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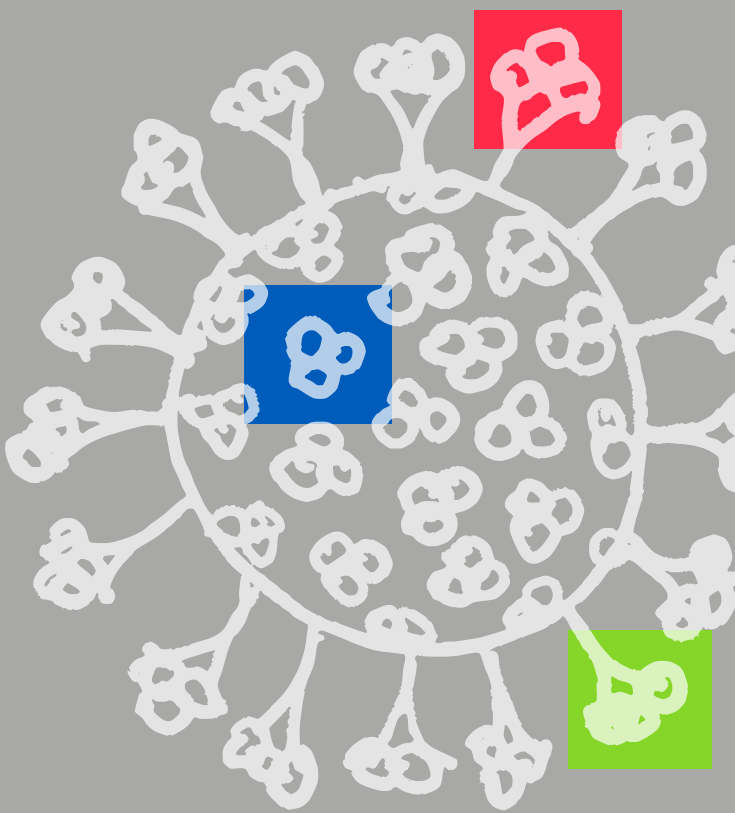

STEINHAUSER VERLAG

Bernd Sebastian Kamps
Christian Hoffmann
COVID Reference
Sixth Edition 2021.6
CR 2021.6.04, uploaded on
3 February 2021
Copy-Editor: Rob Camp

Published 13 January 2021

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Editors, authors, publishing house and translators
have received *no support from third parties* to realize this textbook.

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COVID Reference

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Edition 2021.6

Steinhäuser Verlag

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ISBN: 978-3-942687-53-9

CR 2021.6.04 – Uploaded on 3 February 2021

Preface

In not even a year after the discovery of COVID-19, several vaccines have been licensed and hundreds of millions of people will be vaccinated within months. Soon the number of severe COVID-19 cases will plummet, people at risk will no longer die, and after the vaccination of younger people, Long COVID will retreat too.

There are no analogies to describe COVID-19 vaccine development. A bizarre Christmas gift? A bit dull! A feat that greatly exceeds the achievements of the moon landings? Obvious and dreadfully banal! The only thing history will remember is that by creating COVID vaccines in record time, science has given us a humbling demonstration of what it can achieve.

Nevertheless, we are not out of the woods yet. Nature, too, has demonstrated that it can strike back. If the new English *B117* variant (also called B.1.1.7, VOC-202012/01, 501Y Variant 2 or 20B/501Y.V1) or other mutants are confirmed to increase transmissibility of SARS-CoV-2 on a global scale, the race may be on again. *In 2021, let's perfect our skills of physical distancing and continue to wear face masks. Let's take it as a convenient rehearsal exercise for future pandemics. It isn't fun but it must be done.*

Bernd Sebastian Kamps & Christian Hoffmann

13 January 2021

Preface to the First Edition

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 http://www.CovidReference.com and come back every day for the **Daily Top 10 Papers** on COVID-19. Each citation comes with a short comment and a link to the full-text article.

1. Epidemiology

Bernd Sebastian Kamps
Stefano Lazzari

In December 2019, several patients from Wuhan, People's Republic of China, developed pneumonia and respiratory failure reminiscent of the 2003 SARS epidemic (WMHC 2019, www.SARSReference.com). In early January 2020, a new betacoronavirus, later named SARS-CoV-2, was isolated from bronchoalveolar lavage fluid samples (Zhou 2020). The virus spread first within China (Yu X 2020) and then to several countries in Asia before reaching Iran and Italy where it caused major outbreaks. During the first 11 weeks of the pandemic, almost two-thirds of the first cases in affected countries were in people reported to have recently travelled from only three affected countries (China, Iran, or Italy), showing how international travel from a few countries with substantial SARS-CoV-2 transmission might have seeded outbreaks around the world (Dawood 2020).

Despite some early successes in containment, SARS-CoV-2 eventually took hold in both Europe and North America during the first two months of 2020: in Italy around the end of January, in Washington State around the beginning of February, followed by New York City later that month (Worobey 2020 - see also Figure 6, Deng X 2020, McNeil Jr DG). In Brazil, it was found that there had been more than 100 international virus introductions, with 76% of Brazilian strains falling into three clades that were introduced from Europe between 22 February and 11 March 2020 (Candido 2020).

Between then and the time of this writing (13 January 2021), SARS-CoV-2 has spread to every corner of the world. Almost 100 million people have been diagnosed with SARS-CoV-2 infection and two million people have died of COVID-19, the disease caused by SARS-CoV-2. Not all cases, in particular if asymptomatic, have been diagnosed and the true number of both infections and deaths is probably much higher.

In the autumn of 2020, several new SARS-CoV-2 variants emerged that have a substantial transmission advantage. Find more information in *Mutations and vaccine efficacy,* page 212.

Table 1. Seroprevalence data 2020

		Sample collection		
Italy*	Nationwide	May 25-July 15	2,5%	Sabbadini 2020
Italy	Lodi (red zone)		23%	Percivalle 2020
Spain	Nationwide Madrid		5,0% >10%	Pollán 2020
Spain	Madrid		11%	Soriano 2020
Switzerland	Geneva		5,0-11%	Stringhini 2020
Netherlands	Nationwide	Aprl 2020	1,2-4%	Vos 2020
Denmark	Faroe Islands		0,6%	Petersen 2020
Germany	Kupferzell 'hotspot'	March	12%	Santos-Hövener 2020
UK	UK London South West		6% 13% 3%	Ward 2020
China	Wuhan	March 9-April 10	3,2-3,8%	Xu X 2020
US	New York City San Francisco Bay area	March 23-April 1 April 23-27	6,9% 1,0%	Havers 2020
US	New York State		14%	Rosenberg 2020
US	NYC, Health care personnel		13,7%	Moscola 2020
US	Nationwide in patients receiving dialysis	July 2020	8,3%	Anand 2020
US			1-23%	Bajema 2020
India	Mumbai	July	57%	Malani 2020
Brazil	Nationwide	May-June	1,4-6,4%	Hallal 2020
Brazil	Manaus	March-August	66%	Buss 2020

* Note that Italy's national survey results are preliminary and probably an underestimate. The country only managed to collect 40% of the planned samples, with many people refusing to be tested. Insiders never believed these figures and favored a seropositivity rate of 5-10% like in Spain or France. Later estimates of COVID-19 prevalence in Italy by Francesca Bassi and colleagues set it at 9%, corresponding to almost 6 million Italians (Bassi 2020).

.

Relatively few large scale seroprevalence studies have been completed but the available seroprevalence data show that only a few places, like Mumbai and Manaus, have reached a high prevalence in the population, close to the level required for herd immunity, estimated to be around 60% (see Table 1). [Herd immunity is defined as the proportion of a population that must be immune to an infectious disease, either by natural infection or vaccination, to

provide indirect protection (herd protection) to the rest of the population who are not otherwise immune to the disease (D'Souza 2020, Adam 2020). As shown in Table 1, countries hit hardest by the COVID-19 pandemic have higher seroprevalence rates but, without an effective vaccine, no country can count on any kind of herd immunity soon.

The articles cited in Table 1 show some interesting findings:

- **Wuhan** – Seropositivity for IgM and IgG antibodies was low (3,2%-3,8%) even in a highly affected city like Wuhan (Xu X 2020).
- **New York City** – In NYC, the prevalence of SARS-CoV-2 among health care personnel was 13,7% (5523/40.329 individuals tested) (Moscola 2020), similar to the prevalence among adults randomly tested in New York State (14,0%) (Rosenberg 2020).
- **UK** – Black, Asian and minority ethnic (BAME) individuals were between two and three times more likely to have had SARS-CoV-2 infection compared to white people. An interesting trend: young people aged 18-24 had the highest rates (8%), while older adults aged 65 to 74 were least likely to have been infected (3%).
- **Mumbai** – In a cross-sectional survey in Mumbai, the prevalence of SARS-CoV-2 antibodies was around 57% in the slum areas of Chembur, Matunga and Dahisar, and 16% in neighboring non-slum areas (Malani 2020). In some places of the world, herd immunity may be within reach.
- **Geneva** – Young children (5–9 years) and older people (≥ 65 years) had significantly lower seroprevalence rates than other age groups (Stringhini 2020).
- **Faroe Islands** – At the beginning of the pandemic, small islands tended to have low seropositivity rates.

Careful interpretation of these data is required. We have few nationwide population-based seroprevalence studies, the sensitivity and specificity of serological tests being used can vary from place to place, and some people might have been infected without showing detectable levels of antibodies at the time of the study. Based on all available serological studies, WHO has estimated that around 10% of the world population, or 760 million people, may have been infected by October 2020.

The mean incubation period of SARS-CoV-2 infection is around 5 days (Li 2020, Lauer 2020, Nie X 2020). The serial interval – 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). SARS-CoV-2 is highly contagious, with an estimated basic reproduction number R_0 of around 2,5-3,0 (Chan 2020, Tang B 2020, Zhao 2020). [R_0 indicates the average number of infections one case can generate over the course of the infectious period in a naïve, uninfected population. Read the guide by David Adam (Adam 2020) for more valuable information on R_0.]

Prevention

SARS-CoV-2 is easily transmissible both by symptomatic and asymptomatic individuals, thrives in closed and densely inhabited environments, and is amplified by so-called 'superspreader' events.

The five golden rules to minimize the risk of SARS-CoV-2 infection

1. Wear face masks in public spaces.
2. Keep a distance of 2 (*two!*) meters to other people.
3. Avoid **crowded** places (of more than 5-10 people).
4. Avoid in particular **crowded** and **closed** spaces (even worse: air-conditioned closed places where air is being moved around).
5. Avoid in all circumstances - **crowded**, **closed** and **noisy** spaces where people must shout to communicate. These are SARS-CoV-2's preferred playgrounds.

Find below a detailed discussion of SARS-CoV-2 transmission (pages 69) and its prevention (page 117).

As with the earlier SARS and MERS outbreaks (Shen Z 2004, Cho SY 2016), the spread of SARS-CoV-2 is characterized by the occurrence of so-called "super-spreader events", where one source of infection is responsible for a large number of secondary infections (Wang L 2020). This phenomenon is well-described in a study of SARS-CoV-2 transmission in Hong Kong (Adam DC 2020). The authors analyzed all clusters of infection in 1038 cases that occurred between January and April 2020 and concluded that 19% of cases were responsible for causing 80% of the additional community cases, with large clusters originating at bars, weddings, and religious ceremonies. Interestingly, reduced delays in confirmation of symptomatic cases did not influence the rate of transmission (suggesting higher rate of transmission at or before

symptom onset), whereas rapid contact tracing and quarantine of contacts was very effective in terminating the transmission chain. Other authors (Endo 2020) have also estimated a *k* of 0.1 outside China, meaning that only 10% of infected individuals transmit the virus (*k* or "*dispersion factor*" describes, in mathematical models, how much a disease tends to cluster).

Over-dispersion, with few infected people causing most secondary infections, could explain some puzzling aspects of the early stages of the COVID-19 pandemic. For example, why the early introductions in Europe of SARS-CoV-2 in December 2019 (France) and again in January 2020 (France, Germany) did not result in earlier major outbreaks in Europe. Or why the large outbreak in Northern Italy in February 2020 did not lead to a similar rapid spread of the virus in the rest of the country.

Understanding the reasons underlining superspreader events can be key to the success of preventive measures, so the big question is, "Why do some COVID-19 patients infect many others, whereas most don't spread the virus at all?" (Kupferschmidt 2020). It is possible that some individuals simply shed more virus than others, or that there is much more shedding at a specific moment of higher contagiousness in the natural history of the infection, possibly when viral load is at its peak. Environmental conditions also play a role, with crowded, closed places where people talk loudly, shout, sing or exercise being at higher risk, possibly because of the higher production and diffusion of small particles like aerosols. A "superspreader" in a "superspreading setting" may result in a very large number of infections, as seen in the Shincheonji church cluster in South Korea where, in March 2020, one single person was estimated to have generated more than 6000 cases.

A better understanding of superspreader events may help in defining the most effective measures to reduce SARS-CoV-2 transmission. We will explore below the most common "hotspots" of SARS-CoV-2 infection, where the likelihood of multiple infections is higher.

Hotspots of SARS-CoV-2 Transmission

The following settings were, are or could be catalyzers of SARS-CoV-2 outbreaks:

- Hospitals and other health care centers
- Long-term care facilities
- Homes (also including intense social life with friends and colleagues)
- Leisure facilities (e.g., bars, restaurants clubs, choirs, discos, etc.)
- Workplaces
- Schools

- Universities
- Family meetings (e.g., birthdays, marriages, funerals)
- Mass and family gatherings
    - Sport events
    - Religious gatherings
- Closed and densely populated spaces
    - Prisons
    - Homeless shelters
    - Cruise ships, aircraft carriers and military vessels (closed spaces)

Hospitals

During the first months of the SARS-CoV-2 pandemic, when suspicion of the disease was low, transmission in hospitals and other health care centers (including doctors' offices) played a prominent role in the origin of local outbreaks. This was reminiscent of both SARS and of the more recent largest MERS outbreak outside of the Arabian Peninsula which occurred in the Republic of Korea in 2015, where 184 of 186 cases were nosocomial infections (Korea Centers for Disease Control and Prevention 2015). Hospitals, as many other places where potentially infected strangers meet, can be a favorable environment for the propagation of SARS-CoV-2 (Wison 2020). Within the first 6 weeks of the epidemic in China, 1716 cases and at least 5 deaths were confirmed among health care workers (HCWs) (Wu 2020). In some instances, hospitals could have been even the main COVID-19 hub, facilitating transmission between health workers and uninfected patients (Nacoti 2020).

One study of the hospital environment reports that the virus was widely present in the air and on object surfaces in both the intensive care units and general wards, implying a potentially high infection risk for hospital staff. Contamination was greater in ICUs (Self 2020). Viral RNA has been found on floors, computer mice, trash cans, sickbed handrails, and was detected in the air up to approximately 4 m from patients (Guo 2020). The virus was also isolated from toilet bowl and sink samples, suggesting that viral shedding in stool could also be a potential route of transmission (Young 2020, Tang 2020). However, most of these studies have evaluated only the presence of viral RNA, not its infectivity.

Although nosocomial spread of SARS-CoV-2 is well documented, appropriate hospital infection control measures can prevent nosocomial transmission (Chen 2020, Nagano 2020, Callaghan 2020). This was nicely demonstrated by the case of a person in her 60s who travelled to Wuhan on Dec 25, 2019, returned to the US on Jan 13, 2020, and transmitted SARS-CoV-2 to her hus-

band. 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, shortage of PPEs, and sub-optimal respiratory and hand hygiene after close contact with patients have all been associated with an increased risk of infection in HCWs (Ran 2020). At one time, during the early epidemic in March 2020, around half of 200 cases in Sardinia, Italy, were among hospital staff and other health care workers. On 14 April, the CDC reported that 9282 healthcare personnel had been infected with SARS-COV-2 in the US.

Table 2. Percentage of healthcare workers (HCWs) found to be SARS-CoV-2 infected

		Sample collection	SARS-CoV-2 positive	
UK	435 inpatients at London teaching hospital	2 March–12 April	15% (case fatality rate: 36%)	Rickman 2020
UK	1718 HCWs participating in 5148 at-risk tracheal intubations	23 March–2 June 2020	10,7%	El-Boghdadly 2020
USA	3477 symptomatic employees in the University of Washington medical system	12 March–23 April	5,3%	Mani 2020
China	Wuhan: 44 672 HCWs	Until 11 February	3,8%	Wu 2020
USA	1992 HCWs in COVID-19 units	12 February–9 April	5,4%	Vahidy 2020
Italy	5444 active HCWs	17 April–20 May	6,9%	Calcagno 2021
UK	London, 200 patient-facing HCWs	26 March–8 April	20% seroconverted during the study period	Houlihan 2020
China	Wuhan, 9684 HCWs in Tongji Hospital	1 January–9 February	0,9%	Lai X 2020

Health care workers from COVID-19 units have a higher risk of infection with SARS-CoV-2 (5,4%) than those from non–COVID units (0,6%) (Vahidy 2020). In a prospective cohort study in London, 25% of HCWs were already seropositive

at enrolment (26 March to 8 April) and a further 20% became seropositive within the first month of follow-up (Houlihan 2020). However, a Chinese study of 9684 HCWs in Tongji Hospital showed a higher rate of infection in non-first-line staff (93/6574, 1,4%) compared to those who worked in fever clinics or wards (17/3110, 0,5%) (Lai X 2020). In Italy, seroprevalence was highest in laboratory personnel (18/175, 10,3%), followed by nurse assistants (44/520, 8,5%), nurses (150/1983, 7,6%) and doctors (55/755, 7,3%) (Calcagno 2021). Interpretation: those who worked in clinical departments other than fever clinics and wards may have had less access to, or have neglected to adopt, adequate protective measures.

SARS-CoV-2 outbreaks have also been documented in dialysis units (Schwierzeck 2020, Rincón 2020). The prevalence of SARS-CoV-2 antibodies was lower among personnel who reported always wearing a face covering while caring for patients (6%), compared with those who did not (9%) (Self 2020).

The risk factors for SARS-CoV-2 infection in HCWs have been summarized in a review (Chou 2020). There is evidence that more consistent and regular use of recommended PPE measures was associated with decreased risk of infection. Association was stronger for masks but was also observed for gloves, gowns, and eye protection, as well as hand hygiene. Some evidence was found that N95 respirators might be associated with higher reduction of risk for infection than surgical masks. Evidence also indicates an association with certain exposures (such as involvement in intubations, direct contact with infected patients, or contact with bodily fluids).

Long-term care facilities

Long-term care facilities (LTC) are high-risk settings for infectious respiratory diseases. The first important study published in May 2020 reported an outbreak in a skilled nursing facility in King County, Washington, US, where 167 cases of COVID-19 (101 residents, 50 health care personnel and 16 visitors) were diagnosed within less than three weeks from the identification of the first case: (McMichael 2020) (Table 3).

Among residents (median age: 83 years), the case fatality rate was 33,7%. Chronic underling conditions included hypertension, cardiac disease, renal disease, diabetes mellitus, obesity, and pulmonary disease. The study demonstrated that once introduced in a long-term care facility, often by a care worker or a visitor, SARS-CoV-2 has the potential to spread rapidly and widely, with devastating consequences.

By mid-April 2020, more than 1300 LTC facilities in the US had identified infected patients (Cenziper 2020, CDC 200311). As most residents had one or more chronic underling conditions, COVID-19 put them at very high risk for premature death. Later studies found a high percentage of asymptomatic residents (43%) during the two weeks prior to testing (Graham 2020b), extraordinarily high seropositivity rates (72%; Graham 2020a), and a higher infection rate in residents (9,0%) than in LTC staff (4,7%) (Marossy 2020).

Table 3. 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

A national survey covering 96% of all LTC facilities in Italy found that in Lombardy, the epicenter of the Italian epidemic, 53,4% of the 3045 residents who died between 1 February and 14 April were either diagnosed with COVID-19 or presented flu-like symptoms. Among the 661 residents hospitalized during the same period, 199 (30%) were positive by RT-PCR test.

As soon as a single case is detected among residents of a nursing facility, it is recommended to test all residents, as many of them may be asymptomatic. After an outbreak at an LTC nursing facility for veterans in Los Angeles, USA, all residents, regardless of symptoms, underwent serial (approximately weekly) SARS-CoV-2 RT-PCR testing. Nineteen of 99 (19%) residents had positive test results for SARS-CoV-2 (Dora 2020). Fourteen of the 19 residents with COVID-19 were asymptomatic at the time of testing. Among these, eight developed symptoms 1-5 days after specimen collection and were later classified as pre-symptomatic.

Mortality in LTCs is almost always high. In a study from Ontario, Canada, the incidence rate ratio for COVID-19–related death was more than 13 times higher than the one seen in community-living adults older than 69 years during a similar period (Fisman 2020). In another study in Ontario that included 78.607 residents of 618 nursing homes, 5218 (6,6%) were infected with SARS-CoV-2 and 1452 (1,8%) died of COVID-19 as of May 20, 2020. The case fatality rate was 27,8% (1452/5218) (Brown 2020). Of note, COVID-19 mortality in homes with low crowding (by number of occupants per room and number of bathrooms in the house) was less than half (1,3%) than that of homes with high crowding (2,7%).

In one UK investigation involving 394 residents and 70 staff in 4 nursing homes in central London, 26% of residents died over a two-month period (Graham 2020). It is estimated that residents in LTC facilities contributed 30–60% of all COVID-19 deaths in many European countries (O'Driscoll 2020, ECDC 2020; see also the statement to the press by Hans Henri P. Kluge, WHO Regional Director for Europe). Excess mortality data suggests that in several countries many deaths in long-term care facilities might have occurred in patients not tested for COVID-19, which are often not included in the official national COVID-19 mortality statistics (Buonanno 2020).

Homes

Reported infection rates at home varied widely (between 6% and 32%) between studies. In Spain, in an analysis of 551 outbreaks during the summer of 2020, social settings such as family gatherings or private parties accounted for 14% of cases (854/6208). SARS-CoV-2 positive cases linked to leisure venues such as bars, restaurants, or clubs were even more frequent (NCOMG 2020) (see next section).

An early study noted that children were as likely to be infected as adults (Bi Q 2020). However, two groups found that the odds of infection among children and young people was only about 25% that among the elderly (≥ 60 years old) (Jing QL 2020) and adults (Li W 2020). In addition, secondary attack rate in contacts who were spouses of index cases was 27,8% compared to 17,3% in other adult members in the households (Li W 2020). A study from Spain confirmed that children in quarantined family households had a similar probability as adults to become infected by SARS-CoV-2. In 381 first-reported PCR-positive adult cases and 1084 contacts (672 children, 412 adults), SARS-CoV-2 seroprevalence rates were 18% (118/672) in children and 19% (77/335) in adult contacts (Brotons 2020).

It has been objected that some studies might underestimate true transmission rates if index cases were isolated in or outside of the home (Sun 2020). In one study from Zhuhai, China, 32,4% (48 of 148) of household contacts of 35 index cases were infected (Wu J 2020).

In an excellent retrospective cohort study of 1114 PCR-confirmed index cases from Singapore, Vernon Lee and colleagues identified 7518 close contacts (1779 household contacts, 2231 work contacts, and 3508 social contacts) (Ng OT 2020). The secondary clinical attack rates were 5,9% for household contacts and 1,3% for non-household contacts (Table 4).

Table 4. COVID outbreak in a long-term care facility

	Particular risk factors	Odds ratio
Household contacts	Sharing a bedroom	5.38
Secondary attack rate: 5,9%	Being spoken to by an index case for 30 min or longer	7.86
Non-household contact	Exposure to more than one case	3.92
Secondary attack rate: Work contacts, 1,3% social contacts, 1,3%	Being spoken to by an index case for 30 min or longer	2.67
	Sharing a vehicle with an index case	3.07

Leisure venues (bars, clubs, choirs, karaokes, discos, etc.)

In Spain, an analysis of 551 outbreaks from mid-June to 2 August linked 1230 of 6208 cases (20%) to leisure venues such as bars, restaurants, or clubs (NCOMG 2020). Data from Japan showed that of a total of 61 COVID-19 clusters, 10 (16%) were in restaurants or bars; 7 (11%) in music-related events, such as live music concerts, chorus group rehearsals, and karaoke parties; 5 (8%) in gymnasiums; and 2 (3%) in ceremonial functions (Furuse 2020). Among a cluster of 108 cases in Osaka, Japan, 51 cases seemed to have been infected after a single visit to a live music club (Sugano 2020). In South Korea, superspreading events in nightclubs in downtown Seoul were linked to a local resurgence of cases (Kang 2020). In Hong Kong, an explosive summer outbreak was best explained by the sudden increase in social gatherings after the easing of public health measures, especially gatherings at eateries (To 2020).

College trips and summer camps represent another environment for efficient SARS-CoV-2 transmission. In one case, a spring break trip from Austin to Mexico resulted in 14 asymptomatic and 50 symptomatic cases (Lewis 2020). CDC reported an outbreak with 260 (44%) out of 597 attendees of an overnight

summer camp in Georgia becoming infected in June 2020 (Szablewski 2020). The camp adopted most of CDC's suggested preventive measures for Youth and Summer Camps but wearing cloth masks and opening windows and doors for increased ventilation in buildings were not implemented. ☹

Choirs, too, are places of efficient SARS-CoV-2 transmission. On 8 March 2020, the Amsterdam Mixed Choir gave a performance of Bach's St John Passion in the city's Concertgebouw Auditorium. Days later, the first singers developed symptoms and in the end 102 of 130 choristers were confirmed to have COVID-19. One 78-year-old choir member died, as did three partners of choir members; some singers required intensive care (The Guardian, 17 May). On 9 March, members of the Berlin Cathedral Choir met for their weekly rehearsal. Three weeks later, 32 out of 74 choir members were positive for SARS-CoV-2 (NDR 2020). All recovered. On 10 March 2020, 61 members of a Skagit County choir in Washington met for a 2,5-hour practice. A few weeks later, researchers reported 32 confirmed and 20 probable secondary COVID-19 cases (attack rate = 53,3% to 86,7%); three patients were hospitalized, and two died. The authors conclude that transmission was likely facilitated by proximity (within 6 feet) during practice and increased viral diffusion by the act of singing (Hamner 2020).

These data suggest that any noisy, closed, and stagnant air environment (e.g., discos, pubs, birthday parties, restaurants, meat processing facilities, etc.) where people stand, sit, or lie close together are ideal conditions for generating large SARS-CoV-2 outbreaks. If they need to shout for communication, the situation may become explosive.

Workplaces

As early as January 2020, SARS-CoV-2 was found to spread during workshops and company meetings (Böhmer 2020). A few weeks later, an outbreak of SARS-CoV-2 infection was reported from a call center in South Korea where 94 out of 216 employees working on the same floor were infected, an attack rate of 43,5% (Park SY 2020). Particularly instructive is the case of a scientific advisory board meeting held in Munich, Germany, at the end of February. Eight dermatologists and 6 scientists (among them the index patient) met in a conference room of about 70 m^2 with a U-shaped set-up of tables separated by a central aisle > 1 meter wide. During the meeting, that lasted 9.5 hours, refreshments were served in the room four times. In the evening, the participants had dinner in a nearby restaurant and shook hands for farewell, with a few short hugs (no kisses!). Finally, the index patient shared a taxi with three colleagues for about 45 min. Outcome: the index patient infected at least 11 of the 13 other participants. These individuals infected an additional 14 persons

either in a hospital or at home (Hijnen 2020). In the presence of an infected individual, workplaces can be important amplifiers of local transmission.

In May 2020, outbreaks with hundreds of infected individuals were reported from meat-packing plants in Germany (DER SPIEGEL), the US (The Guardian) and France (Le Monde) as well as from other countries. In March and April, 25,6% (929) of employees of a meat processing facility in South Dakota, USA and 8,7% (210) of their contacts were diagnosed with COVID-19; two employees died (Steinberg 2020). The highest attack rates occurred among employees who worked < 6 feet (2 meters) from one another at the production line. Another study reported 16.233 COVID-19 cases and 86 COVID-19–related deaths among workers in 239 facilities (Waltenburg 2020). The percentage of workers with COVID-19 ranged from 3,1% to over 20% per facility (Waltenburg 2020).

Promiscuity, noise, cold and humid conditions are currently favored as explanations for these unusual outbreaks. In Spain, the above-mentioned analysis of 551 outbreaks linked around 500 out of 6208 cases (8%) to occupational settings, in particular, workers in the fruit and vegetable sector and workers at slaughterhouses or meat processing plants (NCOMG 2020). One study suggested that by 21 July 2020, in the USA alone, livestock processing plants may have been associated with 236.000 to 310.000 COVID-19 cases (6 to 8% of total US cases) and 4300 to 5200 deaths (3 to 4% of total US cases) (Taylor 2020).

Schools and nurseries

Schoolchildren usually play a major role in the spread of respiratory viruses, including influenza. However, while the SARS-CoV-2 virus has been detected in many children, they generally experience milder symptoms than adults, need intensive care less frequently and have a low death rate. An analysis of data from Canada, China, Italy, Japan, Singapore and South Korea found that susceptibility to infection in individuals under 20 years of age was approximately half that of adults aged over 20 years, and that clinical symptoms are manifest in 21% of infections in 10-to-19-year-olds, rising to 69% of infections in people aged over 70 years (Davis 2020).

However, the role of children in SARS-COV-2 transmission is still unclear. Early in the pandemic, several studies suggested that children rarely transmit the infection. In a small COVID-19 cluster detected in the French Alps at the end of January, one person returning from Singapore infected eleven other people, including a nine-year-old schoolboy. The researchers closely tracked and tested all contacts (Danis 2020). The boy had gone to school while showing COVID-19 symptoms and was estimated to have had more than 60 high-risk close contacts. No one was found positive to the coronavirus, though

many had other respiratory infections. Also, no trace of the virus was found in the boy's two siblings who were on the same Alpine vacation.

A study by the Institut Pasteur in April 2020 (before the school closure in France) that included 510 primary school children concluded that "it appears that the children did not spread the infection to other students, or to teachers or other staff at the schools". Another study in 40 patients less that 16 years old in Geneva, Switzerland (Posfay-Barbe 2020) also concluded that unlike with other viral respiratory infections, children do not seem to be a major vector of SARS-CoV-2 transmission, with most pediatric cases described inside familial clusters and no documentation of child-to-child or child-to-adult transmission."

However, a review of 14 published studies (Rajmil 2020) was less categorical, simply concluding that children are not transmitters to a greater extent than adults. A metanalysis of published evidence (Viner 2020) states that there is insufficient evidence to conclude whether transmission of SARS-CoV-2 by children is lower than by adults.

CDC reported in September on twelve children who acquired COVID-19 in three different child-care facilities in Utah. It documented transmission from these children to at least 12 (26%) of 46 non-facility contacts and that transmission was observed from two of three children with confirmed, asymptomatic COVID-19. In addition, several studies have found that both symptomatic and asymptomatic children can shed the SARS-CoV-2 virus for several days or weeks after infection (Liu M 2020, Han 2020). However, qualitative positive or negative findings for molecular detection of virus may not necessarily correlate with infectivity (DeBiasi 2020).

In the early autumn of 2020, if and how to re-open schools was a hot worldwide debate. In Taiwan, authorities established general guidelines, including a combination of strategies such as active campus-based screening and access control; school-based screening and quarantine protocols; student and faculty quarantine when warranted; mobilization of administrative and health center staff; regulation of dormitories and cafeterias; and reinforcement of personal hygiene, environmental sanitation, and indoor air ventilation practices (Cheng SY 2020). Most European countries initially decided to reopen schools, considering the possible increase in infections as being less damaging than the loss of education in schoolchildren. At the time of this writing (early December), the reopening of schools in European countries does not seem to have contributed substantially to national epidemics. In Germany, only a few and mostly small COVID-19 school outbreaks have been reported (Otte im Kamps E 2020). It can indeed be difficult to determine if children were infected at home, at school (by their peers or by their teachers), or outside during

social or sport gatherings. In some school clusters, the index cases identified were teachers and/or parents (Torres 2020), so school prevention should focus on enforcing preventive measures and avoiding new cases among teachers.

Whatever the rules, mind the exceptions. In Poland, within 2 weeks of the reopening of a nursery, a cluster of 29 persons emerged: 8 were children attending the nursery, and 12 were children's family members who did not enter the facility. The high attack rates were explained by the prolonged close contact between very young children who are, of course, less able to adjust to control measures (Okarska-Napierała 2020).

In any case, close monitoring of school clusters will provide much needed additional data that might help clarify the role of children of different ages in the spread of the virus, and whether schools can be considered hotspots or not of SARS-CoV-2 transmission. Meanwhile, at the peak of the second wave, several countries, including Austria, Poland, Greece, Italy, and the US, have imposed again temporary full or partial school closures (UNESCO).

Universities

Reopening of US universities after the summer of 2020 was not always smooth. North Carolina university fully opened its campus for the first time since transitioning to primarily remote learning in March. Consistent with CDC guidance at that time, steps were taken to prevent the spread of SARS-CoV-2 on campus (i.e., daily symptom checks, use of masks in all indoor common spaces and classrooms, physical distancing of ≥ 6 feet in indoor and outdoor settings). These steps were not sufficient. Within 3 weeks, 670 laboratory-confirmed cases were identified. Student gatherings and congregate living settings, both on and off campus, likely contributed to the rapid spread (Wilson 2020).

Again in North Carolina, at Duke University, the COVID-19 prevention strategy included risk reduction behaviors, but also frequent SARS-CoV-2 PCR testing using pooled samples and contact tracing. Of 10.265 students who were tested a total of 68.913 times, 84 had positive results (Denny 2020). Of these, 51% were asymptomatic, and some had high viral loads. This testing approach allowed campus to remain open for 10 weeks of classes without substantial outbreaks among residential or off-campus populations. Importantly, no evidence from contact tracing linked transmission with in-person classes.

To prevent SARS-CoV-2 from entering campuses, a two-week quarantine at home before entering a closed college campus was ordered. It was not sufficient, as shown by an investigation among US Marine Corps. Around 2% of

recruits who had had negative results for SARS-CoV-2 at the beginning of a supervised quarantine tested positive within two weeks (Letizia 2020). Most recruits who tested positive were asymptomatic, and no infections were detected through daily symptoms monitoring. The author's short conclusion: "Transmission clusters occur within platoons."

Family meetings

All family meetings – in small or enlarged circles (e.g., birthdays, marriages, funerals, Christmas, Easter, 年夜饭, Eid al-Adha, Thanksgiving, etc.) have the potential for triggering explosive local epidemics. In rural Maine, USA, a marriage with 53 guests was the start of a cluster 177 cases, with seven hospitalizations and seven deaths (Mahale 2020). *(Editor's note: Do you want to contribute to 7 deaths through your marriage?)*

Mass gatherings

Sports events

Sports events can expose both sportswomen, sportsmen and spectators to SARS-CoV-2. In an unintentional experiment, the German national team of amateur boxers proved that 100% transmission rates can be achieved in a few days. In a training camp, some of the 18 athletes and 7 coaches and supervisors started having flu-like symptoms. Four days later, all 25 persons tested positive for SARS-CoV-2 (Anonymous 2020). In the USA, a recreational ice hockey game was played between two teams, each consisting of 11 players (typically six on the ice and five on the bench at any given time). The players were men aged 19–53 years. During the 5 days after the game, 15 persons (14 of the 22 players and a rink staff member) experienced signs and symptoms compatible with COVID-19 (Atrubin 2020).

Sports events can expose entire populations to SARS-CoV-2. A football match played in Milan, Italy on 19 February 2020 has been described as "Game zero" or "a biological bomb". The match was attended by 40.000 fans from Bergamo and 2.500 from Valencia, Spain, and was played just two days before the first positive case of COVID-19 was confirmed in Lombardy. A few weeks later, 35% of Valencia's team members tested positive for the coronavirus, as did several Valencia fans. By mid-March, there were nearly 7000 people in Bergamo who had tested positive for the coronavirus with more than 1000 deaths, making Bergamo the most heavily hit province during the initial COVID-19 epidemic in Italy.

Other sports events have been implicated in the SARS-CoV-2 spread, including the match between Liverpool and Atletico Madrid, held at Anfield stadium

on 11th March and attended by 3000 supporters from Madrid, the center of the pandemic in Spain, and the Cheltenham horseracing festival, with races attracting crowds of over 60.000 people (Sassano 2020). Most national and international large sporting events, cancelled or postponed in the first half of 2020, resumed during the summer months, though with closed doors or major limitations in the number of spectators. Large sports events including tens of thousands of spectators might not take place for several years to come.

Religious gatherings

Several mass religious events have been associated with explosive outbreaks of COVID-19 in South Korea, USA, France, and many more places. As mentioned above, in April 2020, 5212 coronavirus cases were related to an outbreak at the Shincheonji Church in South Korea, accounting for about 48,7% of all infections in the country at that time.

The annual gathering of the Christian Open Door Church, held on 17-24 February in Mulhouse, France and attended by about 2500 people, became the first significant cluster in France. After a parishioner and 18 family members tested positive on 1 March, a flurry of reported cases brought the existence of a cluster to light. According to an investigative report by France Info, more than 1000 infected members from the rally in Mulhouse contributed to the start of the COVID-19 epidemic in France. Many diagnosed cases and deaths in France as well as Switzerland, Belgium and Germany were linked to this gathering.

Another report described 35 confirmed COVID-19 cases among 92 attendees at church events in Arkansas, USA, during March 6–11. The estimated attack rates ranged from 38% to 78% (James 2020). In Frankfurt, Germany, one of the first post-lockdown clusters started during a religious ceremony held on 10 May. As of 26 May, 112 individuals were confirmed to be infected with SARS-CoV-2 (Frankfurter Rundschau). *Editor's note: May we suggest that going to church does not protect you from SARS-CoV-2?*

Huge religious mass gatherings should probably be postponed. Gatherings that attract millions of pilgrims from many countries (with pilgrims typically > 50 years old and often suffering from a chronic disease such as diabetes or cardiovascular disease [Mubarak 2020]) clearly have the potential to create giga-spreading events, saturating designated hospital wards and ICU capacity within days. Reducing the number of pilgrims and excluding foreign pilgrims is therefore a wise decision (Khan 2020, Ebrahim 2020). Events attended by even more people, such as the Sabarimala annual 41-day Hindu pilgrimage (average attendance: 25 million people) would need even more careful planning (Nayar 2020).

Closed and densely populated spaces

Prisons

According to WHO, people deprived of their liberty, such as people in prisons and other places of detention, are more vulnerable to COVID-19 outbreaks (WHO 200315). People in prison are forced to live in close proximity, which may facilitate infection, amplification and spread of infectious diseases within and beyond prisons. The global prison population is estimated at 11 million and prisons are in no way "equipped" to deal with COVID-19 (Burki 2020).

By August 2020, 90 of the largest 100 cluster outbreaks in the US had occurred in prisons and jails (Wang 2020). In US prisons, COVID-19 attack rates can be high. By June 6, 2020, there had been 42.107 cases and 510 deaths among 1,3 million prisoners (Saloner 2020, Wallace 2020). Among 98 detained persons in Louisiana who were quarantined because of virus exposure, 71 (72%) had SARS-CoV-2 infection identified through serial testing, among them 45% without any symptoms at the time of testing (Njuguna 2020). In July 2020, more than one-third of the inmates and staff (1600 people) in San Quentin Prison tested positive. Six died (Maxmen 2020). Still in July 2020, the rate of COVID-19 among incarcerated individuals in Massachusetts was nearly 3 times that of the general population and 5 times the US rate (Jiménez 2020). The strongest risk factor for SARS-CoV-2 infection among 10.304 incarcerated persons in Connecticut was dormitory housing (odds ratio 35.3) (Kennedy 2020). Reducing the incarcerated population ("de-carceration") is an important and urgent strategy for mitigating viral transmission in prisons and jails (Wang 2020).

Homeless shelters

Shelters for homeless people can also become hotspots of SARS-CoV-2 transmission. Testing 1192 residents and 313 staff members in 19 homeless shelters from 4 US cities (see table online) found infection rates of up to 66% (Mosites 2020). In another report from Boston, Massachusetts, 147/408 (36%) homeless shelter residents were found to be positive. Of note, 88% had no fever or other symptoms at the time of diagnosis (Baggett 2020).

In yet another study of 14 homeless shelters in King County, Washington, researchers divided the number of positive cases by the total number of participant encounters, regardless of symptoms. Among 1434 encounters, 29 (2%) cases of SARS-CoV-2 infection were detected across 5 shelters. Eighty-six percent of persons with positive test results slept in a communal space rather than in a private or shared room (Rogers 2020).

Cruise ships, aircraft carriers, etc.

Cruise ships carry many people in confined spaces. On 3 February 2020, 10 cases of COVID-19 were reported on the Diamond Princess cruise ship off the cost of Japan. Within 24 hours, all sick passengers were isolated and removed from the ship and the rest of the passengers quarantined on board. Over time, more than 700 of the 3700 passengers and crew tested positive (around 20%). One study suggested that without any intervention 2920 individuals (79%) could have been infected (Rocklov 2020). The study also estimated that an early evacuation of all passengers on 3 February would have been associated with only 76 infections. For cruise ships, SARS-CoV-2 may spell disaster – carrying village-loads of people from one place to another may not be a viable business model for years to come.

Big navy vessels such as aircraft carriers can become floating petri dishes for emerging viral respiratory diseases. Already in 1996, an outbreak of influenza A (H3N2) occurred aboard a navy ship. At least 42% of the crew became ill within few days, although 95% had been appropriately vaccinated (Earhart 2001). Since the beginning of the year, several outbreaks of COVID-19 on military ships have been reported, facilitated by the small, enclosed areas of work and the lack of private quarters for the crew. The largest outbreaks have been reported on the *USS Theodore Roosevelt* and the French aircraft carrier *Charles-de-Gaulle*.

On the *Theodore Roosevelt,* in late March, 1271 crew members (26,6%) tested positive for SARS-CoV-2. Among them, 76,9% (978/1271) had no symptoms when they tested positive and 55% developed symptoms during the clinical course. Among the 1331 crew members with suspected or confirmed COVID-19, 23 (1,7%) were hospitalized, 4 (0,3%) received intensive care, and 1 died. Crew members who worked in confined spaces appeared more likely to become infected. The authors concluded that SARS-CoV-2 transmission is facilitated by close-quarters conditions and by asymptomatic and pre-symptomatic infected crew members (Kasper 2020). A previous report had found that preventive measures reduced the risk of infection: among 382 service members, those who reported taking preventive measures had a lower infection rate than did those who did not report taking these measures (e.g., wearing a face-covering, 56% versus 81%; avoiding common areas, 54% versus 68%; and observing social distancing, 55% versus 70%, respectively) (Payne 2020).

On the French aircraft carrier *Charles-de-Gaulle*, a massive epidemic was confirmed on 17 April. Among the 1760 sailors, 1046 (59%) were positive for SARS-CoV-2, 500 (28%) presented symptoms, 24 (1,3%) sailors were hospitalized, 8 required oxygen therapy and one was admitted to intensive care.

Smaller clusters have also been reported on 5 other US military vessels, and in one each from France, Taiwan, and Holland. However, given usual security policies and communication restrictions of national armies and navies, it is possible that other unreported clusters of cases and even deaths might have occurred.

Special Aspects of the Pandemic

The COVID-19 pandemic and the international response to it have highlighted several specific aspects, including both successes and failures. Lessons learned from different countries should be used to inform the preparedness for, and management of, future pandemics (by coronaviruses, influenza viruses or by as yet unknown pathogens):

- First outbreak (China)
- Surprise or unpreparedness (Italy)
- Unwillingness to prepare (UK, USA, Brazil)
- Partial preparedness (France)
- Preparedness (Germany)
- Herd immunity? (Sweden)
- Deferred beginning (South America)
- Splendid isolation (New Zealand, Australia)
- Unknown outcome (Africa)

First outbreak (China)

China was caught by surprise by the COVID-19 outbreak – as any other nation would have been – but "thanks" to the experience of the SARS outbreak in 2003 (Kamps-Hoffmann 2003), was prepared for it. At first, the epidemic spread within Wuhan and Hubei Province (December 2019, Li Q 2020) and then nationwide to all provinces in January 2020, favored by travelers departing from Wuhan before the Chinese Spring Festival (Zhong 2020, Jia JS 2020). However, within 3 weeks from the identification of the new virus, the government ordered the lockdown of more than 50 million people in Wuhan and the surrounding province of Hubei, as well as strict quarantine measures and travel restrictions for hundreds of millions of Chinese citizens. This astonishing first in human history achieved what even specialists did not dare to dream: curbing an epidemic caused by a highly contagious virus (Lau 2020).

As early as four weeks after the Wuhan lockdown, there was evidence that strict containment measures were capable of curbing a SARS-CoV-2 epidemic as shown in Figure 1.1. The lesson from China: it is possible to lockdown entire provinces or countries and lockdown works. Some health authorities in the Western Hemisphere followed the example of China (Italy, for example, ordered a lockdown as early as 18 days after the diagnosis of the first autochthonous case), other governments did not. It cannot be overemphasized that China has basically managed to control the spread of SARS-CoV-2 since March. How was that possible (Burki 2020)?

