepsis Campaign.

Stam HJ, Stucki G, Bickenbach J. **Covid-19 and Post Intensive Care Syndrome**: A Call for Action. J Rehabil Med. 2020 Apr 14. PubMed: https://pubmed.gov/32286675. Full-text: https://doi.org/10.2340/16501977-2677.

Overview of post-intensive case survivors who have been mechanically ventilated.

Lyons C, Callaghan M. **The use of high-flow nasal oxygen in COVID-19**. Anaesthesia. 2020 Apr 4. PubMed: https://pubmed.gov/32246843. Full-text: https://doi.org/10.1111/anae.15073.

Well-balanced review about the pros and cons of high-flow nasal oxygen.

Smereka J, Puslecki M, Ruetzler K, et al. **Extracorporeal membrane oxygenation in COVID-19**. Cardiol J. 2020 Apr 14. pii: VM/OJS/J/68313. PubMed: https://pubmed.gov/32285929. Full-text: https://doi.org/10.5603/CJ.a2020.0053.

A brief review on ECMO.

https://www.esicm.org/blog

Pragmatic recommendations from Italy on mechanical ventilation and management of sepsis.

Endotracheal intubation, bronchoscopy, airway management and staff safety

Cook TM, El-Boghdadly K, McGuire B, McNarry AF, Patel A, Higgs A. **Consensus guidelines for managing the airway in patients with COVID-19**. Anaes-

thesia. 2020 Mar 27. PubMed: https://pubmed.gov/32221970. Fulltext: https://doi.org/10.1111/anae.15054

Luo M, Cao S, Wei L, et al. **Precautions for Intubating Patients with COVID-19.** Anesthesiology. 2020 Mar 19. PubMed: https://pubmed.gov/32195703. Fulltext: https://doi.org/10.1097/ALN.0000000000003288

Lentz RJ, Colt H. **Summarizing societal guidelines regarding bronchoscopy during the COVID-19 pandemic**. Respirology. 2020 Apr 11. PubMed: https://pubmed.gov/32277733. Full-text: https://doi.org/10.1111/resp.13824.

Loftus RW, Dexter F, Parra MC, Brown JR. **Importance of oral and nasal decontamination for patients undergoing anesthetics during the COVID-19 era**. Anesth Analg. 2020 Apr 3. PubMed: https://pubmed.gov/32250978. Full-text: https://doi.org/10.1213/ANE.0000000000004854.

Cheung JC, Ho LT, Cheng JV, Cham EYK, Lam KN. **Staff safety during emergency airway management for COVID-19 in Hong Kong.** Lancet Respir Med. 2020 Feb 24. pii: S2213-2600(20)30084-9. Fulltext: https://doi.org/10.1016/S2213-2600(20)30084-9

Triage for intensive-care treatment

Swiss Academy Of Medical Sciences. **COVID-19 pandemic: triage for intensive-care treatment under resource scarcity.** Swiss Med Wkly. 2020 Mar 24;150:w20229. PubMed: https://pubmed.gov/32208495. https://doi.org/10.4414/smw.2020.20229

Procedures

An P, Ye Y, Chen M, Chen Y, Fan W, Wang Y. **Management strategy of novel coronavirus (COVID-19) pneumonia in the radiology department: a Chinese experience.** Diagn Interv Radiol. 2020 Mar 25. PubMed: https://pubmed.gov/32209526. Fulltext: https://doi.org/10.5152/dir.2020.20167

Pragmatic recommendations for patient care in the radiology department

Zhang Y, Zhang X, Liu L, Wang H, Zhao Q. **Suggestions for infection prevention and control in digestive endoscopy during current 2019-nCoV pneumonia outbreak in Wuhan, Hubei province, China**. Endoscopy. 2020 Apr;52(4):312-314. PubMed: https://pubmed.gov/32212122. Full-text: https://doi.org/10.1055/a-1128-4313

Brief workflow to prevent SARS-CoV-2 transmission in the endoscopy center

Casini A, Alberio L, Angelillo-Scherrer A, et al. **Thromboprophylaxis and laboratory monitoring for in-hospital patients with Covid-19 - a Swiss consensus statement by the Working Party Hemostasis**. Swiss Med Wkly. 2020 Apr 11;150:w20247. PubMed: https://pubmed.gov/32277760. Full-text: https://doi.org/Swiss Med Wkly. 2020;150:w20247

Thromboprophylaxis and laboratory monitoring

Tay JK, Koo ML, Loh WS. **Surgical Considerations for Tracheostomy During the COVID-19 PandemicLessons Learned From the Severe Acute Respiratory Syndrome Outbreak**. JAMA Otolaryngol Head Neck Surg. Published online March 31, 2020. doi:10.1001/jamaoto.2020.0764.

How to perform a tracheostomy

Hanley B, Lucas SB, Youd E, Swift B, Osborn M. **Autopsy in suspected COVID-19 cases.** J Clin Pathol. 2020 Mar 20. pii: jclinpath-2020-206522. PubMed: https://pubmed.gov/32198191.
Fulltext: https://doi.org/10.1136/jclinpath-2020-206522

European Centre for Disease Prevention and Control. **Considerations related to the safe handling of bodies of deceased persons with suspected or confirmed COVID-19.** Stockholm: ECDC; 2020.
https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-safe-handling-of-bodies-or-persons-dying-from-COVID19.pdf.

Recommendations for conducting autopsies

9. Comorbidities

A comorbidity chapter is coming soon. In the meantime, click the full-text links and read the following articles.

Cardiovascular diseases and diabetes

Bavishi C, Maddox TM, Messerli FH. **Coronavirus Disease 2019 (COVID-19) Infection and Renin Angiotensin System Blockers**. JAMA Cardiol. 2020 Apr 3. pii: 2764299. PubMed: https://pubmed.gov/32242890. Full-text: https://doi.org/10.1001/jamacardio.2020.1282

Bonow RO, Fonarow GC, O´Gara PT, Yancy CW. **Association of Coronavirus Disease 2019 (COVID-19) With Myocardial Injury and Mortality**. JAMA Cardiol. 2020 Mar 27. pii: 2763844. PubMed: https://pubmed.gov/32219362. Full-text: https://doi.org/10.1001/jamacardio.2020.1105

Clerkin KJ, Fried JA, Raikhelkar J, et al. **Coronavirus Disease 2019 (COVID-19) and Cardiovascular Disease.** Circulation. 2020 Mar 21. Fulltext: https://doi.org/10.1161/CIRCULATIONAHA.120.046941

Daniels MJ, Cohen MG, Bavry AA, Kumbhani DJ. **Reperfusion of STEMI in the COVID-19 Era - Business as Usual**? Circulation. 2020 Apr 13. PubMed: https://pubmed.gov/32282225. Full-text: https://doi.org/10.1161/CIRCULATIONAHA.120.047122

Fried JA, Ramasubbu K, Bhatt R, et al. **The Variety of Cardiovascular Presentations of COVID-19**. Circulation. 2020 Apr 3. PubMed: https://pubmed.gov/32243205. Full-text: https://doi.org/10.1161/CIRCULATIONAHA.120.047164