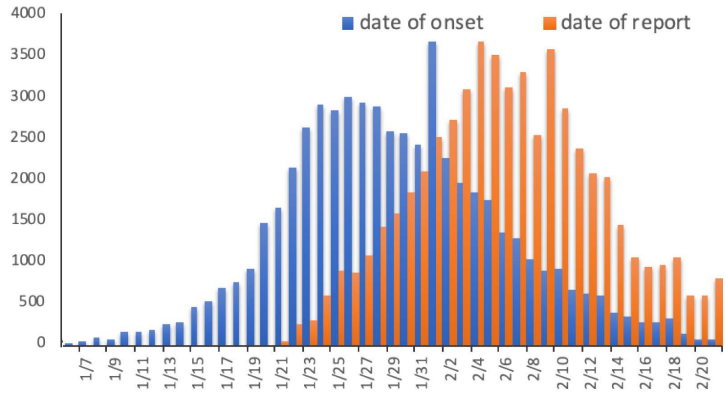

Figure 1.1. The Chinese outbreak in January/February 2020. Epidemic curves by symptom onset and date of report on 20 February 2020 for laboratory confirmed COVID-19 cases for all of China. Modified from *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19),* 16-24 February 2020. https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19)

Preparedness (Taiwan, Vietnam, Japan)

On 7 June, **Taiwan** (24 million people with a population density of 650/km^2) had reported only 443 cases and 7 deaths. Most SARS-CoV-2 infections were not autochthonous. As of 6 April 2020, 321 imported cases were reported in Taiwanese citizens who had travelled to 37 different countries for tourism, business, work, or study (Liu JY 2020). From the beginning, Taiwan drew on its SARS experience to focus on protecting health care workers' safety and strengthening pandemic response (Schwartz 2020 + The Guardian, 13 March 2020). An early study suggested that identifying and isolating symptomatic patients alone might not suffice to contain the spread of the virus and recommended more generalized measures such as social distancing (Cheng HY 2020). Big data analytics were used in containing the epidemic. On one occa-

sion, authorities offered self-monitoring and self-quarantine to 627.386 people who were potential contacts of more than 3000 passengers of a cruise ship. These passengers had disembarked at Keelung Harbor in Taiwan for a 1-day tour five days before the COVID-19 outbreak on the Diamond Princess cruise ship on February 5th, 2020 (Chen CM 2020).

Vietnam did remarkably well too. One hundred days after the first SARS-CoV-2 case was reported in Vietnam on January 23rd, only 270 cases had been confirmed, with no deaths. Although there was a high proportion of asymptomatic and imported cases as well as evidence for substantial pre-symptomatic transmission, Vietnam controlled the spread of SARS-CoV-2 through the early introduction of mass communication, meticulous contact-tracing with strict quarantine, and international travel restrictions (Pham QT 2020).

Finally, in **Japan**, public adherence to the rules, along with cluster tracing and a ban on mass gatherings, helped bring the outbreak under control. Where widespread mask use and hygiene is a normal part of etiquette, combatting SARS-CoV-2 is a lot easier (Looi 2020).

Experiences from these countries show that effective testing and contact tracing, combined with physical distancing measures, can keep the pandemic at bay and an economy open. Health is the key to wealth.

Surprise or unpreparedness (Italy)

Italy was the first European country struck by the pandemic. Although the first local case was diagnosed only on 20 February, the force of the outbreak suggests that the virus had been circulating for weeks among asymptomatic or pauci-symptomatic people, possibly from as early as 1 January (Cereda 2020,Gámbaro 2020). Genome analysis of SARS-CoV-2 isolates suggests that the virus was introduced on multiple occasions (Giovanetti 2020).

However, it was not straightforward to decipher the subtle signs of coming events, in Italy like elsewhere. During the winter flu season, COVID-19 deaths in elderly people could easily be interpreted as flu deaths. And the rapid spread of SARS-CoV-2 among the most active social age group – young people crowded in bars, restaurants and discos –would not have caused visible life-threatening symptoms. Before being detected, the epidemic had plenty of time (at least a month) to grow.

One additional reason for the delay in recognizing the encroaching epidemic in Italy might have been the Italian 'suspected COVID-19 case definition". Following the suspected case definition recommended at that time by WHO, it included the epidemiological criteria of 'history of travel to China or contact

with a person coming from China' before requesting a PCR test. A strict application of this case definition discouraged testing of suspected pneumonia cases where the link with China was not clear (which would eventually happen everywhere after the first asymptomatic chains of infections). The young anesthesiologist in Codogno who eventually requested the PCR test for Mattia, the Italian patient #1, did it "under her own responsibility since not in line with MOH guidelines".

It is as yet unclear why the epidemic took such a dramatic turn in the northern part of Italy, especially in Lombardy (Gedi Visual 2020), while other areas, especially the southern provinces, were relative spared. Over-dispersion might be an explanation (see above). Of note, healthcare in Italy is run regionally and, for a long time, the Lombardy Region has favored the development of a mostly private and hospital-centered health system, with great facilities but poor community-based services. This meant that COVID-19 patients were quick to run to the hospital, even with minor symptoms, resulting in overcrowded emergency services and major nosocomial spread. A more decentralized and community-based system like in the Veneto Region (plus maybe a bit of luck) could have greatly reduced the mortality from COVID-19 in Lombardy.

In addition, since 2006 Italy had not updated nor implemented the national pandemic preparedness plan. The lack of preparedness and the overlap of responsibilities hampered considerably the initial coordination of the national response between the regions and the central government.

Unwillingness to prepare, or simple denial (UK, Iran, USA, Brazil)

In the **United Kingdom**, clumsy political maneuvering delayed the start of effective lockdown measures by a week or more. As the epidemic doubled in size about every 7 days (Li 2020), around 50% and 75% of all deaths might have been prevented had lockdown or social distancing measures been ordered one or two weeks earlier, respectively. Early data from Ireland and the United Kingdom seem to confirm this assumption. Each day of delay increased mortality risk by 5 to 6% (Yehya 2020). The consequences were dramatic (Stoke 2020, Maxmen 2020).

Like in **Iran**, where the regime covered up news of the coronavirus for three days to avoid impacting on the turnout at parliamentary elections on 21 February, domestic politics (or paranoia, see BMJ, 6 March 2020) influenced the epidemic response in the **USA**. Scientific advice from CDC and other national public health institutions was ignored (The Lancet 2020). The US is now the

country with the highest number of cases and deaths. Without their unprecedented vacuum in leadership (NEJM Editors 2020), most of these deaths could have been prevented. And **Brazil**, which is also not an example of good governance performance, has become the country with the second highest number of deaths in the world.

Partial preparedness (France)

France was partially prepared. During the first national outbreak near Mulhouse, hospitals were overwhelmed. Despite the updated and well-structured pandemic plan, personal protective equipment was in short supply all over the country; in particular, face masks were sorely lacking after a quixotic decision by the Hollande government to greatly reduce the planned stocks of 1,7 billion protective masks (surgical and FFP2) available in 2009 and considered too expensive, to only 145 million surgical masks in 2020 ("*Nous n'allons pas gérer des stocks de masques, c'est coûteux, parce qu'il faut les détruire tous les cinq ans.*")[1] (Le Monde 200506).

However, France, thanks to Italy, had an important advantage: time. It had several weeks to learn from the events in Lombardy. When, on the weekend of 21 March, virtually from one day to the next, patients started pouring into the hospitals of the Greater Paris Region, the number of available intensive care unit beds had already been increased from 1400 to 2000. Furthermore, two years before, in a simulation of a major terrorist attack**,** France had tested the use of a high-speed TGV train for transporting casualties. At the height of the COVID 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 high-speed trains as well as 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.

Good virologists, huge lab network, family doctors (Germany)

Germany's fatality rate is lower than in other countries. It is assumed that the main reason for this difference is simply more tests. While other countries were conducting a limited number of tests in older patients with severe disease, 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 and isolate, the lower the fatality rate and the spread of infection.

[1] „*We are not going to manage mask stocks, it is expensive, because we have to destroy them every five years.*"

Furthermore, in Germany's public health system, SARS-CoV-2 testing was not restricted to central laboratories as in many other nations but could be conducted at quality-controlled laboratories throughout the country. Thanks to reliable PCR methods that had been developed by the end of January from the Drosten group at Berlin's Charité (Corman 2020), within a few weeks the 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.

Finally, another important reason for the low mortality in Germany might be 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.

As a result of these first-wave distinctive features, the case-fatality rate (CFR) of COVID was 0,7% in Germany, compared with CFRs as high as 9,3% and 7,4% in Italy and the Netherlands, respectively (Sudharsanan 2020, Fisman 2020). Age distribution of cases may explain as much as 66% of the variation of SARS-CoV-2 cases across countries (Sudharsanan 2020).

Did the experience of the first SARS-CoV-2 wave help Germany during the second wave? It didn't! (Figure 1.2)

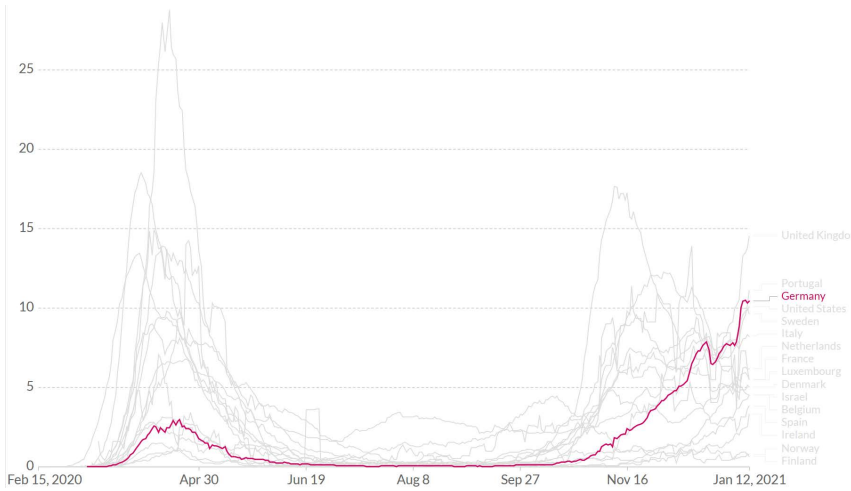

Figure 1.2. Daily new confirmed COVID-19 **deaths** per million people. Rolling 7-day average. Source: Our World in Data +Johns HopkinsUniversity CSSE COVID-19 Data.

Christmas

At the beginning of December 2020, Ireland seemed to be a model of a "good" pandemic. In early January, the country of about 5 million inhabitants recorded 50,000 new SARS-CoV-2 cases in just one week. Ireland is the place in the world where the virus is now spreading the fastest, with 1323 new daily cases detected per million inhabitants (Figure 1.3). The English variant B117 is currently responsible for more than 40% of new cases. The reason for the sudden resurgence is probably linked to a combination of factors: Christmas, pubs, restaurants, relaxation due to having done so well (less masks, distancing).

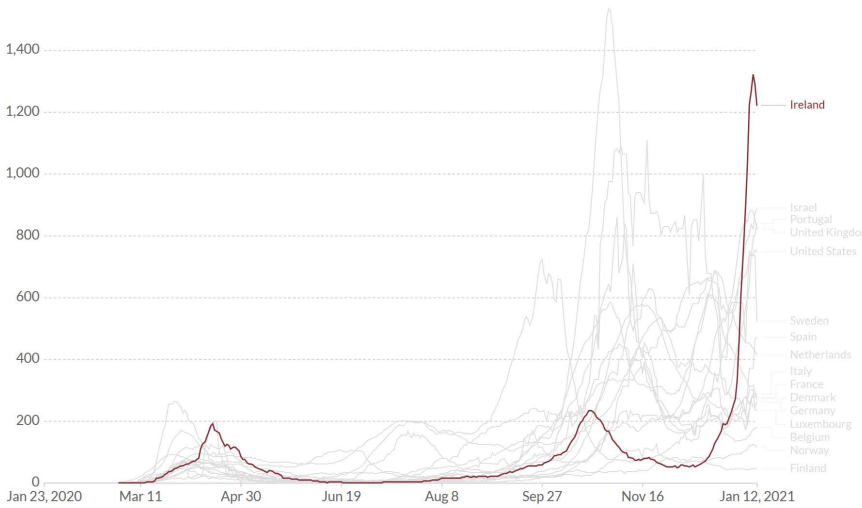

Figure 1.3. Daily new confirmed COVID-19 **cases** per million people. Rolling 7-day average. Source: Our World in Data +Johns HopkinsUniversity CSSE COVID-19 Data.

Herd immunity? Not yet! (Sweden)

Sweden has never really imposed a lockdown, counting on the population to adopt individual physical distancing and other protective measures to curb the transmission of SARS-CoV-2. The price was high (Habib 2020). In October 2020, Sweden had a death rate 10 times higher than Norway and five times higher than Denmark, with most deaths occurring in care homes and immigrant communities. Still worse, Sweden did not benefit economically of its no-lockdown approach as its economic performance contracted at a similar rate as countries in the rest of Europe (Financial Times, 10 May 2020).

At the end of the summer, some people speculated that the winter would have reduced the mortality gap between Sweden and Norway or Denmark. Would Sweden, after accepting many deaths in spring, see fewer of them in the future? Would a (still low!) level of community immunity help slow down the epidemic in winter? After all, cell phone data had shown that Swedes traveled less during the summer than, for example, Norwegians or Danes, and might have imported less infections from summer vacation hotspots.

In any case, it did not work. Simply allowing a deadly pathogen to spread to reach herd immunity is a thing of the past (Aschwanden 2020, Randolph 2020). We have now much better knowledge and tools, even in the absence of vaccines, to at least mitigate, if not suppress completely, any potential pandemic. We just need to apply them widely and consistently (Bedford 2020).

Deferred beginning, then major impact (South America)

The first case of COVID-19 in Latin America was reported on 26 February in Brazil and by early April all countries had reported at least one imported case. However, in the initial months of 2020, the number of cases was low in South America compared to Europe or Asia (Haider 2020). As a matter of fact, the local epidemics took off roughly 4 weeks later than in Europe (see www.worldometers.info/coronavirus).

However, the epidemic accelerated during the month of May when South America become the epicenter of the coronavirus pandemic according to WHO. In September, Latin America, home to around 8% of the world's population, accounted for over a quarter of all confirmed COVID-19 cases and nearly a third of all related deaths. There was, however, wide variation between countries, with **Brazil** and **Mexico** having some of the worst epidemics in the world, while Uruguay infection rates were comparable to the best performing countries in Asia or Europe (Taylor 2020).

According to Marcos Espinal and colleagues from WHO/PAHO, there are several factors in Latin America that make this pandemic more difficult to manage: inequality, belts of poverty surrounding big cities, informal economies, and difficult areas of access. Here, as elsewhere, leadership and sound public health policies made a difference. Both Brazil's and Mexico's presidents have been widely criticized for playing down the threat of COVID-19, not taking action to slow its spread, and suggesting alternative ineffective ways of protection (for example, the use of traditional scarves (?) instead of face masks).

However, other countries have performed much better, managing to keep infections low. **Cuba** and **Costa Rica**, for example, have enforced strict testing, isolation and quarantine measures. The most successful country so far

has been **Uruguay** that managed, though a mix of effective testing, contact tracing, isolation and quarantine, to keep infection rates very low without generalized lockdowns. The President simply asked, rather than ordered, people to stay home for their own well-being and that of fellow citizens (Taylor 2020).

Splendid isolation (New Zealand, Australia)

Australia, New Zealand, French Polynesia, Fiji, New Caledonia and Papua New Guinea and Oceania are among the least hit areas in the world. Geographically isolated islands or island states should be the ideal candidates for elimination trials. However, even **New Zealand**, which viewed itself in the post-elimination stage and where public life had returned to near normal (Baker 2020), was suddenly called back into COVID-19 reality when new autochtonous cases were discovered in August 2020.

In **Australia**, transmission was initially driven by multiple SARS-CoV-2 importations by international travelers which accounted for over half of locally acquired cases (Seemann 2020). However, on 20 June, the State of Victoria reported a spike in community transmitted cases, apparently following lax implementation of quarantine measures, that resulted in a large outbreak with more than 20.000 cases and 800 deaths and the imposition of strict lockdown measures in the State and a night curfew in Melbourne. Easing of restrictions only started mid-September, following a major decrease in the number of new cases.

Both Australia and New Zealand have considered a strategy of COVID-19 elimination, i.e., the absence of sustained endemic community transmission in the country. Later outbreaks raised the question of whether elimination is a reasonable goal (Hewyood 2020). The elimination of any infectious disease is an ambitious objective, requiring strong public health measures and substantial resources. In principle, a zero-case scenario of not less than three months would be the condition for declaring a state or country SARS-CoV-2-free. Then, strict travel and border restrictions and quarantine measures must be implemented over a prolonged period, as the virus continues to spread around the world. It looks like international travel to New Zealand and Australia may continue to be banned for quite some time.

Africa: The unknown outcome

The high transmissibility of SARS-CoV-2, combined with the scarcity of crucial health equipment, facilities and human resources, and the challenges of

implementing widespread case isolation (Wells 2020), was expected to result in a devastating impact of COVID-19 on African countries.

These predictions have not materialized. There has been no COVID-19 explosion in Africa, although seroprevalence data are comparable to those from European countries like France, Italy and Spain. However, the burden and outcomes associated with COVID-19 shows substantial variations across African countries [Twahirwa 2020]. (There is no 'one' Africa!)

In April-June 2020, the crude prevalence of anti–SARS-CoV-2 IgG among blood donors in **Kenya** was 5,6% (174/3098). It was highest in urban counties, Mombasa (8,0%), Nairobi (7,3%) and Kisumu (5,5%) (Uyoga 2020). Of note, Kenya had reported only 341 deaths by the end of that period. The authors conclude that the sharp contrast between the reported COVID-19 cases and deaths suggests that the disease might be attenuated in Africa.

Has time come to hypothesize an "African demographic exception"? In the Democratic Republic of the Congo and Malawi, for instance, only 2-3% of the population is older than 65 years (Kalk 2020), in sharp contrast to Europe at 20,5% or Lombardy at 26%. If > 65-year-old SARS-CoV-2 infected individuals are 100 times more likely to die from COVID-19 than a 25-year-old, we should expect two different epidemics. Simply, the age pyramid might make the difference.

The SARS-CoV-2 pandemic: Past and Future

Natural course of a 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 Americas. The first huge outbreaks occurred in regions with cold winters (Wuhan, Iran, Northern Italy, the Alsace region in France).

Fifty years ago, the course of the COVID-19 pandemic would have been different, with slower global spread but higher burden due to limited diagnostic and therapeutic capacities and no option of nation-wide lockdowns (see also a report of the influenza pandemics in 1957 and 1968: Honigsbaum 2020). According to one (controversial) simulation, in the absence of effective treatment and with a mortality rate of around 0,5%, without interventions COVID-19 would have resulted in 7 billion infections and 40 million deaths globally 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. Another model predicted that 80% of the US population

(around 260 million people) would have contracted the disease. Of those, 2,2 million Americans would have died, including 4% to 8% of those over age 70 (Ferguson 2020). In Germany alone, the SARS-CoV-2 pandemic could have resulted in 730.000 deaths (Barbarossa 2020) with 500.000 deaths each in France, Italy, Spain and the UK.

The 2020 Lockdowns

Fortunately, the world has been spared from a freely circulating SARS-CoV-2. If humanity can change the climate, why shouldn't we 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, at first, almost all governments considered saving hundreds of thousands lives more important than avoiding a massive economic recession. First in China, six weeks later in Italy and another a week later in most Western European countries, and later in the US and in many other countries in the world, unprecedented experiments of gigantic dimensions were started: ordering entire regions or the whole nation to lockdown. By the first week of April, 4 billion people worldwide were under some form of lockdown — more than half of the world's population.

Lockdowns in Europe were generally less strict than in China, allowing the continuation of essential services and industries and the circulation of people when justified. People were generally compliant to mandatory stay-at-home orders, even in the US. Based on location data from mobile devices, in 97,6% of US counties these orders were associated with decreased median population movement (Moreland 2020). Lockdowns were generally also well accepted. During the week of May 5–12, 2020, a survey among 2402 adults In New York City and Los Angeles found widespread support of stay-at-home orders and non-essential business closures, and a high degree of adherence to COVID-19 mitigation guidelines (Czeisler 2020).

Lockdowns were also successful in slowing down the pandemic. In New York City, SARS-CoV-2 prevalence varied substantially between boroughs between 22 March and 3 May 2020 (for example, Manhattan: 11,3%; South Queens: 26,0%). These differences in prevalence correlate with antecedent reductions in commuting-style mobility between the boroughs. Prevalence was lowest in boroughs with the greatest reductions in morning movements out of and evening movements into the borough (Kissler 2020). According to one study, between 12 and 15 million individuals in Europe had been infected with SARS-CoV-2 by May 4th, representing only between 3,2% and 4,0% of the population (Flaxman June 2020). Projected percentages of the total population infected ranged from a low of 0,76% in Austria to a high of 8,0% in Belgium. In South

America, lockdowns were successful, too, although they worked best among the wealthy and less well among the less wealthy who had to choose at times between the risk of dying from COVID or dying from hunger.

Curfews

Lockdowns are effective but frighteningly costly. The spring lockdown cost most countries around 10% of their PIB with unforeseeable economic, political and also health consequences; in exchange, they can "flatten the curve" and did succeed in keeping seroprevalence rates low, somewhere between 1% and 10%. Generalised lockdowns are clearly not a viable, long-term model for the future.

Might curfews be a less costly alternative, both economically and socially? In French Guiana, an French overseas *département*, a combination of curfews and targeted lockdowns in June and July 2020 was sufficient to avoid saturation of hospitals. On weekdays, residents were first ordered to stay at home at 11 p.m., then at 9 p.m., later at 7 p.m., and finally at 5 p.m. On weekends, everyone had to stay at home from 1 p.m. on Saturday (Andronico 2020). Whether curfews can be successfully adapted to other areas than French Guiana, is not known. French Guiana is a young territory with a median age of 25 years and the risk of hospitalization following infection was only 30% that of France. About 20% of the population had been infected with SARS-CoV-2 by July 2020 (Andronico 2020). Following Belgium and Germany, France has just implemented now a night curfew in Paris and a few other major cities. Be prepared to see more curfews orders over the coming six months.

The "second wave"

There is no real pandemic in Africa, a never-ending wave in the Americas, and now a second wave in Europe. The worst may be yet to come (The Lancet 2020) with more people dying and every death leaving 10 more people mourning a grandparent, parent, sibling, spouse, or child (Verdery 2020). Will the SARS-CoV-2 pandemic follow the scenario of the 1918 influenza pandemic (Horton 2020) with a much worst second wave? In anticipating local epidemics, politicians should prepare for the worst, at least until spring 2021.

There are important differences between the springtime "first wave" of COVID-19 epidemics in Europe and the late-summer and autumn second wave. An important feature of this second wave of infections is its widespread nature, as opposed to earlier, more localized outbreaks (e.g., northern Italy, Madrid, Spain or Mulhouse, France.) More populated and better-connected municipalities were generally affected earlier by the SARS-CoV-2 epidemic,

and less populated municipalities at a later stage of the epidemic (de Souza 2020).

Relaxation of mitigation measures and the decreased burden of diseases during the summer led to a resumption of "normal life" behaviors and a resurgence of infections. Initially, the diffusion of the virus may have appeared to have few negative effects, only to lead to deadly outbreaks weeks or months later (Thomas 2020). Public health messaging must stress that apparent lulls in disease progress are not necessarily indicators that the threat has subsided, and that areas "passed over" by past outbreaks could be impacted at any time.

A second major difference in this second wave is the role played by different age groups in the spread of the virus. Understanding whether increasing incidence is predominantly occurring in specific age groups is important for identifying opportunities to prevent or reduce transmission (Oster 2020). In the French Bouches-du-Rhône department, which includes Marseille, the first signs of the second wave were detected in wastewater on July 13[2]. Three weeks later, the first post-lockdown rise in new SARS-CoV-2 infections was seen in young adults 20 to 29 years old; and again a few weeks later, infection rates increased in older age groups. In Spain (NCOMG 2020), Switzerland (see Figure 1.4) and other European countries, the second wave looked equally triggered mostly by transmission among young adults in leisure venues such as bars, restaurants, discos or clubs during the summer of 2020.

In the USA, the summer COVID-19 dynamic was comparable. During June–August 2020, SARS-CoV-2 incidence was highest in persons aged 20–29 years, who accounted for > 20% of all confirmed cases. Across the southern United States, in June 2020, increases in percentage of positive SARS-CoV-2 test results among adults aged 20–39 years preceded increases among those aged ≥ 60 years by 4–15 days (Boehmer 2020, Oster 2020). First the kids, then the parents and, finally the grandparents – with unknown outcome?

[2] SARS-CoV-2 can be detected in wastewater using RT-qPCR. In one study, the total load of gene equivalents in wastewater correlated with the cumulative and the acute number of COVID-19 cases reported in the respective catchment areas [Westhaus 2020]. Note that wastewater is no route for SARS-CoV-2 transmission to humans! All replication tests were negative tests.

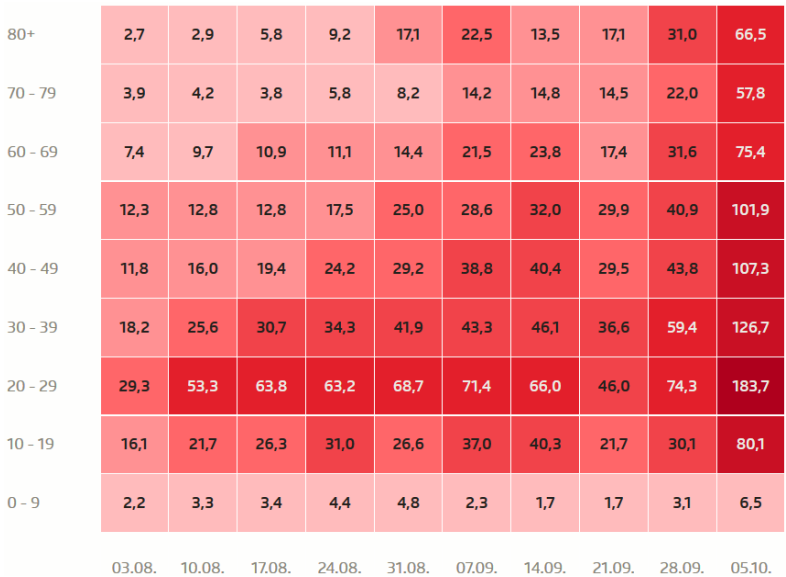

Figure 1.4. Weekly positive SARS-CoV-2 tests in Switzerland by age group (August 3 through October 5).Source: SRF, *So entwickeln sich die Corona-Zahlen in der Schweiz* (https://www.srf.ch/news/schweiz/coronavirus-so-entwickeln-sich-die-corona-zahlen-in-der-schweiz; accessed 12 October 2020).

Herd immunity: Not yet!

Herd immunity, the notion introduced to a wider public by a foolish politician, may not be on the agenda for a long time. Herd immunity, also known as *indirect protection*, *community immunity*, or *community protection*, refers to the protection of susceptible individuals against an infection when a sufficiently large proportion of immune individuals exist in a population (Omer 2020). As for now, not a single country is anywhere close to reaching herd immunity. Even in past hotspots like Wuhan, the prevalence of SARS-CoV-2 IgG positivity was 9,6% among 1021 people applying for a permission to resume work (the SARS-CoV-2 nucleic acid test needed to be negative) (Wu X 2020).

A French study projected 2,8 million or 4,4% (range: 2,8–7,2) prevalence of infections in France. In Los Angeles, the prevalence of antibodies was 4,65% (Sood 2020). (And even this low number may be biased because symptomatic persons may have been more likely to participate.) A nationwide coronavirus antibody study in Spain showed that about 5% of the population had contracted the virus. These infection rates are clearly insufficient to avoid a second wave of a SARS-CoV-2 epidemic (Salje 2020). Achieving herd immunity

without overwhelming hospital capacity would require an unlikely balancing of multiple poorly defined forces (Brett 2020).

Vaccines: In sight, finally! But be patient!

Very few experts expected safe and effective vaccines to become available Christmas 2020. Modern technologies, massive funding, and both international cooperation and competition are making it a reality. However, even if vaccines are confirmed effective and safe soon, mass vaccination for COVID-19 will be a huge logistic challenge and nobody should expect vaccines to have a noticeable impact on the SARS-CoV-2 pandemic before at least the summer of next year. In the meantime, people will need to be patient and continue to practice and observe the established alternative ways of protection.

'Variolation' – Finding of the year?

Reducing the viral SARS-CoV-2 inoculum might not only reduce the probability of infection but also favor an asymptomatic infection while still generating immunity. A few papers (Bielecki 2020, Ghandi 2020; see also the comments to the paper by Rasmussen 2020, Brosseau 2020) suggested that if facial masking may help in reducing the size of the viral inoculum, universal facial masking might ensure that a greater proportion of new infections are asymptomatic. If universal masking could be proved to be a form of 'variolation' (inoculation), it would be an additional argument in favor of mandatory mask wearing.

Outlook

At the beginning of autumn, many feared a second COVID-19 wave comparable to the devastating influenza pandemic during the autumn and winter 1918/1919 (Soper 1919). Fortunately, physical distancing (Bedford 2020) and – when needed – partial selective lockdowns have not allowed SARS-CoV-2 to play out its full potential. Now, with the first vaccine (BioNTech/Pfizer) approved in the UK and more vaccines and more approvals in other countries within reach, we can be confident: SARS-CoV-2 will not be like H1N1 and we have the tools and capacities to get through the winter (Bedford 2020).

A month ago, we asked ourselves, 'How long will SARS-CoV-2 stay with us? How long will it be before we return to pre-COVID-19 normalcy? For how long a combination of physical distancing, enhanced testing, quarantine, and contact tracing will be needed?' We were skeptical, saying that 'even vaccines might not have a substantial impact on the pandemic before 2024, if ever.' We felt – and still feel today – that before mass vaccination, classical infection

control measures are the only way to reduce the number of infections and avoid healthcare systems from breaking down, leaving patients with other morbidities – common emergencies and surgery, cancer treatment, management of patients with chronic diseases – stranded and abandoned in a medical no-man's land.

In December 2020, however, we see the light at the end of the SARS-CoV-2 tunnel. The prospects are bright – vaccines for 99% of people who are at risk of severe COVID-19. This new future is still a few months away, though, and how long the vaccine-induced immunity will last is still unknown. Bright prospects should not induce the fateful error of lowering the guards. No nation should repeat this winter the errors of the past summer. Summer 2020 taught us that post-lockdown epidemic dynamics may be driven by younger adults with gradual 'spill-over' into older age groups. The formula 'young adults -> parents -> grandparents -> death' is clearly a simplistic model for the European second wave. SARS-CoV-2 is introduced and spread in communities via all conceivable routes. Yet, there are simple rules and behaviors than can minimize the need for local lockdowns and economic hardship.

In situations of intense SARS-CoV-2 community transmission, the prevention triad is simple:

1. Stop people from meeting each other in large gatherings.
2. If they MUST meet, have them wear face masks.
3. In any case reduce the time infected or suspected infectious people meet any other people at all: test as much as possible, isolate cases quickly and track the close contacts.

In transmission hotspots, restrictive social-distancing measures will need to continue to be combined with widespread testing and contact tracing to slow down the pandemic (Giordano 2020 + less realistic, Peto 2020). People should concentrate on the essential activities of providing food and shelter as well as continuing their job, school and university activities. All 'après-work' and 'après-school' activities should be reduced to a minimum (no evening bars, no night life).

In such social slowdowns, people will need to avoid prolonged meetings with people from outside their inner-core "friends-and-family-bubble", in particular social events which bring people from many different families together (e.g., marriages, funerals, religious events). Even inside the inner-core "friends-and-family-bubble", meetings should be restricted to a handful of people. Economically, a social slowdown implies the temporary closure of places where foreigners, strangers or simply unacquainted people meet: discos, amusement parks, bars, restaurants, brothels and many more. In a situa-

tion of intense SARS-CoV-2 community transmission, strangers must not come together.

Coronaviruses have come a long way (Weiss 2020) and will stay with us for a long time. Questions still abound: When will we be able to move freely around the world as we did before? Will we wear face masks for years? When we will see the impact of COVID-19 vaccines on the spread of the virus? How long will it take to vaccinate the majority of the global population? Will the immunity be long-lasting? Will the virus mutate, forcing new control measures and a new rush for a more effective vaccine? Will there be any nightlife event with densely packed people dancing and shouting and drinking in any city in the world anytime soon? Nobody knows.

The French have an exquisitely precise formula to express unwillingness for living in a world you do not recognize: "*Un monde de con*!" Fortunately, we are slowly but surely capable of walking out of this "*monde de con*" thanks to a scientific community that is larger, stronger, and faster than at any time in history. (BTW, some politicians who were skeptical of science habe been ousted out of office. It is hight time!)

As of today, we still do not know how long-lasting, how intense, and how deadly this pandemic will be. We are walking on moving ground and, in the coming months and years, we will need to continue to be flexible, resilient, and inventive, looking for and finding solutions nobody would have imagined just a few months ago. Sure enough though, science will lead the way out. If we could leap five years into the future and read the story of COVID-19, we would not believe our eyes.

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2. Transmission

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Summary

The fundamental engines that drive the COVID-19 pandemic are now well established (Lee EC 2020, Madewell 2020). A summary (Meyerowitz 2020):

1. Respiratory transmission is the dominant mode of transmission.
2. Vertical transmission occurs rarely; transplacental transmission has been documented.
3. Direct contact and transmission through fomites (inanimate objects) are presumed but are likely only an unusual mode of transmission.
4. Although live virus has been isolated from saliva and stool and viral RNA has been isolated from semen and blood donations, there are no reported cases of SARS-CoV-2 transmission via fecal–oral, sexual, or bloodborne routes. To date, there is one cluster of possible fecal–respiratory transmission.
5. Cats and ferrets can be infected and transmit to each other, but there are no reported cases to date of transmission to humans; minks transmit to each other and to humans.

For everyday life, the following five *rules of thumb* are helpful:

1. Avoid crowded places (more than 5-10 people). The more people are grouped together, the higher the probability that a superspreader (see page 84) is present who emits infectious particles tens or hundreds times more than a 'normally' contagious individual.
2. Avoid in particular **crowded** and **closed** spaces (the worst: air-conditioned closed places where 'old air' is being moved around). In a room where a SARS-CoV-2 infected individual is coughing frequently, viable virus can be isolated from samples collected 2 to 4,8 meters away.
3. Avoid in all circumstances **crowded**, **closed** and **noisy** spaces where people must shout to communicate. Noise from machines or music in a closed environment creates the perfect setting for a superspreader event.
4. Outside crowded, closed or noisy spaces, keep a **distance of 2 meters** to other people.
5. Always wear a **fask mask in public spaces**.

Introduction

Viruses have substantially influenced human health, interactions with the ecosphere, and societal history and structures (Chappell 2019). In a highly connected world, microbial evolution is boosted and pathogens exploit human behaviors to their own benefit (Morens 2013). This was critically shown during the SARS epidemic in 2003 (Kamps-Hoffmann 2003), the outbreak of Middle East Respiratory Syndrome coronavirus (MERS-CoV) (Zaki 2012), the last great Ebola epidemic in West Africa (Arwady 2015, Heymann 2015) and the Zika epidemic in 2015-2017 (Fauci 2016). Over the same time period, more virulent strains of known respiratory pathogens – H5N1 influenza virus, tuberculosis, avian H7N9 influenza virus – have emerged (Kamps-Hoffmann 2006, Jassal 2009, Gao 2013).

The Virus

SARS-CoV-2, Severe Acute Respiratory Syndrome coronavirus 2, is a highly transmissible 'complex killer' (Cyranoski 2020) that forced half of humanity, 4 billion people, to bunker down in their homes in the early spring of 2020. The respiratory disease rapidly evolved into a pandemic (Google 2020). In most cases, the illness is asymptomatic or paucisymptomatic and self-limited. A subset of infected individuals has severe symptoms and sometimes prolonged courses (Garner 2020). Around 10% of infected people need hospitalization and around one third of them treatment in intensive care units. The overall mortality rate of SARS-CoV-2 infection seems to be less than 1%.

Coronaviruses are tiny spheres of about 70 to 80 nanometers (a millionth of a millimeter) on thin-section electron microscopy (Perlman 2019). Compared to the size of a human, SARS-CoV-2 is as small as a big chicken compared to the planet Earth (El País). The *raison d'être* of SARS-CoV-2 is to proliferate, like that of other species, for example *H. sapiens sapiens* who has been successful in populating almost every corner of the world, sometimes at the expense of other species. SARS-CoV-2, for now, seems to be on a similarly successful track.

SARS-CoV-2's global success has multiple reasons. The new coronavirus hijacks the human respiratory system to pass from one individual to another when people sneeze, cough, shout and speak. It is at ease both in cold and in warm climates; and, most importantly and unlike the two other deadly coronaviruses SARS-CoV and MERS-CoV, it manages to get transmitted to the next individual before it develops symptoms in the first one (see below, Asymptomatic Infection, page 86). There is no doubt that SARS-CoV-2 has a bright fu-

ture – at least until the scientific community develops a safe vaccine (see the chapter *Vaccines*, page 171) and efficient drugs.

SARS-CoV-2 and its kin

SARS-CoV-2 is a **coronavirus** like

- SARS-CoV (cousin from the 2002/2003 epidemic),
- MERS-CoV (Middle East Respiratory Syndrome coronavirus),
- and a group of so-called CAR coronoviruses (for Community-Acquired Respiratory CoVs: 229E, OC43, NL63, HKU1).

The CAR group of viruses are highly transmissible and produce about 15 to 30% of common colds, typically in the winter months. On the contrary, SARS-CoV and MERS-CoV have case fatality rates of 10% and 34%, respectively, but they never achieved pandemic spread. SARS-CoV-2, from a strictly viral point of view, is the shooting star in the coronavirus family: it combines high transmissibility with high morbidity and mortality.

SARS-CoV-2 is a **virus** like other commonly known viruses that cause human disease such as hepatitis C, hepatitic B, Ebola, influenza and human immunodeficiency viruses. (Note that the differences between them are bigger than those between humans and amebas.) With the exception of influenza, these viruses have a harder time infecting humans than SARS-CoV-2. **Hepatitis C virus (HCV)**, a major cause of chronic and often fatal liver disease, is mainly transmitted by percutaneous exposure to blood, by unsafe medical practices and, less frequently, sexually. The **human immunodeficiency virus (HIV)**, in addition to exposure to blood and perinatal transmission, also exploits sexual contact as a potent transmission route. **Hepatitis B virus (HBV)** is an even more versatile spreader than HCV and HIV as it can be found in high titers in blood, cervical secretions, semen, saliva, and tears; even tiny amounts of blood or contaminated secretions can transmit the virus. Ideal infection environments for HBV include, for example, schools, institutions and hospitals where individuals are in close and prolonged contact.

Of note, apart from HIV and hepatitis B and C, most viral diseases have no treatment. For example, there is no treatment for measles, polio, or smallpox. For influenza, decades of research have produced two specific drugs which have not been able to demonstrate reduced mortality – despite tests on thousands of patients. After 35 years of research, there is still no vaccine to prevent HIV infection.

Ecology of SARS-CoV-2

SARS-CoV-2 is present at the highest concentrations in the respiratory tract early in disease and then increases in the lower respiratory tract (Zhu N 2020, Wang 2020, Huang 2020, Wölfel 2020). The virus has also been found, albeit at low levels, in the kidney, liver, heart, brain, and blood (Puelles 2020). Outside the human body, the virus is more stable at low temperature and low humidity conditions, whereas warmer temperatures and higher humidity shorten the half-life (Matson 2020). It has also been shown to be detectable as an aerosol (in the air) for up to three hours, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel (van Doremalen 2020). As expected, viral RNA was more likely to be found in areas immediately occupied by COVID-19 patients than in other hospital areas (Zhou J 2020). Another study documented contamination of toilets (toilet bowl, sink, and door handle) and air outlet fans (Ong SWX 2020). This is in line with the experience from MERS where many environmental surfaces of patients' rooms, including points frequently touched by patients or healthcare workers, were contaminated by MERS-CoV (Bin 2016).

Person-to-person transmission

Person-to-person transmission of SARS-CoV-2 was established within weeks of identification of the first cases (Chan JF 2020, Rothe 2020). Shortly after, it was suggested that asymptomatic individuals would probably account for a substantial proportion of all SARS-CoV-2 transmissions (Nishiura 2020, Li 2020). Viral load can be high 2-3 days before the onset of symptoms and almost half of all secondary infections are supposed to be caused by pre-symptomatic patients (He 2020).

A key factor in the transmissibility of SARS-CoV-2 is the high level of viral shedding in the upper respiratory tract (Wolfel 2020), even among paucisymptomatic patients. Pharyngeal virus shedding is very high during the first week of symptoms, with a peak at > 7 x 10^8 RNA copies per throat swab on day 4. Infectious virus was readily isolated from samples derived from the throat or lung. That distinguishes it from SARS-CoV, where replication occured mainly in the lower respiratory tract (Gandhi 2020); SARS-CoV and MERS-CoV infect intrapulmonary epithelial cells more than cells of the upper airways (Cheng PK 2004, Hui 2018).

The shedding of viral RNA from sputum appears to outlast the end of symptoms and seroconversion is not always followed by a rapid decline in viral load (Wolfel 2020). This contrasts with influenza where persons with asymptomatic disease generally have lower quantitative viral loads in secretions

from the upper respiratory tract than from the lower respiratory tract and a shorter duration of viral shedding than persons with symptoms (Ip 2017).

A review summarized the evidence of human SARS-CoV-2 transmission (Meyerowitz 2020):

1. Respiratory transmission is the dominant mode of transmission.
2. Vertical transmission occurs rarely; transplacental transmission has been documented.
3. Direct contact and fomite transmission are presumed but are likely only an unusual mode of transmission.
4. Although live virus has been isolated from saliva and stool and viral RNA has been isolated from semen and blood donations, there are no reported cases of SARS-CoV-2 transmission via fecal–oral, sexual, or bloodborne routes. To date, there is 1 cluster of possible fecal–respiratory transmission.
5. Cats and ferrets can be infected and transmit to each other, but there are no reported cases to date of transmission to humans; minks transmit to each other and to humans.

Routes of Transmission

SARS-CoV-2 is spread predominantly via virus-containing droplets through sneezing, coughing, or when people interact with each other for some time in close proximity (usually less than one metre) (ECDC 2020, Chan JF 2020, Li Q 2020, Liu Y 2020, Lu J 2020). Direct contact or fomite transmission is suspected and may occur in some cases. Sexual, fecal–oral, and bloodborne transmission are theorized but have not been documented (Meyerowitz 2020).

Respiratory transmission

The upper respiratory tract is the usual initial site of viral replication, with subsequent descending infection (Wölfel 2020). The ideal transmission setting for SARS-CoV-2 is a **crowded**, **closed** and **noisy** space where people must shout to communicate. Shouting or speaking loudly emits a continuous flow of large droplets or fine aerosols laden with virions. Although aerosol lingers in the air for minutes, capable of infecting people at a distance, the ideal transmission setting (the 'SARS-CoV-2 jackpot' from the virus's point of view) are people shouting at one another at a short distance, inhaling deep into theirs lungs the exhalations of the person they are speaking to/shouting at for 5, 10, 20 minutes or longer. Noisy machines, loud music or high spirits during exuberant gatherings in crowded and closed environments are there-

fore the perfect conditions for exceptionally efficient SARS-CoV-2 transmission.

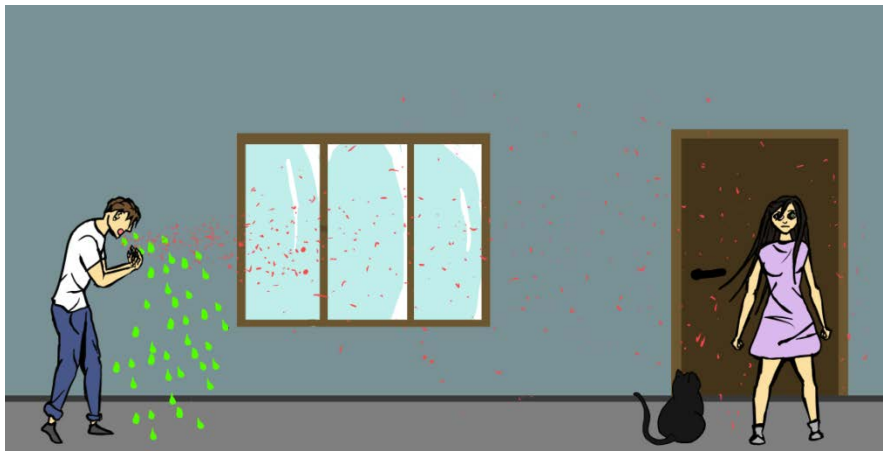

Figure 1. Transmission of SARS-CoV-2. 1) After coughing, sneezing, shouting and even after speaking – particularly loud speaking–, large droplets (green) drop to the ground around the young man. 2) In addition, some droplets, small and lightweight enough (red), are transported by air currents over longer distances (WHO 20200709). The second – aerosol – transmission is now recognized as a possibly relevant transmission route in SARS-CoV-2. Adapted from Morawska 2020. Art work: Félix Prudhomme – IYENSS.

SARS-CoV-2 is transmitted via (macro-)droplets greater than 5-10 µm in diameter, commonly referred to as **respiratory droplets**, and via smaller particles, < 5µm in diameter, which are referred to as droplet nuclei or **aerosols**. The almost century-old dichotomy (Wells 1934) "droplets vs. aerosol transmission" has been challenged by SARS-CoV-2. It is now accepted that there is no real evidence that SARS-CoV-2 pathogens should be carried *only in large droplets* (Fennelly 2020). At the beginning of the pandemic, aerosol transmission of SARS-CoV-2 was generally not accepted; however, within months, it became evident that some COVID-19 clusters, for example in choirs (Hamner 2020, Miller 2020), shopping malls (Cai J 2020), restaurants (Li Y 2020 + Lu J 2020), meat processing plants (Günter 2020, The Guardian) or vertically aligned flats connected by drainage pipes in the master bathrooms (Kang M 2020, Gormley 2020), were best explained by aerosol transmission (Ma J 2020).

On July 9 2020, WHO updated its information about SARS-CoV-2 transmission (WHO 20200709), "There have been reported outbreaks of COVID-19 in some closed settings, such as restaurants, nightclubs, places of worship or places of work where people may be shouting, talking, or singing. In these outbreaks,

aerosol transmission, particularly in these indoor locations where there are crowded and inadequately ventilated spaces where infected persons spend long periods of time with others, cannot be ruled out." In the preceding days, a group of more than 200 scientists led by Lidia Morawska and Donald K. Milton had published a three-page warning: *It is Time to Address Airborne Transmission of COVID-19* (see also LM's first alert on 10 April and the overviews by Prather, Wang and Schooley as well as Jayaweera 2020 et al.).

A single cough from a person with a high viral load in respiratory fluid (2,35 × 10^9 copies per ml) may generate as many as 1,23 × 10^5 copies of viruses that can remain airborne after 10 seconds, compared to 386 copies of a normal patient (7,00 × 10^6 copies per ml) (Wang Y 2020). (And masking can block around 94% of the viruses that may otherwise remain airborne after 10 seconds). A demonstration of aerosol production visualizes speech-generated oral fluid droplets and underlines that even normal speaking may be an important mode of transmission (Bax 2020). The authors provide videos showing speech droplets emitted by four people, when speaking the phrase "spit happens" with the face positioned about 10–15 cm behind a thin sheet of intense green laser light (video: https://www.youtube.com/watch?v=ooVjNth4ut8). Previously, experimental support for aerosol transmission of SARS-CoV-2 came from studies that visualized droplet formation at the exit of the mouth during violent expiratory events such as sneezing and coughing (Scharfman 2016, Bourouiba 2020; see also the video). These studies showed that the lifetime of a droplet could be considerably longer than previously assumed. When analyzed with highly sensitive laser light scattering, loud speech was found to be able to emit thousands of oral fluid droplets per second which could linger in the air for minutes (Anfinrud 2020, Stadnytskyi 2020; see also the movies showing the experimental setup and the critical comment by Abbas 2020). Loud and persistent shouting as would be usual in noisy, closed and stagnant air environments (meat packing facilities, discos, pubs, etc.) is now believed to produce the same number of droplets as produced by coughing (Chao 2020). Speech and other vocal activities such as singing have also been shown to generate air particles, with the rate of emission corresponding to voice loudness (Asadi 2019).

Of note, during the 2003 SARS epidemic, an airborne route of transmission also appeared to be a plausible explanation for the so-called Amoy Garden outbreak. On that occasion, the virus was aerosolized within the confines of very small bathrooms and may have been inhaled, ingested or transmitted indirectly by contact with fomites as the aerosol settled (WHO 2003).

Morawska, Milton et al. suggested the following measures to mitigate airborne transmission of SARS-CoV-2:

- Provide sufficient and effective ventilation (supply clean outdoor air, minimize recirculating air) particularly in public buildings, workplace environments, schools, hospitals, and retirement care homes

  Infrastructure may have to be adjusted, for example, Heating, Ventilation and Air Conditioning Systems (HVAC) in buildings and on ships (Correia 2020, Gormley 2020). In one study, viral RNA was detected in ventilation exhaust filters located at least 50 m from patient room vent openings (Nissen 2020).
- Supplement general ventilation with airborne infection controls such as local exhaust, high efficiency air filtration, and germicidal ultraviolet lights.
- Avoid overcrowding, particularly in public transport and public buildings.

A precautionary approach to COVID-19 prevention is shown in Table 1.

Table 1. Reducing the transmission of SARS-CoV-2

Transmission route	Prevention
1. (Macro-)Droplets (> 5 µm)	Face masks + social distancing
2. Aerosol (micro-droplets, ≤ 5µm)	• Face masks • Improved ventilation (open doors and windows; upgrade ventilation systems) • Improved air filtering • Avoidance of crowded and closed spaces
3. Fomites	Handwashing

For mechanical systems, organizations such as ASHRAE (the American Society of Heating, Ventilating, and Air Conditioning Engineers) and REHVA (the Federation of European Heating, Ventilation and Air Conditioning Associations) have provided guidelines based on the existing evidence of airborne transmission (Morawska 2020b).

The evidence for aerosol transmission and resulting recommendations for prevention have been sublimely summarized by Prather et al. in five sentences: "Respiratory infections occur through the transmission of virus-containing droplets (>5 to 10 µm) and aerosols (≤5 µm) exhaled from infected individuals during breathing, speaking, coughing, and sneezing. Traditional respiratory disease control measures are designed to reduce transmission by droplets produced in the sneezes and coughs of infected individuals. Howev-

er, a large proportion of the spread of coronavirus disease 2019 (COVID-19) appears to be occurring through airborne transmission of aerosols produced by asymptomatic individuals during breathing and speaking (Morawska 2020, Anderson 2020, Asadi 2019). Aerosols can accumulate, remain infectious in indoor air for hours, and be easily inhaled deep into the lungs. For society to resume, measures designed to reduce aerosol transmission must be implemented, including universal masking and regular, widespread testing to identify and isolate infected asymptomatic individuals (Prather 2020)."

Recognizing that SARS-CoV-2 is transmitted via aerosol has far-reaching consequences – personal, professional, societal and economic – in situations of community COVID-19 outbreaks. At the personal level (reminder: 20% of infected individuals are thought to transmit 80% of SARS-CoV-2 cases, so minimizing the probability of coming close to such super-spreader invidivuals is imperative), people might wish to avoid prolonged meetings with people from outside their inner-core "friends-and-family-bubble"; inside the bubble, meetings should be restricted to a handful of people.

At the professional level, healthcare workers will require nothing short of optimal protection. As N95 respirators achieve better filtration of airborne particles than medical masks, they should be recommended for all inpatient care of patients with COVID-19, not only during aerosol generating procedures (Dau 2020). Guideline recommendations that do not support N95 use for all inpatient COVID-19 management should consider reevaluating the existing data.

At the societal level, the attendance of important biographic events such as weddings, baptisms, circumcisions and funerals may need to be limited to a handful of intimate friends and family (probably less than 10). Religious services and recreational activities such as team sport and choir singing may not be possible.

At the economic level, all activities which bring numerous people from outside the "friends-and-family-bubbles" together may be banned during new community outbreaks. Future curfews or lockdowns would target places where strangers or simply unacquainted people meet: discos, amusement parks, bars, restaurants, brothels and many more. Other activities such as meat processing plants might need major restructuring before resuming work. Foreigners, strangers or simply unacquainted people may not meet for some time. SARS-CoV-2 will thus continue to impact cultural and economic life – theaters, cinemas, bars, restaurants, shops, etc – for some time to come.

In the meantime, the discussion about SARS-CoV-2 and aerosols continues. Even the droplet/aerosol terminology has been questioned by advocates of a

new distinction between aerosols and droplets using a size threshold of 100 μm, not the historical 5 μm (Prather 2020). The authors argue that this size more effectively separates their aerodynamic behavior, ability to be inhaled, and efficacy of interventions. Viruses in droplets (larger than 100 μm) typically fall to the ground in seconds within 2 m of the source and can be sprayed like tiny cannonballs onto nearby individuals. Even a fourth transmission route has been hypothesized: *aerosolized fomites.* In this case, virus would remain viable in the environment, on materials like paper tissues and on the bodies of living animals, long enough to be aerosolized on non-respiratory dust particles that can transmit infection through the air to new mammalian hosts (Asadi 2020). In retrospective, we will one day understand that transmission of viruses is not the only conceptual framework upset by the SARS-CoV-2 virus.

Fomites

At the beginning of the SARS-CoV-2 pandemic, it was unclear to what extent transmission via fomites (e.g., elevator buttons, hand rails, restroom taps) were epidemiologically relevant. [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.] SARS-CoV-2 seemed omnipresent in the spaces inhabited by infected individuals where a protein-rich medium like airway secretions could protect the virus when it was expelled and could enhance its persistence and transmission by contaminated fomites (Pastorino 2020). The transmission sequence included virus-laden droplets from SARS-CoV-2 infected people land on surfaces; there the virus would be detectable for up to four hours on copper, up to 24 hours on cardboard and up to two to three days on plastic and stainless steel (van Doremalen 2020, Aboubakr 2020, Joonaki 2020); and finally, other people coming into contact with these droplets, touching their nose, mouth or eyes (Wang Y 2020, Deng W 2020) and getting infected. Some studies reported that SARS-CoV-2 environmental contamination around COVID-19 patients was extensive, and hospital infection prevention and control procedures should account for the risk of fomite, and potentially airborne, transmission of the virus (Santarpia 2020). On a cruise ship, SARS-CoV-2 RNA was detected in 10% from cabins 1-17 days after SARS-CoV-2 infected individuals left their cabins (Yamagishi 2020). Find an overview of studies assessing viral RNA on surfaces and in air samples in Meyerowitz 2020, Table 1.

However, in real-world settings, SARS-CoV-2 RNA levels are markedly lower on environmental surfaces than in the human nasopharynx (Lui G 2020, Jiang FC 2020) and in the rare cases where fomite transmission has been discussed,

respiratory transmission could not be excluded (Cai J 2020, Bae SH 2020). Some authors now question the role of fomites in SARS-CoV-2 transmission and suggest that the chance transmission through inanimate surfaces might be less frequent than hitherto assumed (Mondelli 2020) and less likely to occur in real-life conditions, provided that standard cleaning procedures and precautions are enforced. Transmission through fomites would occur only in instances where an infected person coughs or sneezes on the surface, and someone else touches that surface soon after the cough or sneeze (within 1–2 h) (Goldman 2020). Another group estimated risk of infection from touching a contaminated surface at less than 5 in 10.000 after repeatedly sampling 33 surfaces in public places like liquor and grocery stores, banks, gas stations, laundromats, restaurants and on metro doors and crosswalk buttons (Harvey 2020). Twenty-nine of 348 (8.3 %) surface samples were positive for SARS-CoV-2. These authors suggest that fomites might play only a minimal role in SARS-CoV-2 community transmission.

In conclusion, direct contact and fomite transmission are likely to be only an unusual mode of transmission and on the basis of currently available data, we should assume that the levels of viral RNA or live virus transiently remaining on surfaces are unlikely to cause infection, especially outside of settings with known active cases (Meyerowitz 2020). It is important to stress that this finding should not persuade anyone to refrain from the ritual of regular and thorough handwashing; however, it could calm fears of people who are anxious about touching things in everyday life (doorknobs, keys, money, smartphones, etc.).

Mother-to-child

Vertical transmission occurs rarely. SARS-CoV-2 IgM has been reported in neonates (Zeng H 2020, Dong L 2020) but there is no consensus on the interpretation of this finding (Kimberlin 2020). Although SARS-CoV-2 was detected in breast milk (Groß 2020), no confirmed transmissions to infants from breast milk have been reported (Marín Gabriel 2020, Chambers 2020). Find more information in the chapter *Pediatrics*, page 430.

Cats and dogs et al.

SARS-CoV-2 can infect domestic animals, including cats, dogs, and ferrets (Shi J 2020, Richard 2020, Garigliany 2020). SARS-CoV-2 has been transmitted from their owners to cats and dogs (Newman 2020, Garigliany 2020) but there is currently no evidence of transmission from domestic pets to humans. When inoculated with SARS-CoV-2, cats could transmit the virus to other cats (Halfmann 2020) and although none of the cats showed symptoms, all shed

virus for 4 to 5 days and developed antibody titers by day 24. In another report, two out of fifteen dogs from households with confirmed human cases of COVID-19 in Hong Kong were found to be infected. The genetic sequences of viruses from the two dogs were identical to the virus detected in the respective human cases (Sit 2020). In still another paper, 919 companion animals in northern Italy at the height of the spring 2020 epidemic were tested for SARS-CoV-2. Although no animals tested PCR positive, 3.3% of dogs and 5.8% of cats had measurable SARS-CoV-2 neutralizing antibody titers, with dogs from COVID-19 positive households being significantly more likely to test positive than those from COVID-19 negative households (Patterson 2020).

Evidence of infection of animals with SARS-CoV-2 has been shown experimentally both *in vivo* and *in vitro* for monkeys, ferrets, rabbits, foxes, and hamsters (Edwards 2020). While computational models also predicted infectivity of pigs and wild boar (Santini 2020), one study suggested that pigs and chickens could not be infected intranasally or oculo-oronasally by SARS-CoV-2 (Schlottau 2020). The expression pattern of ACE2 among various mammalian species that are close to human beings has recently been described (Sun K 2020).

At present, it seems unlikely that animals are potential intermediate hosts in the chain of human–pet–human transmission. Only special circumstances, such as the high animal population densities encountered on mink farms, might put humans at risk of animal-to-human transmission (Oreshkova 2020, Munnink 2021, Zhou P 2021). Persons with COVID-19 should be advised to avoid contact with animals. Companion animals that test positive for SARS-CoV-2 should be monitored and separated from persons and other animals until they recover (Newman 2020).

Hypothetical modes of transmission

Live virus can rarely be isolated from stool and saliva and SARS-CoV-2 RNA has been isolated from semen and blood donations; however, in early December 2020, there were no reported cases of SARS-CoV-2 transmission via fecal–oral, sexual, or bloodborne routes.

Stool, urine

There is currently no evidence for relevant fecal–oral SARS-CoV-2 transmission. Although a high concentration of ACE2 receptors in the small bowel (Gu J 2020) and prolonged presence of SARS-CoV-2 viral RNA in fecal samples have been reported (Wu Y 2020, Chen 2020, Du W 2020), live virus has only rarely been detected in stool (Wang W 2020, van Doorn 2020, Sun J 2020, Parasa 2020). This finding should not interfere with the usual precautions when

handling the stools of patients infected with coronavirus. Sewage from hospitals should also be properly disinfected (Yeo 2020). Fortunately, antiseptics and disinfectants such as ethanol or bleach have good activity on human coronaviruses (Geller 2012). During the SARS-CoV outbreak in 2003, where SARS-CoV was shown to survive in sewage for 14 days at 4°C and for 2 days at 20°C (Wang XW 2005), environmental conditions could have facilitated this route of transmission.

Sweat

Immunofluorescence and immunohistochemical analyses detected SARS-CoV-2 spike proteins in three of five patients. In these cases, the virus resided primarily in the sweat glands and sweat ducts with apparently higher amounts in the former than in the latter; in contrast, the virus was rarely detected in the epidermis or sebaceous glands (Liu J 2020). The authors concluded that it was "important to further assess the potential risk of viral transmission via perspiration and skin contact." *(Editor's note: This paper will not change my standard protection measures.)*

Blood products

SARS-CoV-2 is rarely detected in blood (Wang W 2020, Wolfel 2020). After screening of 2430 donations in real-time (1656 platelet and 774 whole blood), authors from Wuhan found plasma samples positive for viral RNA from 4 asymptomatic donors (Chang 2020). 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). In early December 2020, there was no evidence of replication-competent virus isolated from blood samples and no documented case of bloodborne transmission.

Organ donation

In March 2020, US transplant centers began to report potential donor-derived SARS-CoV-2 transmission to the Organ Procurement and Transplantation Network (OPTN). For 8 potential donor-derived SARS-CoV-2 transmissions reported to the OPTN during March–May 2020, the available evidence suggest that the most likely source of transmission was community or healthcare exposure, not the organ donor (Jones 2020).

Sexual transmission

It is unknown whether purely sexual transmission is possible. Scrupulously eluding infection via fomites and respiratory droplets during sexual inter-

course would suppose remarkable acrobatics many people might not be willing to perform. Reassuringly, SARS-CoV-2 doesn't seem to be present in semen (Guo L 2020). Studies published until today showed viral RNA, but no infectious virus in semen (Li 2020) and viral RNA in vaginal fluid on only one occasion (Scorzolini 2020, Qiu L 2020). In a small study from Orléans, France, there was no transmission among discordant partners among five couples who continued sex while one partner was in the period of infectiousness (Prazuck 2020).

Human corpses

A recent stuy found that nasopharyngeal viral RNA stability in 79 corpses showed no time-dependent decrease. Maintained infectivity was supported by virus isolation up to 35 hours post-mortem (Heinrich 2020). There was no correlation between the post-mortem interval (time of death until cooling at 4°C; median 17,8 hours) and the viral RNA loads of corpses. According to the authors, their data indicate potentially high infectivity of human corpses, requiring careful and conscious handling.

Transmission Event

Transmission of a virus from one person to another depends on four variables:

1. The nature of the **virus**;
2. The nature of the **transmitter;**
3. The nature of the **transmittee** (the person who will become infected);
4. The transmission **setting**.

Virus

In order to stay in the evolutionary game, all viruses have to overcome a series of challenges. They must attach to cells; fuse with their membranes; release their nucleic acid into the cell; manage to make copies of themselves; and have the copies exit the cell to infect other cells. In addition, respiratory viruses must make their host cough and sneeze to get back into the environment again. Ideally, this happens before the hosts realize that they are sick. This is all the more amazing as SARS-CoV-2 is more like a piece of computer code than a living creature *in sensu strictu* (its 30,000 DNA base pairs are a mere 100,000th of the human genetic code). That doesn't prevent the virus from being ferociously successful:

- It attaches to the human angiotensin converting enzyme 2 (ACE2) receptor (Zhou 2020) which is present not only in nasopharyngeal and oropharyngeal mucosa, but also in lung cells, such as in type II pneumocytes. SARS-CoV-2 thus combines the high transmission rates of the common coronavirus NL63 (infection of the upper respiratory tract) with the severity of SARS in 2003 (lower respiratory tract);
- It has a relatively long incubation time of around 5 days (influenza: 1-2 days), thus giving it more time to spread;
- It is transmitted by asymptomatic individuals.

All viruses mutate. Mutations within coronaviruses, and indeed all RNA viruses, can arrive as a result of three processes. First, mutations arise intrinsically as copying errors during viral replication, a process which may be reduced in SARS-CoV-2 relative to other RNA viruses, due to the fact that coronavirus polymerases include a proof-reading mechanism (van Dorp 2020). Second, genomic variability might arise as the result of recombination between two viral lineages co-infecting the same host. Third, mutations can be induced by host RNA-editing systems, which form part of natural host immunity. Based on epidemiological data, a SARS-CoV-2 variant carrying the Spike protein amino acid change D614G has been associated with increased infectivity (Korber 2020). In one study, the D614G also exhibited significantly faster droplet transmission between hamsters than the WT virus, early after infection (Hou YJ 2020). However, after analyzing 46.723 SARS-CoV-2 genomes isolated from patients worldwide, one group could not identify a single recurrent mutation which was convincingly associated with increased viral transmission.

In the autumn of 2020, several new SARS-CoV-2 variants emerged that have a substantial transmission advantage. Find more information in *Mutations and vaccine efficacy,* page 212.

Transmittor

The mean incubation of SARS-CoV-2 infection is around 5 days (Lauer 2020, Li 2020, Zhang J 2020, Pung 2020), comparable to that of the coronaviruses causing SARS or MERS (Virlogeux 2016). Almost all symptomatic individuals will develop symptoms within 14 days of infection (Bai Y 2020). Both symptomatic and asymptomatic individuals can transmit SARS-CoV-2 (Bai Y 2020, Qian G 2020, Chau NVV 2020, Luo L 2020). About half of secondary cases are acquired from persons who are presymptomatic at the time of transmission (Shresta 2020, Yang L 2020, Xu XK 2020). Viral shedding may not be distinguishable

between symptomatic and asymptomatic individuals (Lee S 2020, Long QX 2020).

Infectiousness, measured by the detection of cultivatable virus, seems to start around two days before symptom onset, peaks around a day before symptom onset, and declines rapidly within a week (He X 2020, Lauer 2020). It is still unknown how many days infected people can transmit the virus, although some authors suggest that the infectivity window might be as short as one day (Goyal 2020) as SARS-CoV-2 viral load in the respiratory tract rapidly decreases after symptom onset (Wölfel 2020, Guo L 2020, To KK 2020). The duration of SARS-CoV-2 RNA shedding may go on for weeks and sometimes for months (Sun J 2020); however, multiple studies have found virtually no viable virus in patients with mild or moderate disease after 10 days of symptoms despite frequent ongoing RNA shedding (Wölfel 2020, Singanayagam 2020, Perera 2020). Ten days after symptom onset, the probability of culturing virus declined to 6.0% (Singanayagam 2020). In other studies, no viable virus was detected beyond 8 or 9 days after symptom onset and with SARS-CoV-2 RT-PCR cycle threshold (Ct) values > 24 (Bullard 2020, Arons 2020). In a study from Taiwan, there was zero transmission to 852 contacts who were exposed to the index case after day 6 of symptom onset (Cheng HY 2020). Requiring a negative RNA test as late as 21 days after the onset of symptoms to declare the end of quarantine as practised has no scientific basis.

The minimum human infectious dose is unknown. A phylogenetic-epidemiological model estimated the number of virions needed to start an infection at around 10^1-10^3 (Popa 2020).

Symptom severity of the index may have an impact on transmission probability. In one study of 3410 close contacts of 391 SARS-CoV-2 infected index cases, the secondary attack rate increased with the severity of index cases, from 0.3% for asymptomatic to 3,3% for mild, 5,6% for moderate, and 6,2% for severe or critical cases (Luo L 2020). Fever and expectoration were associated with an increased risk for infection in their close contacts (6,7% and 13,6%, respectively). SARS-CoV-2 transmission probably correlates with higher viral loads which in turn is associated with more frequent isolation of infectious virus (Singanayagam 2020). A recent study found that the incidence of COVID-19 among close contacts of a symptomatic index case was 3,85 times higher than for close contacts of an asymptomatic index case (Sayampanathan 2020).

SARS-CoV-2 transmission certainly correlates with a still ill-defined "**super-spreader** status" of the infected individual. An analysis of 772 complete SARS-CoV-2 genomes from the early Boston area epidemic found numerous introductions of the virus, a small number of which led to most cases (Lemieux 2020). For unknown reasons, some individuals are remarkably con-

tagious, capable of infecting dozens or hundreds of people, possibly because they breathe out many more particles than others when they talk (Asadi 2019), shout, cough or sneeze. Transmission of SARS-CoV and MERS-CoV as well occurred to a large extent by means of super-spreading events (Peiris 2004, Hui 2018). Super-spreading has been recognized for years to be a normal feature of disease spread (Lloyd-Smith 2005). Several groups suggest that 80% of secondary transmissions could be caused by around 10% to 20% of infectious individuals (Bi Q 2020, Adam 2020, Miller 2020, Sun 2020). A value called the dispersion factor (k) describes this phenomenon. The lower the k is, the more transmission comes from a small number of people (Kupferschmidt 2020, Tufekci 2020; if you like the FT, read also *To beat Covid-19, find today's superspreading 'Typhoid Marys'*). While SARS was estimated to have a k of 0.16 (Lloyd-Smith 2005) and MERS of 0.25, in the flu pandemic of 1918, in contrast, the value was about one, indicating that clusters played less of a role (Endo 2020). For the SARS-CoV-2 pandemic, the dispersion factor (k) is currently thought to be higher than for SARS and lower than for the 1918 influenza (Endo 2020, Miller 2020, On Kwok 2020, Wang L 2020). A study of 1407 transmission pairs that formed 643 transmission clusters in mainland China identified 34 super-spreaders, with 29 super-spreading events occurring outside households (Xu XK 2020).

A mobility network model mapped the hourly movements of 98 million people from neighborhoods to points of interest (POIs) such as restaurants and religious establishments. After connecting 57.000 neighborhoods to 553.000 POIs, the model predicted that a small minority of "superspreader" POIs account for a large majority of infections (Chang S 2020) and that restricting maximum occupancy at each POI (for example, restaurants, gyms, cafes, etc.) (Ma KC 2020, Cyranoski 2020) is more effective than uniformly reducing mobility. [The model also correctly predicted higher infection rates among disadvantaged racial and socioeconomic groups: disadvantaged groups cannot reduce mobility as sharply as other groups and the POIs they visit are more crowded.]

Transmission is more likely when the infected individual has few or no symptoms because while people experiencing symptoms may self-isolate or seek medical care, those with no or mild symptoms may continue to circulate in the community. Asymptomatic individuals have therefore an outsized influence on maintaining the epidemic (Lee EC 2020). **Asymptomatic transmission** of SARS-CoV-2 – proven a few weeks after the beginning of the pandemic (Bai Y 2020) – has justly been called the Achilles' heel of the COVID-19 pandemic (Gandhi 2020). As shown during an outbreak in a skilled nursing facility, the percentage of asymptomatic individuals can be as high as 50% early

(Arons 2020; most of these individuals would later develop some symptoms). Importantly, SARS-CoV-2 viral load was comparable in individuals with typical and atypical symptoms, and in those who were pre-symptomatic or asymptomatic. Seventeen of 24 specimens (71%) from pre-symptomatic persons had viable virus by culture 1 to 6 days before the development of symptoms (Arons 2020), suggesting that SARS-CoV-2 may be shed at high concentrations before symptom development.

Note that although SARS-CoV-2 is highly transmissible, given the right circumstances and the right prevention precautions, **zero transmission** is possible. 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).

To what extent **children** contribute to the spread of SARS-CoV-2 infection in a community is unknown. Infants and young children are normally at high risk for respiratory tract infections. The immaturity of the infant immune system may alter the outcome of viral infection and is thought to contribute to the severe episodes of influenza or respiratory syncytial virus infection in this age group (Tregoning 2010). Until now, however, there is a surprising absence of pediatric patients with COVID-19, something that has perplexed clinicians, epidemiologists, and scientists (Kelvin 2020). In particular, children younger than 10 years seem to be less susceptible than adults (around 50%) (Zhang J 2020, Jing QL 2020, Li W 2020, Gudbjartsson 2020, Davies 2020, Rosenberg 2020).

Although a retrospective study among individuals hospitalized in Milan showed that only about 1% of children and 9% of adults without any symptoms or signs of SARS-CoV-2 infection tested positive for SARS-CoV-2 (Milani 2020) – suggesting a minor role of children in transmission –, children can be the source for important outbreaks. Twelve children who acquired SARS-CoV-2 infection in child-care facilities – all with mild or no symptoms – transmitted the virus to at least 12 (26%) of 46 non-facility contacts (Lopez 2020). Family gatherings are well-known settings for widespread SARS-CoV-2 transmission. In an outbreak that occurred during a 3-week family gathering of five households, an adolescent aged 13 years was the suspected primary patient. Among the 14 persons who stayed in the same house, 12 experienced symptoms (Schwartz 2020). Of note, none of the additional six family members who maintained outdoor physical distance without face masks during two longer visits (10 and 3 hours) to the family gathering developed symptoms.

Patients with profound **immunosuppression** (for example recipients of hematopoietic stem cell transplants or chimeric antigen receptor (CAR) T cell therapy) may shed viable SARS-CoV-2 for at least 2 months (Aydillo 2020).

In any potential transmission setting, **face coverings** reduce the transmission of SARS-CoV-2. Among 139 clients exposed to two symptomatic hair stylists with confirmed COVID-19 while both the stylists and the clients wore face masks, not a single symptomatic secondary case was observed; among 67 clients tested for SARS-CoV-2, all tests were negative (Hendrix 2020). At least one hair stylist was infectious: all four close household contacts (presumably without masks) became ill. In Germany, face masks may have reduced the daily growth rate of reported infections by around 47% (Mitze 2020). Unfortunately, face masks don't work everywhere – and not for everyone. In some countries, infected individuals claimed the right to not wear face coverings in the name of liberty (they forgot that an individual's liberty ends where it infringes on the liberties of others). Interestingly, social distancing compliance might be predicted by individual differences in **working memory** (WM) capacity. WM retains a limited amount of information over a short period of time at the service of other ongoing mental activities. Limited WM capacity constrains mental functions while extended capacities are often associated with better cognitive and affective outcomes. The hidden message in the paper by Weizhen Xie et al: if the guy sitting next to you in the bus does not wear a mask, don't insist. His working memory capacity is poor (Xie W 2020). Change seats.

Transmittee

Upon exposure to SARS-CoV-2, the virus may come in contact with cells of the upper or lower respiratory tract of an individual. After inhalation, larger respiratory droplets are filtered by the nose or deposited in the oropharynx, whereas smaller droplet nuclei are carried by the airstream into the lungs where their site of deposition depends on their mass, size and shape and is governed by various mechanisms (Dhand 2020).

A high percentage of SARS-CoV-2 seronegative individuals have SARS-CoV-2 reactive T cells. This is explained by previous exposure to other coronaviruses ("common cold" coronaviruses) which have proteins that are highly similar to those of SARS-CoV-2. It is still unclear whether these cross-reactive T cells confer some degree of protection, are inconsequential or even potentially harmful if someone who possesses these cells becomes infected with SARS-CoV-2 (Braun 2020, Grifoni 2020, Sagar 2020, Meyerholz 2020b).

Numerous cell entry mechanisms of SARS-CoV-2 have been identified that potentially contribute to the immune evasion, cell infectivity, and wide spread of SARS-CoV-2 (Shang J 2020). Susceptibility to SARS-CoV-2 infection is probably influenced by the host genotype. This would explain the higher percentage of severe COVID-19 in men (Bastard 2020, Zhang Q 2020, Piccin-

inni 2020) and possibly the similar disease course in some twins in the UK (The Guardian, 5 May 2020).

The "right" genotype may not be sufficient in the presence of massive exposure, for example by numerous infected people and on multiple occassions as might happen, for example, in health care institutions being overwhelmed during the beginning of an epidemic. It is known from other infectious diseases that viral load can influence the incidence and severity of disease. Although the evidence is limited, high infection rates among health workers have been attributed to more frequent contact with infected patients, and frequent exposure to excretia with high viral load (Little 2020).

Rigorous social distancing might not only slow the spread of SARS-CoV-2 in a cohort of young, healthy adults but also prevent symptomatic COVID-19 while still inducing an immune response (Bielecki 2020). After an outbreak in two Swiss army companies (company 2 and 3, see Table 2), 62% of tested soldiers were found to have been exposed to SARS-CoV-2 and almost 30% had COVID-19 symptoms. In company 1 where strict distancing and hygiene measures (SDHMs) had been implemented after the outbreak in companies 2 and 3, only 15% had exposure to SARS-CoV-2, but none of them had COVID-19 symptoms. (The Swiss army SDHMs: keep a distance of at least 2 m from each other at all times; wear a surgical face mask in situations where this can not be avoided [e.g., military training]; enforce a distance of 2 m between beds and during meals; clear and disinfect all sanitary facilities twice daily; separate symptomatic soldiers immediately.)

Table 2: Baseline characteristics of the study population on March 31, 2020

	Company 1	Company 2	Company 3	Company 2+3
Soldiers	154	200	154	354
Tested*	88	130	51	181
Exposed to SARS-CoV-2**	13/88 (15%)	83/130 (64%)	30/51 (59%)	113/181 (62%)
COVID-19***	0 (0%)	54/200 (27%)	48/154 (31%)	102/354 (29%)

* More than 50% of the soldiers of all companies were sampled on April 14.

** On April 14, detection of SARS-CoV-2 in nasopharyngeal swabs or by positive serology test for immunoglobulin A, G or M.

*** Symptomatic patients between March 11 and May 3, 2020.

The authors cautiously suggested that quantitatively reducing the viral inoculum received by SARS-CoV-2 virgin recipients not only reduced the probability of infection but also could have caused asymptomatic infections in

others while still being able to induce an immunological response (Bielecki 2020), and idea that was later echoed by Monica Gandhi and George W. Rutherford (Ghandi 2020).

If genes offer no protection, behavior may. In the coming winter 2020/2021 months, face covering is paramount. After a year of SARS-CoV-2 experience, masks have been shown to decrease transmission both in health care settings and in the wider community (Chu DK 2020, Chou R 2020, Lee JK 2020). In March 2020, the Mass General Brigham, the largest health care system in Massachusetts (12 hospitals, > 75,000 employees), implemented universal masking of all HCWs and patients with surgical masks. During the pre-intervention period, the SARS-CoV-2 positivity rate increased exponentially, with a case doubling time of 3.6 days. During the intervention period, the positivity rate decreased linearly from 14.65% to 11.46% (Wang X 2020). In Paris, in a 1500-bed adult and a 600-bed pediatric setting of a university hospital, the total number of HCW cases peaked on March 23rd, then decreased slowly, concomitantly with a continuous increase in preventive measures (including universal medical masking and PPE) (Contejean 2020). In Chennai, India, before the introduction of face shields, 12/62 workers were infected while visiting 5880 homes with 31,164 persons (222 positive for SARS-CoV-2). After the introduction of shields among 50 workers (previously uninfected) who continued to provide counseling, visiting 18,228 homes with 118,428 persons (2682 positive), no infection occurred (Bhaskar 2020). These preventive measures are not new to medicine – surgeons have been using personal protective equipment (PPE) for more than a century (Stewart 2020). The wearing of masks by adults also remains critical to reducing transmission in child-care settings (Link-Gelles 2020). Under certain circumstances, it is even recommended between household members (Wang Y 2020).

Masks work even with super-emittors. By measuring outward emissions of micron-scale aerosol particles by healthy humans performing various expiratory activities, one group found that both surgical masks and unvented KN95 respirators reduced the outward particle emission rates by 90% and 74% on average during speaking and coughing. These masks similarly decreased the outward particle emission of a coughing super-emitter, who for unclear reasons emitted up to two orders of magnitude more expiratory particles via coughing than average (Asadi 2020).

After visualizing the flow fields of coughs under various mouth covering scenarios, one study (Simha 2020) found that

1. N95 masks are the most effective at reducing the horizontal spread of a cough (spread: 0.1 and 0.25 meters).

2. A simple disposable mask can reduce the spread to 0.5 meters, while an uncovered cough can travel up to 3 meters.
3. **Coughing into the elbow is** not very effective. Unless covered by a sleeve, a bare arm cannot form the proper seal against the nose necessary to obstruct airflow and a cough is able to leak through any openings and propagate in many directions.

Although the data regarding the effectivity of face masks is now clear, will everyone understand, i.e., even individuals with a still functioning working memory? If some individuals continue to put themselves at risk of SARS-CoV-2 infection (as well as their friends and relatives in case of infection), what factors might influence risk for COVID-19 exposure among young adults? In a remote US county, the drivers of behaviors were low severity of disease outcome; peer pressure; and exposure to misinformation, conflicting messages, or opposing views regarding masks (Wilson 2020). A scientifically inspired national prevention policy will be needed to counter misinformation and – let's speak frankly for just two seconds! – address human stupidity. First, public health officials need to ensure that the public understands clearly when and how to wear cloth face coverings properly. Second, innovation is needed to extend physical comfort and ease of use. Third, the public needs consistent, clear, and appealing messaging that normalizes community masking (Brooks 2020). A small adaption in our daily lives relies on a highly effective low-tech solution that can help turn the tide.

Transmission setting

The transmission setting, i.e., the actual place where the transmission of SARS-CoV-2 occurs, is the final element in the succession of events that leads to the infection of an individual. High population density that facilitates super-spreading events is key to widespread transmission of SARS-CoV-2. Transmission clusters, partly linked to super-spreader events, have been reported since the very beginning of the SARS-CoV-2 pandemic. For detailed information about SARS-CoV-2 hotspots, see the chapter *Epidemiology, Transmission Hotspots,* page 23. Suffice it to present here a list of important outbreaks which have beem reported in predominantly indoor settings:

- Hospitals (at the beginning of the pandemic; Houlihan 2020)
- Nursing facility, King County, Washington, 28 February (McMichael 2020)
- Business meeting, Southern Germany, 20-21 January (Rothe 2020)

- Medical advisory board meeting, Munich, Germany, 20-21 (Hijnen 2020)
- Cruise Ship, Yokohoma, Japan, 4 February (Rocklov 2020)
- Church meeting, Daegu, Korea, 9 and 16 February (Kim 2020)
- Religious gathering, Mulhouse, France, 17-24 February (Kuteifan 2020, Gerbaud 2020)
- Aircraft carriers: Theodore Roosevelt (Payne 2020) + Charles-de-Gaulle, March (Le Monde)
- Prisons (Kennedy 2020, Maxmen 2020, Wang EA 2020)
- Meat-processing and livestock plants (Günther 2020, Waltenburg 2020, Taylor 2020)
- Homeless shelters (Baggett 2020, Mosites 2020)
- Call center (Park SY 2020)
- Marriage (Mahale 2020)
- Funeral (Kant 2020)
- Choir (Hamner 2020, Alsveld 2020)
- Concert (Plautz 2020)
- Nightclubs (Kang 2020, Muller 2020)
- Bars (Chau NVV)
- Restaurants (Lu J 2020)
- Sports meetings (Atrubin 2020)

Indoor environments

As with other respiratory viruses, the majority of SARS-CoV-2 infections occur at home where people live in close contact many hours a day, meeting multiple individuals (Read 2014). In a study from South Korea, household contacts accounted for 57% of identified secondary infections, despite exhaustive tracking of community contacts (Park YJ 2020). Globally, the secondary attack-rates (SARs) in households is around 20% (Madewell 2020), spouses being twice as likely to be infected as other adult household members. Household SARs also seem to be higher from symptomatic index cases than asymptomatic index cases, and to adult contacts than child contacts. With suspected or confirmed infections referred to isolate at home, household transmission will continue to be a significant source of transmission (Madewell 2020). Other settings which favor daily close and prolonged contact include nursing homes,

prisons (Njuguna 2020), homeless shelters and worker dormitories where infection rates in excess of 60% have been reported. The risk of indoor transmission may be up to 20 times higher that transmission of SARS-CoV-2 in an outdoor setting (Bulfone 2020).

Indoor environments are SARS-CoV-2's preferred playgrounds. In one modeling study, the authors estimated that viral load concentrations in a room with an individual who was coughing frequently were very high, with a maximum of 7,44 million copies/m^3 from an individual who was a high emitter (Riediker 2020). However, regular breathing from an individual who was a high emitter was modeled to result in lower room concentrations of up to 1248 copies/m^3. They conclude that the estimated infectious risk posed by a person with typical viral load who breathes normally was low and that only a few people with very high viral load posed an infection risk in the poorly ventilated closed environment simulated in this study.

Viable virus from air samples was isolated from samples collected 2 to 4.8 meters away from two COVID-19 patients (Lednicky 2020). The genome sequence of the SARS-CoV-2 strain isolated was identical to that isolated from the NP swab from the patient with an active infection. Estimates of viable viral concentrations ranged from 6 to 74 TCID50 units/L of air. During the first months of the pandemic, most clusters were found to involve fewer than 100 cases, with the exceptions being in healthcare (hospitals and elderly care), large religious gatherings and large co-habitation settings (worker dormitories and ships). Other settings with examples of clusters between 50–100 cases in size were schools, sports, bars, shopping centers and a conference (Leclerc 2020).

Closed doors and windows and poor ventilation favored SARS-CoV-2 transmission in churches and bars (James 2020, Furuse Y 2020). Opening windows and allowing better air movement may lead to lower secondary household transmission (Wang Y 2020).

Transportation in closed spaces – by bus, train or aircraft – has been shown to transmit SARS-CoV-2 at various degrees, depending on face mask use and time of travel. One paper describes a **bus** ride in a vehicle 11.3 meters long and 2.5 meters wide with 49 seats, fully occupied with all windows closed and the ventilation system on during the 2,5-hour trip. Among the 49 passengers (including the driver) who shared the ride with the index person, eight tested positive and eight developed symptoms. The index person sat in the second-to-last row, and the infected passengers were distributed over the middle and rear rows (Luo K 2020). An even more informative paper describes 68 individuals (including the source patient) taking a bus on a 100-minute round trip to attend a worship event. In total, 24 individuals (35%) received a diagnosis of

COVID-19 after the event. The authors were able to identify seats for each passenger and divided bus seats into high-risk and low-risk zones (Shen Y 2020). Passengers in the high-risk zones had moderately but non-significantly higher risk of getting COVID-19 than those in the low-risk zones. On the 3-seat side of the bus, except for the passenger sitting next to the index patient, none of the passengers sitting in seats close to the bus window developed infection. In addition, the driver and passengers sitting close to the bus door also did not develop infection, and only 1 passenger sitting by an operable window developed infection. The absence of a significantly increased risk in the part of the bus closer to the index case suggested that airborne spread of the virus may at least partially explain the markedly high attack rate observed. Lesson learned for the future? If you take the bus, choose seats near a window – and open it!

To answer the question how risky **train** traveling is in the COVID-19 era, one group analyzed passengers in Chinese high-speed trains. They quantified the transmission risk using data from 2334 index patients and 72,093 close contacts who had co-travel times of 0–8 hours from 19 December 2019 through 6 March 2020. Unsurprisingly, travelers adjacent to an index patient had the highest attack rate (3.5%) and the attack rate decreased with increasing distance but increased with increasing co-travel time. The overall attack rate of passengers with close contact with index patients was 0.32% (Hu M 2020).

A review about in-flight transmission of SARS-CoV-2 found that the absence of large numbers of confirmed and published in-flight transmissions of SARS-CoV was encouraging but not definitive evidence that fliers are safe (Freedman 2020). At present, based on circumstantial data, strict use of masks appears to be protective. In previous studies, SARS-CoV-2 transmission has been described onboard aircrafts (Chen J 2020, Hoehl 2020). Note that if you don't wear a mask, business class will not protect you from infection. A Vietnamese group report on a cluster among passengers on VN54 (Vietnam Airlines), a 10-hour commercial flight from London to Hanoi on March 2, 2020 (at that time, the use of face masks was not mandatory on airplanes or at airports) (Khanh 2020). Affected persons were passengers, crew, and their close contacts. The authors traced 217 passengers and crew to their final destinations and interviewed, tested, and quarantined them. Among the 16 persons in whom SARS-CoV-2 infection was detected, 12 (75%) were passengers seated in business class along with the only symptomatic person (attack rate 62%). Seating proximity was strongly associated with increased infection risk (risk ratio 7.3, 95% CI 1.2–46.2). Even more intriguing: a 7.5 h flight to Ireland, with a passenger occupancy of 17% (49/283 seats). The flight-associated attack rate was 9.8–17.8%, leading to 13 cases (Murphy 2020). A mask was worn during

the flight by nine cases, not worn by one (a child), and unknown for three. Spread to 46 non-flight cases occurred country-wide.

The risk may be increased during long-distance flights, for example a 18-hour flight from Dubai to Aukland. Among the 7 eventually-infected passengers, 2 were probably index case-patients infected before the flight, 4 were probably infected during the flight, and the remaining passenger was probably infected while in New Zealand under "managed isolation and quarantine". All 7 passengers were seated in aisle seats within 2 rows of where the presumed index case-patient(s) were seated (Swadi 2021).

Temperature and climate

SARS-CoV-1 (2003): The transmission of coronaviruses can be affected by several factors, including the climate (Hemmes 1962). Looking back to the 2003 SARS epidemic, we find that the stability of the first SARS virus, SARS-CoV, depended on temperature and relative humidity. A study from Hong Kong, Guangzhou, Beijing, and Taiyuan suggested that the SARS outbreak in 2002/2003 was significantly associated with environmental temperature. The study provided some evidence that there was a higher possibility for SARS to reoccur in spring than in autumn and winter (Tan 2005). It was shown that SARS-CoV remained viable for more than 5 days at temperatures of 22–25°C and relative humidity of 40–50%, that is, typical air-conditioned environments (Chan KH 2011). However, viability decreased after 24 h at 38°C and 80–90% relative humidity. The better stability of SARS coronavirus in an environment of low temperature and low humidity could have facilitated its transmission in subtropical areas (such as Hong Kong) during the spring and in air-conditioned environments. It might also explain why some Asian countries in the tropics (such as Malaysia, Indonesia or Thailand) with high temperature and high relative humidity environment did not have major community SARS outbreaks (Chan KH 2011).

SARS-CoV-2 (2020): At the beginning of the pandemic, is was unclear as to whether and to what extent climatic factors influence virus survival outside the human body and might influence local epidemics. SARS-CoV-2 is not readily inactivated at room temperature and by drying like other viruses, for example herpes simplex virus. One study mentioned above showed that SARS-CoV-2 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 (van Doremalen 2020). A few studies suggested that low temperature might enhance the transmissibility of SARS-CoV-2 (Wang 2020b, Tobías 2020) and that the arrival of summer in the northern hemisphere could reduce the transmission of the COVID-19. In one study, af-

ter comparing 50 cities *with* (Wuhan, China; Tokyo, Japan; Daegu, South Korea; Qom, Iran; Milan, Italy; Paris, France; Seattle, US; and Madrid, Spain ; n=8) and *without* an important SARS-CoV-2 epidemic (n=42) in the first 10 weeks of 2020, areas with substantial community transmission of the virus had distribution roughly along the 30° N to 50° N latitude corridor with consistently similar weather patterns, consisting of mean temperatures of 5 to 11 °C combined with low specific and absolute humidity (Sajadi 2020). Cold working environments have been proposed to be considered as an occupational risk factor for COVID-19 (Cunningham 2020).

A possible association of the incidence of COVID-19 and both reduced solar irradiance and increased population density has been discussed (Guasp 2020). It was also reported that simulated sunlight rapidly inactivated SARS-CoV-2 suspended in either simulated saliva or culture media and dried on stainless steel plates while no significant decay was observed in darkness over 60 minutes (Ratnesar-Shumate 2020). However, another study concluded that transmission was likely to remain high even at warmer temperatures (Sehra 2020) and the epidemics in Brazil and India and the southern US – areas with high temperatures – soon tempered hopes that COVID "simply disappears like a miracle". Warm and humid summer conditions alone are not sufficient to limit substantially new important outbreaks (Luo 2020, Baker 2020, Collins 2020).