Kaiser UB, Mirmira RG, Stewart PM. **Our Response to COVID-19 as Endocrinologists and Diabetologists**. J Clin Endocrin Metabol, 105, May 2020, published 31 March 2020. https://doi.org/10.1210/clinem/dgaa148

Meng J, Xiao G, Zhang J, et al. **Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension**. Emerg Microbes Infect. 2020 Dec;9(1):757-760. PubMed: https://pubmed.gov/32228222. Full-text: https://doi.org/10.1080/22221751.2020.1746200

Patel AB, Verma A. **COVID-19 and Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers: What Is the Evidence?** JAMA. 2020 Mar 24. Fulltext: https://doi.org/10.1001/jama.2020.4812

Schiffrin EL, Flack J, Ito S, Muntner P, Webb C. **Hypertension and COVID-19**. Am J Hypertens. 2020 Apr 6. pii: 5816609. PubMed: https://pubmed.gov/32251498. Full-text: https://doi.org/10.1093/ajh/hpaa057

Vaduganathan M, Vardeny O, Michel T, McMurray JV, Pfeffer MA, Solomon SD. **Renin–Angiotensin–Aldosterone System Inhibitors in Patients with**

Covid-19. NEJM, March 30, 2020. DOI: 10.1056/NEJMsr2005760. Fulltext: https://www.nejm.org/doi/full/10.1056/NEJMsr2005760?

HIV infection and immunosuppression

Bashyam AM, Feldman SR. **Should patients stop their biologic treatment during the COVID-19 pandemic.** J Dermatolog Treat. 2020 Mar 19:1-2. Fulltext: https://doi.org/10.1080/09546634.2020.1742438

Bousquet J, Akdis C, Jutel M, et al. **Intranasal corticosteroids in allergic rhinitis in COVID-19 infected patients: An ARIA-EAACI statement**. Allergy. 2020 Mar 31. PubMed: https://pubmed.gov/32233040. Full-text: https://doi.org/10.1111/all.14302

Conforti C, Giuffrida R, Dianzani C, Di Meo N, Zalaudek I. **COVID-19 and psoriasis: Is it time to limit treatment with immunosuppressants?** A call for action. Dermatol Ther. 2020 Mar 11:e13298. Fulltext: https://doi.org/10.1111/dth.13298

EACS & BHIVA **Statement on risk of COVID-19 for people living with HIV (PLWH)**. https://www.eacsociety.org/home/covid-19-and-hiv.html

Favalli EG, Ingegnoli F, De Lucia O, Cincinelli G, Cimaz R, Caporali R. **COVID-19 infection and rheumatoid arthritis: Faraway, so close**! Autoimmun Rev. 2020 Mar 20:102523. PubMed: https://pubmed.gov/32205186. Fulltext: https://doi.org/10.1016/j.autrev.2020.102523

Figueroa-Parra G, Aguirre-Garcia GM, Gamboa-Alonso CM, Camacho-Ortiz A, Galarza-Delgado DA. **Are my patients with rheumatic diseases at higher risk of COVID-19?** Ann Rheum Dis. 2020 Mar 22. PubMed: https://pubmed.gov/32205336. Fulltext: https://doi.org/10.1136/annrheumdis-2020-217322

Joob B, Wiwanitkit V. SARS-CoV-2 and HIV. J Med Virol. 2020 Mar 27. PubMed: https://pubmed.gov/32220066. Fulltext: https://doi.org/10.1002/jmv.25782

Pasha SB, Fatima H, Ghouri YA. **Management of Inflammatory Bowel Diseases in the Wake of COVID-19 Pandemic**. J Gastroenterol Hepatol. 2020 Apr 4. PubMed: https://pubmed.gov/32246874. Full-text: https://doi.org/10.1111/jgh.15056

Torres T, Puig L. **Managing Cutaneous Immune-Mediated Diseases During the COVID-19 Pandemic**. Am J Clin Dermatol. 2020 Apr 10. pii: 10.1007/s40257-020-00514-2. PubMed: https://pubmed.gov/32277351. Full-text: https://doi.org/10.1007/s40257-020-00514-2.

U.S. Department of Health and Human Services. **Interim Guidance for COVID-19 and Persons with HIV.** https://aidsinfo.nih.gov/guidelines/html/8/covid-19-and-persons-with-hiv--interim-guidance-/554/interim-guidance-for-covid-19-and-persons-with-hiv

Zhu F, Cao Y, Xu S, Zhou M. **Co-infection of SARS-CoV-2 and HIV in a patient in Wuhan city, China** [published online ahead of print March 11, 2020]. J Med Virol. https://doi.org/10.1002/jmv.25732

Oncology

Coles CE, Aristei C, Bliss J, et al. **International Guidelines on Radiation Therapy for Breast Cancer During the COVID-19 Pandemic**. Clin Oncol (R Coll Radiol). 2020 May;32(5):279-281. PubMed: https://pubmed.gov/32241520. Full-text: https://doi.org/10.1016/j.clon.2020.03.006

Dholaria B, Savani BN. **How do we plan hematopoietic cell transplant and cellular therapy with the looming COVID-19 threat?** Br J Haematol. 2020 Mar 16. Fulltext: https://doi.org/10.1111/bjh.16597

Francesco C, Pettke A, Michele B, Fabio P, Helleday T. **Managing COVID-19 in the oncology clinic and avoiding the distraction effect.** Ann Oncol. 2020 Mar 19. pii: S0923-7534(20)36373-0. Fulltext: https://doi.org/10.1016/j.annonc.2020.03.286

Jin XH, Zheng KI, Pan KH, Xie YP, Zheng MH. **COVID-19 in a patient with chronic lymphocytic leukaemia**. Lancet Haematol. 2020 Apr;7(4):e351-e352. Full-text: https://doi.org/10.1016/S2352-3026(20)30074-0

Liang W, Guan W, Chen R, et al. **Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China.** Lancet Oncol. 2020 Mar;21(3):335-337. Fulltext: https://doi.org/10.1016/S1470-2045(20)30096-6

The Lancet Oncology. **COVID-19: global consequences for oncology**. Lancet Oncol. 2020 Apr;21(4):467. PubMed: https://pubmed.gov/32240603. Full-text: https://doi.org/10.1016/S1470-2045(20)30175-3

Ueda M, Martins R, Hendrie PC, et al. **Managing Cancer Care During the COVID-19 Pandemic: Agility and Collaboration Toward a Common Goal.** J Natl Compr Canc Netw. 2020 Mar 20:1-4. Fulltext: https://doi.org/jnccn1804COVID

Xia Y, Jin R, Zhao J, Li W, Shen H. **Risk of COVID-19 for cancer patients.** Lancet Oncol. 2020 Mar 3. Fulltext: https://doi.org/10.1016/S1470-2045(20)30150-9

Transplantation

Andrea G, Daniele D, Barbara A, et al. **Coronavirus Disease 2019 and Transplantation: a view from the inside**. Am J Transplant. 2020 Mar 17. PubMed: https://pubmed.gov/32181969. Fulltext: https://doi.org/10.1111/ajt.15853

Guillen E, Pineiro GJ, Revuelta I, et al. **Case report of COVID-19 in a kidney transplant recipient: Does immunosuppression alter the clinical presentation?** Am J Transplant. 2020 Mar 20. PubMed: https://pubmed.gov/32198834. Fulltext: https://doi.org/10.1111/ajt.15874

Kumar D, Manuel O, Natori Y, et al. COVID-19: **A Global Transplant Perspective on Successfully Navigating a Pandemic.** Am J Transplant. 2020 Mar 23. PubMed: https://pubmed.gov/32202064. Fulltext: https://doi.org/10.1111/ajt.15876

Zhong Z, Zhang Q, Xia H, et al. **Clinical characteristics and immunosuppressants management of coronavirus disease 2019 in solid organ transplant recipients**. Am J Transplant. 2020 Apr 13. PubMed: https://pubmed.gov/32282986. Full-text: https://doi.org/10.1111/ajt.15928

Dialysis

Basile C, Combe C, Pizzarelli F, et al. **Recommendations for the prevention, mitigation and containment of the emerging SARS-CoV-2 (COVID-19) pandemic in haemodialysis centres.** Nephrol Dial Transplant. 2020 Mar 20. pii: 5810637. PubMed: https://pubmed.gov/32196116. Fulltext: https://doi.org/10.1093/ndt/gfaa069

Other comorbidities

Dave M, Seoudi N, Coulthard P. **Urgent dental care for patients during the COVID-19 pandemic**. Lancet. 2020 Apr 3. pii: S0140-6736(20)30806-0. PubMed: https://pubmed.gov/32251619. Full-text: https://doi.org/10.1016/S0140-6736(20)30806-0

Little P. **Non-steroidal anti-inflammatory drugs and covid-19**. BMJ. 2020 Mar 27;368:m1185. PubMed: https://pubmed.gov/32220865. Fulltext: https://doi.org/10.1136/bmj.m1185

Yao H, Chen JH, Xu YF. **Patients with mental health disorders in the COVID-19 epidemic**. Lancet Psychiatry. 2020 Apr;7(4):e21. Full-text: https://doi.org/10.1016/S2215-0366(20)30090-0

Wang H, Li T, Barbarino P, et al. **Dementia care during COVID-19**. Lancet. 2020 Apr 11; 395(10231):1190-1191. PubMed: https://pubmed.gov/32240625. Full-text: https://doi.org/10.1016/S0140-6736(20)30755-8

10. Pediatrics

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SARS-CoV-2 infection in children

SARS-CoV-2 infection in children and adolescents is a major factor in spreading of the COVID-19 disease worldwide and key to the development of herd immunity. Children have an often asymptomatic or less severe COVID 19 disease course than adults. In this regard COVID is strikingly different from other virus-induced respiratory diseases, which can be fatal in infants (e.g. RSV). The CoV-2 pandemic causes anxiety to seek medical care and leads to collateral damage to children because parents avoid hospitals despite their children having an emergency (Lazzerini 2020).

At this stage (13 April 2020), great caution is advised when interpreting the data that have been collected on children so far, (e.g., data on the Wuhan children were published more than once). Some children were seen in children's hospitals, some in Internal Medicine Departments. The Chinese health system is ranked number 144 out of 191 member countries of the World Health Organization. Delivery of medical service in China is largely dependent on economic income, resulting in a large bias regarding inclusion and exclusion of children into regis-

tries/studies and in under- or over-estimation of important facts like disease severity, outcome, treatment effects.

Commonly circulating coronaviruses in children: tropism, incubation period and spreading

The first International Corona Virus Conference was organized by Volker Termeulen in Würzburg/Germany in 1980. At the time only one human coronavirus, HCoV2229E, was known to be associated with the common cold. (Weiss 2020) Commonly circulating human coronaviruses can be isolated from 4-8% of all children with acute respiratory tract infections, which tend to be mild, unless the child is immunocompromised (Ogimi 2019). Seven coronaviruses circulate among humans: α-Coronaviruses HCoV2-229e, -HKU1; β-Coronaviruses HCoV2-NL63, -OC43; MERS-CoV, SARS-CoV and SARS-CoV-2 that have originally derived from bats (NL63, 229e, SARS-CoV), Dromedary Camels (229e, MERS-CoV), cattle (OC43), pangolins (SARS-CoV-2) (Zimmermann 2020). There appear to be re-infections with the earlier described common COV despite the fact that most individuals seroconvert to human coronaviruses. In many children there are co-infections with other viruses such as Adeno-, Boca-, Rhino-, RSV-, Influenza- or Parainfluenza virus. There seems to be a cyclic pattern with seasonal outbreaks between December and May or March to November in the southern hemisphere.

A characteristic of the single-strand RNA coronaviruses is the capability of rapid mutation and recombination leading to novel coronaviruses that can spread from animals to humans. They have caused epidemics leading to significant case fatality rates (10% in SARS-CoV, Hong Kong 2002; more than 30% in MERS-CoV, Saudi Arabia 2012). Because of the high case fatality rate, both SARS-COV and MERS-COV have a low potential for long-

term sustained community transmission. Accordingly, no human SARS-CoV infections have been reported since July 2003.

It is estimated that in SARS-CoV-2 one person infects 2-3 other persons. In clusters (e.g. nosocomial outbreaks) this number might be much higher. In both SARS-CoV and MERS-CoV, super-spreading events with one individual infecting up to 22 (SARS) or even 30 individuals (MERS) have been reported, especially in nosocomial outbreaks. In SARS-CoV a total of 41 children were reported with no deaths. Similarly, in MERS-CoV only 38 children were reported in two studies, with two deaths (Zimmermann 2020).

Epidemiology of COVID-19 in children

In April 6 the US CDC reported 2572 (1.7%) children under 18 years among 149,082 reported cases from 12 February to 2 April 2020. The availability of data was extremely limited (less than 10% available on symptoms, 13% on underlying conditions, 33% on whether children were hospitalized or not). Three deaths were reported to the CDC but no details were given. The median age was 11 and they were 57% males. 15 children were admitted to an ICU (≤2%). Children <1 year accounted for the highest percentage (15-62%) of hospitalization (CDC 2020). The Chinese CDC report (Dong 2020) comprises 2,143 pediatric patients from January 16 to February 8 2020. Only 731 children (34.1%) were laboratory confirmed cases. The median age was 7 years with 56.6% boys, less than 5% were classified as severe and less than 1% as critical. One Chinese 10 month-old child who had been infected with CoV-2 was reported to have died with intussuception and multi-organ failure (Lu X 2020). The Korean Center for Disease Control and Prevention reported on 20 March that 6.3% of all COVID-19 cases were children under 19 years of age; again, the children had a mild form of the disease (Korean Center for Disease Control and Prevention. Press releases,

https://www.cdc.go.kr). Italian data published on 18 March showed that only 1.2% of the 22,512 Italian cases with COVID-19 were children; no deaths were reported in this and in the Spanish cohort from Madrid (2 March to 16 March) (Livingstone 2020, Tagarro 2020). As of 15 April 2020, in Germany 41 centers reported 65 pediatric hospital admissions, about one third had an underlying disease, mostly pulmonary or cardiac diseases. One child died (a 2 year-old child from a consangineous genetic background with encephalopathy and a history of juvenile idiopathic arthritis and weekly methotrexate treatment; personal communication, U. Neudorf, University Childrens Hospital, Essen) (www.dgpi.de).