One group found a significant negative association between UVI and COVID-19 deaths, indicating evidence of the protective role of Ultraviolet-B (UVB) in mitigating COVID-19 deaths (Moozhipurtath 2020). If confirmed via clinical studies, the possibility of mitigating COVID-19 deaths via sensible sunlight exposure or vitamin D intervention would be attractive.

End of Quarantine

Infectiousness peaks around a day before symptom onset and declines within a week of symptom onset, and no late linked transmissions (after a patient has had symptoms for about a week) have been documented (Meyerowitz 2020). After suspected or confirmed SARS-CoV-2 infection, people should quarantine until

- 10 days since symptoms first appeared

  and

- 24 hours with no fever without the use of fever-reducing medications

  and

- Other symptoms of COVID-19 are improving (exception: loss of taste and smell which may persist for weeks or months after recovery and need not delay the end of isolation).

(Note that these recommendations **do not** apply to immunocompromised persons or persons with severe COVID-19. Find more information at https://bit.ly/3qB62IR [CDC]).

Health authorities should know that SARS-CoV-2 infected individuals do not need to be **quarantined** for weeks. Persistently positive RT-PCRs generally do not reflect replication-competent virus. SARS-CoV-2 infectivity rapidly decreases to near-zero after about 10 days in mild-to-moderately-ill patients and 15 days in severely-to-critically-ill and immunocompromised patients (Rhee 2020). Of note, RT-PCR cycle threshold (Ct) values (a measure for viral load) correlated strongly with cultivable virus. In one study, the probability of culturing virus declined to 8% in samples with Ct > 35 and to 6% (95% CI: 0.9–31.2%) 10 days after onset; it was similar in asymptomatic and symptomatic persons (Singanayagam 2020). A meta-analysis of 79 studies (5340 individuals) concluded that no study detected live virus beyond day 9 of illness, despite persistently high viral loads (Cevik 2020). In individuals who had mildly or moderately symptomatic SARS-CoV-2 infection and who present no symptoms for at least two days, a positive RT-PCR test 10 days or more after the first symptoms does not indicate infectiousness ('post-infectious PCR-positivity'; Mina 2020).

In most countries (for example, Germany, USA), health authorities do not require a negative SARS-CoV-2 RT-PCR test to end the quarantine. Autorities in Italy or other countries that even in late November continued quarantining people at home for two, three, four weeks or longer because of continuously positive RT-PCR results should take note.

Prevention

Find a detailed discussion of SARS-CoV-2 prevention in the corresponding chapter on page 117.

For everyday life, the following five *rules of thumb* are helpful:

1. Avoid **crowded** places (more than 5-10 people). The more people are grouped together, the higher the probability that a superspreader is present who emits infectious particles tens or hundreds times more than a 'normally' contagious individual. Avoid funerals, and postpone religious services including weddings, baptisms, circumcisions, as well as team sports and choir singing until after the pandemic.

2. Avoid in particular **crowded** and **closed** spaces (even worse: air-conditioned closed places where 'old air' is being moved around). In a room where a SARS-CoV-2 infected individual is coughing frequently, viable virus can be isolated from samples collected 2 to 4,8 meters away. Strangers or unacquainted persons should not meet in crowded or closed spaces.
3. Avoid in all circumstances **crowded**, **closed** and **noisy** spaces where people must shout to communicate. Shouting or speaking loudly emits a continuous flow of aerosols that linger in the air for minutes. Intimate conversation in a noisy and crowded room, with people shouting at one another at a distance of 30 centimeters, inhaling deep into theirs lungs the exhalations of the person they are speaking to/shouting at for 5, 10, 20 minutes or longer is, from the virus's point of view, the best conceivable transmission setting. Noise from machines or music around people grouped in a closed environment also creates the perfect setting for a superspreader event.
4. Outside crowded, closed or noisy spaces, keep a **distance of 2 meters** to other people.
5. Always wear a **fask mask in public spaces**. A face mask is a highly effective low-tech solution that can help contain local SARS-CoV-2 outbreaks. Face masks are not new to medicine – surgeons have been using them for more than a century. Next time you are unhappy when wearing a face mask, watch this video and enjoy the fact that unlike the doctors who might one day treat you for COVID-19 or other ailments, you won't never have to put on and remove protective gear in a hospital.

Those who doubt the effectiveness of face masks might extract precious information from Figure 2. The cumulative number of confirmed COVID-19 cases in different countries – presented per million population – is intriguing. What did Japan, South Korea, Taiwan and Vietnam right that the other countries didn't? The most probable explanation is

- Better testing
- Efficient contract tracing and isolation
- Early use of face masks

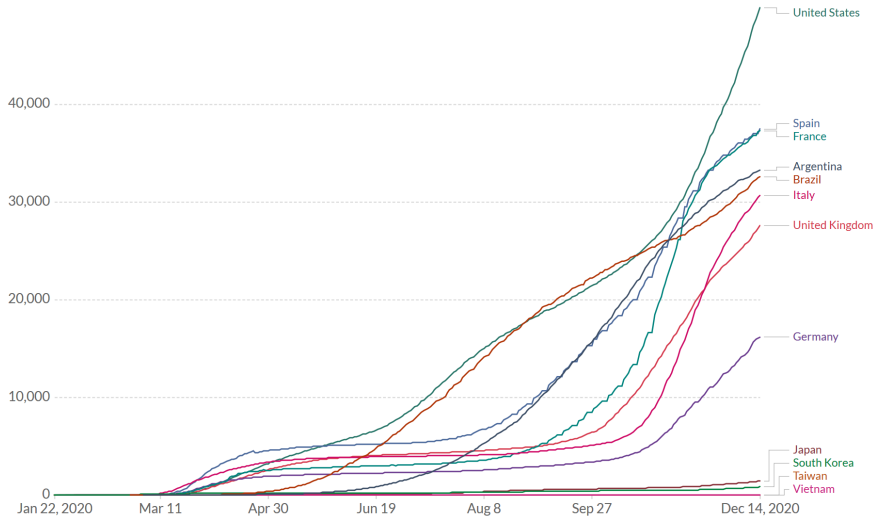

Figure 2. Cumulative confirmed COVID-19 cases per million people. What did Japan, South Korea, Taiwan and Vietnam do right that the other countries didn't? Better testing, efficient contract tracing and isolation, and early use of face masks. Source: Our World in Data.

Outlook

Almost a year after the first SARS-CoV-2 outbreak in China, the transmission dynamics driving the pandemic are coming into focus. It now appears that a high percentage (as high as 80%?) of secondary transmissions could be caused by a small fraction of infectious individuals (10 to 20%?; Adam 2020); if this is the case, then the more people are grouped together, the higher the probability that a superspreader is part of the group.

It is now acknowledged that aerosol transmission plays an important role in SARS-CoV-2 transmission (Morawska 2020b, WHO 20200709, Prather 2020); if this is the case, then building a wall around this same group of people and putting a ceiling above them further enhances the probability of SARS-CoV-2 infection.

It finally appears that shouting and speaking loudly emits thousands of oral fluid droplets per second which could linger in the air for minutes (Anfinrud 2020, Stadnytskyi 2020, Chao 2020, Asadi 2019, Bax 2020); if this is the case, then creating noise (machines, music) around people grouped in a closed environment would create the perfect setting for a superspreader event.

Over the coming months, the scientific community will try and

- unravel the secrets of super-spreading;
- advance our understanding of host factors involved in the successful "seeding" of SARS-CoV-2 infection;
- elucidate the role of young adults in the genesis of the second European SARS-CoV-2 wave;
- continue to describe the conditions under which people should be allowed to gather in larger groups.

SARS-CoV-2 vaccines will have to be rolled out safely and affordably to billions of people. In the meantime there will be no return to a "normal" pre-2020 way of life, and the best prevention scheme is a potpourri of physical distancing (Kissler 2020), intensive testing, case isolation, contact tracing, quarantine (Ferretti 2020) and as a last (but not impossible) resort, local lockdowns and curfews. If thanks to the history-making ultra-fast development of potent vaccines the pandemic should be over earlier than most people feared, SARS-CoV-2 will have taught the world a lesson of value for future pandemics: face masks are a simple and powerful tool to mitigate the impact of infectious respiratory diseases.

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3. Prevention

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Introduction

In the absence of an effective vaccine or antiviral treatment, prevention through public health measures remains the mainstay of SARS-COV-2 infection control and pandemic impact mitigation. Effective preventive measures for respiratory infections exist and have been standard practices for many years. However, uncertainties about the role and importance of different transmission routes in the spread of SARS-COV-2 (see chapter *Transmission*) complicate the selection of the most efficient and effective mix of personal and public health measures to be implemented, and of the prevention messages to be communicated to the public.

The basic COVID-19 preventive strategies include: the identification and isolation of infectious cases and quarantine for suspected cases and close contacts; changes in individual behaviors including physical and social distancing, use of face masks and hand hygiene; public health measures like travel restrictions, bans on mass gatherings and localized or nationwide lockdowns when the other measures prove ineffective in halting the spread of the virus. Specific prevention measures can be simple recommendations left to the decision of the individual or mandatory measures to be implemented under control by the public health authorities. Preventive measures can therefore be applied at the personal, community or societal level.

In this chapter we will review the available scientific evidence on the effectiveness of these measures in reducing the spread of SARS-COV-2.

Prevention at the personal level

Good respiratory hygiene/cough etiquette.

Good respiratory hygiene refers to measures aimed at containing respiratory secretions and reducing their spread in the environment or to other people (Chavis, 2019). Traditionally, they include:

- Covering your mouth and nose with a tissue or with your elbow when coughing or sneezing; and safe disposal of the tissue once used.
- Use of a surgical or tissue face mask.
- Perform hand hygiene often, and always after contact with potentially contaminated objects/materials.

Good respiratory hygiene and cough etiquette are usually recommended for individuals with signs and symptoms of a respiratory infection. However, given the established risk of SARS-COV-2 infection from asymptomatic individuals, public health authorities all over the world have recommended these measures for everybody when in public places. This is not without controversy, in particular on the use of masks in the absence of symptoms.

Face masks

The use of face masks to reduce the risk of infection is an established medical and nursing procedure. It is therefore surprising that it has created such a debate in the context of COVID-19. The initial recommendation by WHO and other health authorities that masks should only be used by health workers and symptomatic patients resulted in controversy among the experts and widespread confusion among the public. This advice was contradictory with the images of people regularly wearing masks in all settings from countries in Asia that successfully managed to contain the pandemic. In addition, the existence of different types of masks greatly complicated communication efforts.

Face masks can prevent transmission of respiratory viruses in two ways:

1. When worn by healthy individuals they are protecting them from infection by reducing the exposure of the mouth and nose to viral particles present in the air or on contaminated hands;
2. When worn by an infected person they perform source control, by reducing the amount of virus dispersed in the environment while coughing, sneezing or talking.

Different types of masks perform these tasks differently, which also dictates the situations in which they should be used. Masks most currently used include:

- **N95 (or FFP2) masks,** designed to block 95% of very small particles. They reduce the wearer's exposure to particles including aerosols and large droplets. They also reduce the patient or other bystanders' exposure to particles emitted by the wearer (unless they are equipped with a one-way valve to facilitate breathing).
- **Surgical masks** only filter effectively large particles. Being loose fitted, they will reduce only marginally the exposure of the wearer to droplets and aerosols. They do, however, limit considerably the emission of saliva or droplets by the wearer, reducing the risk of infecting other people.

- **Cloth masks** will stop droplets that are released when the wearer talks, sneezes, or coughs. As recommended by WHO, they should include multi-layers of fabric. When surgical or N95 masks are not available, cloth masks can still reduce the risk of SARS-COV-2 transmission in public places.

If masks are protective, why they were not widely recommended at the beginning of the pandemic? Whether due to poor communication, fear of shortage of essential medical supplies, or under-appreciation of the role of asymptomatic carriers in spreading the virus, the initial reluctance in promoting mask use and the resulting controversy was clearly not helpful in combating the pandemic and contributed to a general undermining of the credibility of national and international public health authorities.

It was only on 5 June, months into the pandemic, that WHO released updated guidance on the use of masks (further updated in December 2020), recognizing the role that face masks can play in reducing transmission from asymptomatic carriers in particular settings. This was a few days after the publication of a comprehensive review and meta-analysis of observational studies showing a significant reduction in risk of infection with all types of masks (Chu 2020). Surgical masks were also shown to work in a hamster model (Chan JF 2020). Other authors, based on reviews or modelling, recommend wearing suitable masks whenever infected persons may be nearby (Meselson 2020, Prather 2020, Zhang 2020). (See also the discussion on droplets and aerosol, page 73.)

While there is now a general acceptance, some controversy on the use of masks continues, including on the potential negative effects of wearing masks on health, for example on cardiopulmonary capacity (Fikenzer, 2020). Regardless of the controversy and the mounting "No-Mask" movements, face masks are clearly "here to stay". The view of people wearing face masks in public, which in the past surprised and at times amused Western travelers to Asian countries will be a common sight worldwide for months and maybe for years to come.

Hand Hygiene

The role of fomites in transmission of SARS-CoV-2 remains unclear but cannot be excluded. (Although objects can be easily contaminated by infected droplets and contaminate hands, it is extremely challenging to prove such transmission.) In any case, frequent handwashing is known to disrupt the transmission of respiratory diseases since people routinely make finger-to-nose or finger-to-eye contact (Kwok, 2015). Handwashing for 30 seconds with ordinary soap is always recommended when there is a contact with a poten-

tially infected item and regularly whenever possible (ex. when returning home). If water and soap are not available (ex. in public places), use of hydroalcoholic solutions or gel is recommended. These solutions have been shown to efficiently inactivate the SARS-CoV-2 virus in 30 seconds (Kratzel, 2020) and can be home-made using a WHO recommended formulation. Hand-hygiene has the added advantage of preventing infections from many other respiratory pathogens. Unfortunately, both water for handwashing and hydroalcoholic solutions are often not available in resource-poor settings (Schmidt, 2020)

Physical/Social distancing and avoiding crowded conditions

Physical distancing means keeping a safe distance from others. The term is often confused with the more common "**social distancing**", usually imposed during lockdowns, that means reducing social contacts as much as possible by staying home and keeping away from others to prevent the spread of COVID-19.

Social distancing has been unequivocally shown to contribute to reducing the spread of SARS-CoV-2. In Wuhan and Shanghai, daily contacts were reduced 7-8-fold during the social distancing period, with most interactions restricted to the household (Zhang J 2020b, Du Z 2020). Social distancing can be an individual choice, but it is usually imposed by health authorities during localized or generalized "Lockdowns" or "stay-at-home orders". We will expand on the issues related to lockdowns and social distancing in the sections below.

With the end of lockdowns and the restart of economic and social activities, physical distancing in public places should become an important behavioral aspect of everyday life and an essential measure to reduce the spread of SARS-CoV-2. Keeping a safe distance from others seems like a straightforward recommendation but defining what can be considered a "safe distance" is in fact quite complex. In a published meta-analysis (Chu, 2020), the authors estimated that the risk of being infected with SARS-CoV-2 is reduced to 13% for those standing at 1 m and further reduced to only 3% beyond that distance. Based on this evidence, the WHO and ECDC recommend a minimum interpersonal distance of 1 m, although other agencies and countries suggest 1.5 m (Australia, Italy, Germany), 1.8 m (US CDC), or even 2 meters (Canada, China, UK) (BBC News, 2020).

Some authors suggest that even 2 meters might not be sufficient and that being "safe" would depend on multiple factors related to both the individual and the environment. These could include infecting viral load, duration of exposure, number of individuals present, indoor versus outdoor settings, level of ventilation, and whether face coverings are worn or not (Qureshi 2020,

Jones 2020). In crowded conditions, including public transport (e.g. trains, buses, metros), physical distancing is often impossible and the use of a protective mask is usually mandatory.

Figure 1. Jones NR et al. Two meters or one: what is the evidence for physical distancing in covid-19? BMJ. 2020 Aug 25;370:m3223. Reproduced with permission.

Speak quietly, don't shout (or sing)!

Traditionally, visible droplets produced during coughing and sneezing are considered the main carriers of respiratory viruses. It emerged only later that normal speech also yields large quantities of particles that are too small to be visible but are large enough to carry a variety of communicable respiratory pathogens and can remain airborne for longer periods. The rate of particle emission during normal human speech is positively correlated with the loudness (amplitude) of vocalization, ranging from approximately 1 to 50 particles per second (0.06 to 3 particles per cm^3), regardless of the language spoken (English, Spanish, Mandarin, or Arabic) (Asadi 2019). However, a small fraction of individuals behaves as "speech superemitters," consistently releasing many more particles than their peers.

These data may help explain the occurrence of some super-spreaders events (e.g. choirs, parties and festivals, slaughterhouses, sport events, religious celebrations, family gatherings, etc.) that are disproportionately responsible for

outbreaks of COVID-19 (See Epidemiology section). While research will continue to study super-spreaders events, people should abide to a very simple rule: **Regardless of physical distance, speak quietly, don't shout!**

Household hygiene

Several studies suggest the possibility of aerosol and fomite transmission of SARS-CoV-2, since the virus can remain viable and infectious in aerosols for hours and on surfaces up to several days (Doremalen 2020, Chin 2020). Though transmission of SARS-COV-2 from contaminated surfaces has not been clearly documented, traditional good home hygiene measures like cleaning floors and furniture, keeping good ventilation and the general disinfection of frequently used objects (e.g. door and window handles, kitchen and food preparation areas, bathroom surfaces, toilets and taps, touchscreen personal devices, computer keyboards, and work surfaces) are recommended to prevent transmission, particularly where confirmed or suspected COVID-19 cases are present (CDC 2020, WHO 20200515).

SARS-CoV-2 is sensitive to ultraviolet rays and heat (Chin 2020). Sustained heat at 56°C for 30 minutes, 75% alcohol, chlorine-containing disinfectants, hydrogen peroxide disinfectants and chloroform can effectively inactivate the virus. Common detergents and sodium hypochlorite (bleach) can also be used effectively (Kampf 2020). To avoid poisoning, disinfectants should always be used at the recommended concentrations, wearing appropriate PPE and should never be mixed. US CDC reported a substantial increase in calls to the poison centers in March 2020 associated with improper use of cleaners and disinfectants; many cases were in children <5 years old (MMWR 2020).

Chemoprophylaxis (not there yet!)

In the future, antiviral drugs may be used to reduce viral shedding in suspected cases and as a prophylactic treatment of contacts. As for now, unfortunately, no such drugs are available.

Prevention at the community/societal levels

Widespread testing, quarantine, and intensive contact tracing

Tedros Adhanom Ghebreyesus didn't get everything right in the SARS-CoV-2 pandemic, but he was right when he recommended: "**Test! Test! Test!**" (WHO, 16 March 2020). Indeed, identification, and testing of suspected cases, isolation and care for those confirmed, and tracing, testing and quarantine of close contacts are critical activities to try to break the chain of transmission in any epidemic. They worked well, for example, in responding to the 2003

SARS outbreak and many countries in Asia successfully applied them to COVID-19 (Li 2020, Lam 2020, Park 2020). The South Korea experience has been nicely summarized in an article in The Guardian.

However, despite the early availability of **sensitive and specific PCR tests** (Sheridan 2020), many countries in Europe and elsewhere were initially caught by surprise. Unprepared, they struggled at first to provide sufficient testing, isolation, and contact tracing capacities to keep up with the pace of spread of SARS-CoV-2. Initially, in Italy, the lack of laboratory capacities led to limiting PCR tests to symptomatic patients only, missing many asymptomatic cases. Other countries, like Germany, fared better in diagnostics but implementing contact tracing proved difficult everywhere when the epidemic reached its peak, due to the large number of potential contacts of asymptomatic cases and their relatively long incubation period.

Ensuring sufficient testing capacities paired with the development of new rapid diagnostic tests (see section on Diagnosis) will continue to be an essential measure in facing future COVID-19 clusters. Use of rapid "point of care" tests, advanced pooled testing strategies (Mallapaty, 2020) and the use of saliva samples could facilitate the task by allowing the rapid testing of large number of people, as China has done by testing all the population of large urban areas like Wuhan (more than 10 million people) in less than 2 weeks.

Isolation (separation of ill or infected persons from others) and **quarantine** (the restriction of activities or separation of persons who are not ill, but who may be exposed to an infectious agent or disease) are essential measures to reduce the spread of COVID-19. Unless a patient is hospitalized, quarantine and isolation are usually done at home or in dedicated facilities like hotels, dormitories, or group isolation facilities (CDC 2020). Given the uncertainty about the infectivity of the suspected individual, preventive measures are similar for both isolation of confirmed cases and quarantine of contacts. Basically, you are required to stay at home or in the facility and avoid non-essential contacts with others, including household members, for a set period to avoid spreading the infection.

The long incubation and high pre-symptomatic infectivity of COVID-19 puts family members of infected individuals at particular risk (Little 2020). The infection rate found for household members varies between 11% and 32% (Bi Q 2020, Wu J 2020). These differences are probably due to different isolation measures implemented inside the family homes. Ideally, people in isolation should have access to a separate bedroom (and bathroom), personal protection equipment (PPE) and should not have contacts with people at high risk of serious COVID-19 disease.

The period of isolation and quarantine required before suspected or confirmed cases can be considered no more infectious is still being debated. Initially, the requirement for a confirmed case was to have clinically recovered and to have two negative RT-PCR results on sequential samples taken at least 24 hours apart (WHO 2020). This second criteria proved challenging in countries with limited testing capacities and even when tests are available, some patients can continue to have positive PCR results for weeks or months after the cessation of symptoms and infectivity, leading to prolonged, probably unnecessary isolation periods.

Updated WHO criteria for releasing COVID-19 patients from isolation were published in June (WHO 20200617). Based on data showing the rarity of the presence of vital virus after 9 days from symptom onset (Cevic 2020), the new recommendation is to limit the isolation period to:

- 10 days after symptom onset, plus at least 3 additional days without symptoms for symptomatic patients.
- 10 days after positive test for SARS-CoV-2 for asymptomatic cases.

However, several countries, (e.g. Italy), continue to apply the earlier testing criteria including a negative PCR test, which can result in individual being kept in isolation for a longer period.

Recommended quarantine period for contacts and for travelers has not changed and remains set at 14 days, though several countries have reduced it to 10 days (e.g. Switzerland).

Contact tracing can be effective in reducing the risk of spread of the virus (Keeling 2020) but it is a complex and resource intensive exercise. It is most effective when implemented early in the outbreak, before there is sustained community transmission. Once cases are soaring, identifying and monitoring all the potential contacts using only the public health resources becomes close to impossible and additional measures like physical distancing, face masks and localized lockdowns become necessary (Cheng 2020). WHO has published detailed guidance on contact tracing for COVID-19 and alternative approaches to contact tracing that results in resource-saving measures have been suggested (ECDC, April 2020).

As stated by several authors, (Steinbrook, 2020, Salathé 2020) in countries that have managed to bring the pandemic under control a necessary step in "reopening" society was to have sufficient testing and contact tracing capacities to successfully contain the outbreaks that will inevitably occur as social restrictions are removed or relaxed. The coming winter months will show which countries have learned this important lesson.

Tracking apps

Mobile phone data reveal astonishing details about population movements. According to an analysis by Orange, a French phone operator, data from its telephone subscribers revealed that 17% of the inhabitants of Grand Paris (Métropole du Grand Paris, 7 million people) left the region between March 13 and 20 – just before and after the implementation of the French lockdown measures (Le Monde, 4 April 2020). Again, mobile phone data from individuals leaving or transiting through the prefecture of Wuhan between 1st and 24th January 2020 showed that the distribution of population outflow from Wuhan accurately predicted the relative frequency and geographical distribution of SARS-CoV-2 infections throughout China until 19 February 2020 (Jia JS 2020).

Numerous countries have tried to harness the power of the smartphone to design and target measures to contain the spread of the pandemic (Oliver 2020). In addition to the dissemination of COVID-19 information and prevention messages, the use of smartphones in support to contact tracing has been promoted widely. This contact tracing system (better named "exposure notification") would basically use an application to detect if the phone has come in close distance for a set period of time from another phone of a person diagnosed with SARS-COV-2 and therefore potentially infectious. It will then give a warning message prompting the owner to seek medical assistance, self-isolation, and testing.

The deployment of these tracking applications has faced several hurdles, including the need for inter-operability across platforms (Google, Apple) and across countries (unfortunately, each European country has developed its own app); the possibility of false-positive alerts; and the need for a majority of the population to download and regularly activate the app to be truly effective. The need to preserve the privacy of the users forced less performing technical solutions (e.g. decentralized data systems with data only stored in each phone vs centralized database; preference for less-accurate Bluetooth connection over GPS geo-localization; voluntary decision required on the sharing of data on positivity; time-limited storage of collected data, etc.) As a result, in June, Norway's health authority had to delete all data gathered via its Covid-19 contact-tracing app and suspend its further use following a ruling by the Norwegian Data Protection Authority.

A few months into their introductions, most COVID-19 tracking apps have failed to deliver as expected. In almost all countries only a small proportion of the population have downloaded the app (only Qatar, Israel, Australia, Switzerland, and Turkey have seen downloads above the minimum threshold of 15% of the population) and probably even less people are regularly activat-

ing it. More importantly, the success of a tracking application should not be measured by the number of downloads but by the number of contacts detected, which so far have been relatively few (due to privacy concerns, the total number of contacts is not available in countries where information is decentralized).

Several countries, including France and Germany, have started to provide additional services with the app, including for accessing laboratory services and receiving test results. Maybe, with these improvements, tracking applications will become more efficient and their use will increase in future, though they will probably continue to be only a support rather that a replacement for a traditional "manual" contact tracing system.

Mandatory use of face masks

Wearing a face mask to protect self and others from SARS-CoV-2 infection may be considered an individual choice (see above). However, as of 6 May 2020, more than 150 countries had made wearing a mask in some settings a mandatory requirement as a collective preventive public health measure. Mandatory settings range from "everywhere in public" to only indoor public places, public transportation, shops, workplaces, schools, etc. Children and people with breathing difficulties are often exempted from the mandatory use of face masks (US CDC 2020, WHO 2020, ECDC 2020). As a result, the global number of people regularly wearing masks in public has soared, reaching the peak of 80-90% of the population in most countries in Asia but also in Italy, France, and Spain. Surprisingly, mask acceptance has increased to the point of being branded as a fashion items.

As shown in the chart, authorities in Asia have mandated the use of face masks in public at the early stages of the pandemic, which contributed to reduced spread and the sharp drops in infections. As mentioned earlier, in many other parts of the world, conflicting advice with misleading or incomplete information about the usefulness of masks has caused confusion among the population and a late adoption of this preventive measure. In addition, a growing "no-Masks" movement has gathered momentum, staging rallies in several countries. Regardless, as new infections have started to increase again following the summer reopening, mandatory mask requirements have been introduced again in most European countries and is becoming a norm in most public places.

Figure 2. Source: YouGov.com. Reproduced with permission.

Ban on mass gatherings

Recognizing their potential role in generating explosive clusters of SARS-CoV-2 infections, (McCloskey 2020, Ebrahim 2020) most countries have implemented nationwide bans of mass gathering like sporting and cultural events, concerts, religious celebrations, rallies and political demonstrations, etc. Several important international mass gatherings events have been cancelled or postponed in 2020, including the Tokyo Olympic Games, Euro football championship, Formula 1 Grand Prix races, the Eurovision Song Contest, Geneva Motor Show, Christian Holy Week events in Rome, Umrah pilgrimage to Mecca, and many others. Most sport events have resumed, but without public.

It is currently uncertain under which conditions events that require the contemporaneous presence of large numbers of people in restricted or closed spaces (discos, bar, etc.) can be resumed without the risk of resulting in a super spreader event. The limited reopening of these premises during the summer holidays has been associated with a resurgence of the spread of the virus observed all over Europe. WHO has published key recommendations for mass gatherings in the context of COVID-19. Unless the risk of SARS-COV-2

spread is reduced significantly, postponing or cancelling of planned large event is likely to continue in the months to come.

Localized and nationwide Lockdowns

Lockdowns (or "stay-at-home orders") are restrictions of movements of the whole population, ordered by a government authority to suppress or mitigate an epidemic or pandemic. They differ from quarantine in that all residents are supposed to stay at home, except for those involved in essential tasks, while quarantine is usually limited to people suspected to be infected.

Lockdowns and social distancing have been used for centuries in the fight against epidemics, as famously illustrated in the *Decameron*, a book by Bocaccio, an Italian writer, which contains tales told by a group of young people sheltering in a villa outside Florence to escape the Black Death of 1348. However, the 2020 nationwide lockdowns which ordered almost 4 billion people in 90 countries to stay at home were unprecedented in human history (see also The First Eight Months, page 453). For the first time, lockdowns were imposed initially in a whole city of 10 million people (Wuhan), then to the whole province of Hubei (60 million people), finally to a whole country (Italy, followed by most other European countries.) Though countries opted for more (China) or less (Europe) strict confinement measures, lockdowns were clearly effective in decreasing the infection rate to less than 10% (Cowling 2020).

How strict such measures can be has been shown in Hong Kong (Normile 2020). The recipe: hospitalize all those who test positive, even if asymptomatic, and order two weeks of self-quarantine to all close contacts, monitored by the compulsory use of electronic wristbands. A website even displays the location of infected people in Hong Kong at all times: https://chp-dashboard.geodata.gov.hk/covid-19/en.html. Such strict measures can be very effective but would not be acceptable or feasible in most countries. Indeed, one of the limitations of generalized lockdowns is that they can never be 100% complete. People occupied in essential services (e.g. health, security, transport, communication, food production and delivery, etc.) will need to be allowed to move and work, and sick people will need to continue to access health services.

Generalized lockdowns are blunt prevention tools, affecting the whole healthy population to reduce the risk of transmission from the relatively few potentially infectious individuals (Hsiang 2020). They impose a major economic and social burden on the affected populations, while also preventing at times access to prevention and treatment for other health conditions (Charlesworth 2020). They have been described as a type of "induced coma"

for the whole society and economy, though few benefits are also noted, for example on pollution levels (UNDP 2020). Various authors (Marshall 2020, Pierce 2020, Williams 2020, Galea 2020) have emphasized the combined impact of the pandemic, social distancing and closures on the mental health of the population. In addition, implementing generalized lockdowns in low-income countries is particularly difficult. People in the informal economy without social net benefits may be forced to choose between the risk of infection and risking of falling into poverty and hunger (ILO, 2020).

In fact, widespread testing, isolation and quarantine, combined with population behavioral changes (physical distancing, use of masks, hand hygiene) – that have a less disruptive social and economic impact – have been shown to successfully contain COVID-19 if applied widely and consistently (Cowling 2020). In summary, the tighter you control the infected individuals and trace and isolate the close contacts, the less restriction you will have to impose on the uninfected.

The hope is for countries to learn this lesson and, being better prepared, to be able to avoid in future the need for generalized lockdowns to respond to COVID-19 (and other epidemics). However, the resurgence of COVID-19 in Europe is showing how difficult it is to balance health and economic/social imperatives. Until a sufficiently large proportion of the population is immunized through vaccination or infection, localized or even generalized temporary lockdowns might continue to be required in the fight against this pandemic.

Travel bans/border closures

It has long been recognized that both land, sea and air travel can be efficient and rapid routes for the international spread of a pandemic virus (Hufnagel 2004, Hollingsworth 2007). The conditions for restricting movements of people and goods between countries in case of a public health emergency are therefore described in the WHO International Health Regulations adopted by all WHO member states in 2005 (IHR 2005).

As of 18 June 2020, almost all (191) countries had taken some measures that restrict people's movement since the COVID-19 pandemic began. Measures ranged from control of entry onto the territory of a State to control of movement within a territory, comprising of partial or total border closures (125 countries) and international flight suspensions (122 countries).

As pointed out by some authors (Habibi 2020), these measures may be in breach of the IHR 2005, not being grounded on "scientific principles, scientific evidence, or advice from WHO". Several scientific studies have indeed

shown how the limited effectiveness of the imposition of travel bans and border closures in slowing down the introduction and spread of an epidemic or pandemic virus (like influenza or Ebola), while carrying many damaging and even potentially counterproductive effects (Brownstein 2006, Mateus 2014, Poletto 2014).

In fact, widespread travel restrictions and border closures have not prevented SARS-COV-2 from reaching quickly just about every country on the planet (see section on Epidemiology). Though Italy was the first in Europe to impose a travel ban on China, it was also the first European country to experience a major COVID-19 outbreak. Australia has imposed a total travel ban since 24 March that contributed initially to stop the spread of the virus but did not prevent returning citizens and poorly-trained quarantine guards to break the rules and cause a major outbreak in Melbourne.

One reason why travel bans are usually ineffective is that you cannot prevent everybody from entering a country. Some people (e.g. citizens, long-term residents, diplomats, air or ship crews, health personnel, businessmen, etc.) are usually exempted and able to travel under national or international agreements. Others (e.g., illegal migrants) can cross borders unofficially.

Some authors have also pointed out how the travel bans and border closures can restrict the movement of health personnel, vital health equipment and supplies (e.g. medicines, PPEs, testing reagents and equipment), particularly needed in countries with limited resources (Devi 2020). Others suggest that early detection, hand washing, self-isolation, and household quarantine will likely be more effective than travel restrictions at mitigating this pandemic (Chinazzi 2020).

On the other hand, the economic damage of travel bans has been substantial. The activities of airlines, airports, travel agents, hotels and resorts have basically come to a halt at the peak of the pandemic. Eurocontrol has recorded a 90% drop in air passenger in Europe at the end of April. This figure has improved with the reopening of borders but is still at -50% compared to 2019 as of mid-July. In May, the UN World Tourism Organization (UNWTO) projected the potential economic loss for the tourist industry worldwide at US$ 910 billion to US$ 1.2 trillion, with 100-120 million jobs at risk.

Generalized travel bans and border closures can reduce the risk of spread of a pandemic virus but, like generalized lockdowns, are blunt tools. They affect a large number of uninfected individuals, cause a substantial impact on the economy and on trade, and can result in an erroneous and dangerous false sense of security in the population and the authorities. Regular screening and quarantine for all travelers remain the most effective ways to avoid local

transmission of a virus by imported cases. Hopefully, once this is understood, international travel will finally be allowed to resume in a safe, controlled environment.[3]

Vaccinate for seasonal influenza and (almost there) for COVID-19

Several authors (Richmond 2020, Jaklevic 2020, Singer 2020, Rubin 2020, Maltezoua 2020) and public health agencies are recommending expanding seasonal flu vaccination in the context of the COVID-19 pandemic. This follows concerns about the potential "double epidemic" of COVID-19 and seasonal flu during the winter months (Balakrishnan 2020, Gostin 2020). There are indeed many similarities (but also a few important differences) between the two diseases (Solomon 2020, Zayet 2020, Faury 2020) which may complicate the differential diagnosis for symptomatic patients, e.g. similar transmission routes, similar symptoms for mild cases , similar high-risk groups for severe complications and mortality. A "double epidemic" could overburden both primary care services and hospitals, require a major increase in diagnostic requests, lead to unnecessary isolation and quarantine of influenza cases and even increase stigma and discrimination of anyone presenting with symptoms of a respiratory infection (Rubin 2020). The possibility of COVID-19 and flu co-infection should also *not* be ruled out (Kim 2020). Combined SARS-CoV-2 and flu diagnostic tests, as recently approved by the FDA and being evaluated in some countries in Europe, could be useful in quickly identifying the pathogen(s) involved from a single sample.

Increasing coverage of seasonal influenza vaccination among high-risk groups is a good public health measure on its own, as influenza is estimated to cause close to 10 million hospitalizations and between 294,000 and 518,000 deaths every year (Paget 2019, CDC-US). It is also an essential measure in the response to COVID-19 to avoid a potential breakdown of health care systems and the related increase in mortality and morbidity.

Unfortunately, the regular uptake of flu vaccination in high-risk groups (> 65 years of age) has been in the past largely insufficient, averaging around 50% in OECD countries. Along with efforts to increase coverage in the recommended risk groups, additional measures being suggested include reducing the recommended age for vaccination from 65 to 60 years, universal vaccination of children aged 6 months to 17 years, mandatory vaccination for all

[3] COI declaration: The contributing author has been stranded since March 2020, unable to to join, as planned, his far away relatives due to COVID-19 travel restrictions.

health-care workers, including all workers and visitors of long-term care facilities (Balakrishnan 2020, Gostin 2020, CDC).

However, widespread implementation of these additional measures will not be simple. The usual misguided concerns about the safety of vaccines and more recent social media fake news reports about the possibility of flu vaccine causing COVID-19 will need to be addressed. Reduced healthcare seeking behaviors due to fear of SARS-CoV-2 infection could also be a challenge. In addition, despite efforts by vaccine manufacturers and a major increase in flu vaccine production capacities in the last decade, due in part to preparation for a possible flu pandemic (Rockman 2020), vaccine availability is unlikely to be sufficient to meet such an increase in demand, at least for the coming northern hemisphere flu season in 2020-21.

The definition of the composition of the seasonal flu vaccine is agreed by a WHO advisory group of flu experts. Based on an analysis of data from flu surveillance, laboratory and clinical studies collected through the WHO Global Influenza Surveillance and Response System (GISRS), the group makes recommendations on the composition of the new influenza vaccine. The advisory group meetings are held in February (for the northern hemisphere's seasonal influenza vaccine) and in September (for the southern hemisphere's vaccine) to allow sufficient time (7-9 months) to produce the required doses of vaccine (Dunning 2020).

Influenza vaccine effectiveness can vary from season to season depending on the similarity or "match" between the flu vaccine and the flu viruses spreading in the community. During those years when the flu vaccine is not well matched to circulating influenza viruses, effectiveness can be as low as 20%, rising to 60% for the years when there is a good match. However, even less effective influenza vaccines have been shown to reduce considerably the burden of severe cases of influenza, admission to ICUs, and flu-related deaths (Thompson 2018, Ferdinands 2019).

Several recent studies have reported that indicators of influenza activity have been declining substantially in 2020 in both the northern (e.g. in Asia and the US) and the southern hemispheres, including in countries that implemented limited lockdown measures (Soo 2020, Olsen 2020, Itaya 2020). The decreased influenza activity was closely associated with the introduction of interventions to reduce SARS-CoV-2 transmission (Choe 2020). This is really good news, as the evidence on the effectiveness of public health interventions in slowing the spread of influenza has been otherwise limited (Fong 2020, Xiao 2020, Ryu 2020). If these findings are confirmed during the coming winter season in the northern hemisphere, not only we would avoid the dangers of a "dual epidemic" but will have confirmation on the effectiveness of non-

pharmaceutical interventions. They could become standard interventions, in addition to vaccination, for reducing the health burden of seasonal influenza and other respiratory infections in high-risk groups.

On the down side, the limited detection and isolation of circulating flu viruses by the WHO surveillance system will reduce the availability of updated and robust data for the decision on the composition of the flu vaccine for 2021-22, raising the danger of a poor match between future influenza vaccines and circulating flu viruses.

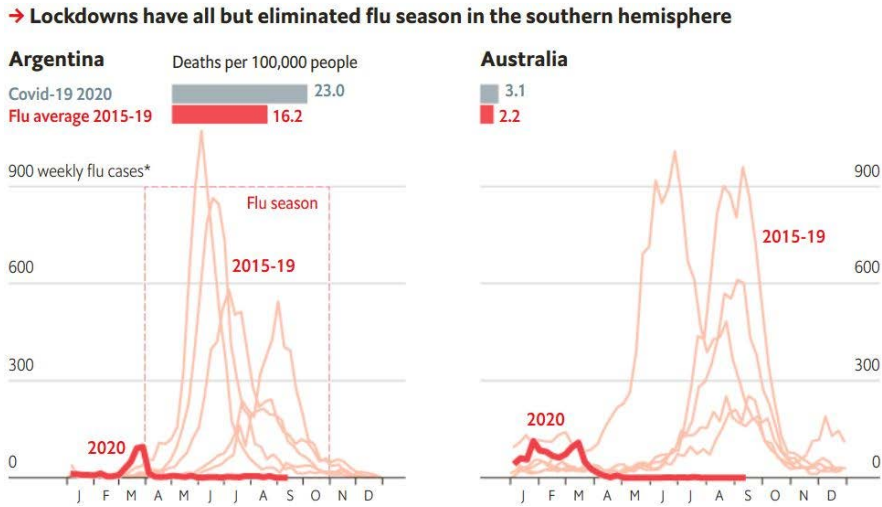

Figure 3. *The southern hemisphere skipped flu season in 2020 – Efforts to stop covid-19 have had at least one welcome side-effect.* The Economist 2020, published 12 September. Full-text: https://www.economist.com/graphic-detail/2020/09/12/the-southern-hemisphere-skipped-flu-season-in-2020. Reproduced with permission.

Additional potential good news could come from research on the effects of influenza vaccination on the severity of SARS-CoV-2 infection. Among the few studies available, a recent paper (Fink 2020) reports on the analysis of data from 92,664 confirmed COVID-19 cases in Brazil showing that patients who received a trivalent influenza vaccine during the last campaign (March 2020) experienced on average 8% lower odds of needing intensive care treatment, 18% lower odds of requiring invasive respiratory support and 17% lower odds of death. Similar conclusions were reached in another pre-print paper modelling COVID-19 mortality data and recent influenza vaccination coverage in the USA (Zanettini 2020).

More studies are clearly required before reaching conclusions, but the available evidence does suggest that increasing coverage of influenza vaccination could result in both direct and indirect benefits in terms of reduced morbidity and mortality from both COVID-19 and influenza. In addition to the long-term benefits of expanding influenza vaccine production and uptake, these efforts will be of great value for rolling-out the COVID-19 vaccines, since production, distribution and promotion of uptake for the new vaccines will face similar challenges and will need to prioritize the same vulnerable populations (Jaklevic 2020, Mendelson 2020).

Containment or mitigation of COVID-19?

Public health interventions to control an outbreak or an epidemic aim at achieving two separate but linked objectives (Zhang 2020, OECD 2020):

- To **contain** the spread by minimizing the risk of transmission from infected to non-infected individuals, eventually **suppressing** transmission and ending the outbreak.
- To **mitigate** the impact by slowing the spread of the disease while protecting those at higher risk. While not halting the outbreak, this would "flatten the epidemic curve", reduce disease burden and avoid a peak in health care demand. In case of new emerging pathogens, it would also buy time to develop effective treatments or vaccines (Djidjou-Demasse 2020).

Containment strategies rely heavily on case detection and contract tracing, isolation, and quarantine. They are usually applied most successfully in the early stages of an outbreak or epidemic, when the number of cases is still manageable by the public health system (Hellewell 2020). When containment measures are insufficient or applied too late, **mitigation** becomes the only option, usually through the imposition of generalized preventive measures like closing of non-essential activities, social distancing, mandatory mask use, or lockdowns (Parodi 2020, Walker 2020).

During the first months of the COVID-19 pandemic, several countries (China, Vietnam, South Korea, Australia, and New Zealand) have shown how the implementation of a well-timed, comprehensive package of aggressive and combined containment and mitigation policies can be effective in suppressing the COVID-19 epidemic, at least in the short-term. Other countries (most countries in Europe) have not been able to suppress transmission but have managed, at least temporarily, to mitigate the impact and bring the spread of SARS-COV-2 down to acceptable levels during the summer months. In others

the pandemic is raging with no end in sight (e.g., US, Brazil, most of Latin America) and a second wave of infections is now becoming evident in several European countries. In any case, as long as the virus is actively spreading anywhere in the world, no country can feel safe (as shown by the recent outbreaks in Victoria, Australia and in New Zealand). The fight against SARS-CoV-2 is far from over.

Conclusion

Despite the rapid progress of the last few months, the widespread availability of an effective vaccine or antiviral treatments still a few months away. Meanwhile, countries are still struggling to find the right mix of preventive measures (and the right balance between health and socio-economic priorities) to build an effective response to the COVID-19 pandemic.

Finding the right prevention mix means identifying what are the most cost-effective measures that can be widely implemented to reduce or halt the transmission of the virus. For this, we need a better understanding of how this virus spreads and how effective the different preventive measures are. Only more research and better science will provide this information.

However, finding the right balance also means recognizing that some measures can be effective, but carry very high social, economic, political, educational, and even health costs. These are political decisions. For example, many European countries have tried very hard to avoid imposing again strict generalized lockdowns, border closures or travel bans. These measures are simply too costly for society to be acceptable.

The best scenario is to be able to respond to new cluster of cases or the acceleration of the spread of the virus, due to "superspreader" events or a relaxation of individual preventive measures, through localized time-limited public health measures, their effectiveness being judged by better and timely monitoring of the spread of the virus. Even in the absence of COVID-19 vaccines or treatments and comprehensive knowledge of the immune response to SARS-CoV-2, countries can navigate pathways to reduced transmission, decreased severe illness and mortality, and less economic disruption in the short and longer term (Bedford 2020). It is not ideal, it is not being "back to normal", but while we wait for the widespread availability of the new "silver bullets" it is probably the best option we have right now to contain this pandemic.

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4. Virology

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Introduction

In January 2020, a novel virus later named severe acute respiratory syndrome coronavirus (SARS-CoV-2) was isolated from the broncho-alveolar fluid of a patient in Wuhan, People's Republic of China, suffering from what became known as coronavirus disease 2019 (COVID-19). SARS-CoV-2 is highly transmissible and pathogenic. Until present (October 2020), it has infected tens of millions of individuals, causing more than a million deaths and debilitating the economy.

Coronaviruses (CoV) are large, spherical, enveloped RNA viruses with distinct protruding spike glycoproteins visible on the viral surface. The name is derived from the Latin "corona", which means crown or halo, referencing the characteristic morphology when viewed under an electron microscope (Zuckerman 2009, Perlman 2020). Structural proteins include envelope (E), matrix (M), and nucleocapsid (N).CoV contain a single strand of positive-sense RNA. Their genome size ranges from c. 26 to 32 kilobases, placing them among the known RNA viruses with the largest genomes.