Natural course and risk factors for complications

The incubation period is believed to be 3-7 days (range 1-14 days) (She 2020), the clinical onset 5-8 days after infection with the virus. At 10 days after onset of symptoms hyperinflammation may set in and cause a more severe and potentially fatal disease, especially in high risk groups. The clinical manifestation is believed to last for 1-2 weeks, longer in complicated cases. Due to the paucity of data it is as yet unclear which group of children may be at a higher risk for development of complications, e.g. children with underlying chronic pulmonary or cardiac disease, severe neurologic deficits, immunosuppressed or critically ill children etc. Analogous to influenza there might be genetic susceptibility in some children (Clohisey 2019). Interestingly, in a flash survey from 25 countries with 10,000 children with cancer at risk and 200 tested, only 9 were found to be CoV-2 positive. They were asymptomatic or had mild disease (Hrusak 2020).

Pathophysiology and immunopathology

It is unclear why COVID-19 in children is associated with a less severe disease course.

The tissue expression pattern of the receptor for CoV-2 angiotensin converting enzyme (ACE2) and the transmembrane serine protease TMPRSS2 (essential for CoV-2 cell entry) as well as the tissue tropism of CoV-2 in childhood are unknown. ACE2 is expressed on cells of the airways, the lungs, mucosal cells (lids, eyelids, nasal cavities), intestines and on immune cells (monocytes, lymphocytes, neutrophils) (Molloy 2020, reviewed in Brodin 2020). It needs to be clarified whether there is neurotropism (e.g. affecting the developing brain of newborns).

The main target of CoV-2 is the respiratory tract. As respiratory infections are extremely common in children it is to be expected that there are other viruses present in the respiratory tract of young children concomitantly with the coronavirus, which may limit its growth and the number of CoV-2 copies in the respiratory tract of children. Systematic viral load measurements in the respiratory tract of different viruses in children are underway. Key to the later immunopathologic stages of COVID-19 pneumonia is the macrophage activation syndrome (MAS)-like hyperinflammatory phase with a cytokine storm and acute respiratory distress syndrome ARDS, usually within 10-12 days after symptom onset. In general, children are not less prone to develop ARDS during respiratory tract infections than adults. In the H1N1 flu pandemic in 2009, being under the age of 1 year was a significant risk factor for developing a severe form of the infection and ARDS (Bautista 2010). Why ARDS is less common in children compared to adults with COVID is unclear.

Regarding childhood immunity, an explanation for the milder disease course in children could be age-related differences in immune responses to CoV-2 between adults and children. In the innate immune response damaged lung cells induce inflamma-

tion by macrophages and granulocytes. Based on influenza animal models it has been proposed that BCG vaccination (done in the first week of life in some countries) may enhance non-specific innate immunity in children to infections like COVID-19 (so-called trained immunity) (Moorlag 2019).

In the adaptive response cytotoxic T cells play an important role in regulating responses to viral infections and control of viral replication. Children could benefit from the fact that the cytotoxic effector function of CD8 T cells in viral infection in children may be less detrimental compared to adults. Immune dysregulation with exhaustion of T cells has been reported in adults with COVID-19 infection. Children could benefit from the fact that the cytotoxic effector function of CD8 T cells in viral infection in children may be less detrimental compared to adults. Regarding humoral immunity CoV-2 maternal antibodies are transferred to the child via placenta or breast milk but may not include anti CoV-2 antibodies, if the mother is naïve to CoV-2 or infected late in pregnancy. In mothers with COVID-19 pneumonia serum and throat swabs of their newborns were negative for CoV-2 but virus-specific IgG antibodies were detected (Zeng H 2020). Thus, neonates may benefit from placental transmission of virus-specific antibodies from pre-exposed mothers. In SARS-CoV-2 the child itself may mount a significant humoral response to one of the immunodominat epitopes, e.g. the crown-like spike proteins giving the coronaviruses their name. Data regarding seroprevalence and quality of the immune response in children are lacking.

Transmission

Contraction of COVID-19 in a pregnant woman may have an impact on fetal outcome, namely fetal distress, potential preterm birth or respiratory distress if the mother gets very sick. As of yet there is no evidence that SARS-CoV-2 can be transmitted

vertically from mother to child. Amniotic fluid, cord blood, neonatal throat swabs all tested negative in a small cohort (Chen 2020). Schwartz reviewed 5 publications from China and was able to identify 38 pregnant women with 39 offspring among whom 30 were tested for COVID-19 and all of them were negative (Schwartz 2020). Transmission by breastfeeding has not yet been reported and there are no case reports of detection of CoV-2 in breast milk.

SARS-CoV-2 in children is transmitted through family contacts and mainly through respiratory droplets. Prolonged exposure to high concentrations of aerosols may facilitate transmission. (She 2020). Favoring successful virus spread is the fact that virus shedding starts 24-48 hours prior to any symptoms.

SARS-CoV-2 may also be transmitted through the digestive tract. ACE2 is also found in upper esophageal and epithelial cells as well as intestinal epithelial cells in the ileum and colon (She 2020). SARS-CoV-2 RNA can be detected in the feces of patients (Holshue 2020). Cai revealed that viral RNA is detected from feces of children at a high rate (and can be excreted as long as 2-4 weeks) (Cai et al 2020). However, direct evidence of a fecal to oral transmission has not yet been documented.

Diagnosis and classification

Testing for the virus is only necessary in clinically suspect children. If the result is initially negative, repeat nasopharyngeal or throat swab-testing of upper respiratory tract samples or testing of lower respiratory tract samples should be done. Sampling of the lower respiratory tract (induced sputum or bronchoalveolar lavage) is more sensitive (Han 2020). This is not always possible in critically ill patients and in young children.

Diagnosis is usually made by real time polymerase chase reaction RT-PCR on respiratory secretions and available within 4 hours. For SARS-CoV, MERS-CoV and SARS-CoV-2, higher viral loads

have been detected in samples from lower respiratory tract compared with upper respiratory tract. Stool samples cannot be used for routine diagnosis. In rare cases positive PCRs in blood have been reported.

Serologic testing for CoV-2 antibodies in children who are symptomatic is currently not useful but may be helpful to assess immunity in children in the future. As in other viral infections, a CoV-2 specific IgG antibody response will mount within 2-3 weeks after infection and may or may not indicate protective immunity (yet to be determined). In case it indicates protective immunity, this will be extremely important for the assessment of CoV-2 epidemiology and herd immunity.