The family *Coronaviridae* belongs to the order Nidovirales, suborder Cornidovirineae. Subfamily Orthocoronavirinae includes four genera: alpha-, beta-, delta- and gammacoronavirus. Genera alpha- and betacoronavirus contain several human-pathogenic subgenera and species. SARS-CoV-2 is a previously unknown betacoronavirus in subgenus Sarbecovirus, like its close relative, severe acute respiratory syndrome-related coronavirus (SARS-CoV). Other notable beta-CoV are Middle East respiratory syndrome-related CoV (MERS-CoV) in subgenus Merbecovirus as well as human CoV HKU1 and human CoV OC43, species Betacoronavirus 1, both in subgenus Embecovirus.

Species in of the family Coronaviridae infect various species of animals – humans, other mammals, and birds - causing a broad spectrum of different diseases. Human CoV are primarily respiratory pathogens but may cause enteric disease. Respiratory illness caused by human CoV HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63 is usually mild and "common cold"-like and thus not of major public health concern (Korsman 2012). The highly pathogenic CoV affecting humans cause severe acute respiratory infections often resulting in serious disease and death is was caused by the novel SARS-CoV and

MERS-CoV. There is strong evidence that these viruses emerged recently from animal reservoirs, originating in bats and transmitted to man via intermediate host species. Intra- and inter-species transmission of CoVs, and genetic recombination events contribute to the emergence of new CoV strains.

The following sections will review coronaviruses in general with a more detailed appraisal of the origin, evolution, virological structure and pathogenesis of SARS-CoV-2 to expand knowledge pertaining to COVID-19 and prospective anti-viral and vaccine therapies.

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The complete chapter will be available soon.

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Taxonomy

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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.

Genomic variation

MacLean O, Orton RJ, Singer JB, et al. **No evidence for distinct types in the evolution of SARS-CoV-2**. Virus Evolution. Full-text: https://doi.org/10.1093/ve/veaa034

Do not overinterpret genomic data! In this paper, authors discuss the difficulty in demonstrating the existence or nature of a functional effect of a viral mutation, and advise against overinterpretation.

Zhang X, Tan Y, Ling Y, et al. **Viral and host factors related to the clinical outcome of COVID-19.** Nature (2020). Full-text: https://doi.org/10.1038/s41586-020-2355-0

Viral variants do not affect outcome. This important study on 326 cases found at least two major lineages with differential exposure history during the early phase of the outbreak in Wuhan. Patients infected with these different clades did not exhibit significant differences in clinical features, mutation rates or transmissibility.

Day T, Gandon S, Lion S, et al. **On the evolutionary epidemiology of SARS-CoV-2.** Curr Biol 2020, June 11. Full-text: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7287426

Outstanding essay about what little is currently known about the evolution of SARS-CoV-2. At present, there is a lack of compelling evidence that any existing variants impact the progression, severity, or transmission of COVID-19.

Gussow AB, Auslander N, Faure G, Wolf YI, Zhang F, Koonin EV. **Genomic determinants of pathogenicity in SARS-CoV-2 and other human coronaviruses.** Proc Natl Acad Sci U S A. 2020 Jun 30;117(26):15193-15199. PubMed: https://pubmed.gov/32522874. Full-text: https://doi.org/10.1073/pnas.2008176117

This in-depth molecular analysis reconstructs key genomic features that differentiate SARS-CoV-2 from less pathogenic coronaviruses.

Korber B, Fischer WM, Gnanakaran S, et al. **Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus**. Cell July 02, 2020. Full-text: https://doi.org/10.1016/j.cell.2020.06.043

A SARS-CoV-2 variant carrying the Spike protein amino acid change D614G (caused by an A-to-G nucleotide mutation at position 23,403 in the Wuhan reference strain) has become the most prevalent form in the global pandemic within a month, indicating a fitness advantage (better transmission).

Plante JA, Liu Y, Liu J, et al. **Spike mutation D614G alters SARS-CoV-2 fitness.** Nature 2020, published 26. October. Full-text: https://doi.org/10.1038/s41586-020-2895-3

D614G enhances replication on human lung epithelial cells and primary human airway tissues through an improved infectivity of virions.

Yurkovetskiy L, Wang X, Pascal KE, et al. **Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant.** Cell 2020, published 15 September. Full-text: https://doi.org/10.1016/j.cell.2020.09.032

D614G is more infectious than the ancestral form on human lung cells, colon cells, and on cells expressing ACE.

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 beta-coronaviruses. 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. 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.

Xiao K, Zhai J, Feng Y, et al. **Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins**. Nature. 2020 May 7. PubMed: https://pubmed.gov/32380510. Full-text: https://doi.org/10.1038/s41586-020-2313-x

In a wildlife rescue center, authors found a coronavirus in 25 Malayan pangolins (some of them were very sick), showing 90-100% amino acid identity with SARS-CoV-2 in different genes. Comparative genomic analysis suggested that SARS-CoV-2 might have originated from the recombination of a Pangolin-CoV-like virus with a Bat-CoV-RaTG13-like virus. As the RBD of Pangolin-CoV is virtually identical to that of SARS-CoV-2, the virus in pangolins presents a potential future threat to public health. Pangolins and bats are both nocturnal animals, eat insects, and share overlapping ecological niches, which make pangolins the ideal intermediate host. *Stop the illegal pangolin trade!*

Zhang T, Wu Q, Zhang Z. **Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak**. Curr Biol. 2020 Mar 13. 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.

Zhou H, Chen X, Hu T, et al. **A Novel Bat Coronavirus Closely Related to SARS-CoV-2 Contains Natural Insertions at the S1/S2 Cleavage Site of the Spike Protein.** Curr Biol. 2020 May 11. PubMed: https://pubmed.gov/32416074. Full-text: https://doi.org/10.1016/j.cub.2020.05.023

A novel bat-derived coronavirus was identified from a metagenomics analysis of samples from 227 bats collected from Yunnan Province in 2019. Notably, RmYN02 shares 93.3% nucleotide identity with SARS-CoV-2 at the scale of the complete genome and 97.2% identity in the lab gene, in which it is the closest relative of SARS-CoV-2 reported to date. However, RmYN02 showed low sequence identity (61.3%) in the receptor binding domain and might not bind to ACE2.

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. 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 print and tissue paper after 3 hours, from treated wood and cloth on day 2, from glass and banknotes on day 4, stainless steel and plastic 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. 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. 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).

Chan KH, Sridhar S, Zhang RR, et al. **Factors affecting stability and infectivity of SARS-CoV-2**. J Hosp Infect. 2020 Jul 8. PubMed: https://pubmed.gov/32652214. Full-text: https://doi.org/10.1016/j.jhin.2020.07.009

Dry heat is bad, damp cold is good (for the virus). Dried SARS-CoV-2 virus on glass retained viability for over 3-4 days at room temperature and for 14 days at 4°C, but lost viability rapidly at 37°C. SARS-CoV-2 in solution remained viable for much longer under the same different temperature conditions.

Cell tropism, ACE expression

Chu H, Chan JF, Yuen TT, et al. **Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study**. Lancet Microbe April 21, 2020. Full-text: https://doi.org/10.1016/S2666-5247(20)30004-5

An elegant study, explaining distinct clinical features of COVID-19 and SARS. Investigation of cell susceptibility, species tropism, replication kinetics, and virus-induced cell damage from both SARS-CoVs, using live infectious virus particles. SARS-CoV-2 replicated more efficiently in human pulmonary cells, indicating that SARS-CoV-2 has most likely adapted better to humans. SARS-CoV-2 replicated significantly less in intestinal cells (might explain lower diarrhea frequency compared to SARS) but better in neuronal cells, highlighting the potential for neurological manifestations.

Hou YJ, Okuda K, Edwards CE, et al. **SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract.** Cell, May 26, 2020. Full-text: https://doi.org/10.1016/j.cell.2020.05.042

This study quantitated differences in ACE2 receptor expression and SARS-CoV-2 infectivity in the nose (high) vs the peripheral lung (low). If the nasal cavity is the initial site mediating seeding of the lung via aspiration, these studies argue for the widespread use of masks to prevent aerosol, large droplet, and/or mechanical exposure to the nasal passages.

Hui KPY, Cheung MC, Perera RAPM, et al. **Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures**. Lancet Respir Med. 2020 May 7. PubMed: https://pubmed.gov/32386571. Full-text: https://doi.org/10.1016/S2213-2600(20)30193-4

More insights into the transmissibility and pathogenesis. Using *ex vivo* cultures, the authors evaluated tissue and cellular tropism of SARS-CoV-2 in human respiratory tract and conjunctiva in comparison with other coronaviruses. In the bronchus and in the conjunctiva, SARS-CoV-2 replication competence was higher than SARS-CoV. In the lung, it was similar to SARS-CoV but lower than MERS-CoV.

Shang J, Ye G, Shi K. **Structural basis of receptor recognition by SARS-CoV-2**. Nature 2020, March 30. Full-text: 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.

Sungnak W, Huang N, Bécavin C,et al. **SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes**. Nature Medicine, Published: 23 April 2020. Full-text: https://www.nature.com/articles/s41591-020-0868-6

Another elegant paper, confirming the expression of ACE2 in multiple tissues shown in previous studies, with added information on tissues not previously investigated, including nasal epithelium and cornea and its co-expression with TMPRSS2. Potential tropism was analyzed by surveying expression of viral entry-associated genes in single-cell RNA-sequencing data from multiple tissues from healthy human donors. These transcripts were found in specific respiratory, corneal and intestinal epithelial cells, potentially explaining the high efficiency of SARS-CoV-2 transmission.

Spike protein

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.

Watanabe Y, Allen JD, Wrapp D, McLellan JS, Crispin M. **Site-specific glycan analysis of the SARS-CoV-2 spike**. Science. 2020 May 4. PubMed: https://pubmed.gov/32366695. Full-text: https://doi.org/10.1126/science.abb9983

The surface of the envelope spike is dominated by host-derived glycans. These glycans facilitate immune evasion by shielding specific epitopes from antibody neutralization. SARS-CoV-2 S gene encodes 22 N-linked glycan sequons per protomer. Using a site-specific mass spectrometric approach, the authors reveal these glycan structures on a recombinant SARS-CoV-2 S immunogen.

Cai Y, Zhang J, Xiao T, et al. **Distinct conformational states of SARS-CoV-2 spike protein.** Science 21 Jul 2020. Full-text: https://doi.org/10.1126/science.abd4251

The authors report two cryo-EM structures, derived from a preparation of the full-length S protein, representing its pre-fusion (2.9Å resolution) and post-fusion (3.0Å resolution) conformations, respectively, and identify a structure near the fusion peptide – the fusion peptide proximal region (FPPR), which may play a critical role in the fusogenic structural rearrangements of S protein.

Ke Z, Oton J, Qu K, et al. **Structures and distributions of SARS-CoV-2 spike proteins on intact virions.** Nature 2020, published 17 August. Full-text: https://doi.org/10.1038/s41586-020-2665-2

More on how SARS-CoV-2 Spike (S) proteins function and how they interact with the immune system. This work extends the knowledge of the structures, conformations and distributions of S trimers within virions.

Toelzer C, Gupta K, Yadav SK, et al. **Free fatty acid binding pocket in the locked structure of SARS-CoV-2 spike protein**. Science 21 Sep 2020. Full-text: https://doi.org/10.1126/science.abd3255

The structure of the SARS-CoV-2 S glycoprotein. The RBDs tightly bind the essential free fatty acid (FFA) linoleic acid (LA) in three composite binding pockets. The LA-binding pocket presents a promising target for future development of small molecule inhibitors that, for example, could irreversibly lock S in the closed conformation and interfere with receptor interactions.

Turoňová B, Sikora M, Schürmann C, et al. **In situ structural analysis of SARS-CoV-2 spike reveals flexibility mediated by three hinges.** Science 2020, published 18 August. Full-text: https://science.sciencemag.org/content/early/2020/08/17/science.abd5223

This work shows that the stalk domain of S contains three hinges, allowing S to scan the host cell surface, shielded from antibodies by an extensive glycan coat.

Binding to ACE

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.

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. 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 experiments 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, this paper shows 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). The 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.

Starr TN, Greaney AJ, Hilton SK, et al. **Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding**. Cell August 11, 2020. Full-text: https://doi.org/10.1016/j.cell.2020.08.012

The authors have systematically changed every amino acid in the RBD and determine the effects of the substitutions on Spike expression, folding, and ACE2 binding. The work identifies structurally constrained regions that would be ideal targets for COVID-19 countermeasures and demonstrates that mutations in the virus which enhance ACE2 affinity can be engineered but have not, to date, been naturally selected during the pandemic.

Yang J, Petitjean SJL, Koehler M. **Molecular interaction and inhibition of SARS-CoV-2 binding to the ACE2 receptor.** Nat Commun 11, 4541 (2020). Full-text: https://doi.org/10.1038/s41467-020-18319-6

How the receptor binding domain serves as the binding interface within the S-glycoprotein with the ACE2 receptor. Kinetic and thermodynamic properties of this binding pocket.

Cell entry

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. 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.

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.

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.

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. 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.

Cantuti-Castelvetri L, Ojha R, Pedro LD, et al. **Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity.** Science 2020, published 20 October. Full-text: https://doi.org/10.1126/science.abd2985

Neuropilin-1 (NRP1), known to bind furin-cleaved substrates, significantly potentiates SARS-CoV-2 infectivity, an effect blocked by a monoclonal blocking antibody against NRP1.

Daly JL, Simonetti B, Klein K, et al. **Neuropilin-1 is a host factor for SARS-CoV-2 infection.** Science 2020, published 20 October. Full-text: https://doi.org/10.1126/science.abd3072

More on how S binds to cell surface neuropilin-1 (NRP1) and neuropilin-2 (NRP2) receptors.

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. 15 May 2020: Vol. 368, Issue 6492, pp. 779-782. Full-text: https://doi.org/10.1126/science.abb7498

Using cryogenic electron microscopy, the 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. The authors determined a 2.9-angstrom-resolution structure of the RNA-dependent RNA polymerase (also known as nsp12), which catalyzes the synthesis of viral RNA, in complex with two cofactors, nsp7 and nsp8.

Hillen HS, Kokic G, Farnung L et al. **Structure of replicating SARS-CoV-2 polymerase**. Nature 2020. Full-text: https://doi.org/10.1038/s41586-020-2368-8

The cryo-electron microscopic structure of the SARS-CoV-2 RdRp in active form, mimicking the replicating enzyme. Long helical extensions in nsp8 protrude along the exiting RNA, forming positively charged 'sliding poles'. These sliding poles can account for the known processivity of the RdRp that is required for replicating the long coronavirus genome. A nice video provides an animation of the replication machine.

Chen J, Malone B, Llewellyn E, et al. **Structural basis for helicase-polymerase coupling in the SARS-CoV-2 replication-transcription complex.** Cell 2020, 27 July, 2020. Full-text: https://doi.org/10.1016/j.cell.2020.07.033

A cryo-electron microscopic structure of the SARS-CoV-2 holo-RdRp with an RNA template-product with two molecules of the nsp13 helicase and identify a new potential target for future antiviral drugs.

Wolff G, Limpnes RW, Zevenhoven-Dobbe JC, et al. **A molecular pore spans the double membrane of the coronavirus replication organelle**. Science 06 Aug 2020: eabd3629. Full-text: https://doi.org/10.1126/science.abd3629

Coronavirus replication is associated with virus-induced cytosolic double-membrane vesicles, which may provide a tailored microenvironment for viral RNA synthesis in the infected cell. Visualization of a molecular pore complex that spans both membranes of the double-membrane vesicle and would allow export of RNA to the cytosol. Although the exact mode of function of this molecular pore remains to be elucidated, it would clearly represent a key structure in the viral replication cycle that may offer a specific drug target.

Animals and animal models

Bao L, Deng W, Huang B, et al. **The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice**. Nature. 2020 May 7. PubMed: https://pubmed.gov/32380511. Full-text: https://doi.org/10.1038/s41586-020-2312-y

In transgenic mice bearing human ACE2 and infected with SARS-CoV-2, the pathogenicity of the virus was demonstrated. This mouse model will be valuable for evaluating antiviral therapeutics and vaccines as well as understanding the pathogenesis of COVID-19.

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.

Chandrashekar A, Liu J, Martinot AJ, et al. **SARS-CoV-2 infection protects against rechallenge in rhesus macaques**. Science. 2020 May. PubMed: https://pubmed.gov/32434946. Full-text: https://doi.org/10.1126/science.abc4776

No re-infection in macaques. Following initial viral clearance, 9 rhesus macaques were re-challenged on day 35 with the same doses of virus that were utilized for the primary infection. Very limited viral RNA was observed in BAL on day 1 after re-challenge, with no viral RNA detected at subsequent timepoints. These data show that SARS-CoV-2 infection induced protective immunity against re-exposure in nonhuman primates.

Halfman PJ, Hatta M, Chiba S, et al. **Transmission of SARS-CoV-2 in Domestic Cats**. NEJM May 13, 2020. Full-text: https://www.nejm.org/doi/full/10.1056/NEJMc2013400

Three domestic cats were inoculated with SARS-CoV-2. One day later, an uninfected cat was cohoused with each of the inoculated cats. All six cats became infected and developed antibody titers but none showed any symptoms. Cats may be a silent intermediate host.

Rockx B, Kuiken T, Herfst S, et al. **Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model**. Science 17 Apr 2020. Full text: https://science.sciencemag.org/content/early/2020/04/16/science.abb7314

Macaques may serve as a model to test therapeutic strategies. Virus was excreted from nose and throat in the absence of clinical signs, and was detected in type I and II pneumocytes *in foci* of diffuse alveolar damage and in ciliated epithelial cells of nasal, bronchial, and bronchiolar mucosae. In SARS-CoV infection, lung lesions were typically more severe, while they were milder in MERS-CoV infection, where virus was detected mainly in type II pneumocytes.

Munster VJ, Feldmann F, Williamson BN, et al. **Respiratory disease in rhesus macaques inoculated with SARS-CoV-2**. Nature 2020. Full-text: https://doi.org/10.1038/s41586-020-2324-7

SARS-CoV-2 caused respiratory disease in 8 rhesus macaques, lasting 8-16 days. High viral loads were detected in swabs as well as in bronchoalveolar lavages. This "model" recapitulates COVID-19, with regard to virus replication and shedding, the presence of pulmonary infiltrates, histological lesions and seroconversion.

Sia SF, Yan L, Chin AWH. et al. **Pathogenesis and transmission of SARS-CoV-2 in golden hamsters**. Nature 2020. Full-text: https://doi.org/10.1038/s41586-020-2342-5

In most cases, you don't need monkeys. Golden Syrian hamsters may also work. SARS-CoV-2 transmitted efficiently from inoculated hamsters to naïve hamsters by direct contact and via aerosols. Transmission via fomites in soiled cages was less efficient. Inoculated and naturally-infected hamsters showed apparent weight loss, and all animals recovered with the detection of neutralizing antibodies.

Sit TH, Brackman CJ, Ip SM et al. **Infection of dogs with SARS-CoV-2**. Nature 2020. Full-text: https://www.nature.com/articles/s41586-020-2334-5

Two out of fifteen dogs (one Pomeranian and one German Shepherd) from households with confirmed COVID-19 cases in Hong Kong were found to be infected. Both dogs remained asymptomatic but later developed antibody responses detected using plaque reduction neutralization assays. Genetic analysis suggested that the dogs caught the virus from their owners. It still remains unclear whether infected dogs can transmit the virus to other animals or back to humans.

Dinnon KH, Leist SR, Schäfer A et al. **A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures**. Nature, August 27, 2020. Full-text: https://doi.org/10.1038/s41586-020-2708-8

Unfortunately, standard laboratory mice do not support infection with SARS-CoV-2 due to incompatibility of the S protein to the murine ortholog (mACE2) of the human receptor. This work has developed a recombinant virus (SARS-CoV-2 MA) that could utilize mACE2 for entry. This model may be helpful in studying COVID-19 pathogenesis.

Muñoz-Fontela C, Dowling WE, Funnell SGP, et al. **Animal models for COVID-19**. Nature. 2020 Sep 23. PubMed: https://pubmed.gov/32967005. Full-text: https://doi.org/10.1038/s41586-020-2787-6

Mice, hamsters, ferrets, minks, cats, pigs, fruit bats, monkeys: a variety of murine models for mild and severe COVID-19 have been described or are under development. All will be useful for vaccine and antiviral evalu-

ation and some share features with the human disease. Review (performed by a huge international collaboration).

Vaccine (see also Immunology)

Le TT, Andreadakis Z, Kumar A, et al. **The COVID-19 vaccine development landscape**. Nature reviews drug discovery. 09 April 2020. 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!

Callaway E. **The race for coronavirus vaccines: a graphical guide, Eight ways in which scientists hope to provide immunity to SARS-CoV-2**. Nature 2020, 28 April 2020. 580, 576-577. Full-text: https://doi.org/10.1038/d41586-020-01221-y

Fantastic graphic review on current vaccine development. Easy to understand, it explains different approaches such as virus, viral-vector, nucleic-acid and protein-based vaccines.

Zhu FC, Li YH, Guan XH. **Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial**. Lancet May 22, 2020. Full-text: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31208-3/fulltext

Open label Phase I trial of an Ad5 vectored COVID-19 vaccine, using the full-length spike glycoprotein. A total of 108 healthy adults aged between 18 and 60 years from Wuhan, China, were given three different doses. ELISA antibodies and neutralising antibodies increased significantly and peaked 28 days post-vaccination. Specific T cell response peaked at day 14 post-vaccination. Follow up is still short and authors are going to follow up the vaccine recipients for at least 6 months, so more data will be obtained. Of note, adverse events were relatively frequent, encompassing pain at injection sites (54%), fever (46%), fatigue (44%) and headache (39%). Phase II studies are underway.

Pathogenesis

Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. **Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19**. Cell May 15, 2020. Full-text: https://doi.org/10.1016/j.cell.2020.04.026

Incredible in-depth analysis of host response to SARS-CoV-2 and other human respiratory viruses in cell lines, primary cell cultures, ferrets, and COVID-19 patients. Data consistently revealed a unique and inappropriate inflammatory response to SARS-CoV-2 which is imbalanced with regard to controlling virus replication versus activation of the adaptive immune response. It is defined by low levels of type I and III interferons juxtaposed to elevated chemokines and high expression of IL-6. The authors propose that reduced innate antiviral defenses coupled with exuberant inflammatory cytokine production are the defining and driving features of COVID-19. Given this dynamic, treatments for COVID-19 have less to do with the IFN response and more to do with controlling inflammation.

Bordoni V, Sacchi A, Cimini E. **An inflammatory profile correlates with decreased frequency of cytotoxic cells in COVID-19**. Clinical Infectious Diseases 2020, May 15. Full-text: https://doi.org/10.1093/cid/ciaa577

The increase in inflammatory mediators is correlated with a reduction of innate and adaptive cytotoxic antiviral function. The authors found a lower perforin+ NK cell number in 7 intensive care unit (ICU) patients compared to 41 non-ICU patients, suggesting an impairment of the immune cytotoxic arm as a pathogenic mechanism.

Grifoni A, Weiskopf D, Ramirez SI, et al. **Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals**. Cell 2020. Full-text: https://doi.org/10.1016/j.cell.2020.05.015

Cellular response is a major knowledge gap. This important study identified circulating SARS-CoV-2–specific CD8 and CD4 T cells in 70-100% of 20 COVID-19 convalescent patients, respectively. CD4 T cell responses to spike protein were robust and correlated with the magnitude of IgG titers. Of note, the authors detected SARS-CoV-2–reactive CD4 T cells in 40-60% of unexposed individuals, suggesting cross-reactive T cell recognition between circulating seasonal coronaviruses and SARS-CoV-2.

Li H, Liu L, Zhang D, et al. **SARS-CoV-2 and viral sepsis: observations and hypotheses**. Lancet. 2020 May 9;395(10235):1517-1520. PubMed: https://pubmed.gov/32311318. Full-text: https://doi.org/10.1016/S0140-6736(20)30920-X

Brief but nice review and several hypotheses about SARS-CoV-2 pathogenesis. What happens during the second week - when resident macrophages initiating lung inflammatory responses are unable to contain the virus after SARS-CoV-2 infection and when both innate and adaptive immune responses are inefficient to curb the viral replication so that the patient would recover quickly?

Shen B, Yi X, Sun Y, et al. **Proteomic and Metabolomic Characterization of COVID-19 Patient Sera**. Cell May 27, 2020. Full-text: https://www.sciencedirect.com/science/article/pii/S0092867420306279

Molecular insights into the pathogenesis of SARS-CoV-2 infection. The authors applied proteomic and metabolomic technologies to analyze the proteome and metabolome of sera from COVID-19 patients and several control groups. Pathway analyses and network enrichment analyses of the 93 differentially expressed proteins showed that 50 of these proteins belong to three major pathways, namely activation of the complement system, macrophage function and platelet degranulation. It was found that 80 significantly changed metabolites were also involved in the three biological processes revealed in the proteomic analysis.

Tay MZ, Poh CM, Rénia L et al. **The trinity of COVID-19: immunity, inflammation and intervention**. Nat Rev Immunol (2020). Full-text: https://www.nature.com/articles/s41577-020-0311-8

Brilliant overview of the pathophysiology of SARS-CoV-2 infection. How SARS-CoV-2 interacts with the immune system, how dysfunctional immune responses contribute to disease progression and how they could be treated.

Vabret N, Britton GJ, Gruber C, et al. **Immunology of COVID-19: current state of the science**. Immunity 2020, May 05. Full-text: https://www.cell.com/immunity/fulltext/S1074-7613(20)30183-7

Fantastic review on the current knowledge of innate and adaptive immune responses elicited by SARS-CoV-2 infection and the immunological pathways that likely contribute to disease severity and death.

Other key papers

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. 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.

Thao TTN, Labroussaa F, Ebert N, et al. **Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform**. Nature. 2020 May 4. PubMed: https://pubmed.gov/32365353. Full-text: https://doi.org/10.1038/s41586-020-2294-9

An important technical advance, enabling the rapid generation and functional characterization of evolving RNA virus variants. The authors show

the functionality of a yeast-based synthetic genomics platform to genetically reconstruct diverse RNA viruses (which are cumbersome to clone and manipulate due to size and instability). They were able to engineer and resurrect chemically-synthetized clones of SARS-CoV-2 only a week after receipt of the synthetic DNA fragments.

Gordon DE, Hiatt J, Bouhaddou M, et al. (Total: 200 authors) **Comparative host-coronavirus protein interaction networks reveal pan-viral disease mechanisms.** Science 2020, published 15 October. Full-text: https://doi.org/10.1126/science.abe9403

A group of 200 researchers uncovers molecular processes used by coronaviruses MERS, SARS-CoV1 and SARS-CoV2 to manipulate host cells.

5. Variants

Bernd Sebastian Kamps

Published 31 January

With viruses, some mutations emerge while others recede. Rarely does one or more mutations confer a "selective advantage" to a new variant, for example enhanced transmissibility. Such variants can then become the new dominant virus.

Introduction

Over the last two months, several new SARS-CoV-2 variants have been described that are more transmissible, may escape both natural and vaccine-induced immunity and could impact COVID-19 morbidity and mortality. It is too early to assert that these variants will create *a new pandemic within the pandemic*, however, in countries like England, South Africa, Brazil, Ireland, Portugal and Israel, they may have modified the dynamic of the latest outbreaks for the worse. More transmissible SARS-CoV-2 variants will replace older variants – everywhere! Countries where the prevalence of these new variants is still low should anticipate rapid spread within the next weeks and months and plan ahead accordingly, ie closing/restricting borders, etc.

The current *trio infernale*:

- B117 (first described in England; Rambaut 2020)
- B1351 (first described in South Africa; Tegally 2020)
- P1 (first described in Brazil; Faria 2021)

Of note, although these variants evolved independently in different places around the globe, they share key mutations which are involved in receptor binding. This viral evolution is a normal process known from seasonal coronaviruses (Wong AHM 2017, Eguia 2020, Kistler 2020) and has recently been reproduced *in vitro* (Zahradnik 2020). Convergent evolution suggests that under the pressure of an increasing number people having developed antibodies against SARS-CoV-2, the virus is developing a more perfect configuration. The new variants represent now (31 January):

- The vast majority of newly isolated strains in England (B117), South Africa (B1351), Amazonia (P1; Fiocruz 20210129)
- More than half of newly isolated strains in Ireland
- As many as half of newly isolated strains in Portugal and Israel (B117)

The variant mutations may affect the COVID-19 pandemic in multiple ways:

- Increased transmissibility
- Increased severity of illness
- Diminished protection from previous SARS-CoV-2 infection
- Diminished response to vaccines
- Diminished response to monoclonal antibodies

A 30% to 40% higher mortality after infection with B117 has recently been described (Horby 2021); however, these are preliminary data that need to be confirmed. In any case, a higher rate of transmission will lead to more COVID-19 cases, increase the number of persons who need clinical care, exacerbate the burden on an already strained health care system, and finally result in more deaths (Galloway 2020). The increased transmissibility of new variants may therefore require an even more rigorous implementation of vaccination and mitigation measures (e.g., distancing, masking, and hand hygiene) to control the spread of SARS-CoV-2. These measures will be more effective if they are instituted sooner rather.

Epidemiology

Figure 1 shows the daily new confirmed COVID-19 cases in selected European countries. The increased transmissibility of B117 has led to an increased number of infections in several countries, which in turn has led to higher hospitalisation and death rates. Find more information about B117 on page 185.

At the end of January 2021, most countries had incomplete information on the true percentage of people infected with the new variants as only very few samples had been sequenced. The new variants, in particular B117, are expected to expand over the coming weeks and months. Figure 2 presents an overview of information on B117, B1351 and P1. For detailed epidemiological information on laboratory-confirmed cases in Europe, find the weekly ECDC COVID-19 surveillance report (https://covid19-surveillance-report.ecdc.europa.eu) and the weekly country overview (https://covid19-country-overviews.ecdc.europa.eu).

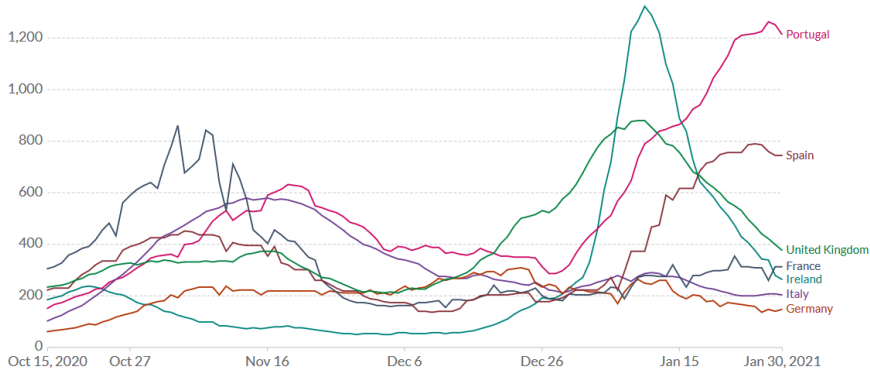

Figure 1. 31 January: Portugal, Spain, United Kingdom, France, Ireland, Italy, Germany. Daily new confirmed COVID-19 cases per million people (rolling 7-day average). Source: Our World in Data – Johns Hopkins University CSSE COVID-19 Data.

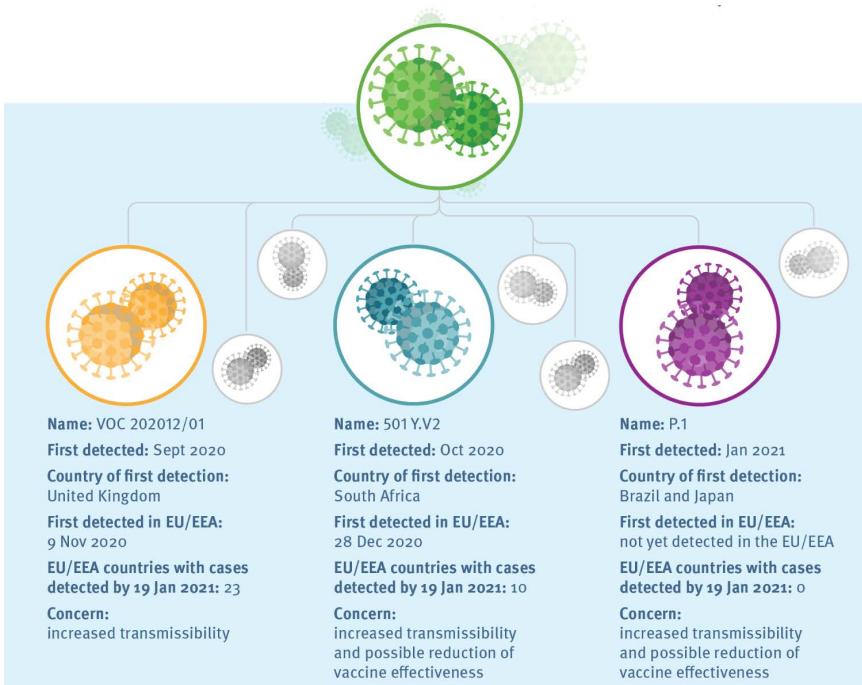

Figure 2. Mutation of SARS-CoV-2. VOC 202012/01: **B117** – 501Y.V2: **B1351**. Source and copyright: ECDC Infographics, 21 January.

United Kingdom

In the UK, the first case of the B117 variant (ie, 20B/501Y.V1 or VOC 202012/01) was retrospectively dated to 20 September. In Kent, a county in South East England, cases continued to increase during a lockdown in November, despite having the same restrictions as other regions. When, on 2 December, England lifted its lockdown, the proportion in England of B117 continued to increase sharply in Kent and then rapidly in Greater London and other parts of the southeast (Kirby 2021), rising to over 70% at the beginning of January 2021. Areas with the highest B117 incidence coincided with areas now reporting high levels of patient hospitalisation (Gravagnuolo 2021). In December, the seven-day notification rate rose from 162 cases per 100.000 population at the end of week 49, to 345 for week 51 and 595 for week 53 of 2020 (corresponding to a 3,7-fold increase in week 53 compared to week 49 2020; see Figure 3) (ECDC 20210121).

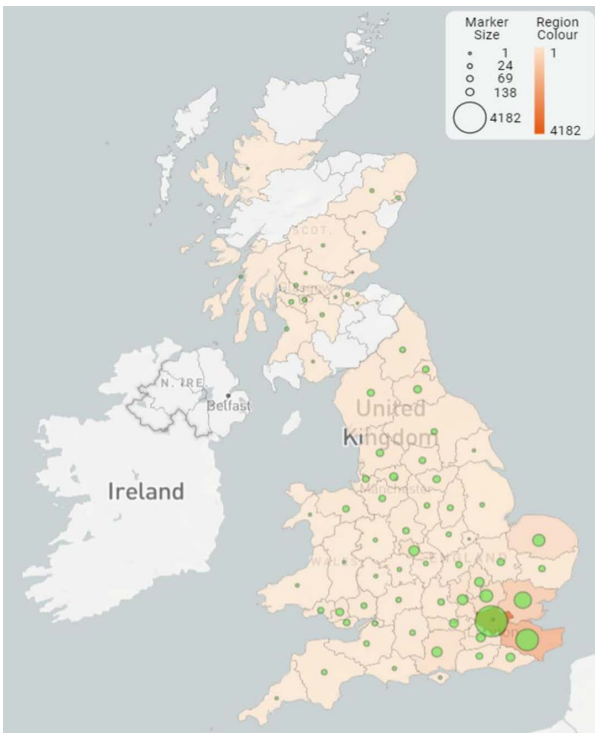

Figure 3. Distribution of VOC 202012/01 cases confirmed by sequencing, UK, 1 September 2020 to 9 January 2021. Source and copyright: European Centers for Disease Control, Stockholm (ECDC 20210121)

The good news from the UK: lockdowns are efficient against the SARS-CoV-2 variant too. After a peak of the rolling 7-day average on 9 January, the numbers started decreasing (Figure 4).

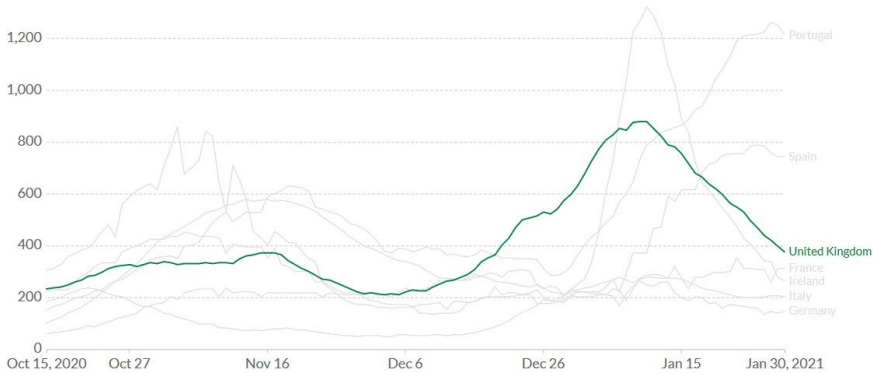

Figure 4. United Kingdom, 31 January. Lockdowns are efficient against B117. After a peak of the rolling 7-day average on 10 January, the number of daily new confirmed SARS-CoV-2 cases decreased rapidly. Source: Our World in Data – Johns Hopkins University CSSE COVID-19 Data.

South Africa

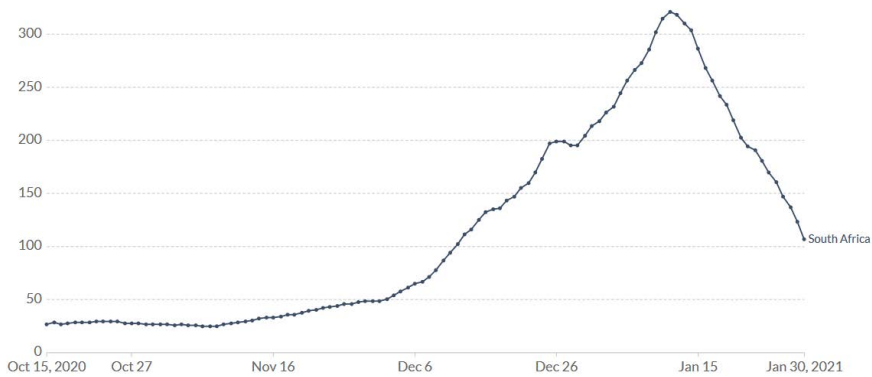

Figure 5. South Africa, 31 January. Lockdowns are efficient against B1351. After a peak of the rolling 7-day average on 11 January, the number of daily new confirmed SARS-CoV-2 cases decreased rapidly. Source: Our World in Data – Johns Hopkins University CSSE COVID-19 Data.

In South Africa, B1351 (also called 501Y.V2) was first detected in early October 2020 and is now the most prevalent variant in the country. Like B117, it has an increased transmissibility. As of 31 January, B1351 has been identified in more than two dozen countries. One cluster of this variant is currently being investigated in France. In addition to France, Israel and the UK have also reported cases or clusters of non-travel-related B1351 cases (ECDC 20210121). Lockdowns are efficient against B1351. After a peak of the rolling 7-day average on 11 January, the numbers decreased rapidly (Figure 5). Find more information about B1351 on page 187.

Brazil

In January 2021, the P1 variant was identified in 42% (13 out of 31) of RT-PCR positive samples collected between 15 and 23 December in Manaus, Amazonia, Brazil (Faria 2021). At the time, Manaus was experiencing an upsurge in COVID-19 cases. P1 has 10 mutations in the spike protein (Faria 2021) and some, including N501Y and E484K, have been reported in B117 and B1351, the variants first detected in the UK and South Africa. Find more information about P1 and on the situation in Manaus in January 2021 on page 190.

Israel

At the end of January, due to a swift start of vaccination targeted toward older ages, a majority of people over 60 were fully vaccinated by the end of January. As a result, 75% of all recent new infections have occurred in people younger than 30 years. B117 represents now around 40% of all new infections. It may have been partly responsible for a late decline in the number of new cases (Figure 6, red line) and deaths (black line) after the 27 December lockdown.

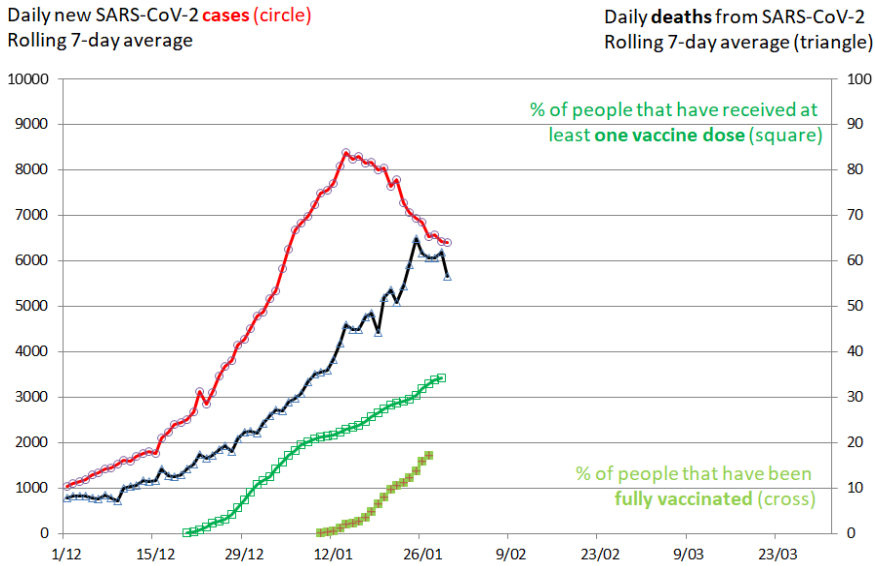

Figure 6. SARS-CoV-2 cases in Israel. 31 January 2021. Impact of mass vaccination on the pandemic. The rolling 7-day average of new SARS-CoV-2 cases is shown in red circles (left vertical axis), the rolling 7-day average of deaths as a plain black line (right vertical axis). The percentage of people that have received at least one vaccine dose is shown in green squares. The percentage of people that have been fully vaccinated is shown in green-yellow crosses. As the country entered a third lockdown on 27 December, the evolution of daily new cases and deaths are influenced by the lockdown measures, transmissibility and the vaccination campaign.

Ireland

In Ireland, the proportion of B117 was low as late as early December 2020 and Ireland seemed to be a model of a "good" pandemic response. One month later, in early January, B117 accounted for 46% of samples tested (ECDC 20210121). The country of approximately 5 million inhabitants recorded 50.000 new SARS-CoV-2 cases in just one week and was the place in the world where the virus was spreading the fastest, with 1323 new daily cases detected per million inhabitants. The reason for the sudden resurgence is probably linked to a combination of factors: Christmas, pubs, restaurants, relaxation due to having done so well (less masks, less distancing) ... plus B117. Good news also from Ireland: lockdowns are efficient against B117. After a peak of the rolling 7-day average on 10 January, the numbers decreased rapidly.

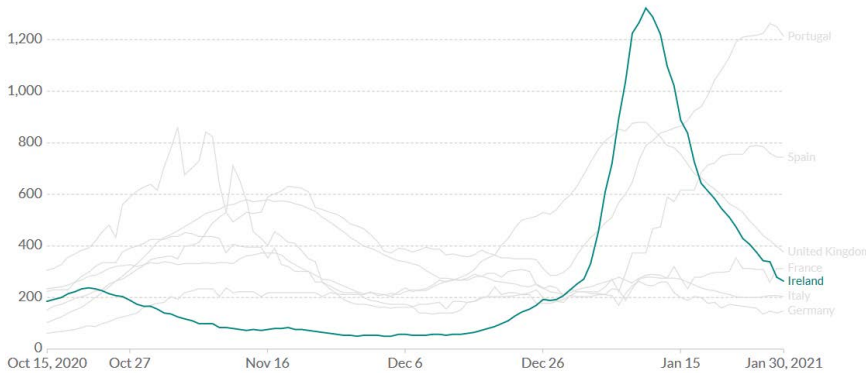

Figure 7. Ireland, 31 January. Lockdowns are efficient against B117. After a peak of the rolling 7-day average on 10 January, the number of daily new confirmed SARS-CoV-2 cases decreased rapidly. Source: Our World in Data – Johns Hopkins University CSSE COVID-19 Data.

Paris, France

According to health authorities in Paris (Assistance publique – Hôpitaux de Paris, AP-HP), the B117 variant of SARS-CoV-2 represented 9,4% of 1080 cases detected in Île-de-France (the Greater Paris area) between January 11 and 21 (Le Monde 20210126). Three weeks earlier, a quick survey had found a national rate of only 2,5%.

Denmark

At the end of January, while Denmark was still in lockdown and cases declining rapidly, B117 was continuing to increase in frequency. On 24 January, the Danish COVID-19 Genome Consortium published the percentage of B117 samples of the previous 4 weeks: 2,4%, 4,0%, 7,4%, and 12,1% (DCGC 20210124).

Netherlands

In the Netherlands, random sampling of SARS-CoV-2 positive samples indicated that 10% of the samples identified in week 1 of 2021 were B117 (ECDC 20210121). Amsterdam and Rotterdam are B117 hotspots.