Table 1. COVID classification in children (Shen 2020)

1	Asymptomatic without any clinical symptoms
2	Mild fever, fatigue, myalgia and symptoms of acute respiratory tract infections,
3	Moderate pneumonia, fever and cough, productive cough, wheezing but no hypoxemia
4	Severe fever, cough, tachypnea, oxygen saturation less than 92%, somnolence
5	Critical quick progress to acute respiratory distress syndrome ARDS or respiratory failure

Laboratory and radiology findings

Laboratory and/or radiology studies in outpatient children who have mild disease are not indicated. Upon admission to the hospital the white blood cell count is usually normal. In a minority of children decreased lymphocyte counts have been documented. In contrast, adults (with hyperinflammation and cytokine release syndrome) often have an increase in neutrophils and lymphopenia. The inflammation parameters C-reactive protein and procalcitonin can be slightly elevated or normal while there

are elevated liver enzymes, creatine kinase CK-MB and D-dimers in some patients. LDH appears to be elevated in severe cases and can be used to monitor severe disease.

A chest X-ray should only be done in children with moderate or more severe disease as CT scans mean a very high radiation exposure for the child and should only be done in complicated or high-risk cases. In the beginning of the pandemic in China, children all received CT scans even when they were asymptomatic and oligosymptomatic; surprisingly, they displayed very severe changes. On chest radiography there are bilateral patchy airspace consolidations and so-called ground-glass opacities. CT scans were more impressive than chest x-ray examinations. In 20 children with CT, 16 (80%) had some abnormalities (Xia 2020).

Symptoms and signs

Children and adolescents

The clinical presentation of the disease appears somewhat similar to influenza. In the largest clinical trial of 171 children from Wuhan fever was reported in 41% (71 of 171), cough in over 50% (83 of 171), tachypnea in 28% (49 of 171). In 27 of the patients there were no symptoms at all (15.8%). At initial presentation very few children required oxygen supplementation (4 of 171, 2.3%). Other symptoms like diarrhea, fatigue, runny nose and vomiting were observed only in less than 10% of the children (Lu 2020). In the case series from Zhejiang as many as 10 out of 36 patients (28%) had no symptoms at all. None of the children had an oxygen saturation below 92% (Qiu 2020).

Neonates and infants

Zeng reports 33 newborns born to mothers with COVID-19 in Wuhan. Three of the 33 infants (9%) presented with early-onset SARS-CoV-2 infection. In 2 of the 3 neonates there were radiolog-

ical signs of pneumonia. In one child disseminated intravascular coagulation was described but eventually all children had stable vital signs three weeks after the infection when the report was published (26 March 2020) (Zeng L 020). In a second cohort, 9 infants aged 1 month to 9 months were described without any severe complications (Wei 2020). Whether there may be complications of COVID-19 in newborns and infants long-term cannot be judged at this stage of the pandemic. At present it is not recommended to separate healthy newborns from mothers with suspicion of COVID-19 (CDC-2 2020). Clearly a preterm or newborn that has been exposed to CoV-2 needs to be closely monitored by the hospital and/or the primary care pediatrician. If there are signs of COVID (e.g. poor feeding, unstable temperature, tachy/dyspnea) it needs to be hospitalized and tested and lab examinations and chest x-ray to be done. Testing for CoV-2 is not useful before day 5 because of the incubation period. There needs to be a strict hygiene as much as possible in this mother-child setting.

Management

Infection control

Early identification of COVID-19 and quarantine of contacts is imperative. In the in- and outpatient setting it is advised to separate children who have infectious diseases from healthy non-infectious children. Nosocomial outbreaks have played a role in the clustering of COVID-19. Thus it is advised to admit children with COVID-19 to the hospital only if an experienced pediatrician feels it is medically necessary (e.g. tachypnea, dyspnea, oxygen levels below 92%). In the hospital the child with COVID-19 or suspicion of COVID-19 needs to be isolated in a single room or admitted to a COVID-19-only ward in which COVID-19 exposed medical personnel maintains distance as well (e.g. no shifts on other wards). The presence of one parent is not negotiable in the

care of the sick child both for emotional reasons as well as for help in nursing of the child.

During the peak phase of the COVID-19 epidemic, precautions in the outpatient and hospital setting include entrance control, strict hand and respiratory hygiene, daily cleaning and disinfection of the environment, and provision of protection (gloves, mask, goggles) for all medical staff when taking care of a COVID-19 or a suspected COVID-19 case (Wang 2020). In neonatal intensive care units (NICU), negative pressure rooms and filtering of exhaust would be ideal (Lu Q 2020). Respirators with closed circuit and filter systems should be used. Aerosol generating procedures, e.g. intubation, bronchoscopy, humidified inhalations/nebulization should be avoided as much as possible.

Supportive treatment (respiratory support, bronchodilatation therapy, fever, superinfection, psychosocial support)

Having the child sitting in an upright position will be helpful for breathing. It might be useful to have physiotherapy. Insufflation of oxygen via nasal cannula will be important to children as it will increase lung ventilation and perfusion. In neonates high flow nasal cannula (HFNC) has been utilized widely due to its superiority over other non-invasive respiratory support techniques.

The clinical use and safety of inhaling different substances in COVID-19 is unclear. In other common obstructive and infectious childhood lung diseases, e.g. in bronchiolitis, the American Academy of Pediatrics is now recommending against the use of bronchodilators (Dunn 2020). Regarding the inhalation of steroids as part of maintenance therapy for asthma bronchiale there is no evidence to discontinue this treatment in children with COVID-19.

There is a large controversy over the extent of antipyretics usage in children. Still, in a child with COVID-19 who is clinically affected by high-degree fever, paracetamol or ibuprofen may be useful. There is no restriction despite initial WHO warnings of using ibuprofen, there is no evidence that the use of paracetamol or ibuprofen is harmful in COVID-19 in children (Day 2020).

The differentiation between CoV-2-induced viral pneumonia and bacterial superinfection is difficult unless there is clear evidence from culture results or typical radiological findings. Bacterial superinfection will be treated according the international and national guidelines (Mathur 2018).

The virus outbreak brings psychological stress to the parents and family as well as medical staff; therefore, social workers and psychologists should be involved when available.

Treatment of respiratory failure

The treatment of pediatric acute respiratory distress syndrome (pARDS) is reviewed elsewhere (Allareddy 2019). For neonates with pARDS high-dose pulmonary surfactant replacement, nitric oxide inhalation, and high-frequency oscillatory ventilation might be effective. In critically ill neonates, continuous renal replacement and extracorporeal membrane oxygenation need to be implemented if necessary.

COVID-19-specific drug treatment

As of yet there are no data from controlled clinical trials and thus there is currently no high-quality evidence available to support the use of any medication to treat COVID-19. The drugs listed below are repurposed drugs and there is limited or almost no pediatric experience. In the case of a severe or critically ill child with COVID the pediatrician has to make a decision whether to try a drug or not. If initiation of a drug treatment is decid-

ed, children should be included into clinical trials (https://www.clinicaltrialsregister.eu) if anyhow possible. However, there are only very few, if any, studies open for recruitment in children.

When to treat with drugs

Under the lead of the German Society for Pediatric Infectiology (DGPI) an expert panel has proposed a consensus on when to start antiviral or immunomodulatory treatment in children (Table 2, https://dgpi.de/stellungnahme-medikamentoese-behandlung-von-kindern-mit-covid-19/).

Inhibitors of viral RNA synthesis

Remdesivir (GS-5734) is available as 150 mg vials. Child dosing is

- <40 kg: 5 mg/kg i.v. loading dose, then 2.5 mg/kg i.v. QD for 9 days
- ≥40 kg: 200 mg loading dose, then 100 mg QD for 9 days

Remdesivir is an adenosine nucleotide analogue with broad-spectrum antiviral activity against various RNA viruses. The compound undergoes a metabolic mechanism, activating nucleoside triphosphate metabolite for inhibiting viral RNA polymerases. Remdesivir has demonstrated *in vitro* and *in vivo* activity in animal models against MERS and SARS-CoV. Remdesivir showed good tolerability and a potential positive effect in regard to decrease of the viral load and mortality in Ebola in Congo in 2018 (Mulangu 2019). In Europe this drug has rarely been used in children so one should be extremely careful. It can be obtained through compassionate use programs (https://rdvcu.gilead.com).

Table 2. Consensus on antiviral or immunomodulatory treatment in children

Disease severity in child	Intervention
Mild or moderate disease pCAP, upper respiratory tract infection, no need for oxygen	Treat symptomatically No need for antiviral or immunomodulatory treatment
More severe disease and risk groups* pCAP, need for oxygen	Treat symptomatically consider antiviral therapy
Critically ill, admitted to ICU	Treat symptomatically Consider antiviral therapy Consider immunomodulatory treatment
Secondary HLH (hemophagocytic lymphohistiocytosis)	Treat with immunomodulatory or immunosuppressive drugs

* Congenital heart disease, immunosuppression, inborn/acquired immunodeficiencies, cystic fibrosis, chronic lung disease, chronic neurological/kidney/liver disease, diabetes/metabolic disease

Lopinavir/r (LPV/r, Kaletra®) is a co-formulation of lopinavir and ritonavir, in which ritonavir acts as a pharmacokinetic enhancer (booster). It is available as 200/50 and 100/25 mg tablets or 133.3/33.3 mg capsules in some countries. There is a liquid preparation with an unpleasant taste (5 ml = 400/100 mg). Liquid has to be kept in the fridge. LPV/r contains 42% ethanol, 153 mg/ml and proprylene glycol which is toxic to preterms/neonates.

Dosing for liquid:

- ≥14 days (postnatal age) and >42 weeks (postmenstrual age) to 6 months (postnatal age):
  - 16/4 mg/kg or 300/75 mg/m^2 BID

- ≥6 months-18 years: 230/57.5 mg/m^2 BID
  - <15 kg 12/3 mg/kg BID
  - ≥15-40 kg: 10/2.5 mg/kg BID (max. 400/100mg BID)

Dosing for tablet:

- 15-25 kg or 0.5-0.9 m^2: 200/50 mg BID
- 25-35 kg or 0.9-1.4 m^2): 300/75 mg BID
- >35 kg or ≥1.4 m^2: 400/100mg BID.

Lopinavir/r should be taken with meals. It has a well-characterized safety, tolerability and toxicity profile. Adverse events include significant drug interactions, pancreatitis, hepatotoxicity, QT and PR interval prolongation.

LPV/r is an HIV-1 protease inhibitor successfully used in HIV infected children as part of highly active antiretroviral combination therapy (PENTA Group 2015). In the SARS epidemics, LPV/r was recommended as a treatment. A recent study in adult COVID-19 patients did not show an effect regarding the primary endpoint in a controlled clinical trial (see the Treatment chapter, page 166). Despite the fact that there is a large experience with LPV/r in HIV it is questionable whether its use in COVID-19 is effective at all.

Inhibitors of viral entry

Hydroxychloroquine (HCQ, Quensyl®) is available as 200 mg tablets. Dosing is day 1 loading dose: 6.5 mg/kg (max. 400 mg) BID; then 3 mg/kg (max. 200 mg) BID for 5-10 days.

Chloroquine (CQ, Resochin junior®, Resochin®) is available as 81 or 250 mg tablets. Dosing is day 1 loading: 8 mg/kg (max 500 mg) BID; then 4 mg/kg (max. 250 mg) BID for 10 days. Oral solution of HCQ or CQ can be produced by the pharmacy. Adverse events: gastrointestinal effects, including nausea, vomiting, diar-

rhoea and abdominal discomfort, myopathy, cardiotoxic effects, including rhythm disorders (such as a prolonged QT interval) and the development of cardiomyopathy. It is useful to do an ECG before starting therapy. Both drugs bind strongly to melanin and can deposit in melanin-containing tissues which might explain retinopathy that occurs in high cumulative doses (Schrezenmeier 2020)

The efficacy of HCQ in rheumatic diseases has been characterized by a significant time delay by weeks to months because the drug needs to accumulate in the tissues. The half-lives of the two drugs are comparably long (40–60 days) and plasma, blood and serum concentrations of HCQ/CQ can vary individually. Little information is available concerning drug concentrations in 'deep' organs, e.g. the lung. It is unclear to what extent HCQ/CQ have immunomodulatory effects in a COVID-19 patient with short disease duration. Their antiviral effect comes from lowering the pH of the lysosome and thereby inhibiting the entry of virus particles into the cell (Yao 2020, Zhou 2020). The experience among pediatricians with HCQ/CQ (except pediatriciancs working in malaria) is very limited. Authorities in the US are warning about a widespread use of HCQ/CQ in COVID-19 (https://mailchi.mp/clintox/aact-acmt-aapcc-joint-statement)

Immunomodulatory drug treatment

The rational for immunomodulation in COVID-19 patients comes from a high expression of pro-inflammatory cytokines (Interleukin-1 (IL1) and interleukin-6 (IL6)), chemokines ("cytokine storm") and the consumption of regulatory T cells resulting in damage of the lung tissue as reported in patients with a poor outcome. **Blocking IL-1 or IL-6** can be successful in children with (auto) inflammatory disease (reviewed in Niehues 2019). However, both interleukins are also key to the physiological immune response and severe side effects of immunomodulators

have been reported. In adults with COVID-19, blocking interleukin-1/6 might be helpful (see the Treatment chapter). In the rare situation that the condition of the child deteriorates due to hyperinflammation and that they are resistant to other therapies, tocilizumab or anakinra may be an option.

Steroids (e.g. prednisone, prednisolone) are available as oral solution, tablets or different vials for intravenous application. Dosage in children is 0.5 to 1 mg/kg i.v. or oral BID. Short term use of steroids has few adverse events. Administration of steroids will affect inflammation by inhibiting the transcription of some of the pro-inflammatory cytokines and various other effects. The use of corticosteroids in children and adults with CoV-induced ARDS is controversial (Lee 2004, Arabi 2018, Russell 2020). The corticosteroid-induced decrease of antiviral immunity (e.g. to eliminate CoV-2 viruses) might be disadvantageous in patients with COVID-19. The use of low-dose hydrocortisone may be of advantage in adults with ARDS, whereas its use is controversial in pediatric ARDS.