Portugal

Portugal is currently experiencing the third wave of the pandemic, attributed mainly to Christmas and New Year's festivities (relaxation, less masks, less distancing). On 12 January, the Portuguese National Health Institute (INSA,

Instituto Nacional de Saúde Doutor Ricardo Jorge) had reported 72 B117 cases (INSA 20210112) but according to a paper pre-published on 19 January press report, data collected between 11 and 17 January showed a prevalence of B117 of 13%. The authors anticipate that B117 might reach up to 60% of positive cases in early February 2021 (Borges 2021). Robust public health measures on the horizon. In Lisbon and the Tagus Valley, B117 may already represent half of new cases the most affected by the third wave. Of the 11.600 deaths recorded since the start of the pandemic, nearly 3000 have been recorded in the past two weeks.

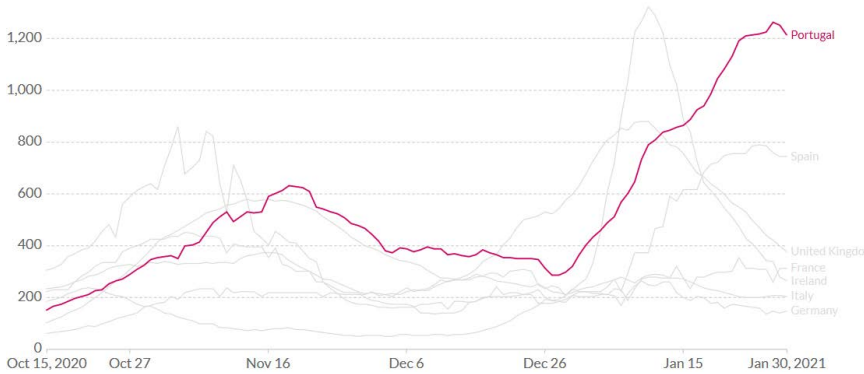

Figure 8. Portugal, 31 January. Daily new confirmed SARS-CoV-2 cases. The third pandemic wave is not yet under control. Source: Our World in Data – Johns Hopkins University CSSE COVID-19 Data.

Transmission

The epidemiological consequences of B117, B1351, P1 and those variants to come are currently not predictable. While it is still unclear whether and to what extent the new variants may cause more severe illness and more deaths (see the discussion of a very preliminary report in the paragraph *Clinical Consequences,* page 180), it is acknowledged that they probably all have a substantial transmission advantage (+ 30%?, + 50%?, + 70%?) (Davies 2020, Volz 2021, Leung 2021). Increased transmission will lead to more SARS-CoV-2 infections and more hospitalizations and might significantly increase the number of deaths over the coming months. Figure 5.2 depicts a simplified scenario showing the number of new deaths every six days from three different viral strains, assuming each strain started from 10.000 infections. It shows how a more infectious virus may lead to more deaths.

Figure 9. A more infectious virus could lead to many more deaths. Simplified, hypothetical scenario showing the number of new deaths every six days from three different virus strains, assuming each strain started from 10.000 infections. Source: Adam Kucharski, Associate Professor, London School of Hygiene and Tropical Medicine.

Clinical Consequences

Initially, there was no indication of increased morbidity or mortality associated with B117. An early cohort study found no statistically significant difference in hospitalisation and 28-day case fatality between cases with B117 and wild-type comparator cases (Public Health England 20201228, Public Health England 20210105). Then, on 21 January, the British NERVTAG (New and Emerging Respiratory Virus Threats Advisory Group) announced preliminary evidence of a "realistic possibility" that infection with B117 might be associated with a 30% - 40% increased risk of death compared to infection with non-B117 viruses (Horby 2021). The increase in case fatality rate would seem to hold across all age groups. As the same report also mentioned that there was no evidence of an increased risk of hospitalization in individuals with B117, we should wait for further results before concluding that the new SARS-CoV-2 variants have an impact on morbidity and mortality. There are as yet no data on different clinical outcomes after infection with B1351 or P1.

Immune escape

The *Question of the Year 2021* is, "Will immunity – natural or vaccine induced – be effective against evolving SARS-CoV-2 variants or will the variants be able to escape human immunity?" In other words: will B117, B1351, P1 or other upcoming variants be able to

- Escape naturally acquired immunity
- Escape vaccine induced immunity
- Escape the effect of monoclonal antibodies

Acquired immunity

There is growing evidence for a long-lived and robust T cell immunity generated after natural SARS-CoV-2 infection (Neidleman 2020). Reinfections with phylogenetically distinct SARS-CoV-2 strains that have been reported were usually milder than the first episode (To 2020, Gupta 2020, Van Elslande 2020, Tillett 2021, Iwasak 2021), with only occasionally more severe disease being reported (Larson 2020). These reports are certainly only the tip of an iceberg of hundreds or thousands of potentially undetected reinfections worldwide; however, today, 9 months after the first COVID-19 wave, there is no documented epidemic of reinfections, not even in countries where B117 has largely replaced the previously circulating SARS-CoV-2 strains. The prospect of reinfection with antigenically distinct variants is real but doesn't seem thus far a major concern.

In this reassuring context, the case of Manaus, Brazil, may need to be interpreted. The city of 2 million in the Amazon region saw a first pandemic wave in spring 2020 and a second one starting at the end of the year. The second catastrophic wave came as a surprise (see the insert on page 192) because in September 2020, a pre-print paper reporting serological data of *blood donors* from Manaus had announced "COVID-19 herd immunity in the Brazilian Amazon" - as much as 66% of the population of Manaus had already have been infected with SARS-CoV-2 (Buss 2020). Three months later, the paper appeared in *Science* with the title "Three-quarters attack rate of SARS-CoV-2 in the Brazilian Amazon during a largely unmitigated epidemic" (Buss 2020b). Today, the question is why can there be such a catastrophic situation in Manaus at the end of January, with 400 people not finding a place in hospital and 73 people not finding an urgently needed place in intensive care units (Leandro Resende, CNN Brasil, 29 January) if "three-quarters" of the population had been immunized during the first first wave? Should we assume that immunity against SARS-CoV-2 wanes after less than a year? Or, worse, that

new variants like P1 are able to evade immunity? Or might it be suspect that there never was a 75% attack rate in Manaus? 40 years ago we would not have used data from blood donors to make predictions on the AIDS pandemic. Extrapolating data from blood donors to the general population may be risky even today. We should wait for more data and hope that the *Lancet* paper published on 27 January (Sabino, Buss 2021) was not a semi-retraction of a *Science* paper (Buss 2020b).

Vaccine-induced immunity

The three vaccines licensed in Europe and the US – Pfizer-BioNTech's Comirnaty as well as the Moderna and the Oxford-AstraZeneca vaccines – all target the spike protein of the virus, where B117, B1351 and P1 have several mutations. Will vaccine-induced neutralizing antibodies be effective against these variants? They probably will, albeit to a lesser extent. Real life results from two clinical vaccine trials – Janssen's ENSEMBLE and Novavax's NVX-CoV2373 – have shown that the level of protection against moderate to severe COVID-19 infection was lower in South Africa where B1351 has been the predominant variant of late. The Janssen vaccine candidate provided a level of protection against moderate to severe COVID-19 infection of 57% in South Africa and 72% in the United States (JNJ 20210129) while the Novavax product provided a level of protection against mild & moderate-to-severe COVID-19 infection of 60% in South Africa and 89,3% in the UK (Novavax 20210128). The Novavax trial also found that their vaccine candidate was more efficient against the original COVID-19 strain (95,6%) than against B117 (85,6%). It is quite possible that SARS-CoV-2 vaccines will need to be reformulated – a challenge most companies have already accepted (see *Outlook*, page 197). Find more information about immune escape on page 187 (B117) and page 189 (B1351).

These real-life data were somewhat anticipated by *in vitro* studies. An analysis of 579 COVID patients samples collected between March and July 2020 suggested that the B117 mutation would not result in immune evasion for a large majority of these COVID patients (Haynes 2021). An analysis of immune sera from individuals vaccinated with the Pfizer-BioNTech vaccine (Comirnaty™) showed that B117 seems unlikely to escape vaccine-mediated protection (Muik 2021). The authors had investigated SARS-CoV-2-S pseudoviruses bearing either the Wuhan reference strain or the B117 lineage spike protein with sera of 16 participants in a previously reported trial with the mRNA-based COVID-19 vaccine Comirnaty. The immune sera had equivalent neutralizing titers to both variants.

In vitro data for B1351 and P1 had already suggested greater concern. Both variants harbor the E484K ("Erik") mutation (Tegally 2020, Voloch 2020) which seems to be the "bad boy on the block". A recently published map of all amino acid mutations to the SARS-CoV-2 spike receptor-binding domain (RBD) shows that the site where mutations tend to have the largest effect on antibody-binding and neutralization is E484 (Greaney 2020). In a study by David H. Ho and colleagues, the serum of 12 people vaccinated with Moderna's vaccine and 10 people vaccinated with the Pfizer-BioNTech vaccine was six to nine times less potent against B1351. Serum from 20 previously infected people was 11 to 33 times less potent (Wang P 2021). E484K accounted for much of the effect.

Escape from monoclonal antibodies

Monoclonal antibodies are a potential treatment for SARS-CoV-2. The receptor binding domain (RBD) of of the virus is the prime target of the neutralizing response during infection (Rogers 2020, Piccoli 2020, Barnes 2020, Robbiani 2020) and most antibodies target this region (Piccoli 2020, Tzou 2020). Recent studies have shown that a single amino-acid mutation can fully escape the recently approved REGN-COV2, which consists of two antibodies targeting distinct structural epitopes (Starr 2021). The authors also identified viral mutations that are selected in a persistently infected patient treated with REGN-COV2, as well as during *in vitro* viral escape selections. Earlier, there was evidence that one of the spike protein mutations, E484K, might affect neutralization by some polyclonal and monoclonal antibodies (Greaney 2020, Weisblum 2020). Now, some variants have been shown to exhibit complete escape from relevant monoclonal antibodies (Wibmer 2021, Wu K 2021, Wang Z 2021). All monoclonal antibodies in clinical use will have to be tested against newly arising variants. Stiff winds ahead.

The Variants

Nomenclature

The pandemic spread of SARS-CoV-2 has resulted in the generation of tens of thousands of viral genome sequences. Earlier there was a need for a rational and dynamic viral nomenclature that would account for the expanding phylogenetic diversity of SARS-CoV-2. Such a scheme has been proposed by Andrew Rambaut et al. and is now generally accepted (Rambaut 2020b). The new variants first discovered in the UK, South Africa and Brazil are called B1.1.1.7, B.1.351, and P.1, respectively (Table 1). For simplicity's sake, they are commonly called B117, B1351 and P1.

Table 1. Adapted from Eric Topol: https://bit.ly/3tcqEsr

Commenly used name	B117	B1351	P1
Where first detected	UK	South Africa	Brazile
Official name	B.1.1.7	B.1.351	P.1
Other names used in the scientific literature	N501Y.V1 20B/501Y.V1 VOC 202012/1	N501Y.V2 20H/501Y.V2 VOC 202012/2	N501Y.V3 VOC 202012/3
Mutations	23	21	17
Spike mutations	8	9	10
Key RBD, spike mutations beyond N501Y in all	69/70 del, P681H, A570D, Y144 del	E484K, K417N, orf1b deletion	E484K, K417N, orf1b deletion
Other mutations, including N-terminal	T716I, S982A, D1118H	L18F, D80A, D215G, Δ242-244, R264I	L18F, T20N, P26S and others
Transmissibility Δ	<40% increased	Not established	Not established
Lethality Δ	Concern raised, not resolved	Not established	Not established
Immune escape	Not established	Probably, extent unclear (in vitro)	Probably, extent unclear (in vitro)
Countries reported (does not necessarily imply local transmission)	70	31	8

SARS-CoV-2 evolution

Viruses evolve (mutate)

- when they come under pressure or
- when they are given weeks and months to do so.

Situations of pressure include areas where an explosive epidemic infects large proportions of the population, killing some, but making most people immune. In these settings or in settings where most people, but not all, are immunized through vaccination, only mutant variants that are able to spread despite

existing post-infection immunity can sustain continuous chains of transmission.

In an entirely different situation, immunodeficient and chronically infected individuals can be silent incubators of accelerated viral evolution. Such infections are rare, and onward transmission from them presumably even rarer, but they are not improbable (Rambaut 2020). High rates of mutations have been reported in immunodeficient or immunosuppressed patients who were chronically infected with SARS-CoV-2. One paper describes the 154-day clinical course of a 45-year-old man with severe antiphospholipid syndrome who was receiving Immunosuppressants medication (Choi 2020). Of interest, amino acid changes were predominantly in the spike gene and the receptor-binding domain, which make up 13% and 2% of the viral genome, respectively, but harbored 57% and 38% of the observed changes. Another report describes the history of an immunocompromised individual with chronic lymphocytic leukemia and acquired hypogammaglobulinemia (Avanzato 2020). In this case, shedding of infectious SARS-CoV-2 was observed up to 70 days.

In the following, we will briefly discuss

- B117 (first detected in England)
- B1351 (first detected in South Africa)
- P1 (first detected in Brazil)

B117

History and epidemiology

On 14 December 2020, the UK reported to WHO the B117 variant (referred to by the UK authorities as *SARS-CoV-2 VOC 202012/01*: *Variant of Concern, year 2020, month 12, variant 01*) (WHO 20201231). This variant was first detected in September 2020. Phylogenetic studies carried out by the UK COVID-19 Genomics Consortium soon showed that the new variant had an unusual accumulation of substitutions and was growing at a large rate relative to other circulating lineages (Volz 2021). Within weeks, B117 began to replace other viral lineages and as early as November/December 2020, it became the dominant strain in England. As of 20 December 2020, the regions in England with the largest numbers of confirmed cases of the variant were London, the South East, and the East of England (Volz 2021). From there, B117 quickly spread all over the country & around the world (Du Z 2021). Between 30 November 2020 and 20 December 2020, 41% of 9321 UK cases that had genomic sequencing data included were B117 (Public Health England 20210105). As of 19 January, B117 had been identified in 60 other countries (Figure 4). After the UK, the three

non-EU/EEA countries reporting most cases were Israel, India and the United States (ECDC 20210121) (Figure 10).

A short graphical guide to B117 has been published in the lay press by Corum & Zimmer.

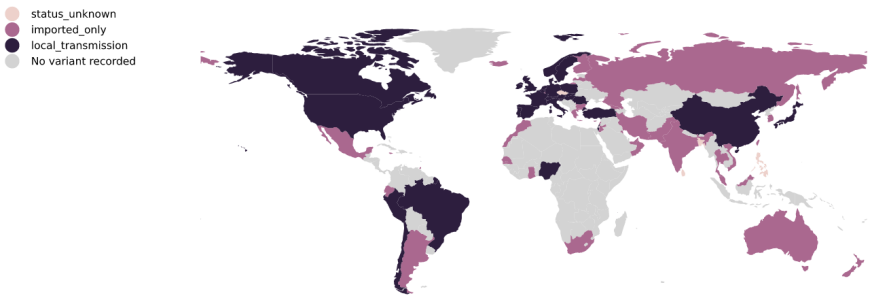

Figure 10. Map of B117 local transmission, 31 January 2021 | Colours indicate reports of imported cases (pink) or of local transmission (darker purple). Data is obtained from news reports and similar sources and is manually maintained. Source and copyright: PANGO lineages, Áine O'Toole and Verity Hill, Rambaut Group, University of Edinburgh.

Virology

B117 has emerged with an unusually large number of mutations in the spike protein (deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H) as well as in other genomic regions (Rambaut 2020). At the moment of discovery, the accrual of 14 lineage-specific amino acid replacements was unprecedented. Three of these mutations are of particular concern:

- Mutation N501Y is one of the six key amino acids interacting with ACE2 receptor and experimental data suggests that it increases ACE2 receptor affinity (Starr 2020). The tyrosine substitution has been shown to have increased binding affinity to the ACE2 receptor (Chan 2020). N501Y has been associated with increased infectivity and virulence in a mouse model (Gu H 2020). [Remember: the receptor binding domain (RBD) of the SARS-CoV-2 spike protein mediates viral attachment to ACE2 receptors. It is a major determinant of host range and a dominant target of neutralizing antibodies.]
- Deletion 69-70 is one of a number of deletions observed in the N terminal domain of the spike protein (McCarthy 2020, Kemp 2020) and is associated with reduced sensitivity to neutralization by SARS-CoV-2 human convalescent serum. It also arose in the mink-associated outbreak in Denmark with the background of the Y453F RBD mutation,

and in humans in association with the N439K RBD mutation, accounting for its relatively high frequency in the global genome data (~3000 sequences) (Rambaut 2020).

- Mutation P681H is immediately adjacent to the furin cleavage site between S1 and S2 in spike. [The S1/S2 furin cleavage site of SARS-CoV-2 is not found in other human coronaviruses and has been shown to promote entry into respiratory epithelial cells and transmission in animal models (Hoffmann 2020).]

Transmission

Preliminary epidemiologic, modelling, phylogenetic and clinical findings suggest that B117 may be significantly more transmissible (+50% to +75%) than previously circulating variants (Leung 2020, Volz 2021).

Clinical consequences

The British NERVTAG (New and Emerging Respiratory Virus Threats Advisory Group) announced in January 2021 preliminary evidence that there is a "realistic possibility" that B117 infection might be associated with a 30% to 40% increased risk of death compared to infection with non-B117 viruses (Horby 2021). These data need to be confirmed. See also the comment on page 180, paragraph *Clinical Consequences*.

Immune escape

The Novavax trial found that their vaccine candidate was more efficient against the original COVID-19 strain (95,6%) than against B117 (85,6%) (Novavax 20210128). These data will now be confronted with *in vitro* studies which suggest that sera from individuals who have been infected with non-B117 lineages show neutralising activity against B117 virus, and *vice versa* (Public Health England 20210115). A small study investigating 16 participants who had received the Pfizer-BioNTech vaccine Comirnaty showed that the immune sera had equivalent neutralizing titers to both the B117 variant and the previous Wuhan reference strain. The authors concluded – maybe prematurely – that these data, together with the combined immunity involving humoral and cellular effectors induced by this vaccine, would make it unlikely that the B117 lineage will escape Comirnaty-mediated protection (Muik 2021).

B1351

History and epidemiology

On 18 December, national authorities in South Africa announced the detection of a new SARS-CoV-2 variant. The earliest detection had been traced back to October 2020 (South African Government 20201218). It emerged in a severely affected metropolitan area, Nelson Mandela Bay, located on the coast of the Eastern Cape Province. B1351 rapidly spread and largely replaced other SARS-CoV-2 viruses circulating in the Eastern Cape, Western Cape, and KwaZulu-Natal provinces. Within weeks it became the dominant lineage in the Eastern Cape and Western Cape Provinces (Tegally 2020, WHO 20201231).

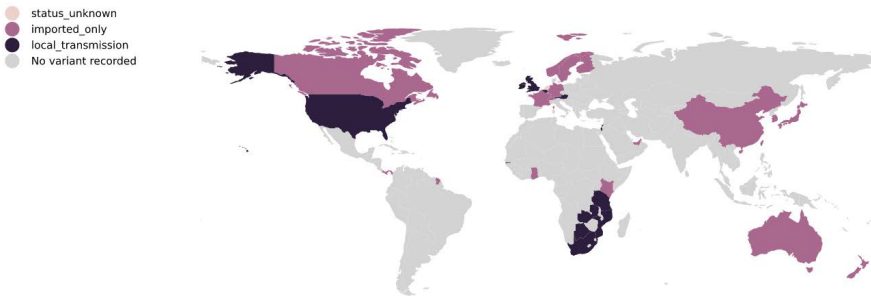

Figure 11. Map of B1351 local transmission, 31 January 2021 | Colours indicate reports of imported cases (pink) or of local transmission (darker purple). Data is obtained from news reports and similar sources and is manually maintained. Source and copyright: PANGO lineages, Áine O'Toole and Verity Hill, Rambaut Group, University of Edinburgh.

As of 19 January 2021, the number of B1351 cases reported outside South Africa was increasing. Most of the other B1351 cases detected in the European Union are travel-related, even though not all are from South Africa. Several B1351 clusters have been found in several French regions, with cases belonging to a number of households, including children attending schools. Of 29 cases identified, only five were travel-associated (returning from Mozambique) (ECDC 20210121) (Figure 11).

Virology

The first description of the B1351 lineage found 8 mutations within two immunodominant domains of the spike protein: one cluster in the N-terminal domain (NTD) that includes four substitutions and a deletion (L18F, D80A, D215G, Δ242-244, and R246I), and another cluster of substitutions including three at important residues in the receptor-binding domain (K417N, E484K and N501Y) (Tegally 2020). Unlike the B117 lineage detected in the UK, B1351 does not contain the deletion at 69/70.

Transmission

While the full significance of the B1351 mutations described above is not yet clear, the genomic and epidemiological data suggest that this lineage may be associated with increased transmissibility (Tegally 2020). A mathematical model has estimated that B1351 could be 50% more transmissible than previously circulating variants in South Africa (Pearson 2021).

Clinical consequences

At this stage, there is no clear evidence to suggest that B1351 has any impact on disease severity. A more precise picture will evolve over the next few months.

Immune escape

Preliminary data of a clinical trial on Novavax's protein-based COVID-19 vaccine candidate NVX-CoV2373 suggest reduced vaccine efficacy against B1351. In the South Africa trial that enrolled over 4400 patients, efficacy was 60% (95% CI: 19,9 – 80,1) for the prevention of mild, moderate and severe COVID-19 disease. That was significantly lower than the 89,3% efficacy found in an analogous trial in the UK (Novavax 20210128). In South Africa, 29 cases were observed in the placebo group and 15 in the vaccine group. During the study period, B1351 was widely circulating in South Africa. Preliminary sequencing data for 27 of 44 COVID-19 events showed that 92,6% (25 out of 27 cases) were B1351 (Novavax 20210128). Equally preliminary data of Janssen's clinical ENSEMBLE trial inform that efficacy of Ad26.COV2.S (Mercado 2020) was 57% in South Africa (which has B1351), 66% in Latin America and 72% in the United States (JNJ 20210129). The excellent news is that this one-shot vaccine candidate *has* efficacy against the variants present in Latin America and South Africa. Of note, Ad26.COV2.S demonstrated complete protection against COVID-19 related hospitalization (no ICU admission, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO)) or death as of day 28 after vaccination.

These real-life data will now be confronted with *in vitro* studies of people aged 18-55 years who had received two 100 µg doses of mRNA-1273 vaccine which showed there was a 6-fold reduction in neutralizing titers against B1351 relative to prior variants. As these neutralizing titers remained above those previously found to be protective in non-human primate challenge studies, the producer of mRNA-1273 was confident that they would remain above levels that are expected to be protective (Moderna 20210125).

Recent research results might be less encouraging for monoclonal antibodies. B1351 has been shown to exhibit complete escape from three classes of ther-

apeutically relevant monoclonal antibodies (Wibmer 2021). In another study, after examining the neutralizing effect of convalescent plasma collected from six adults hospitalized with COVID-19, Tulio de Oliveira, Alex Sigal and colleagues found that mutations in B1351 caused the virus to lose much of its sensitivity to antibodies, with IC50 6 to 200-fold higher relative to first-wave virus (Cele 2021). Finally, a study by David H. Ho and colleagues found that the serum of 12 people vaccinated with Moderna's vaccine and 10 people vaccinated with the Pfizer-BioNTech vaccine was six to nine times less potent against B1351. Serum from 20 previously infected people was 11 to 33 times less potent (Wang P 2021). E484K accounted for much of the effect.

P1

History and epidemiology

On January 6, 2021, the National Institute of Infectious Diseases (NIID) of Japan detected a new variant isolate of SARS-CoV-2 in isolates collected at airport screening from four travelers who arrived in Tokyo from Amazonas, Brazil, on January 2, 2021 at airport screening. The isolate had some mutations found in previously reported variant isolates of concern from the UK and South Africa (NIID 20210112). The new variant isolate had 12 mutations in the spike protein, including N501Y and E484K.

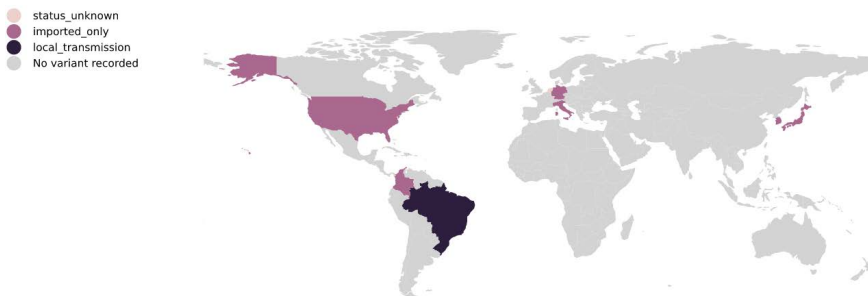

Figure 12. Map of B117 local transmission, 31 January 2021 | Colours indicate reports of imported cases (pink) or of local transmission (darker purple). Data is obtained from news reports and similar sources and is manually maintained. Source and copyright: PANGO lineages, Áine O'Toole and Verity Hill, Rambaut Group, University of Edinburgh.

A few days later, on 12 January 2021, a pre-print article described a variant detected in Manaus, Brazil, identical to the one detected in Japan (Faria 2021). The new variant, P1, was identified in 42% (13 out of 31) of RT-PCR positive samples collected between 15 and 23 December in Manaus (Faria 2021). At the time, Manaus was experiencing an upsurge in COVID-19 cases.

Virology

The new P1 lineage carries 17 unique amino acid changes, 3 deletions, and 4 synonymous mutations, and one 4nt insertion compared to the most closely related available non-P1 sequence (EPI_ISL_722052) (Faria 2021). P1 has 11 amino acid changes in the spike protein compared to its ancestral lineage B.1.1.28 (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F). Some mutations, such as E484K, N501Y and K417T, might influence antibody and vaccine efficacy. The variant is not closely related to B117 or B1351.

The P1 lineage and B117 (first described in the UK - Rambaut 2020) share the spike N501Y mutation and a deletion in ORF1b (del11288-11296, 3675-3677 SGF). P1 and B1351 (first described in South Africa - Tegally 2020) share three mutation positions in common in the spike protein (K417N/T, E484K, N501Y) (Faria 2021). Both the P1 and the B1351 lineage also have the orf1b deletion del11288-11296 (3675-3677 SGF) (Faria 2021).

Transmission

Manaus, the largest city in the Amazon region, has seen a dramatic surge in SARS-CoV-2 infettions since mid-December. As this coincides with a report of more than 40% RT-PCR positive P1 samples collected between 15 and 23 December (Faria 2021), it is tempting to assume that the new variant has led to an increase in transmissibility of the virus. A previous pre-print paper claiming 'herd immunity-like' infection rates in September should be interpreted with caution (see the paragraph *Acquired immunity*, page 181).

Clinical consequences

At this stage, there is no clear evidence to suggest that P1 has any impact on disease severity. A more precise picture will evolve within the next months.

Immune escape

As yet it is unclear if the mutation in the P1 variant affects the ability of antibodies generated through a previous natural infection or through vaccination to recognize and neutralize the virus. In particular the presence of the mutation E484K could indicate a reduction in antibody neutralization (Greaney 2021, Greaney 2021b, Andreano 2020).

Manaus 2021

See also the paragraph *Acquired immunity*, page 181.

Galarraga Gortázar N, Schmidt S. **La pesadilla de morir asfixiado en los hospitales de la Amazonia.** El País 2021, published 24 January. Full-text: https://elpais.com/sociedad/2021-01-23/la-pesadilla-de-morir-asfixiado-en-los-hospitales-de-la-amazonia.html

Más de medio centenar de enfermos mueren sin aire en Manaos, mientras prolifera un mercado paralelo de oxígeno. Es un nuevo y terrible capítulo de la caótica gestión de la pandemia en el Brasil de Bolsonaro.

Brum E. **Un estudio sostiene que Bolsonaro lideró una "estrategia institucional de propagación del virus".** El País 2021, published 23 January. Full-text: https://elpais.com/sociedad/2021-01-23/un-estudio-revela-que-bolsonaro-lidero-una-estrategia-institucional-de-propagacion-del-virus.html

Tras examinar 3.049 normas federales creadas en 2020, la Facultad de Salud Pública de la Universidad de São Paulo y la ONG Conectas Derechos Humanos analizan por qué Brasil supera las 212.000 muertes por covid-19.

Sampedro J. **Vuelve Manaos.** El País 2021, published 18 January. Full-text: https://elpais.com/ciencia/2021-01-18/vuelve-manaos.html

La primera ciudad que alcanzó la inmunidad de rebaño sufre de nuevo un aumento de casos.

Galarraga Gortázar N. **Miles de brasileños siguen la decisión final sobre la vacuna en directo por YouTube.** El País 2021, published 18 January. Full-text: https://elpais.com/sociedad/2021-01-17/miles-de-brasilenos-siguen-la-decision-final-sobre-la-vacuna-en-directo-por-youtube.html

Una enfermera negra recibe en São Paulo la primera dosis de inmunizante minutos después de que las dos inyecciones candidatas fueran aprobadas.

Schimidt S. **Los hospitales de Manaos se quedan sin oxígeno en un segundo colapso sanitario por la pandemia.** El País 2021, published 15 January. Full-text: https://elpais.com/sociedad/2021-01-15/los-hospitales-de-manaos-se-quedan-sin-oxigeno-en-un-segundo-colapso-hospitalario-de-la-pandemia.html

El Gobierno de Bolsonaro y el de Amazonas, que minimizaron la emergencia en Brasil, corren contra reloj para trasladar pacientes a otros Estados y conseguir importar el insumo.

Jucá B, Galindo J. **Brasil llega a 200.000 muertes por coronavirus sin una estrategia clara de vacunación.** El País 2021, published 8 January. Full-text: https://elpais.com/sociedad/2021-01-08/brasil-llega-a-200000-muertes-por-coronavirus-sin-una-estrategia-clara-de-vacunacion.html

Los picos de contagios son más moderados que en el inicio de la crisis, pero la pandemia se ha extendido por todo el territorio.

Galarraga Gortázar N. **La falta de jeringuillas amenaza la vacunación contra la covid en Brasil.** El País 2021, published 4 January. Full-text: https://elpais.com/sociedad/2021-01-03/la-falta-de-jeringuillas-amenaza-la-vacunacion-contra-la-covid-en-brasil.html

Este país, considerado un modelo en inmunización, no ha aprobado aún ninguna vacuna pese a sus casi 200.000 muertos por coronavirus.

Galarraga Gortázar N. **Jair Bolsonaro celebra como un triunfo la suspensión del ensayo de la vacuna china.** El País 2020, published 10 November. Full-text: https://elpais.com/internacional/2020-11-10/jair-bolsonaro-celebra-como-un-triunfo-la-suspension-del-ensayo-de-la-vacuna-china.html

El presidente de Brasil usa el asunto para redoblar su ofensiva contra el fármaco que promueve su rival João Doria, gobernador de São Paulo.

Galarraga Gortázar N. **La caótica gestión lastra la batalla contra el virus en Brasil.** El País 2020, published 19 May. Full-text: https://elpais.com/sociedad/2020-05-18/la-caotica-gestion-lastra-la-batalla-contra-el-virus-en-brasil.html

El boicoteo de Bolsonaro a las cuarentenas y la dimisión de dos ministros de Salud marca la respuesta a la pandemia en el tercer país con más casos y el sexto con más muertos.

Goulart J. **Una médica de urgencias en uno de los epicentros de la pandemia en Brasil: "La gente muere sola, sola, sola."** El País 2020, published 3 May. Full-text: https://elpais.com/sociedad/2020-05-02/una-medica-de-urgencias-en-uno-de-los-epicentros-de-la-pandemia-en-brasil-la-gente-muere-sola-sola-sola.html

La doctora Uildéia Galvão relata desde Manaos las difíciles condiciones de trabajo y el colapso del sistema. Los profesionales no cobran desde febrero

Galarraga Gortázar N, Torrado S, Fowks J. **Los indígenas de la Amazonia lanzan un SOS para reclamar protección ante la pandemia.** El País 2020, published 6 May. Full-text: https://elpais.com/internacional/2020-05-06/los-indigenas-de-la-amazonia-lanzan-un-sos-para-reclamar-proteccion-ante-la-pandemia.html

Las primeras muertes y el avance de los contagios activan las alarmas en la frontera que comparten Brasil, Colombia y Perú.

CAL.20C

In California, the proportion of SARS-CoV-2 cases associated with this variant rose from 3,8% to 25% between mid-November and late December. By then, CAL.20C accounted for 24% of samples in one study, and 36,4% (66/181) of samples in a local Los Angeles cohort (Zhang W 2021). The emerging predominance of this strain is temporally related to the time of onset of the current spike in SARS-CoV-2 infections in Southern California. CAL.20C is defined by mutations in the S protein (L452R, S13I, W152C) and in the ORF1a (I4205V) and ORF1b protein (D1183Y).

Mutations

Do you know "what mutations define a variant, what impact they might have (with links to papers and resources), and where variants are found"? If you don't, the excellent web site https://covariants.org, by Emma Hodcroft et al., will provide you a primer (Figure 13). Note that the indicated values may be biased. For example, for the week of 2021-01-11, CoVariants indicated 63% of N511Y variant in France while the government estimates it at around 10%. The reason: many countries are preferentially sequencing samples that are suspected to belong to the *infernale* trio B177, B1351 and P1.

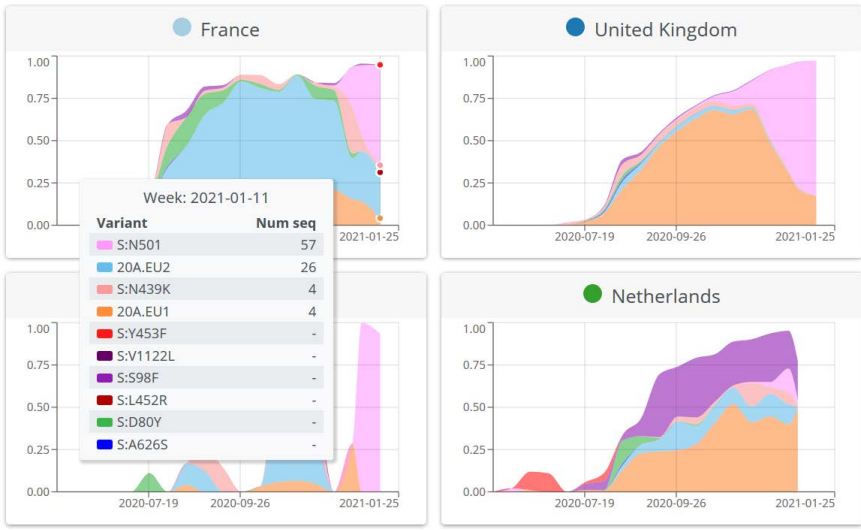

Figure 13. Overview of variants in countries. Insert: France, 11 January. The graphs show for each country, the proportion of total number of **sequences** (*not cases*), over time, that fall into defined variant groups. Source and copyright: CoVariants.org, by Emma Hodcroft et al.

In the coming revision of this chapter, we will give you a detailed overview of the most important mutations:

- N501Y ("Kelly")
- E484K ("Eric")
- K417T ("Knut")
- N439K ("Nick")
- E406W ("Ewen")

- P618H (“Paho”)
- S477N (“Sean”)
- D614G (“Deng”)

Prevention and Care

In the coming months, the new SARS-CoV-2 variants will confront many countries with a novel wave of viral spread. Once a more contagious variant has established itself, stabilizing the number of new infections will become increasingly difficult (Priesemann 2021), leading to a spiral of increasing number of infections, hospitalisations and deaths. The increased transmissibility of SARS-CoV-2 variants has far-reaching public health consequences. Non-pharmaceutical interventions (NPIs = everything from mask wearing to lockdowns) which were sufficient to control previous SARS-CoV-2 lineages may need to be reinforced to control B117, B1351 and P1. Fortunately, as shown in the *Epidemiology* section above (page 172), hard lockdowns, including closing of primary schools, secondary schools, and universities (Davies 2020) are effective against new variants.

Since the population groups driving transmission will not be targeted with vaccination for some months, ECDC recommends that Member States should to be very cautious about relaxing currently enacted NPIs. Non-essential travel should be avoided. Vaccination should focus on protecting those most at risk from severe disease. Find an overview of *Options for response* in ECDC’s Rapid Risk Assessment (ECDC 20210121, page 15), in particular

- Surveillance, testing and detection of the emering variants
- Non-pharmaceutical interventions
    - Community measures
    - Shielding medically and socially vulnerable populations
    - Considerations for school settings
    - Contact tracing for emerging variants
    - Measures for travellers

- Vaccination
    - Availability of COVID-19 vaccines
    - Monitoring breakthrough infections following vaccination, adjustment of vaccination schedules and possible update of vaccine contents due to SARS-CoV-2 variants in circulation
    - Accelerating vaccination campaigns
    - Vaccine effectiveness studies
- Hospital and healthcare preparedness

These recommendations translate into 9 commandments: "Whenever possible:

1. Stay home, if you can
2. Avoid gatherings, both inside and outside your household
3. Avoid enclosed spaces
4. Wear a mask, do not sing or shout!
5. Keep a distance – 2 meters!
6. Ventilate whenever you can
7. Wash hands
8. Disinfect hightouch surfaces (maybe less important?)
9. Get vaccinated as soon as available!"

Or, according to the more joyful words of UN Women: "The pandemic is hard – spread joy:

- Buy someone flowers
- Call a loved one
- Deliver groceries to your neighbour
- Write a greeting card
- Motivate a friend who needs a boost
- Virtually tutor a student
- Make face masks to give away"

Outlook

In a few months, we will know whether B117, B1351 and P1 will

- Change the clinical presentation of COVID-19 and mortality
- Affect the few existing treatment option (corticosteroids, etc.)
- Increase the number of reinfections
- Affect the immune response to vaccines
- Affect the therapeutical benefit from monoclonal antibodies

Fortunately, global genomic surveillance and rapid open-source sharing of viral genome sequences have facilitated near real-time detection, comparison, and tracking of evolving SARS-CoV-2 variants that can inform public health efforts to control the pandemic (Galloway 2020).

Vaccine producers are already at work. Pfizer-BioNTech and Moderna are modifying their vaccine for *emerging* variant booster candidates. They will test an additional (third) vaccine booster dose to study if neutralizing titers against emerging variants can be increased. All companies have started talks with regulators to know what types of clinical trials and safety reviews would be required to authorize new versions of already approved vaccines. Over time, it is possible that, as with seasonal influenza, these adaptive changes in antigenic regions of the virus would give rise to continual reformulation of existing vaccines (Kistler 2020).

We expected 2021 to the year of the SARS-CoV-2 vaccine. We didn't expect it to be the year of a *race* between the virus and SARS-CoV-2 vaccines. Vaccines – and science – will ultimately prevail, but for the coming months, the race will stay close.

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6. Vaccines

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Summary

As of 8 January, three vaccines had been approved in Europe and in the US: **tozinameran** (Comirnaty™) by BioNTech/Pfizer (formerly known as BNT162b2), **mRNA-1273** by Moderna and **ChAdOx1 nCoV-19** by University of Oxford/AstraZeneca. Adverse events such as pain at injection site, fatigue, headache, myalgia and fever were common, but serious adverse events were rare. All three vaccines seem to have a favorable safety profile.

Three other vaccine candidates have been approved (not by FDA or EMA) although data are incomplete or have not yet been published:

- China: BBIBP-CorV (30 December), Sinopharm and the Beijing Institute of Biological Products
- India: Covaxin (3 January), Bharat Biotech
- Russia: Sputnik-V (28 December), Gamaleya Research Institute

In early January 2021, 63 vaccine candidates were in clinical development against SARS-CoV-2 and 172 in pre-clinical development. The World Health Organization (WHO) maintains a twice-weekly updated working document that includes most vaccines in development (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines; accessed 8 January). SARS-CoV-2 vaccine development is a dynamic field (Slaoui 2020, Heaton 2020) which is inaugurating a new future of vaccine science (Ball 2020).

On 8 January 2021, almost 20 million people had received a SARS-CoV-2 vaccine, mostly in the US, China, Israel, England, the United Arab Emirates, and Russia (OurWorldInData.org). In most countries, an effect of mass vaccination in changing the pandemic is not expected to be measurable before the summer.

Approved SARS-CoV-2 vaccines – An overview

In the preface to our November edition, we predicted that "massive nationwide vaccine campaigns would not be available until well into 2021 and that massive global vaccine campaigns were unlikely to have a more than marginal impact on the SARS-CoV-2 pandemic before 2022, if ever." We were wrong. As of 8 January, three vaccines have been approved in Europe and the US: see

above. In Phase III trials, tozinameran and mRNA-1273 had an efficacy of over 94% while ChAdOx1 nCoV-19 had an efficacy of 62-90%. The vaccines are be given in two doses three (nRNA-1273), four (tozinameran) or up to 12 weeks apart (ChAdOx1). Vaccinees will be protected from SARS-CoV-2 infection from about 7 days after the second injection; some protection, especially against severe COVID-19, is provided by the first injection.

Table 1. SARS-CoV-2 vaccines approved in Europe (EMA) and the US (FDA)

Vaccine *Manufacturer*	**Trial Efficacy**	**Storage**	**Administration**	**References**
Tozinameran (formerly: BNT162b2) Comirnaty™ *BioNTech & Pfizer*	95%	–70°C	2 injections 3 weeks apart	Polack 2020 Mulligan 2020 FDA EUA FDA briefing doc Sponsor briefing doc Recommendation for use
mRNA-1273 N.N.™ *Moderna*	94%	–20°C	2 injections 4 weeks apart	Polack 2020 Jackson 2020 FDA EUA FDA briefing doc Sponsor briefing doc Recommendation for use
ChAdOx1 nCoV-19 (formerly: AZD1222) N.N.™ *Oxford University & AstraZeneca*	62-90%	2-8°C (fridge)	2 injections up to 12 weeks apart	Voysey 2020 Folegatti 2020 MHRA Decision

All three vaccines seem to have a favorable safety profile (see below). Although local or systemic side effects are frequent – mostly pain at injection site, fatigue, headache, muscle pain, joint pain, and sometimes fever during the first 24 to 48 hours after vaccination (Folegatti 2020, Voysey 2020, Jackson 2020, Mulligan 2020, Polack 2020, Baden 2020) – more *severe* side effects have been in the single-digit range. As a general rule, side effects appear to be more common after the second dose, and younger adults experience more

side effects than older adults. Importantly, *serious*[4] side effects were equally rare in people who received the vaccine and those who received placebo (Polack 2020, Baden 2020). Cases of anaphylaxis are very rare and may occur in 1 of 100,000 vaccine recipients (see next paragraph). Vaccine recipients should be informed that side effects generally do not signal that the vaccine is unsafe but, on the contrary, that the immune system is mounting a defense response against future exposure to SARS-CoV-2. We will learn to accept fever and aches as signs that the vaccine works, in rare cases even bone, muscle aches and fever that might last 12 hours (Wadman 2020). Mild side effects? Not really side effects. *Vaccine at work*!

On December 8, 2020, within 24 hours after the start of the UK vaccination program, the program reported probable cases of anaphylaxis in two women in their forties, who had known food and drug allergies and were carrying auto-injectable epinephrine (Castells 2020). One week later, a 32-year-old female health care worker in Alaska who had no known allergies presented with an anaphylactic reaction within 10 minutes after receiving the first dose of the vaccine. Since then, several more cases of anaphylaxis associated with the Pfizer mRNA vaccine have been reported after vaccination of almost 2 million health care workers, and the incidence of anaphylaxis associated with the Pfizer SARS-CoV-2 mRNA vaccine appears to be approximately 10 times as high as the incidence reported with all previous vaccines, at approximately 1 in 100,000, as compared to 1 in 1,000,000 (Castells 2020). The CDC recommends that appropriate medical treatment for severe allergic reactions be immediately available in the event that an acute anaphylactic reaction occurs following administration of an mRNA COVID-19 vaccine (CDC 20201231, CDC20210106). In particular, persons without contraindications to vaccination who receive an mRNA COVID-19 vaccine should be observed after vaccination for the following time periods:

[4] Serious adverse events are defined as requiring hospitalization, deemed life-threatening, or resulting in persistent or significant disability/incapacity, another medically important condition, or death. The terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) is not necessarily serious.

- 30 minutes: Persons with a history of an immediate allergic reaction of any severity to a vaccine or injectable therapy and persons with a history of anaphylaxis due to any cause.
- 15 minutes: All other persons

Find detailed information about tozinameran, mRNA-1273 and ChAdOx1 nCOV-19 on page 215.

Dosing schedule

As mentioned above, the vaccines should be given in two doses three (nRNA-1273), four (tozinameran) or up to 12 weeks apart (ChAdOx1). In the current situation of scarce vaccine supply, the UK Joint Committee on Vaccination and Immunisation (JCVI) now recommends that vaccinating more people with the first dose of both the Oxford/AstraZeneca and the BioNTech/Pfizer vaccine should be prioritized above offering others their second dose (JCVI 2020), as this would provide the greatest public health benefits in the short term and save more lives. For the BioNTech/Pfizer vaccine, the second vaccine dose could be offered between 3 to 12 weeks after the first dose. For the University of Oxford/AstraZeneca vaccine, the second dose could be offered 4 to 12 weeks after the first dose. The UK has also just opened the way to offering a vaccine "mix" (first injection of a first vaccine, the second of another) in cases where the second dose of the same vaccine is not available at a vaccination center for one person. Similar recommendations of a maximum of 41 days between the first and the second dose have been issued in Germany.