Tocilizumab (Roactemra®) is available in 80/200/400 mg vials (20 mg/ml). Dosing is

- <30 kg: 12 mg/kg i.v. QD, sometimes repeated after 8 hrs
- ≥30 kg: 8mg/kg i.v. QD i.v. (max. 800 mg)

Adverse events (deriving largely from long term use in chronic inflammatory diseases and use in combination with other immunomodulatory drugs): severe bacterial or opportunistic infections, immune dysregulation (anaphylactic reaction, fatal macrophage activation), psoriasis, vasculitis, pneumothorax, fatal pulmonary hypertension, heart failure, gastrointestinal bleeding, diverticulitis, gastrointestinal perforation (reviewed in Niehues 2019).

Anakinra (Kineret®) is available as 100 mg syringes (stored at 4-8° C). Dosing is 2-4 mg/kg s.c. QD daily as long as hyperinflam-

mation persists. Thereafter, dose reduction by 10-30% per day. Adverse events (deriving largely from long term use in chronic inflammatory diseases and use in combination with other immunomodulatory drugs): severe bacterial or opportunistic Infections, fatal myocarditis, immune dysregulation, pneumonitis, colitis, hepatitis, endocrinopathies, nephritis, dermatitis, encephalitis, psoriasis, vitiligo, neutropenia (reviewed in Niehues 2019).

Immunotherapy

Engineering **monoclonal antibodies** against the CoV spike proteins or against its receptor ACE2 or **specific neutralizing antibodies** against CoV-2 present in convalescent plasma may provide protection but are not generally available yet.

Interferon α has been inhaled by children with COVID-19 in the original cohorts but there are no data on its effect (Qiu 2020). Type-1 interferons (e.g. interferon-α) are central to antiviral immunity. When coronaviruses (or other viruses) invade the host, viral nucleic acid activates interferon-regulating factors like IRF3 and IRF7 which promote the synthesis of type I interferons (IFNs).

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References

Allareddy V, Cheifetz IM. **Clinical trials and future directions in pediatric acute respiratory distress syndrome.** Ann Transl Med. 2019 Oct;7(19):514. PubMed: https://pubmed.gov/31728367. Full-text: https://doi.org/10.21037/atm.2019.09.14

Arabi YM, Mandourah Y, Al-Hameed F, et al. **Corticosteroid Therapy for Critically Ill Patients with Middle East Respiratory Syndrome.** Am J Respir Crit Care Med. 2018 Mar 15;197(6):757-767. PubMed:

https://pubmed.gov/29161116. Full-text: https://doi.org/10.1164/rccm.201706-1172OC

Bautista E, Chotpitayasunondh T, Gao Z, et al. **Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection.** N Engl J Med. 2010 May 6;362(18):1708-19. PubMed: https://pubmed.gov/20445182. Full-text: https://doi.org/10.1056/NEJMra1000449

Brodin P. **Why is COVID-19 so mild in children?** Acta Paediatr. 2020 Mar 25. PubMed: https://pubmed.gov/32212348. Full-text: https://doi.org/10.1111/apa.15271

Cai J, Xu J, Lin D, et al. **A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features.** Clin Infect Dis. 2020 Feb 28. pii: 5766430. PubMed: https://pubmed.gov/32112072. Full-text: https://doi.org/10.1093/cid/ciaa198

CDC COVID-19 Response Team. **Coronavirus Disease 2019 in Children - United States, February 12-April 2, 2020.** MMWR Morb Mortal Wkly Rep. 2020 Apr 10;69(14):422-426. PubMed: https://pubmed.gov/32271728. Full-text: https://doi.org/10.15585/mmwr.mm6914e4

CDC (2). **Considerations for Inpatient Obstetric Healthcare Settings.** April 2020. Full-text: https://www.cdc.gov/coronavirus/2019-ncov/hcp/inpatient-obstetric-healthcare-guidance.html. Accessed 20 April 2020.

Chen H, Guo J, Wang C, et al. **Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records.** Lancet. 2020 Mar 7;395(10226):809-815. PubMed: https://pubmed.gov/32151335. Full-text: https://doi.org/10.1016/S0140-6736(20)30360-3

Clohisey S, Baillie JK. **Host susceptibility to severe influenza A virus infection.** Crit Care. 2019 Sep 5;23(1):303. PubMed: https://pubmed.gov/31488196. Full-text: https://doi.org/10.1186/s13054-019-2566-7

Day M. **Covid-19: European drugs agency to review safety of ibuprofen.** BMJ. 2020 Mar 23;368:m1168. PubMed: https://pubmed.gov/32205306. Full-text: https://doi.org/10.1136/bmj.m1168

Dong Y, Mo X, Hu Y, et al. **Epidemiology of COVID-19 Among Children in China.** Pediatrics. 2020 Mar 16. pii: peds.2020-0702. PubMed: https://pubmed.gov/32179660. Full-text: https://doi.org/10.1542/peds.2020-0702

Dunn M, Muthu N, Burlingame CC, et al. **Reducing Albuterol Use in Children With Bronchiolitis.** Pediatrics. 2020 Jan;145(1). pii: peds.2019-0306. PubMed: https://pubmed.gov/31810996. Full-text: https://doi.org/10.1542/peds.2019-0306

Han H, Luo Q, Mo F, Long L, Zheng W. **SARS-CoV-2 RNA more readily detected in induced sputum than in throat swabs of convalescent COVID-19 patients.** Lancet Infect Dis. 2020 Mar 12. pii: S1473-3099(20)30174-2. PubMed: https://pubmed.gov/32171389. Full-text: https://doi.org/10.1016/S1473-3099(20)30174-2

Holshue ML, DeBolt C, Lindquist S, et al. **First Case of 2019 Novel Coronavirus in the United States.** N Engl J Med. 2020 Mar 5;382(10):929-936. PubMed: https://pubmed.gov/32004427. Full-text: https://doi.org/10.1056/NEJMoa2001191

Hrusak O, Kalina T, Wolf J. **Flash survey on severe acute respiratory syndrome coronavirus-2 infections in paediatric patients on anticancer treatment**. European Journal of Cancer 2020 April 7. Full-text: https://doi.org/10.1016/j.ejca.2020.03.021

Lazzerini M, Barbi E, Apicella A, Marchetti F, Cardinale F, Trobia G. **Delayed access or provision of care in Italy resulting from fear of COVID-19.** Lancet Child Adolesc Health. 2020 Apr 9. pii: S2352-4642(20)30108-5. PubMed: https://pubmed.gov/32278365. Full-text: https://doi.org/10.1016/S2352-4642(20)30108-5

Lee N, Allen Chan KC, Hui DS, et al. **Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients.** J Clin Virol. 2004 Dec;31(4):304-9. PubMed: https://pubmed.gov/15494274. Full-text: https://doi.org/10.1016/j.jcv.2004.07.006

Livingston E, Bucher K. **Coronavirus Disease 2019 (COVID-19) in Italy.** JAMA. 2020 Mar 17. pii: 2763401. PubMed: https://pubmed.gov/32181795. Full-text: https://doi.org/10.1001/jama.2020.4344

Lu Q, Shi Y. **Coronavirus disease (COVID-19) and neonate: What neonatologist need to know.** J Med Virol. 2020 Mar 1. PubMed: https://pubmed.gov/32115733. Full-text: https://doi.org/10.1002/jmv.25740

Lu X, Zhang L, Du H, et al. **SARS-CoV-2 Infection in Children.** N Engl J Med. 2020 Mar 18. PubMed: https://pubmed.gov/32187458. Full-text: https://doi.org/10.1056/NEJMc2005073

Mathur S, Fuchs A, Bielicki J, Van Den Anker J, Sharland M. **Antibiotic use for community-acquired pneumonia in neonates and children: WHO evidence review.** Paediatr Int Child Health. 2018 Nov;38(sup1):S66-S75. PubMed: https://pubmed.gov/29790844. Full-text: https://doi.org/10.1080/20469047.2017.1409455

Molloy EJ, Bearer CF. **COVID-19 in children and altered inflammatory responses.** Pediatr Res. 2020 Apr 3. pii: 10.1038/s41390-020-0881-y. PubMed: https://pubmed.gov/32244248. Full-text: https://doi.org/10.1038/s41390-020-0881-y

Moorlag SJCFM, Arts RJW, van Crevel R, Netea MG. **Non-specific effects of BCG vaccine on viral infections.** Clin Microbiol Infect. 2019 Dec;25(12):1473-1478. PubMed: https://pubmed.gov/31055165. Full-text: https://doi.org/10.1016/j.cmi.2019.04.020

Mulangu S, Dodd LE, Davey RT Jr, et al. **A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics.** N Engl J Med. 2019 Dec 12;381(24):2293-2303. PubMed: https://pubmed.gov/31774950. Full-text: https://doi.org/10.1056/NEJMoa1910993

Niehues T, Ozgur TT. **The Efficacy and Evidence-Based Use of Biologics in Children and Adolescents.** Dtsch Arztebl Int. 2019 Oct 18;116(42):703-710. PubMed: https://pubmed.gov/31711560. Full-text: https://doi.org/arztebl.2019.0703

Ogimi C, Englund JA, Bradford MC, Qin X, Boeckh M, Waghmare A. **Characteristics and Outcomes of Coronavirus Infection in Children: The Role of Viral Factors and an Immunocompromised State.** J Pediatric Infect Dis Soc. 2019 Mar 28;8(1):21-28. PubMed: https://pubmed.gov/29447395. Full-text: https://doi.org/10.1093/jpids/pix093

Paediatric European Network for Treatment of AIDS (PENTA). **Once vs. twice-daily lopinavir/ritonavir in HIV-1-infected children.** AIDS. 2015 Nov 28;29(18):2447-57. PubMed: https://pubmed.gov/26558544. Full-text: https://doi.org/10.1097/QAD.0000000000000862

Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. **Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study.** Lancet Infect Dis. 2020 Mar 25. pii: S1473-3099(20)30198-5. PubMed: https://pubmed.gov/32220650. Full-text: https://doi.org/10.1016/S1473-3099(20)30198-5

Russell CD, Millar JE, Baillie JK. **Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury.** Lancet. 2020 Feb 15;395(10223):473-475. PubMed: https://pubmed.gov/32043983. Full-text: https://doi.org/10.1016/S0140-6736(20)30317-2

Schrezenmeier E, Dorner T. **Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology.** Nat Rev Rheumatol. 2020 Mar;16(3):155-166. PubMed: https://pubmed.gov/32034323. Full-text: https://doi.org/10.1038/s41584-020-0372-x

Schwartz DA. **An Analysis of 38 Pregnant Women with COVID-19, Their Newborn Infants, and Maternal-Fetal Transmission of SARS-CoV-2: Maternal Coronavirus Infections and Pregnancy Outcomes.** Arch Pathol Lab Med. 2020 Mar 17. PubMed: https://pubmed.gov/32180426. Full-text: https://doi.org/10.5858/arpa.2020-0901-SA

She J, Liu L, Liu W. **COVID-19 epidemic: Disease characteristics in children.** J Med Virol. 2020 Mar 31. PubMed: https://pubmed.gov/32232980. Full-text: https://doi.org/10.1002/jmv.25807

Shen K, Yang Y, Wang T, et al. **Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: experts' consensus statement.** World J Pediatr. 2020 Feb 7. pii: 10.1007/s12519-020-00343-7. PubMed: https://pubmed.gov/32034659. Full-text: https://doi.org/10.1007/s12519-020-00343-7

Tagarro A, Epalza C, Santos M, et al. **Screening and Severity of Coronavirus Disease 2019 (COVID-19) in Children in Madrid, Spain.** JAMA Pediatr. 2020 Apr 8. pii: 2764394. PubMed: https://pubmed.gov/32267485. Full-text: https://doi.org/10.1001/jamapediatrics.2020.1346

Wang J, Qi H, Bao L, Li F, Shi Y. **A contingency plan for the management of the 2019 novel coronavirus outbreak in neonatal intensive care units.** Lancet Child Adolesc Health. 2020 Apr;4(4):258-259. PubMed: https://pubmed.gov/32043976. Full-text: https://doi.org/10.1016/S2352-4642(20)30040-7

Wei M, Yuan J, Liu Y, Fu T, Yu X, Zhang ZJ. **Novel Coronavirus Infection in Hospitalized Infants Under 1 Year of Age in China.** JAMA. 2020 Feb 14. pii: 2761659. PubMed: https://pubmed.gov/32058570. Full-text: https://doi.org/10.1001/jama.2020.2131

Weiss SR. **Forty years with coronaviruses.** J Exp Med. 2020 May 4;217(5). pii: 151597. PubMed: https://pubmed.gov/32232339. Full-text: https://doi.org/10.1084/jem.20200537

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 May;55(5):1169-1174. PubMed: https://pubmed.gov/32134205. Full-text: https://doi.org/10.1002/ppul.24718

Yao X, Ye F, Zhang M, 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 Mar 9. pii: 5801998. PubMed: https://pubmed.gov/32150618. Full-text: https://doi.org/10.1093/cid/ciaa237

Zeng H, Xu C, Fan J, et al. **Antibodies in Infants Born to Mothers With COVID-19 Pneumonia.** JAMA. 2020 Mar 26. pii: 2763854. PubMed: https://pubmed.gov/32215589. Full-text: https://doi.org/10.1001/jama.2020.4861

Zeng L, Xia S, Yuan W, et al. **Neonatal Early-Onset Infection With SARS-CoV-2 in 33 Neonates Born to Mothers With COVID-19 in Wuhan, China.** JAMA Pediatr. 2020 Mar 26. pii: 2763787. PubMed: https://pubmed.gov/32215598. Full-text: https://doi.org/10.1001/jamapediatrics.2020.0878

Zhou D, Dai SM, Tong Q. **COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression.** J Antimicrob Chemother. 2020 Mar 20. pii: 5810487. PubMed: https://pubmed.gov/32196083. Full-text: https://doi.org/10.1093/jac/dkaa114

Zimmermann P, Curtis N. **Coronavirus Infections in children including COVID-19.** Ped Inf Dis. Full-text: https://journals.lww.com/pidj/Fulltext/2020/05000/Coronavirus_Infections_in_Children_Including.1.aspx

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