Distribution

Access to a safe vaccine will be unequal, both within countries and between them. Within countries, health authorities have prepared strategic prioritization plans (Lipsitch 2020), offering vaccines first to healthcare workers and people at high risk of severe COVID-19; then maybe to those living in epidemiological hotspots; and, finally, to the rest of the population – if they want to get vaccinated (Schwartz 2020, Bingham 2020). Between countries, there is no doubt that those that produce vaccines will get the vaccine before countries that don't and that countries paying more will get vaccines earlier. On 24 August 2020, wealthy countries had pre-ordered around two billion vaccine doses without knowing which one may prove effective (see an overview of the August situation in Callaway 2020). As it is not acceptable that low-risk people in wealthy countries get the vaccine while health care workers in low- and middle-income countries do not, GAVI, the Vaccine Alliance (a Geneva-based funder of vaccines for low-income countries), the Coalition for Epidem-

ic Preparedness Innovation (CEPI[5]) and the World Health Organization have set up the COVID-19 Vaccines Global Access (COVAX) Facility (Kupferschmidt 20200728, Jeyanathan 2020). COVAX aims to accelerate the development and manufacture of COVID-19 vaccines, and to guarantee fair and equitable access for every country in the world by securing 2 billion vaccine doses which should be enough to protect high risk and vulnerable people, as well as front-line healthcare workers. One billion have already been reserved for 92 low- and middle-income countries (LMICS), which make up half the world's population. All participating countries, regardless of income levels, will have equal access to these vaccines once they are developed. For lower-income nations who would otherwise be unable to afford these vaccines as well as a number of higher-income self-financing countries that have no bilateral deals with manufacturers, COVAX is quite literally a lifeline and the only viable way in which their citizens will get access to COVID-19 vaccines. South Africa and India have asked the World Trade Organization to temporarily suspend intellectual property rights so that COVID-19 vaccines and other new technologies are accessible for poor countries (Usher 2020).

Impact on the Pandemic

The fact that hundreds of millions of people will receive a SARS-CoV-2 vaccine during the first months of 2021 will not end the COVID-19 pandemic (Editorial 20201218). An exception may be countries like Israel that are able to vaccinate more than 1% of their population per day. These situations will provide the answer to the Question of 2021: *When and how will vaccine impact a local epidemic?* Over the coming 10 weeks, it will be highly instructive to update the three curves in Figure 1.

[5] The Coalition for Epidemic Preparedness Innovation (CEPI), an international nongovernmental organization funded by the Wellcome Trust, the Bill and Melinda Gates Foundation, the European Commission, and eight countries (Australia, Belgium, Canada, Ethiopia, Germany, Japan, Norway, and the United Kingdom), is supporting development of vaccines against five epidemic pathogens on the World Health Organization (WHO) priority list (Lurie 2020).

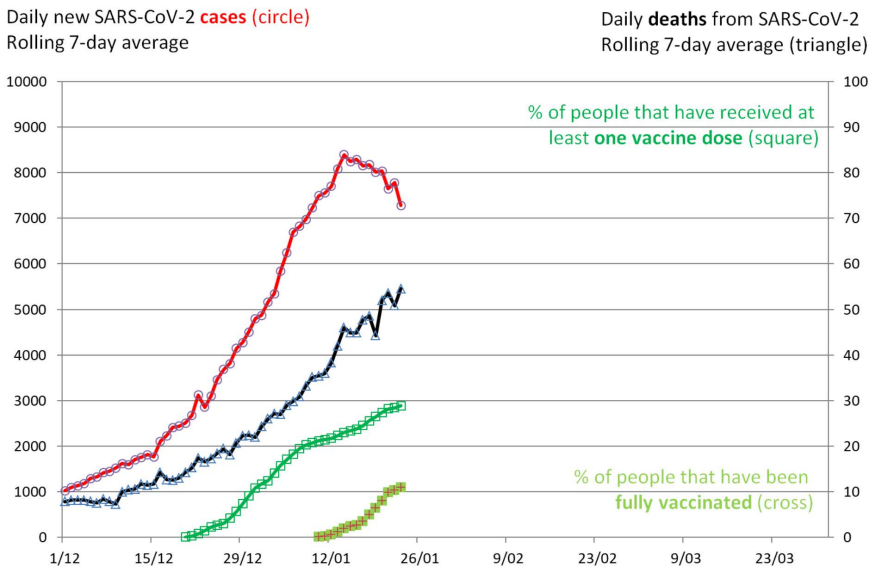

Figure 1. SARS-CoV-2 cases in Israel. Impact of mass vaccination on the pandemic. The rolling 7-day average of new SARS-CoV-2 cases is shown in red circles (left vertical axis), the rolling 7-day average of deaths as a plain black line (right vertical axis). The percentage of people that have received at least one vaccine dose is shown in green squares. The percentage of people that have been fully vaccinated is shown in green-yellow crosses. Please remember that the country entered a third lockdown on 27 December. The evolution of daily new cases and deaths will be influenced both by the lockdown measures and the vaccination campaign. Updated 24 January.

Mutations and vaccine efficacy

Multiple SARS-CoV-2 variants are circulating globally (CDC 2021 V, CDC 2021 NCV, Kupferschmidt 20201220, Kupferschmidt 20201223). In the autumn of 2020, several new variants emerged, most notably:

- In England the new B117 variant (also known as B.1.1.7, 20B/501Y.V1, or VOC 202012/01; ECDC 20201220a, ECDC 20201220b, Lauring 2021) emerged with an unusually large number of mutations. This variant has since been detected in numerous countries around the world (Callaway 2021).

- In South Africa, another variant of SARS-CoV-2 (known as 20C/501Y.V2 or B.1.351 lineage) emerged independently of the B117 lineage. It does share some mutations with B117. Cases attributed to this variant have been detected outside of South Africa.

All variants have multiple mutations in the Spike protein which are involved in receptor binding (Wong AHM 2017, Kistler 2020). This viral evolution is a normal process (all coronaviruses undergo antigenic evolution that erodes neutralizing antibody immunity (Eguia 2020)) – and has recently been reproduced *in vitro* (Zahradnik 2020).

The epidemiological consequences of these new variants are currently not foreseeable (Tufekci 2020, Felter 2021). There is no evidence that they cause more severe illness or increased risk of death; however, they seem to have a substantial transmission advantage (up to +75%) (Public Health England 2020, Volz 2021, Leung 2021). Increased transmission will lead to more SARS-CoV-2 infections and might significantly increase the number of deaths over the coming months (Figure 2).

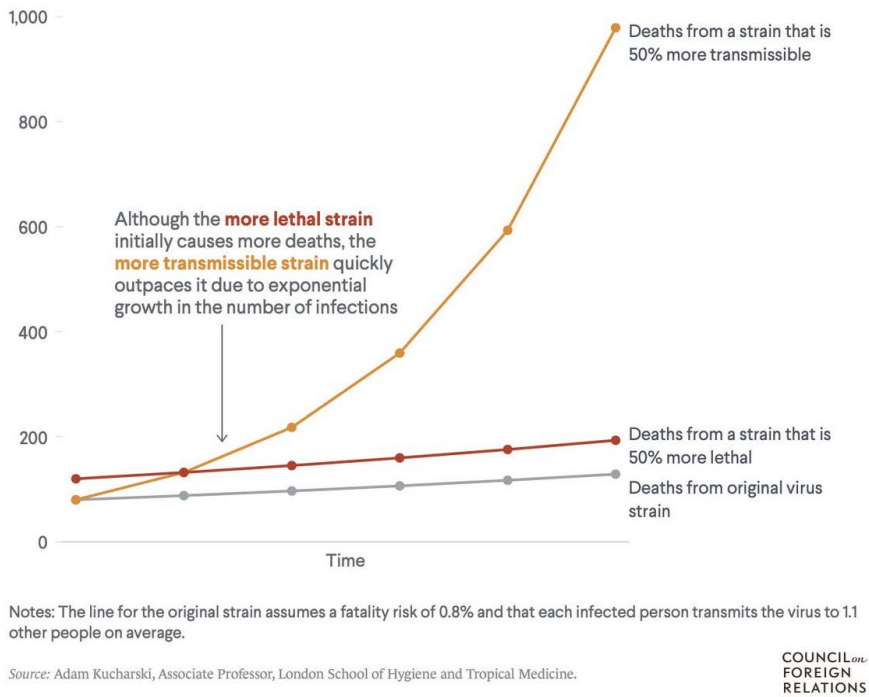

Figure 2. A more infectious virus could lead to many more deaths. Simplified, hypothetical scenario showing the number of new deaths every six days from three different virus strains, assuming each strain started from 10.000 infections. Source: Adam Kucharski, Associate Professor, London School of Hygiene and Tropical Medicine.

Preliminary results suggest that existing mRNA vaccines will maintain efficacy against variants that share the N501Y mutation (Xie X 2020). However, a

recently published comprehensive map of all amino acid mutations to the SARS-CoV-2 spike receptor-binding domain (RBD) show that the site where mutations tend to have the largest effect on antibody-binding and neutralization is E484 (Greaney 2020). The E484K mutation, too, is present in several emerging SARS-CoV-2 lineages (Tegally 2020, Voloch 2020). Intense research is currently investigating vaccine efficacy against variants with multiple mutations; results are expected within weeks. If widely circulating SARS-CoV-2 variants are confirmed to elicit insufficient neutralizing antibody responses after vaccination, existing SARS-CoV-2 vaccines would require reformulating.

Prices

In December 2020, a Belgian minister tweeted the price that the EU had agreed to pay for COVID vaccines (The Guardian). The University of Oxford/AstraZeneca vaccine is the cheapest and Moderna is the most expensive:

1. University of Oxford/AstraZeneca: €1.78 (£1.61)
2. Sanofi/GSK: €7.56
3. Johnson & Johnson: $8.50 (£6.30)
4. CureVac: €10
5. BioNTech/Pfizer: €12
6. Moderna/NIAID: $18

Initially, AstraZeneca had pledged it would provide doses on a cost basis for at least as long as the pandemic lasts and in poorer countries in perpetuity. However, according to a newspaper article, an agreement between AstraZeneca and a Brazilian manufacturer seem to define the "Pandemic Period" as ending on July 1 2021. The period could be extended but only if "AstraZeneca acting in good faith considers that the SARS-COV-2 pandemic is not over" (Financial Times, 8 October 2020).

Questions

After the beginning of mass vaccinations, numerous questions remain (Polack 2020, Rubin 2020):

- Will unexpected safety issues arise when the number grows to millions and possibly billions of people?
- Will side effects emerge with longer follow-up?
- What happens to the inevitable large number of recipients who miss their second dose?

- How long will the vaccine remain effective? Are re-vaccinations needed at regular intervals or might immunity last for years (Dan 2021)?
- Will the vaccines also reduce transmission of SARS-CoV-2 when vaccinated (and protected!) individuals will become infected?
- What about people who were not represented in the recent Phase III trials, such as children, pregnant women, and immunocompromised patients of various sorts?
- Will new SARS-CoV-2 mutations require regular vaccine 'updates'?

Vaccines

Tozinameran

In November 2020, the German company BioNTech and the New York-based Pfizer made history by presenting data which indicated that their vaccine tozinameran (trade name: Comirnaty) had an extraordinary efficacy of over 90%. Tozinameran is a lipid nanoparticle–formulated (Pardi 2015), nucleoside-modified RNA vaccine (Karikó 2008; see also Karikó 2005 + Karikó 2012 + Wired) that encodes a prefusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein (Wrapp 2020). The vaccine was approved on the basis of data from a Phase III trial which demonstrated that two 30 µg doses given three weeks apart conferred 95% protection against COVID-19 in persons 16 years of age or older (Polack 2020). Of 170 confirmed COVID-19 cases, 162 occurred in the placebo group and 8 in the vaccine group. Efficacy was consistent across age, gender, race and ethnicity. In particular, the observed efficacy in adults over 65 years of age was above 94%. Safety over a median of 2 months was similar to that of other viral vaccines.

Researchers involved in the development of tozinameran had previously published Phase I safety and immunogenicity data (Walsh 2020). Two 30 µg doses had been shown to elicit high SARS-CoV-2 neutralizing antibody titers and robust antigen-specific $CD8^+$ and Th1-type $CD4^+$ T cell responses (Sahin 2020, Mulligan 2020).

Data on local and systemic reactions were collected with electronic diaries from participants in a reactogenicity subset of 8183 participants for 7 days after each vaccination. Local and systemic adverse events were reported more often by younger vaccine recipients (16 to 55 years of age) than by older vaccine recipients (older than 55 years of age) and more often after dose 2 than dose 1. Apart from pain at the injection site, the most commonly report-

ed systemic events were fatigue and headache (see Tables 5.2 and 5.3). Most local and systemic reactions occur within the first 1 to 2 days after the injection and resolve within days. In some patients, axillary lymphadenopathy might indicate a robust vaccine-elicited immune response; it generally resolves within 10 days.

In comparison to these normal events, the incidence of *serious* adverse events was similar for tozinameran and placebo (0,6% and 0,5%, respectively).

The major drawback of the BioNTech/Pfizer vaccine is its temperature sensitivity – it must be stored at –70º C (–94º F) while the Moderna/NIAID vaccine can be stored at –20 C (–4º F). In normal refrigerator conditions the BioNTech/Pfizer vaccine can be stored only up to 5 days and the Moderna/NIAID vaccine up to 30 days. In this regard, the University of Oxford/AstraZeneca vaccine presents an important advantage: it can be transported at a temperature between 2 and 8º C (36º F to 46°F) for at least six months and could be administered by physicians, pharmacists or in other already existing healthcare settings.

A press article narrates the background of the Pfizer vaccine development: https://www.nytimes.com/2020/11/21/us/politics/coronavirus-vaccine.html. On 31 December, WHO listed the Comirnaty COVID-19 mRNA vaccine for emergency use, making the Pfizer/BioNTech vaccine the first to receive emergency validation from WHO (WHO 20201231). Countries who do not have the means to rigorously assess the efficacy and safety of vaccines can now take advantage of the WHO EV and begin rolling out their vaccination programs.

Table 2 – Tozinameran (Comirnaty™): local and systemic reactions reported after the **second** injection of tozinameran or placebo (age group: 16-55 years) (FDA briefing document). See also Figure 2 of the paper by Polack et al.

	Tozinameran (Comirnaty™, formerly: BNT162b2)	**Placebo**
Pain at injection site	78%	12%
Fever	16%	0%
Fatigue	59%	23%
Headache	52%	24%
Chills	35%	4%
Myalgia	37%	8%
Arthralgia	22%	5%

Table 3 – Tozinameran (Comirnaty™): **severe** local and systemic reactions reported after the **second** injection of tozinameran or placebo (age group: 16-55 years) (FDA briefing document).

	Tozinameran (Comirnaty™, formerly: BNT162b2)	**Placebo**
Pain at injection site	1,2%	0%
Fever >38.9°	1,2%	0,1%
Fatigue	4,6%	0,7%
Headache	3,2%	0,7%
Chills	2,1%	0%
Myalgia	2,2%	0,1%
Arthralgia	1,0%	0,2%

mRNA-1273

mRNA-1273, developed by Moderna, is a lipid nanoparticle–encapsulated, nucleoside-modified messenger RNA (mRNA)–based vaccine that encodes the SARS-CoV-2 spike (S) glycoprotein stabilized in its prefusion conformation. The vaccine was approved on the basis of data from a Phase III trial which demonstrated that 100 μg taken four weeks apart conferred 94,5% protection against COVID-19 in persons 16 years of age or older (FDA EUA). Of 95 confirmed COVID-19 cases, 90 occurred in the placebo group and 5 in the vaccine group. Subgroup analyses of the primary efficacy endpoint showed similar efficacy point estimates across age groups, genders, racial and ethnic groups, and participants with medical comorbidities associated with high risk of severe COVID-19. The vaccine might also prevent severe COVID-19 and prevent COVID-19 following the first dose, but available data for these outcomes were not sufficient to allow for any firm conclusions.

Researchers involved in the development of mRNA-1273 had previously demonstrated that mRNA-1273 induced potent neutralizing antibody responses (Korber 2020, Widge 2020, Anderson 2020) to SARS-CoV-2 as well as $CD8^+$ T cell responses, and protects against SARS-CoV-2 infection in mice (Corbett 2020) and non-human primates (Corbett 2020b). In early clinical trials, it induced anti–SARS-CoV-2 immune responses in all participants, and no trial-limiting safety concerns were identified (Jackson 2020). Check also this press article at https://www.nytimes.com/interactive/2020/health/moderna-covid-19-vaccine.html.

Side effects (adverse events):

1. Side effects were more frequent after the second dose. After the second dose, moderate-to-severe systemic side effects, such as fatigue, headache, chills, myalgia, and arthralgia, were noted in about 50% of participants in the mRNA-1273 group. The majority of solicited systemic adverse events were grade 1 to grade 2 in severity. In the mRNA-1273 group, the most common grade 3 solicited systemic ARs after the second injection included fatigue, myalgia, headache, and arthralgia.
2. These side effects were transient, starting about 15 hours after vaccination and resolving in most participants by day 2, without sequelae (Baden 2020; see also Tables 4 and 5).
3. With the exception of more frequent, generally mild to moderate reactogenicity in participants < 65 years of age, the safety profile of mRNA-1273 was generally similar across age groups, genders, ethnic and racial groups, participants with or without medical comorbidities, and participants with or without evidence of prior SARS-CoV-2 infection at enrollment.
4. Several participants reported injection site reactions after day 7 that were characterized by erythema, induration, and often pruritis. Consultation with a dermatopathologist suggested that these were most likely dermal hypersensitivity reactions and were unlikely to represent a long-term safety concern.
5. The rate of *serious* adverse events (SAEs) was low, and similar in both vaccine and placebo groups (around 1%). The most common SAEs in the vaccine group which were numerically higher than the placebo group were myocardial infarction (0,03%), cholecystitis (0,02%), and nephrolithiasis (0,02%), although the small numbers of cases of these events do not suggest a causal relationship (FDA Briefing). The most common SAEs in the placebo arm which were numerically higher than the vaccine arm, aside from COVID-19 (0,1%), were pneumonia (0,05%) and pulmonary embolism (0,03%). The incidence of serious adverse events was similar in the vaccine and placebo groups.
6. Throughout the safety follow-up period, there were three reports of facial paralysis (Bell's palsy) in the vaccine group and one in the placebo group. Currently available information is insufficient to determine a causal relationship with the vaccine.

Table 4 – mRNA-1273: local and systemic reactions after the **second** injection of mRNA-1273 or placebo (18-64 years) (FDA Briefing).

	mRNA-1273	Placebo
Pain at injection site	90%	19%
Lymphadenopathy	16%	4%
Fever	17%	0%
Fatigue	68%	25%
Headache	63%	26%
Chills	48%	6%
Myalgia	61%	12%
Arthralgia	45%	11%

Table 5 – mRNA-1273: **severe** local and systemic reactions after the **second** injection of mRNA-1273 or placebo (18-64 years) (FDA Briefing).

	mRNA-1273	Placebo
Pain at injection site	4,6%	0,2%
Lymphadenopathy	0,4%	< 0,1%
Fever	1,6%	< 0,1%
Fatigue	10,6%	0,8%
Headache	5,0%	1,2%
Chills	1,5%	0,1%
Myalgia	10,0%	0,4%
Arthralgia	5,8%	0,3%

ChAdOx1 nCoV-19

ChAdOx1 nCoV-19 (or AZD1222), developed by University of Oxford/AstraZeneca, uses replication-deficient chimpanzee adenovirus vector ChAdOx1, which contains the full-length, unmodified spike protein of SARS-CoV-2. On December 30, UK regulatory authorities approved the vaccine (GOV.UK 20201230). The vaccine was approved in India a few days later.

Researchers involved in the development of ChAdOx1 nCoV-19 had previously published results from a Phase I/II trial showing that in ChAdOx1 vaccine recipients, T cell responses peaked on day 14, anti-spike IgG responses rose by day 28, and neutralizing antibody responses against SARS-CoV-2 were detected in > 90%. Adverse events such as fatigue, headache, and local tenderness commonly occurred, but there were no serious adverse events (Folegatti 2020). A multiplex cytokine profiling and intracellular cytokine staining anal-

ysis demonstrated that ChAdOx1 nCoV-19 vaccination induces a predominantly Th1-type response (Ewer 2020). In a Phase II/III trial ChAdOx1 nCoV-19 appeared to be better tolerated in older adults than in younger adults and has similar immunogenicity across all age groups after a boost dose (Ramasamy 2020, Andrew 2020). Finally, in December, the results from four randomized studies showed that ChAdOx1 had an efficacy of 62-90% (Voysey 2020, Knoll 2020). Importantly, no hospitalizations or severe cases of the disease were reported in participants receiving the vaccine. ChAdOx1 nCoV-19 (AZD1222) needs only normal refrigeration at 2-8°C and is far cheaper than the mRNA vaccines Comirnaty (BioNTech/Pfizer) and mRNA-1273 (Moderna).

In December, AstraZeneca and Gamaleya announced that they would combine their vaccines to see if the combination would deliver a stronger protection than either vaccine on its own. A Phase I trial was registered on Christmas Eve 2020.

In October, an ideal pandemic vaccine was defined as being acceptably safe for everyone, effective in inducing a durable protective immune response, rapidly scalable, stable at room temperature, single dose and cost effective (Bingham 2020). ChAdOx1 nCoV-19 fulfills these requirements. AstraZeneca will soon seek an Emergency Use Listing from the World Health Organization for an accelerated pathway to vaccine availability in low-income countries.

Vaccine platforms

mRNA vaccines

The recently approved mRNA vaccines – tozinameran (Comirnaty™, BioNTech/Pfizer) and mRNA-1273 (Moderna/NIAID) – use a lipid-based nanoparticle carrier system that facilitates *in vivo* delivery and prevents the rapid enzymatic degradation of mRNA. This carrier system is further stabilized by a polyethylene glycol (PEG) 2000 lipid conjugate. PEG 2000 prolongs the half-life of the vaccine by providing a hydrophilic layer to the lipid nanoparticle.

An mRNA vaccine is comparable to software code which instructs human cells (the 'operating system') to produce its own SARS-CoV-2 vaccine. Once introduced into a cell, the mRNA molecule is read by a ribosome and translated into SARS-CoV-2 spike proteins (see Figure 3) which, when released into the body, are recognized by the immune system as *other* and trigger an immune response (Walsh 2020). Another mRNA vaccine currently in Phase III is CvnCoV by CureVac (NCT04652102).

mRNA vaccines have the potential to be truly transformative (Abbasi 2020). BioNTech, Moderna, CureVac and GSK own nearly half of the mRNA vaccine patent applications (Martin 2020).

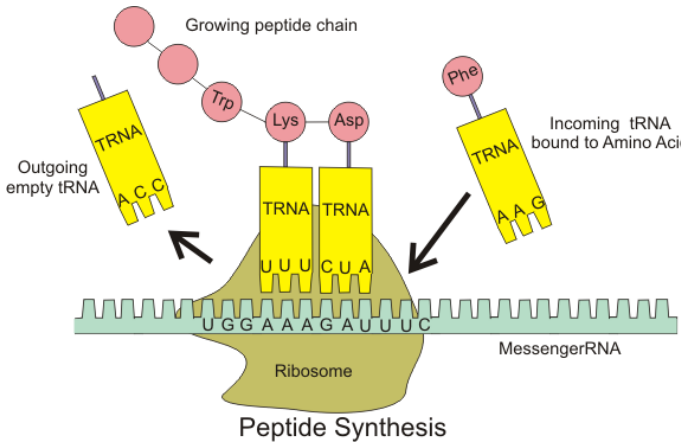

Figure 3. Ribosomes assemble protein molecules whose sequence is controlled by the sequence of messenger RNA molecules. The growing peptide chain (top left) will form the SARS-CoV-2 spike protein. TRNA: transfer RNA.

Replication-incompetent vectors

Another approach is to use recombinant viral vectors in which an antigen of the pathogenic virus is expressed. Such vaccines are typically based on another virus that has been engineered to express the spike protein and has been disabled from replication *in vivo* by the deletion of parts of its genome (Krammer 2020). The majority of these vaccines are based on adenovirus (AdV) vectors. The vectors usually show good stimulation of B cell and T cell responses; however, pre-existing anti-AdV immunity might partially neutralize a candidate vaccine's immunogenicity (Zhu FC 2020b). Before 2020, the Ebola vaccine was the only approved vaccine based on this principle (Henao-Restrepo 2017).

Replication-incompetent vector vaccines (approved or in advanced Phase III development) are being developed/distributed by

- Oxford University / AstraZeneca: ChAdOx1 nCoV-19, also known as AZD1222 – van Doremalen 2020, Folegatti 2020, page 219
- Janssen: AdV26-based vector – Ad26.COV2.S, Mercado 2020; page 227
- CanSino: AdV5 – Convidecia, Zhu FC 2020a, Zhu FC 2020b: page 226
- Gamaleya Research Institute: Ad5/Ad26 – Sputnik V, Logunov 2020; page 225

Other vaccine platforms

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). Two inactivated vaccines protected rhesus monkeys from SARS-CoV-2. The vaccines were well tolerated pre-clinically and no type 2 immunopathology was found in the lungs (see below: pathological immune responses) (Gao Q 2020, Wang H 2020). Inactivated vaccines (in advanced Phase III development) are being developed by

- Sinopharm + Beijing Institute of Biological Products**:** BBIBP-CorV; page 224
- Sinovac Biotech**:** CoronaVac – Zhang Y 2020, Gao 2020; page 224
- Bharat Biotech: Covaxin – Ella 2020; page 225

Find an overview of other platforms used for SARS-CoV-2 vaccine development at Krammer 2020 (the letters in the brackets refer to Figure 3 of the review):

- recombinant protein vaccines based on the spike protein (e), the RBD (f) or on virus-like particles (g); licensed examples include the vaccines against hepatitis B and human papilloma virus.
- replication-competent vector vaccines (i)
- inactivated virus vector vaccines that display the spike protein on their surface (j)
- DNA vaccines (k)

Vaccine candidates in Phase III Trials

On 8 January 2021, 63 vaccine candidates were in clinical development against SARS-CoV-2 and 172 in pre-clinical development. The World Health Organization (WHO) maintains a twice-weekly updated working document that includes most of the vaccines in development (WHO Landscape 2020; accessed 8 January).

Table 1. Vaccines in Phase III trials*

Vaccine candidate *Developers*	Vaccine platform	Type of candidate vaccine	Doses	Schedule
BBIBP-CorV *Sinopharm + Beijing Institute of Biological Products*	Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell)	2	Day 0 + 21
CoronaVac *Sinovac Biotech* (Zhang Y 2020, Gao 2020)	Inactivated virus	SARS-CoV-2 vaccine (inactivated)	2	Day 0 + 14
Covaxin *Bharat Biotech*	Inactivated virus	Whole-Virion Inactivated SARS-CoV-2 Vaccine (BBV152)	2	Day 0 + 14
Sputnik V *Gamaleya Research Institute* (Logunov 2020, Bucci 2020)	Viral vector (Non-replicating)	Adeno-based (rAd26-S+rAd5-S)	2	Day 0 + 21
Ad26.COV2.S *Janssen Pharmaceutical* (Mercado 2020)	Viral vector (Non-replicating)	Ad26.COV2.S	1-2	Day 0 or Day 0 +56
CvnCoV *CureVac* (NCT04652102)	RNA based vaccine	CVnCoV	2	Day 0 + 28
Convidecia *CanSino Biological Inc./Beijing Institute of Biotechnology* (Zhu FC 2020a, Zhu FC 2020b)	Viral vector (Non-replicating)	Recombinant novel coronavirus vaccine (Adenovirus type 5 vector; formerly: CTII-nCoV)	1	Day 0
NVX-CoV2373 *Novavax* (Keech 2020)	Protein subunit	SARS-CoV-2 rS/Matrix M1-Adjuvant (Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M)	2	Day 0 + 21
N. N. *Sinopharm + Wuhan Institute of Biological Products* (Xia S 2020)	Inactivated virus	Inactivated SARS-CoV-2 vaccine (Vero cell)	2	Day 0 + 21
ZF2001 *Anhui Zhifei Longcom + Chinese Academy of Sciences* (NCT04646590)	Protein subunit	Recombinant SARS-CoV-2 vaccine (CHO Cell)	2-3	Day 0 + 28 or Day 0 + 28 + 56

CoVLP *Medicago* (Ward 2020, NCT04636697)	Virus like particle	Coronavirus-Like Particle COVID-19 (CoVLP)	2	Day 0 + 21
SCB-2019 *Clover Biopharmaceuticals* (Press release, NCT04672395)	Protein subunit	SCB-2019 + AS03 or CpG 1018 adjuvant plus Alum adjuvant (Native like Trimeric subunit Spike Protein vaccine)	2	Day 0 + 21

* Source: WHO Landscape 2020

BBIBP-CorV (China)

BBIBP-CorV is an inactivated virus vaccine developed by Sinopharm and the Beijing Institute of Biological Products. On 30 December, Sinopharm announced that the vaccine had an efficacy of almost 80%. A day later, China's health authorities approved the vaccine for general use on the population (31 December 2020: The Guardian, The New York Times). Outside China, the vaccine has been approved in the United Arab Emirates and Bahrain.

CoronaVac (China)

CoronaVac™ is an inactivated virus vaccine developed by Sinovac Biotech. In macaques, the vaccine provided partial or complete protection against a SARS-CoV-2 challenge (Gao 2020). In a Phase I/II trial, CoronaVac was well tolerated and moderately immunogenic in healthy adults aged 18–59 years. Most adverse reactions were mild, with the most common symptom being injection-site pain (Zhang Y 2020). In July 2021, the Chinese government approved CoronaVac for emergency use; it is assumed that the vaccine has been offered to people in high-risk jobs, for example, medical workers and other public service personnel. In Brazil, CoronaVac is being developed in partnership with the Butantan Institute. On 12 January, the government of São Paulo, Brazil, announced the overall effectiveness of the Sinovac vaccine to be 50.38%. The data was obtained with tests carried out on 12,508 volunteers in the country, all health professionals. According to a report of The New York Times (7 January), Sinovac had sold more than 300 million doses, mostly to low- and middle-income countries, accounting for about half of the total production.

Covaxin (India)

Covaxin (formerly known as BBV152), developed by Bharat Biotech (Bharat Biotech, Hyderabad) in collaboration with the Indian Council of Medical Research and the National Institute of Virology, is a whole-virion inactivated SARS-CoV-2 vaccine. In a Phase I/II trial, robust humoral and cell-mediated responses were observed in the Algel-IMDG recipients (Ella 2020). Pain at the injection site resolved spontaneously. The overall incidence rate of local and systemic adverse events in this study was 10%-20% which is lower than the rates for other SARS-CoV-2 vaccine platform candidates and comparable to the rates for other inactivated SARS-CoV-2 vaccine candidates. On 3 January, the Drugs Controller General of India (DCGI) approved the emergency use of Covaxin, making it India's first vaccines against the pandemic, even though Phase III safety and efficacy clinical trials had not been completed. At that time, 22.500 of the 25.800 participants in a Phase III trial had been vaccinated (CTRI/2020/11/028976). Bharat has agreed on a partnership with Ocugen (Pennsylvania) to develop Covaxin for the United States market (Ocugen Press release).

Sputnik V (Russia)

Sputnik V (formerly known as Gam-COVID-Vac), developed by the Gamaleya Research Institute, is a combination of two adenoviruses, Ad5 and Ad26, each carrying an S antigen of the new coronavirus. In a December 14 press release, Gamaleya announced an efficacy of over 90% of their vaccine after analyzing 22.714 volunteers who had received the first dose of the vaccine or placebo. The Gamaleya vaccine candidate might also prevent severe COVID-19 – there were 20 severe cases of coronavirus infection among confirmed cases in the placebo group and no severe cases in the vaccine group. These findings need to be confirmed.

Gam-COVID-Vac still suffers a serious handicap – there are no substantial data published to date. The small Phase I/II trials presented only 38 people. In their recent press release, the researchers from Gamaleya still provide no information on outcomes by age group, and no data on safety. In a late-December tweet, Gamaleya announced an efficacy of over 90 percent in people over 60. Gamaleya's hair-raising communication skills – a premature presidential fake approval, Phase I/II results, press releases and tweets – clearly need coaching.

In December, Gamaleya and AstraZeneca announced that they would combine their vaccines to see if the combination would deliver a stronger protection

than either vaccine on its own. A Phase I trial was registered on Christmas Eve 2020.

Wuhan Institute vaccine (China)

In addition to BBIBP-CorV (from the *Beijing* Institute of Biological Products), Sinopharm also began testing an inactivated virus vaccine developed by the *Wuhan* Institute of Biological Products. In an interim analysis of 2 randomized placebo-controlled trials (96 and 224 healthy adults) showed that the vaccine produced antibodies (Xia S 2020). With the success of BBIBP-CorV, the future of the Wuhan candidate is uncertain. No clinical data.

Convidecia (China)

Convidecia (formerly: CTII-nCoV), developed by CanSino Biologics in partnership with the Institute of Biology at the Chinese Academy of Military Medical Sciences, is based on an adenovirus called Ad5**.** Results from a Phase I safety trial on Convidecia were published in May (Zhu FC 2020a). In July, Phase II trials demonstrated that the vaccine produced significant neutralizing antibody responses to live SARS-CoV-2 (Zhu FC 2020b) and that over 90% of participants showed either cellular or humoral immune responses at day 28 post-vaccination (Zhu 2020). The authors found that older people had a significantly lower immune response, but higher tolerability. Pre-existing immunity to the Ad5 vector and increasing age might partially hamper the specific immune responses to vaccination, particularly for the humoral immune responses. Adverse events such as fever, fatigue, headache, or local site pain were comparable to the ChAdOx1 study above. On 25 June, the Chinese military approved the vaccine for a year as a "specially needed drug." In November, the Chief Executive of CanSino Biologics said in an interview that about 40.000 to 50.000 people had received Convidecia. Since August, Phase III trials have been under way in Russia, Pakistan, Mexico and Chile.

CvnCoV (Germany)

In December 2020, CureVac launched a Phase III trial of its vaccine candidate CvnCoV which will recruit 36.500 volunteers (NCT04652102). The first efficacy data are expected in summer 2021. In a November 2020 press release, the company claimed that CVnCOV might be stable for at least three months at +5°C (+41°F) and up to 24 hours at room temperature. This might give the vaccine an advantage over other mRNA vaccines such as tozinameran or mRNA-1273. It will be interesting to follow a CureVac-Tesla collaboration on creating

mRNA "micro-factories" deployed around the world to make billions of doses of the vaccine. No clinical data.

Ad26.COV2.S (Belgium/US)

Ad26.COV2.S, developed by Janssen Pharmaceutical Companies of Johnson & Johnson, is a recombinant replication-incompetent adenovirus type 26 (Ad26) vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen (Bos 2020). Its potency in eliciting protective immunity against SARS-CoV-2 infection was demonstrated in a non-human primate challenge model (Mercado 2020). Ad26.COV2.S induced robust neutralizing antibody responses and provided complete protection against a SARS-CoV-2 challenge in five out of six rhesus macaques and near-complete protection in one out of six macaques. A Phase III trial studying a 1-dose regimen started in September 2020. In November, Johnson & Johnson announced that they were launching a second Phase III trial to test a two-dose regimen. No clinical data.

NVX-CoV2373 (US)

NVX-CoV2373, developed by Novavax, is a recombinant nanoparticle vaccine (rSARS-CoV-2) composed of trimeric full-length SARS-CoV-2 spike glycoproteins and Matrix-M1 adjuvant (Keech 2020). In a Phase I/II trial, the vaccine induced levels of neutralizing antibodies that closely correlated with anti-spike IgG. After the second vaccination neutralizing antibody responses exceeded values seen in symptomatic COVID-19 outpatients and were of the magnitude seen in convalescent serum from hospitalized patients with COVID-19. Phase III trials are under way in Britain and in the US. In September, Novavax signed a deal with the Serum Institute of India to produce a minimum of one billion doses of its NVX-CoV2373, thereby doubling its potential COVID-19 vaccine manufacturing capacity to two billion doses annually. No clinical data.

CoVLP (Canada)

CoVLP, developed by Medicago, is a coronavirus-like particle COVID-19 vaccine candidate composed of recombinant spike (S) glycoprotein expressed as virus-like particles (VLPs). A Phase I study showed that when combined with an adjuvant made by GSK, the vaccine produced promising levels of antibodies. A Phase II/III trial investigating two doses 21 days apart began in November 2020 (NCT04636697). The trial aims at enrolling more than 30.000 participants. No clinical data.

ZF2001 (China)

ZF2001 is a recombinant subunit candidate vaccine produced by the Chinese company Anhui Zhifei Longcom and the Chinese Academy of Sciences. The vaccine is composed of the receptor-binding domain of the spike protein and an adjuvant. Phase III trials will enroll 29.000 adult volunteers (NCT04646590). Global trials are expected to begin in Uzbekistan, followed by trials in Indonesia, Pakistan and Ecuador. The company announced an annual production capacity of up to 300 million doses. No clinical data.

SCB-2019 (China)

SCB-2019, developed by Clover Biopharmaceuticals, China, is a recombinant subunit vaccine candidate administered with AS03 adjuvant. Clover has produced an S trimer subunit vaccine candidate that resembles the native trimeric viral spike. A Phase II/III trial investigating two doses 21 days apart was to start in December 2020 (NCT04672395). The trial aims at enrolling more than 34.000 participants. No clinical data.

Immunization fundamentals

The SARS-CoV-2 pandemic and the unprecedented research effort to develop multiple vaccines on different platforms is the perfect time for immunologists to be involved in designing the next generation of powerful immunogens (Pollard 2020, Dagotto 2020).

Recovery from infections often induces long-term and sometimes life-long immunity against the causative pathogen. After the resolution of the infection, immunological memory protects against re-infection and is mediated by specific antibodies and T cells.

In contrast, immunizations confer immunity without exposure to virulent pathogens. Immunization can be passive or active. In passive immunization protective antibodies are transferred from a donor into a recipient whereas active immunization induces a protective immune response in the recipient.

Passive immunization against SARS-CoV-2

Passive immunization against SARS-CoV-2 can be achieved with convalescent plasma or with neutralizing monoclonal antibodies.

Convalescent plasma

Treatment with human convalescent plasma (CP) is based on the assumption that protective antibodies against the causative pathogen are present in the

blood of people who have overcome an infectious disease. For example, CP has been used to treat some infectious diseases such as Argentine hemorrhagic fever (Casadevall 2004). CP was also used to treat SARS patients in the 2002/2003 epidemic but not in controlled clinical studies; a later meta-analysis concluded that the treatment was probably safe and perhaps helpful (Mair-Jenkins 2015).

CP could become an option for prevention and treatment of COVID-19 disease when there are sufficient numbers of people who have recovered and can donate immunoglobulin-containing serum (Casadevall 2020). Antibodies that are found in CP are very stable. Pathogen inactivation (using psoralen and UV light) did not impair the stability and neutralizing capacity of SARS-CoV-2-specific antibodies that was also preserved at 100% when the plasma was shock frozen at -30°C after pathogen-inactivation or stored as liquid plasma for up to 9 days (Tonn 2020). However, in a recently published open label randomized controlled trial (the largest to date with results) 464 patients were assigned either to two doses of 200 mL CP or standard of care only. The result was sobering: progression to severe disease at 28 days after enrolment occurred in 44 (19%) participants in the CP arm and 41 (18%) in the control arm (Agarwal 2020).

The major caveat of CP is quantity and quality of antibody titers. In plasma from 149 patients collected on average 39 days after the onset of symptoms, neutralizing titers were extremely variable. Most plasma did not contain high levels of neutralizing activity (Robbiani 2020). There seems to be a correlation between serum neutralizing capacity and disease severity, suggesting that the collection of CP should be restricted to those with more severe symptoms (Chen 2020). Another, unintended, consequence of receiving CP may be that recipients will not develop their own immunity, putting them at risk for re-infection.

In addition, in light of the possibility of antibody-dependent disease enhancement (ADE), safety is still a hypothetical consideration in the ongoing CP trials. One study on macaques found that passive transfer of anti-SARS-CoV-S immunoglobulin 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). In one case, the administration of convalescent plasma to MERS patients raised the possibility of acute lung injury (Chun 2016).

The future development of anti-SARS-CoV-2 convalescent plasma should take into account 1) the potential harms of the non-immune components of conva-

lescent plasma (especially prothrombotic risks); 2) that only donor plasma with detectable titers of neutralizing antibodies be given to trial participants; 3) ensure double-blind designs with placebo controls as the gold standard for future trials; 4) preclude non-immune plasma as a control intervention, because of potential harms and availability of lower risk alternatives such as normal saline (Pathak 2020).

In early January, a randomized, double-blind, placebo-controlled trial of convalescent plasma with high IgG titers against SARS-CoV-2 (n=160) found that severe respiratory disease developed in 13 of 80 patients (16%) who received convalescent plasma and 25 of 80 patients (31%) who received placebo (Libster 2021). Find more information on CP in the *Treatment* chapter, page 359.

Monoclonal antibodies

The development of highly successful monoclonal antibody-based therapies for cancer and immune disorders has created a wealth of expertise and manufacturing capabilities (Biopharma 2020) and neutralizing monoclonal antibodies are now a plausible therapeutic option against infectious diseases (Marston 2018). Monoclonal antibodies against rabies virus and against the respiratory syncytial virus (RSV) are approved for the treatment of patients and other monoclonal antibodies are in advanced stages of clinical trials (Walker 2018). Both protective and pathogenic effects were observed (Wang Q 2016, Chen X 2020). The 'COVID-19 antibodysphere' features companies like Amgen, AstraZeneca, Vir, Regeneron, Lilly and Adagio (Biopharma 2020). However, the future role of monoclonal antibodies as a bridging solution before the general availability of vaccines and efficient antiviral drugs is unclear. These drugs are complex and expensive to produce, leaving people from poor countries locked out (Ledford 2020, Ledford 2020b) and fears have been voiced that they could split the world into the haves and have-nots, like many other drugs before (Cohen 2020). Fortunately, these fears may not materialize. As soon as the first truly effective antiviral drugs become available – as for HSV in 1981, HIV in 1996 and HCV in 2013 – there will be no need for monoclonal antibodies anymore.

In 2020, SARS-CoV-2 neutralizing human monoclonal antibodies were intensely studied (Robbiani 2020, Wec 2020, Ju B 2020, Cohen 2020) and it was shown that REGN-CoV-2, a cocktail of two antibodies, might preclude the appearance of escape mutants (Baum 2020) and decrease virus-induced pathological sequalae in rhesus macaques (Baum 2020b). In November, the FDA issued an emergency use authorization for bamlanivimab (see page 354) and later for the antibody cocktail casirivimab (REGN10933)/imdevimab

(REGN10987) (see page 355) to be administered together for the treatment of mild to moderate COVID-19 in patients of 12 years of age or older (weighing at least 40 kilograms) who are at high risk for progressing to severe COVID-19 (65 years of age or older or certain chronic medical conditions. These antibodies are not authorized for patients who are hospitalized due to COVID-19 or require oxygen therapy due to COVID-19. Find more details on monoclonal antibodies in the *Treatment* chapter, page 354.

Issues to address during vaccine development

Rarely, vaccines can enhance disease rather than protect from disease (Kim 1969, Openshaw 2001). Some vaccine candidates against SARS-CoV-1 or MERS-CoV have caused disease-intensifying immunopathological effects in some pre-clinical models. Reassuringly, at least in the short term, results from the Phase III trials did not show evidence of enhanced respiratory disease after infection (Polack 2020, Voysey 2020, Baden 2020). Antibody-dependent enhanced infections may be unlikely because coronavirus diseases in humans lack the clinical, epidemiological, biological, or pathological attributes of ADE disease exemplified by dengue viruses (DENV) (Halstead 2020). In contrast to DENV, SARS and MERS CoVs predominantly infect respiratory epithelium, not macrophages.

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

Ferrets are susceptible to SARS-CoV and SARS-CoV-2 infections (Kim YI 2020). Weingartl et al. immunized ferrets with a recombinant modified vaccinia virus Ankara (rMVA), which encoded the SARS-CoV S protein (Weingartl 2004). After infection with the virus, high titers of neutralizing antibodies were detectable in the immunized animals. Nevertheless, the immunized infected ferrets developed severe hepatitis while the non-immunized did not (Weingartl 2004).

Type 2 immunopathology in the lungs of immunized mice

Bolles et al. (Bolles 2011) immunized mice with inactivated SARS-CoV with or without adjuvant. The vaccine protected young and old animals from morbidity and mortality after infection with high doses of virus. If the mice were infected with a heterologous virus strain, the immunized animals developed more pronounced inflammatory infiltrates and pulmonary eosinophilia than the non-immunized (Bolles 2011). These results were later confirmed by another working group (Tseng CT 2012). Eosinophilic lung infiltrates were also observed in mice after immunization with a recombinant baculovirus (S pro-

tein) or coronavirus-like particles (VLPs) that expressed SARS-CoV S protein. It is important to note that these are histopathological findings; the immunized mice still had reduced virus titers after infection (Tseng CT 2012, Lokugamage 2008). Nevertheless, the findings are worrying. They are similar to the histopathological changes seen in the 1960s in children who became ill after immunization with a vaccine against RSV (Castilow 2007). Pathological changes in the lungs and even pneumonia after infection with SARS-CoV were also observed in mice in other SARS-CoV vaccine candidates (Yasui 2008).

Similar findings have been reported for vaccine candidates for MERS-CoV. An inactivated MERS-CoV vaccine induced neutralizing antibodies in mice. Nevertheless, after infection, the immunized mice developed an increased type 2 pathology in the lungs with increased eosinophilic infiltrates and increased concentrations of IL-5 and IL-13 (Agrawal 2016). Recent studies suggest that the development of type 2 immunopathology can be influenced by the choice of appropriate adjuvants, e.g. TLR ligands, for inactivated viruses, or recombinant S protein can be avoided (Iwata-Yoshikawa 2014, Honda-Okubo 2015).

Overall, these findings are a clear indication that during the preclinical development of vaccines against SARS-CoV-2, an intensive search should be made for immunopathological changes in the lungs of the immunized animals. It is encouraging that many of the pre-clinical studies published to date on SARS-CoV-2 vaccine candidates explicitly indicate that such changes have been sought and not found.

Type 2 immunopathology in the lungs of immunized primates

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). These findings are similar to those of an earlier study in which macaques were immunized with inactivated SARS-CoV. Three monkeys were protected upon challenge whereas one macaque had lung pathology consistent with antibody-dependent disease enhancement (ADE) (Wang Q 2016). The authors suspect that only antibodies against certain SARS-CoV S epitopes induce the immunopathology. In the previously published SARS-CoV-2 vaccination studies in monkeys, no lung pathology was observed (Gao Q 2020, Wang H 2020, van Doremalen 2020).

Questions for the Future

Despite the rapid and massive roll-out of highly effective vaccines, some important questions remain unanswered.

Correlates of Protection

Knowledge about the immune responses against SARS-CoV-2 is growing rapidly (Vabret 2020); it seems clear that neutralizing antibodies against the S protein can mediate protection. SARS-CoV-2-specific T cells can also be present in people without detectable antibodies against SARS-CoV-2 (Braun 2020, Grifoni 2020, Sekine 2020). Preclinical studies on SARS (Li CK 2008) and MERS (Zhao J 2017) suggest that virus-specific CD4+ (Zhao J 2016) and CD8+ (Channappanavar 2014) T cells can be protective even in the absence of serologically detectable antibodies (Tang F 2011). A challenge still needing to be addressed is the fact 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 usually higher doses or particular immunization schemes in order to generate a protective immune response.

Longevity of the immunological memory against SARS-CoV-2

Ideally, a vaccine induces long-term immunity. In the context of the SARS-CoV-2 pandemic, we will remember that infections with common cold coronaviruses generate only a short-lived immunity. Experiments from the 1980s have shown that just one year after inoculation with coronavirus 229E, the majority of the test subjects could be infected again. They did have milder symptoms than non-inoculated subjects, so a certain protection was seen despite renewed infection (Callow 1990).

Experience from the 2002-2004 SARS-CoV(-1) outbreak, too, suggest that SARS-CoV-2 immune responses will be short-lived. Six years after having suffered SARS disease, antibodies to SARS-CoV were no longer detectable in 21/23 patients (Tang F 2011). In contrast, SARS-CoV-specific T cells were still detectable, which suggests the possibility that T cell memory against coronaviruses may be more long-lasting than serological memory (Tang F 2011). Similar findings have been described for the immune response after MERS disease (Zhao J 2017); however, there is currently no reliable knowledge about the longevity of T cell memory against SARS-CoV 2. There have been reports of renewed SARS-CoV-2 infections after surviving the first infection (To KK 2020).

After almost one year of research, the picture of immunological memory against SARS-CoV-2 is becoming clearer. The kinetics of the neutralizing antibody response to SARS-CoV-2 is typical of an acute viral infection where a peak response is detected 3–4 weeks post-infection, which then wanes (Seow 2020). More than 90% of of infected individuals with mild-to-moderate COVID-19 experience might develop robust IgG antibody responses against the viral spike protein and titers will be relatively stable for months Anti-spike binding titers correlate with neutralization of authentic SARS-CoV-2 (Gudbjartsson 2020, Wajnberg 2020, Alter 2020). Patients with a worse clinical classification may have higher neutralizing antibody titer (Wang X 2020).

However, asymptomatic individuals have a weaker immune response to SARS-CoV-2 infection than patients with severe COVID-19 (Long QX 2020) and humoral immunity against SARS-CoV-2 may not be long-lasting in this large group that composes the majority of infected persons (Ibarrondo 2020, Weis 2020). One group showed that in individuals who develop a low neutralizing antibody response (ID_{50} 100–300), titers can return to baseline over a relatively short period, whereas those who develop a robust neutralizing antibody response maintain titers > 1,000 despite the initial decline (Seow 2020).

After Year 1 of the SARS-CoV-2 pandemic, we realize that although millions of people were infected during spring 2020, there is no sizeable epidemic of *re*-infections. This observation suggests that SARS-CoV-2 infection might confer a solid immunity. Recently, Shane Crotty, Alessandro Sette, Daniela Weiskopf, Jennifer Dan and colleagues have analyzed multiple compartments of circulating immune memory to SARS-CoV-2 in 188 COVID-19 cases, including 43 cases at > 6 months post-infection. The result: Spike-specific memory B cells were more abundant at 6 months than at 1 month post symptom onset. SARS-CoV-2-specific $CD4^+$ T cells and $CD8^+$ T cells declined with a half-life of 3-5 months (Dan 2020). These findings suggest that after SARS-CoV-2 infection (or after vaccination), the vast majority of people could be protected from severe COVID-19 for years.

Pre-existing immune responses against SARS-CoV-2

SARS-CoV-2-specific CD4+ and CD8+ T lymphocytes can be detected in around 20 to 100% of non-exposed healthy volunteers (Braun 2020, Grifoni 2020, Mateus 2020, Bacher 2020). It has been speculated that these cross-reactive T lymphocytes could be protective against SARS-CoV-2 or influence the course of the disease. As a matter of fact, it is known that cross-reactive T cells can influence the course of viral infections both positively and negatively (Ngono 2018). Antibodies that neutralize SARS-CoV-2 have also been detected in uninfected and non-exposed healthy volunteers (Ng KW 2020).

We might not rely on protection without exposure, though. In a recently published pre-print study from Rockefeller University, the authors measured neutralizing activity against SARS-CoV-2 in pre-pandemic sera from patients with prior PCR-confirmed seasonal coronavirus infection. While neutralizing activity against seasonal coronaviruses was detected in nearly all sera, cross-reactive neutralizing activity against SARS-CoV-2 was undetectable (Posten 2020). The authors conclude that while it is possible that there are rare instances of individuals possessing antibodies from prior seasonal HCoV infection who may be able to also target SARS-CoV-2 S, their data would argue against a broad role for pre-existing protective humoral immunity against SARS-CoV-2.

Recently, a provocative concept was introduced by Alexander Scheffold and colleagues. The authors propose the immunological age as an independent risk factor for developing severe COVID-19 (Bacher 2020). Their reasoning:

1. Unexposed individuals harbor SARS-CoV-2-specific memory T cells with marginal cross-reactivity to common cold corona and other unrelated viruses.
2. Low avidity pre-existing T cell memory negatively impacts on the T cell response against neoantigens such as SARS-CoV-2, which may predispose to developing inappropriate immune reactions especially in the elderly.

Find more about this topic in the 7th edition of COVID Reference.

Outlook

This rapid development of SARS-CoV-2 vaccines is the result of a massive global effort, including the parallelization of development and production steps that have traditionally been carried out sequentially (Lurie 2020), the knowledge generated in attempts to develop vaccines against SARS-CoV-1 and MERS-CoV, and innovative techniques (Hekele 2013) that were not available until recently. The speed of SARS-CoV-2 vaccine development was 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 (Arnold 2020). 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. With the sequencing of the genome of SARS-CoV-2, the high homology between the S proteins of the 3 viruses was known and a little later the interaction of SARS-CoV-2 with ACE was confirmed (Hoffmann 2020).

A relevant target structure for immune responses was identified in record time.

In less than a year, we have characterized a novel illness, sequenced a new viral genome, developed diagnostics, produced treatment protocols, and established the efficacy of drugs and vaccines in randomized controlled trials (Editorial 20201121). More people will be able to get vaccinated more quickly than ever before. If we can achieve some kind of pre-COVID-19 'normalcy' by 2022, it would be an historical feat remembered by generations.

Nobody should forget: vaccines are the most potent medical products of all time to prevent morbidity and mortality. Over the last two centuries, no other medical intervention has saved as many lives. Without vaccines, many of today's anti-vaccine activists (Burki 2020) would not have been born (and maybe neither you nor me) because of lack of ancestors – one or more of them would have succumbed to infectious disease before reaching mating age. Vaccines train the body's immune system to recognize and fight pathogens and on the next exposure to the pathogen, the immune system is ready to fight the invader off. The vaccination procedure is simple: introduce certain molecules from the pathogen into the body – the mass equivalent of a few grains of fine salt – and trigger an immune response. Vaccines are 'elegant medicine' – they prevent rather than treat a disease.

At the end of this COVID Year One, virology, biologic chemistry and immunology are the celebrated fields of medicine. Virology explores the structure and functioning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and, together with biologic chemistry, prepares the terrain for future drug development. In the meantime, immunology explores the virus-human interface and describes how the human body fights back and forms a memory after the first encounter with SARS-CoV-2: it examines why most people recover from the infection while a few die and other remain disabled; and it contributes to the understanding of the biological mechanisms that lead to illness and death. Why do older people die from COVID-19 while younger people don't? Why are people with hypertension, diabetes or obesity at increased risk of severe COVID-19? Immunology also tries to elucidate the mystery of superspreader individuals, those few acutely SARS-CoV-2 infected people who are thought to be responsible for the vast majority of transmissions. Finally, immunology will spin out the most powerful antiviral weapon: vaccines.

For quite some time, the SARS-CoV-2 pandemic will continue to be a colossal challenge for healthcare systems and societies. It is also the time of the 'Great Rehearsal'. By coordinating global resources and supra-national structures to react swiftly, science is currently creating the infrastructure to fight any oth-

er new and potentially far deadlier viral disease that emerges in the future. SARS-CoV-2 is not the last pathogen humanity will have to deal with in the 21st century and more enzootic viruses will jump from their animal reservoirs to humans. After this pandemic, hopefully we will be better prepared for future challenges, with new vaccine platforms that can be quickly adapted to newly emerging viral diseases. There is even a final twist to the unexpected events of 2020: the SARS-CoV-2 pandemic is opening up a new era of vaccine development. In 10 years we can expect to have a wide range of new and innovative vaccines (Dolgin 2021) we would not have dared to previously dream of.

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7. Diagnostic Tests and Procedures

Christian Hoffmann

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 just 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 diagnostic testing for COVID-19 in suspected human cases, published by WHO in March and updated on September 11, 2020 (WHO 20200911). Several comprehensive up-to-date reviews of laboratory techniques in diagnosing SARS-CoV-2 have been published recently (Kilic 2020, Loeffelholz 2020).

According to WHO, the decision to test "should be based on both clinical and epidemiological factors", in order to support clinical management of patients and infection control measures. In symptomatic patients, a PCR test should be immediately carried out, especially for medical professionals with symptoms. In particular, this applies to nursing homes and other long-term facilities where large outbreaks with high resident mortalty may occur. In these settings, every day counts: both residents and health-care workers should be tested immediately. In regression analyses among 88 nursing homes with a documented case before facility-wide testing occurred, each additional day between identification of the first case and completion of facility-wide testing was associated with identification of 1.3 additional cases (Hatfield 2020). However, the predictive value of the tests markedly varies with time from exposure and symptom onset. The false-negative rate is lowest 3 days after onset of symptoms, or approximately 8 days after exposure (see below).

In settings with limited resources, however, patients should only be tested if a positive test results in imperative action. It does not necessarily make sense to attempt to ascertain the prevalence of infection by PCR. For example, in a family which was put on quarantine after the infection was confirmed in one

member, not all household contacts have to be tested, especially younger persons with only mild symptoms.

For many countries and regions, there are constantly updated recommendations by authorities and institutions about who should be tested by whom and when: these recommendations are constantly changing and have to be adapted to the local epidemiological situation. The lower the infection rates and the higher the testing capacities, the more patients will be able to be tested.

Specimen collection

Respiratory tract

SARS-CoV-2 can be detected in a wide range of different tissues and body fluids. In a study on 1,070 specimens collected from 205 patients with COVID-19 (Wang X 2020), 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%).

Though respiratory secretions may be quite variable in composition, respiratory samples remain the sample type of choice for diagnostics. Viral replication of SARS-CoV-2 is very high in upper respiratory tract tissues which is in contrast to SARS-CoV (Wolfel 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. Besides nasopharyngeal swabs, samples can 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.

A prospective study in two regional hospitals in Hong Kong examined 563 serial samples collected during the viral shedding period of 50 patients: 150 deep throat saliva (DTS), 309 pooled nasopharyngeal (NP) and throat swabs, and 104 sputum (instructions for deep throat saliva: first clear your throat by gargling with your own saliva, and then spit out the DTS into a sterile bottle). Deep throat saliva produced the lowest viral RNA concentration and a lower RT-PCR positive rate compared to conventional respiratory specimens. Buccal swabs do not work well either. In 11 children positive via nasopharyngeal

swabs, 2 remained negative via buccal swabs. There was a general trend for buccal specimens to contain lower SARS-CoV-2 viral loads compared with nasopharyngeal specimens (Kam 2020).

Nasopharyngeal swabs – practical issues

It is important to carry out the swab process 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 protective clothing should be practiced! Many mistakes occur even just removing the protective mask. Gathering specimens from nasopharyngeal and throat swabs can cause discomfort for patients and put health-care workers at risk. If not performed properly or in patients with complex and delicate anatomy, there is a risk for adverse events such as cerebrospinal fluid leak (Sullivan 2020). There is a very useful video on protection, preparation, equipment, handling, removing personal protective equipment, etc (Marty 2020).

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 any possible cough droplet. Tell the patient that it might 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. Mid-turbinate nasal swabs may be less sensitive (Pinninti 2020). 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 swab process.

In order to minimize the exposure risk to health care workers and depletion of personal protective equipment, we have established swab instructions for patients who are able to do this (ie, most of them!) at home. After appropriate instruction, they can perform the swabs themselves. A courier with the tubes is sent directly to the patient's home, and the courier places the tubes at 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.

Unsupervised home swab collection was comparable to clinician-collected nasopharyngeal swab collection (McCulloch 2020). In one of the largest studies to date, a total of 530 patients with upper respiratory infection were provided with instructions and asked to collect tongue, nasal, and mid-turbinate samples (Tu 2020). A nasopharyngeal sample was then collected from the patient by a healthcare worker. When this NP sample was used as the comparator, the estimated sensitivities of the tongue, nasal, and mid-turbinate samples collected by the patients were 89.8%, 94.0% and 96.2%, respectively.

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 and longer storage can lead 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 stools (see below).

In contrast to many respiratory viruses, SARS-CoV-2 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, Wyllie 2020, Yokota 2020). In a large study on "enhanced" saliva specimens (strong sniff, elicited cough, and collection of saliva/secretions) from 216 patients with symptoms deemed consistent with COVID-19, there was a 100% positive agreement (38/38 positive specimens) and 99.4% negative agreement (177/178 negative specimens).

Fecal shedding

Although no cases of transmission via fecal-oral route have yet been reported, there is also evidence that SARS-CoV-2 is actively replicating in the gastrointestinal tract. Several studies showed prolonged presence of SARS-CoV-2 viral RNA in fecal samples (Chen 2020, Wu 2020). Combining results of 26 studies, a rapid review revealed that 54% of those patients tested for fecal RNA were positive. Duration of fecal viral shedding ranged from 1 to 33 days after a negative nasopharyngeal swab (Gupta 2020). In another meta-analysis of 17 studies, the pooled detection rate of fecal SARS-CoV-2 RNA was 44% and 34% by patient and number of specimens, respectively. Patients who presented with gastrointestinal symptoms (77% vs. 58%) or with a more severe disease (68% vs. 35%) tended to have a higher detection rate.

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 findings remains unclear and there is one study that did not detect infectious virus from stool samples, despite having high virus RNA concentrations (Wolfel 2020). Therefore, the presence of nucleic acid alone cannot be used to define viral shedding or infection potential (Atkinson 2020). For many viral diseases including SARS-CoV or MERS-CoV, it is well known that viral RNA can be detected long after the disappearance of infectious virus.

Specimens other than respiratory and gastrointestinal: blood, urine, breast milk

- Blood – in patients with mild or moderate disease, SARS-CoV-2 is relatively rarely detected in blood (Wang W 2020, Wolfel 2020). In a screening study of 7,425 blood donations in Wuhan, plasma samples were found positive for viral RNA from 2 asymptomatic donors (Chang 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 (Kwon 2020). As with feces, it remains unclear whether detectable RNA in the blood signifies infectivity. In a study of 167 hospitalized patients, SARS-CoV-2 was found in 64 patients at hospital admission, 3 of 106 serum PCR negative patients and 15 of 61 positive patients died (Hagman 2020). However, the clinical significance of SARS-CoV-2 "RNAemia" needs to be defined.
- Urine - None of 72 urine specimens tested positive (Wang X 2020).
- Breast milk – in a case report, SARS-CoV-2 RNA was detected in breast milk samples from an infected mother on 4 consecutive days. Detection of viral RNA in milk coincided with mild COVID-19 symptoms and a SARS-CoV-2 positive diagnostic test of the newborn (Groß 2020). However, this seems to be rare. Among 64 breast milk samples from 18 infected women, SARS-CoV-2 RNA was detected in only one milk sample; the viral culture for that sample was negative. These data suggest that SARS-CoV-2 RNA does not represent replication-competent virus and that breast milk may not be a source of infection for the infant (Chambers 2020. Case reports of transmitted antibodies in breast milk have also been reported (Dong 2020).

- Vaginal fluid - all samples of 10 women with COVID-19 were negative (Saito 2020).
- Semen – Absence of virus in samples collected from 12 patients in their recovery phase (Song 2020).
- Tears and conjunctival secretions - among 40 patients (10 with conjunctivitis) who tested positive by RT-PCR of nasopharyngeal and oropharyngeal swabs, conjunctival swab PCR was positive for 3 patients, among them one with conjunctivitis (Atum 2020). In another study, no SARS-CoV-2 was found in tears (Meduri 2020).

PCR

Dozens of in-house and commercial rRT-PCR assays 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 (Loeffelholz 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 JF 2020).

The limits of detection of commercial kits may differ substantially. However, most comparative studies have shown a high sensitivity and their suitability for screening purposes worldwide:

- In a comparison of 11 different RT-PCR test systems used in seven labs in Germany in March 2020, the majority of RT-PCR assays detected ca 5 RNA copies per reaction (Münchhoff 2020). A reduced sensitivity was noted for the original Charité RdRp gene confirmatory protocol, which may have impacted the confirmation of some cases in the early weeks of the pandemic. The CDC N1 primer/probe set was sensitive and robust for detection of SARS-CoV-2 in nucleic acid extracts from respiratory material, stool and serum from COVID-19 patients.

- Analytical limits of detection for seven SARS-CoV-2 assays using serial dilutions of pooled patient material quantified with droplet digital PCR. Limits of detection ranged from ≤ 10 to 74 copies/ml for commercial high-throughput laboratory analyzers (Roche cobas, Abbott m2000, and Hologic Panther Fusion) and 167 to 511 copies/ml for sample-to-answer (DiaSorin Simplexa, GenMark ePlex) and point-of-care instruments (Abbott ID NOW) (Fung 2020).
- A total of 239 specimens (168 contained SARS-CoV-2) were tested by five test methods (Procop 2020). The assays that lacked a nucleic acid extraction step produced more false-negative reactions than assays that included this step. The false-negative rates were 0% for the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel, 3,5% for TIB MOLBIOL Assay (Roche), 2,4% for Xpert Xpress SARS-CoV-2 (Cepheid), 11,9% for Simplexa COVID-19 Direct Kit (DiaSorin), and 16,7% for the ID NOW COVID-19 (Abbott). Most false negatives were seen in patients with low viral loads.

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 very rare. However, they do occur. Though the analytical specificity of these tests is usually 100%, the clinical specificity is less, due to contamination (a significant problem for NAT procedures) and/or human error in the handling of samples or data (very hard to eliminate entirely). As seen with serology (see below), these false positive results can have substantial effects when prevalence is low (Andrew Cohen, personal communication).

Most PCR assays are designed to detect two or more specific target gene regions. Rarely, an inconclusive result can occur when only one of the targets is detected (< 1%). There are quantitative algorithms to assess and interpret inconclusive PCR results, by combining laboratory, clinical, and epidemiologic data (Yang S 2020).

Another problem of any qualitative PCR is false negative results which can have many causes (review: Woloshin 2020). Incorrect smears are particularly common, but laboratory errors also occur. In a review of 7 studies with a total of 1,330 respiratory samples, the authors estimated the false-negative rate of RT-PCR by day since infection. Over the 4 days before symptom onset, the rate decreased from 100% to 67%. On the day of symptom onset (day 5), the rate was 38%, decreasing to 20% (day 8) and then beginning to increase again

from 21% (day 9) to 66% (day 21). If clinical suspicion is high, infection should not be ruled out on the basis of RT-PCR alone. The false-negative rate is lowest 3 days after onset of symptoms, or approximately 8 days after exposure (Kucirka 2020). Figure 1 illustrates PCR and antibody detection during SARS-CoV-2 infection.

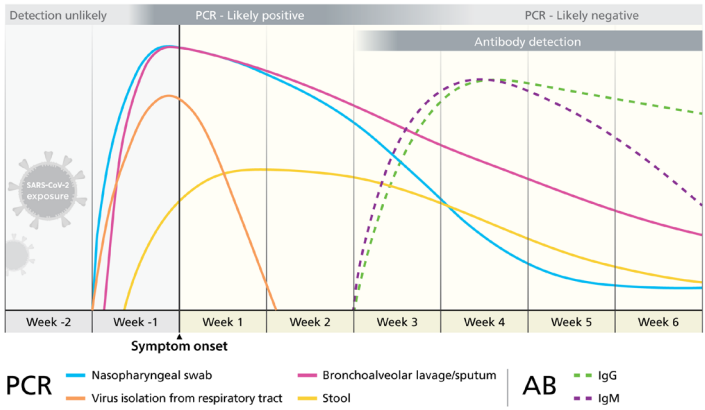

Figure 1. Timeline of diagnostic markers for detection of SARS-CoV-2. AB = Antibody.

Do we need to re-test in the case of a negative PCR? Several studies argue against this strategy, finding very low rates of negative-to-positive conversion with repeated testing (Lepak 2020). Among 20.912 patients, one study analyzed the frequency of SARS-CoV-2 RT-PCR test discordance among individuals initially testing negative by nasopharyngeal swab who were retested on clinical grounds within 7 days. The frequency of subsequent positivity within this window was only 3,5% and similar across institutions (Long 2020). It appears that if the first PCR is negative, a second PCR only yields a small number of positive results.

Several studies have shown that asymptomatic patients also have positive PCR results and can transmit the virus (Bai 2020, Cereda 2020, Rothe 2020). The cycle threshold values of RT-PCR for SARS-CoV-2 ("viral load") in asymptomatic patients are similar to those in symptomatic patients (Lee S 2020, Lavezzo 2020).

In symptomatic patients, 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, PCR was positive even longer: male gender and a severe course (invasive mechanical ventilation) were independent risk factors for prolonged shedding (Xu K 2020). It is of note that immunocompromised patients may shed infectious virus for longer durations than previously recognized. In some of these patients, shedding of infectious SARS-CoV-2 was observed for up to 3-5 months or even longer (Avanzato 2020, Choi 2020).

Several reports from patients have repeatedly gained much media attraction, showing positive results after repeated negative PCR and clinical recovery (Lan 2020, Xiao AT 2020, Yuan 2020). These studies have raised the question of re-activation or re-infection of COVID-19 (see below, chapter *Clinical Presentation*, page 279). However, it seems probable that the results are much more likely due to methodological problems (Li 2020). At low virus levels, especially during the final days of infection, the viral load can fluctuate and sometimes be detectable, sometimes not (Wolfel 2020). Reactivation, and also a rapid reinfection would be very unusual for coronaviruses. In a study on nasal/oropharyngeal swab samples of 176 recovered patients with no fever and with 2 negative RT-PCR results for SARS-CoV-2 RNA 24 hours apart, 32 of 176 samples (18%) tested positive for total SARS-CoV-2 RNA. All had low viral loads and only one of the 32 samples (3,1%) had replicative SARS-CoV-2 RNA (Liotti 2020).

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 asymptomatic patients was similar to that in 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 (2552 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 large cohort (n = 1145) of hospitalized, symptomatic patients from New York, viral loads were measured. In a Cox proportional hazards model adjusting for several confounders, there was a significant independent association between viral load and mortality (hazard ratio 1.07, 95% CI 1.03–1.11, p = 0,0014), with a 7% increase in hazard for each log transformed copy/mL (Pujadas 2020). However, prospective trials are needed to evaluate the role of SARS-CoV-2 viral load as a marker for assessing disease severity and prognosis.

Should we measure viral load? Probably yes. It may be helpful in clinical practice. A positive RT-qPCR result may not necessarily mean the person is still infectious or that they still have any meaningful disease. The RNA could be from non-viable virus and/or the amount of live virus may be too low for transmission.

Cycle threshold (Ct) values

RT-qPCR provides quantification by first reverse transcribing RNA into DNA, and then performing qPCR where a fluorescence signal increases proportionally to the amount of amplified nucleic acid. The test is positive if the fluorescence reaches a specified threshold within a certain number of PCR cycles (Ct value, inversely related to the viral load). Many qPCR assays use a Ct cut-off of 40, allowing detection of very few starting RNA molecules. Some experts (Tom 2020) suggest using this Ct value or to calculate viral load which can help refine decision-making (shorter isolation, etc). Unfortunately, there is still a wide heterogeneity and inconsistency of the standard curves calculated from studies that provide Ct values from serial dilution samples and the estimated viral loads. According to other experts, precautions are needed when interpreting the Ct values of SARS-CoV-2 RT-PCR results shown in COVID-19 publications to avoid misunderstanding of viral load kinetics for comparison across different studies (Han 2020). Caution is needed when regarding Ct values as a surrogate indicator of 'quantity' in a qualitative PCR assay ("viral

load"). Results are not transferable across different assays, different gene targets and different specimen types (Poon 2020).

However, some clinical key studies are listed here:

- In 678 patients with COVID-19, in-hospital mortality was 35,0% with a "high viral load" (Ct < 25; n = 220), 17,6% with a "medium viral load" (Ct 25-30; n = 216), and 6,2% with a "low viral load" (Ct > 30; n = 242). High viral load was independently associated with mortality (adjusted odds ratio 6.05; 95% CI: 2.92-12.52) and intubation (aOR 2,73; 95% CI: 1,68-4,44) in multivariate models (Magleby 2020).
- A prospective serial sampling of 70 patients revealed clinically relevant Ct values, namely a Ct of 24 ("high viral load"), and > 40 ("negative"), occurred 9 and 36 days after symptom onset (Lesho 2020).
- Among 93 household members (including index cases) who tested positive for SARS-CoV-2 by NP swab, Ct values were lowest soon after symptom onset and were significantly correlated with time elapsed since onset; within 7 days after symptom onset, the median Ct value was 26,5, compared with a median Ct value of 35,0 at 21 days after onset (Salvatore 2020).
- Virus culture was attempted from 324 samples (from 253 cases) that tested positive for SARS-CoV-2 by RT-PCR. Ct values correlated strongly with cultivable virus. Probability of culturing virus declined to 8% in samples with Ct > 35 and to 6% (95% CI: 0,9–31,2%) 10 days after onset (Singanayagam 2020).
- A cross-sectional study determined PCR positive samples for their ability to infect cell lines. Of 90 samples, only 29% demonstrated viral growth. There was no growth in samples with a Ct > 24 or duration of symptoms > 8 days (Bullard 2020).

Test systems other than conventional RT-PCR

Access to rapid diagnosis is key to the control of the SARS-CoV-2 pandemic. In the future, point-of-care testing could relieve pressure on centralized laboratories and increase overall testing capacity. Besides PCR, additional potentially valuable amplification/detection methods, such as CRISPR (targeting clustered regularly interspaced short palindromic repeats), isothermal nucleic acid amplification technologies (e.g. reverse transcription loop-mediated isothermal amplification (RT-LAMP), and molecular microarray assays are under development or are in the process of being commercialized. According to WHO on September 11, validation of the analytic and clinical performance of these assays, demonstration of their potential operational utility, rapid

sharing of data, as well as emergency regulatory review of manufacturable, well-performing tests "are encouraged to increase access to SARS-CoV-2 testing" (WHO 20200911).

Point-of-care tests

Point-of-care tests are easy-to-use devices to facilitate testing outside of laboratory settings (Guglielmi 2020, Joung 2020). They are eagerly awaited. But will they be game changers? On May 6, the FDA granted an emergency use authorization for a CRISPR-based SARS-CoV-2 fluorescent assay marketed by Sherlock Biosciences. This straightforward SARS-CoV-2 test combines simplified extraction of viral RNA with isothermal amplification and CRISPR-mediated detection. The results are available within an hour with minimal equipment. First results (n = 202 positive/200 negative samples): sensitivity 93,1%, specificity 98,5% (Joung 2020). However, its use still remains limited to laboratories certified to perform high-complexity tests. There are other reports of an all-in-one dual CRISPR-Cas12a assay (Ding 2020) which allows all components to be incubated in one pot for CRISPR-based nucleic acid detection, enabling simple, all-in-one molecular diagnostics without the need for separate and complex manual operations.

On May 6, FDA also authorized (EUA) Quidel's Sofia 2 SARS Antigen Fluorescent Immunoassay. This test must be read on a dedicated analyzer and detects SARS-CoV-2 nucleocapsid protein from nasopharyngeal swabs in 15 min. According to the manufacturer, the assay demonstrated acceptable clinical sensitivity and detected 47/59 infections (80%). In another study, the so called CovidNudge test had 94% sensitivity and 100% specificity when compared with standard laboratory-based RT-PCR (Gibani 2020). In other studies, sensitivity was much lower. The BIOCREDIT COVID-19 antigen test was 10.000 fold less sensitive than RT-PCR and detected between 11,1 % and 45,7% of RT-PCR-positive samples from COVID-19 patients (Mak 2020).

Besides antigen tests, several rapid nucleic acid amplification tests have been recently released (Collier 2020). The Abbott ID NOW COVID-19 assay (using isothermal nucleic acid amplification of the RdRp viral target) is capable of producing positive results in as little as 5 minutes. In one stuy, results were compared with RT-PCR Cepheid Xpert Xpress SARS-CoV-2 using nasopharyngeal swabs (Basu 2020). Regardless of method of collection and sample type, the rapid test had negative results in a third of the samples that tested positive by PCR when using nasopharyngeal swabs in viral transport media and 45% when using dry nasal swabs. Such "Reverse Transcription Loop-Mediated Isothermal Amplification" tests (RT-LAMP) could be used outside of a central laboratory on various types of biological samples. They can be completed by

individuals without specialty training or equipment and may provide a new diagnostic strategy for combating the spread of SARS-CoV-2 at the point-of-risk (Lamb 2020).

Given the low (or still unproven) sensitivity, these tests may mainly serve as an early adjunctive tool to identify infectious individuals very rapidly, i.e. in the emergency unit. These tests help to avoid bed closure, allow discharge to care homes and expedite access to hospital procedures. Some experts are even more optimistic: the frequent use of cheap, simple, rapid tests is essential, even if their analytic sensitivities are vastly inferior to those of benchmark tests. The key question is not how well molecules can be detected in a single sample - but how effectively infections can be detected in a population by the repeated use of a given test as part of an overall testing strategy - the sensitivity of the testing regimen (Mina 2020).

Diagnosis in the setting of a 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 needs of a growing infected population. Especially in low-prevalence settings, sample pooling is an option to reduce costs and speed results. In this approach, small volumes of samples from multiple patients are combined into a single test, resulting in substantial reagent savings. Several studies have shown that 5-10 samples can be pooled, without compromising the results (Ben-Ami 2020, Schmidt 2020). However, pooling is not that trivial (Mallapaty 2020). There are several caveats and careful and rigorous investigation is necessary to assure that the pooling of specimens does not impact the analytical sensitivity of the assay (review: Clark 2020).

Some studies have investigated whether the diagnosis in high prevalence periods and countries can 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 only make 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 serves as the gold standard method for confirmation of infection. Whenever PCR is available, PCR should be performed.

Serology (antibody testing)

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. 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 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.

In recent weeks it has become clear that serology testing may also aid as a complementary diagnostic tool for COVID-19. The seroconversion of specific IgM and IgG antibodies were observed as early as the 4th day after symptom onset. Antibodies can be detected in the middle and later stages of the illness (Guo L 2020, Xiao DAT 2020). If a person with a highly suspicious COVID-19 remains negative by PCR testing and if symptoms are ongoing for at least several days, antibodies may be helpful and enhance diagnostic sensitivity.

However, antibody testing is not trivial. The molecular heterogeneity of SARS-CoV-2 subtypes, imperfect performance of available tests and cross-reactivity with seasonal CoVs have to be considered (reviews: Cheng 2020, Krammer 2020). According to a Cochrane analysis on 57 publications with 15.976 samples, the sensitivity of antibody tests is too low in the first week from symptom onset to have a primary role in the diagnosis of COVID-19. However, these tests may still have a role in complementing other testing in individuals presenting later, when RT-PCR tests are negative or are not done (Deeks 2020). Antibody tests are likely to have a useful role in detecting previous SARS-CoV-2 infection if used 15 or more days after the onset of symptoms. Data beyond 35 days post-symptom onset is scarce. According to the authors, studies of the accuracy of COVID-19 tests require considerable improvement. Studies must report data on sensitivity disaggregated by time

from onset of symptoms. A practical overview of the pitfalls of antibody testing and how to communicate risk and uncertainty is given by Watson 2020.

Tests

Average sensitivity and specificity of FDA-approved antibody tests is 84,9% and 98,6%, respectively. Given variable prevalence of COVID-19 (1%-15%) in different parts, statistically the positive predictive value will be as low as 30% to 50% in areas with low prevalence (Mathur 2020). A systematic review of 40 studies on sensitivity and specificity was recently published (Lisboa-Bastos 2020), stratified by method of serological testing (enzyme linked immunosorbent assays - ELISAs), lateral flow immunoassays (LFIAs), or chemiluminescent immunoassays - CLIAs). The pooled sensitivity of ELISAs measuring IgG or IgM was 84,3% (95% confidence interval 75,6% to 90,9%), of LFIAs was 66,0% (49,3% to 79,3%), and of CLIAs was 97,8% (46,2% to 100%). According to the authors, higher quality clinical studies assessing the diagnostic accuracy of serological tests for COVID-19 are urgently needed.

A nice overview of the different platforms, including binding assays such as enzyme-linked immunosorbent assays (ELISAs), lateral flow assays, or Western blot–based assays is given by Krammer 2020. In addition, functional assays that test for virus neutralization, enzyme inhibition, or bactericidal assays can also inform on antibody-mediated immune responses. Many caveats and open questions with regard to antibody testing are also discussed.

Antibody testing usually focuses on antigens (proteins). In the case of SARS-CoV-2, different ELISA kits based on recombinant nucleocapsid protein and spike protein are used (Loeffelholz 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. However, detection and quantification of neutralizing antibodies are relatively low-throughput and limited to Biosafety Level 3-equipped research laboratories. To avoid neutralization tests that require live pathogen and a biosafety level 3 laboratory, several studies have proposed tests based on antibody-mediated blockage of the interaction between the ACE2 receptor protein and the receptor-binding domain. The tests achieved 99,93% specificity and 95–100% sensitivity (Tan 2020).

Even with a very high specificity of 99% and above, however, especially in low-prevalence areas, the informative value of antibody testing 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. General antibody screening in these populations will therefore produce a fairly high rate of false positive tests. When assessing anti-SARS-CoV-2 immune status in individuals with low pre-test probability, it may be better to confirm positive results from single measurements by alternative serology tests or functional assays (Behrens 2020).

Some key studies with head-to-head-assessments of different immunoassays

- Abbott, EUROIMMUN and the Elecsys (Roche): The Abbott assay demonstrated the fewest false negative results > 14d post-symptom onset and the fewest false positive results. While the Roche assay detected more positive results earlier after onset of symptoms, none of the assays demonstrated high enough clinical sensitivity before day 14 from symptom onset to diagnose acute infection (Tang 2020).
- Abbott, LIAISON (DiaSorin), Elecsys (Roche), Siemens, plus a novel in-house 384-well (Oxford) ELISA in 976 (!) pre-pandemic blood samples and 536 (!) blood samples with confirmed SARS-CoV-2 infection. All assays had a high sensitivity (92,7-99,1%) and specificity (98,7-99,9%). The most sensitive test assessed was the in-house ELISA. The Siemens assay and Oxford immunoassay achieved 98% sensitivity/specificity without further optimization. However, a limitation was the small number of pauci-symptomatic and asymptomatic cases analyzed (NAEG 2020).
- Abbott, Epitope Diagnostics, EUROIMMUN, and Ortho Clinical Diagnostics: all four immunoassays performed similarly with respect to sensitivity in COVID-19 hospitalized patients, and except for the Epitope assay, also in individuals with milder forms of the infection (Theel 2020). The Abbott and Ortho Clinical immunoassays provided the highest overall specificity, of over 99%.
- Six SARS-CoV-2 antibody assays, namely Beckman Coulter, EUROIMMUN (IgG, IgA), Roche, and Siemens (Centaur, Vista) were assessed for specificity (n = 184), sensitivity (n = 154), and seroconversion in a defined cohort

with clinical correlates and molecular SARS-CoV-2 results. Assay specificity was 99% or greater for all assays except the Euroimmun IgA (95%). Sensitivity at more than 21 days from symptom onset were 84%, 95%, 72%, 98%, 67%, and 96% for Beckman Coulter, Centaur, Vista, Roche, Euroimmun IgA, and Euroimmun IgG, respectively. These findings raised some concerns that seroprevalence studies may vary significantly based on the serologic assay utilized (Zilla 2020).

Indication in clinical practice

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. However, we only measure antibodies when the testing result might have consequences. Patients should be informed about the low positive predictive value, especially in those without any evidence of prior disease or exposition to COVID-19. In these patients, antibody testing is not recommended. Outside epidemiological hot spots, in low prevalence countries like Germany, virtually everybody is still seronegative. If positive, the predictive value is too low.

The kinetics of antibodies

Serologic responses to coronaviruses are only transient. A brilliant systematic review of antibody-mediated immunity to coronaviruses (kinetics, correlates of protection, and association with severity) was recently published (Huang AT 2020).

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 DAT 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 these tests can aid 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%, 93% and 78% 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 83% (12 days) and 65% (14 days), respectively. A higher titer of antibodies was independently associated with severe disease (Zhao 2020). In other studies, however, antibody level did not correlate clearly with clinical outcomes (Ren 2020).

In some patients, IgG occurs even faster than IgM. In a study on seroconversion patterns of IgM and IgG antibodies, the seroconversion time of IgG antibody was earlier than IgM. IgG antibody reached the highest concentration on day 30, while IgM antibody peaked on day 18, but then began to decline (Qu J 2020). The largest study to date reported on acute antibody responses in 285 patients (mostly non-severe COVID-19). Within 19 days after symptom onset, 100% of patients tested positive for antiviral IgG. Seroconversion for IgG and IgM occurred simultaneously or sequentially. Both IgG and IgM titers plateaued within 6 days after seroconversion. The median day of seroconversion for both IgG and IgM was 13 days post-symptom onset. No association between plateau IgG levels and clinical characteristics was found (Long 2020). In a cohort of 30.082 infected individuals with mild to moderate COVID-19 symptoms screened at Mount Sinai Health System in New York City to determine the robustness and longevity of the anti–SARS-CoV-2 antibody response, neutralizing antibody titers against the SARS-CoV-2 spike protein persisted for at least 5 months after infection. The authors plan to follow this cohort over a longer period of time (Wajnberg 2020).

However, there is some evidence that asymptomatic individuals develop less strong antibody responses. Moreover, antibodies disappear from the blood. Your COVID pass expires within a few weeks. Compared to symptomatic patients, 37 asymptomatic patients had lower virus-specific IgG levels in the acute phase (Long Q 2020). IgG levels and neutralizing antibodies started to decrease within 2–3 months after infection. Of note, 40% became seronegative (13% of the symptomatic group) for IgG in the early convalescent phase. Among 19 health care workers who had anti–SARS-CoV-2 antibodies detected at baseline, only 8 (42%) had antibodies that persisted above the seropositivity threshold at 60 days, whereas 11 (58%) became seronegative (Patel 2020). A decrease in anti-RBD antibody level was also seen in 15 donors of convalescent plasma (Perreault 2020).

Taken together, antibody testing is not only an epidemiological tool. It may also help in diagnosis. 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

Chest computed tomography

Computed tomography (CT) can play a role in both diagnosis 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 shown significant errors and shortcomings. In view of the high effort and also due to the risk of infection for the staff, many experts strictly reject the general CT screening in SARS-CoV-2 infected patients or in those suspected cases (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 pa-

tients. 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). According to a review of 45 studies comprising 4410 (!) patients, ground glass opacities (GGOs), whether isolated (50%) or coexisting with consolidations (44%) in bilateral and subpleural distribution, were the most prevalent chest CT findings (Ojha 4410). Another 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 M 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 Y 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 Y 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 the 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 respiratory 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, PET and other techniques

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, Qin 2020).

In patients with neurological symptoms, brain MRI is often performed. In 27 patients, the most common imaging finding was cortical signal abnormalities on FLAIR images (37%), accompanied by cortical diffusion restriction or leptomeningeal enhancement (Kandemirli 2020). However, the complex clinical course including comorbidities, long ICU stay with multidrug regimens, respiratory distress with hypoxia episodes can all act as confounding factors and a clear cause-effect relationship between COVID-19 infection and MRI findings will be hard to establish.

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8. Clinical Presentation

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Published 20 January 2021

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 diminishment of smell and taste. In most patients, COVID-19 remains mild or moderate and symptoms resolve within a week and patients typically recover at home. Around 10% of patients remain symptomatic through the second week. The longer the symptoms persist, the higher the risk of developing more severe COVID-19, requiring hospitalization, intensive care and invasive ventilation. 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.

In this chapter we discuss the clinical presentation, including

- The incubation period
- Asymptomatic patients
- Frequent and rare symptoms
- Laboratory findings
- Outcome: Risk factors for severe disease
- Reactivations and reinfections
- Long-term sequelae

The radiological findings are described in the diagnostic chapter, page 249.

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 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 pre-symptomatic patients (He 2020).

Asymptomatic cases

Understanding the frequency of asymptomatic patients and the temporal course of asymptomatic transmission will be crucial for assessing disease dynamics. It is important to distinguish those patients who will remain asymptomatic during the whole time of infection and those in which infection is still too early to cause symptoms (pre-symptomatic). While physicians need to be aware of asymptomatic cases, the true percentage is difficult to assess. To evaluate symptoms systematically is not trivial and the ascertainment process could lead to misclassification. If you do not ask precisely enough, you will get false negative answers. If questions are too specific, the interviewees may give false positive answers (confirmation bias). For example, in a large study, only two thirds of patients reporting olfactory symptoms had abnormal results in objective olfactory testing (see below). What is a symptom? And, is it possible to interview the demented residents of a nursing home? Sweet grandma will say she was fine over the last few weeks.

A nice review addressed the three main methodological issues that hinder attempts to estimate the proportion of asymptomatic or pre-symptomatic individuals. First, incomplete symptom assessment may overestimate the asymptomatic fraction; second, studies with inadequate follow-up misclassify pre-symptomatic individuals; and third, serological studies might identify people with previously unrecognised infection, but reliance on poorly defined antibody responses and retrospective symptom assessment might result in misclassification (Meyerowitz 2020).

In a living systematic review (through June 10, 2020, analyzing 79 studies in a range of different settings), 20% (95% CI 17%–25%) remained asymptomatic

during follow-up, but biases in study designs limit the certainty of this estimate (Buitrago-Garcia 2020). In seven studies of defined populations screened for SARS-CoV-2 and then followed, 31% (95% CI 26%–37%) remained asymptomatic. Another review found that asymptomatic persons seem to account for approximately 40-45% of infections, and that they can transmit the virus to others for an extended period, perhaps longer than 14 days. The absence of COVID-19 symptoms might not necessarily imply an absence of harm as subclinical lung abnormalities are frequent (Oran 2020).

The probable 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 incubation periods between 5,5 and 9,5 days, the authors calculated the true asymptomatic proportion at 17,9% (Mizumoto 2020). The outbreak at the aircraft carrier USS Theodore Roosevelt revealed that 146/736 infected sailors (19,8%) remained asymptomatic for the duration of the study period.

Table 1. Larger studies with defined populations; proportion of asymptomatic patients (LTF = long-term facilities)

	Population, n	Asymptomatic
Kasper 2020	US Aircraft carrier, mainly young sailors and crew members (n=1.271)	43%
Borras-Bermejo 2020	Nursing Homes Spain, residents (n=768) and staff (n = 403)	68% of residents, 56% of staff (including pre-symptomatic)
Feaster 2020	LTFs California, residents and staff (n = 631)	19-86% of residents, 17-31% of staff
Gudbjartsson 2020	Icelandic Population (n = 1,221)	43% (including pre-symptomatic)
Hoxha 2020	LTFs Belgium, residents (n = 4059) and staff (n = 2185)	75% of residents, 74% of staff (including pre-symptomatic)
Lavezzo 2020	(Small town) Vo, Italy, all residents (n = 2812)	43%
Marossy 2020	LTFs London (n = 2455)	51% of residents, 69% of staff

There is no doubt that asymptomatic patients may transmit the virus (Bai 2020, Rothe 2020). In several studies from Northern Italy or Korea, viral loads in nasal swabs did not differ significantly between asymptomatic and symptomatic subjects, suggesting the same potential for transmitting the virus

(Lee 2020). Of 63 asymptomatic patients in Chongquing, 9 (14%) transmitted the virus to others (Wang Y 2020).

Taken together, these preliminary studies indicate that a significant proportion (20-60%) of all COVID-19 infected subjects may remain asymptomatic during their infection. The studies show a broad range, depending on the populations and probably on methodological issues. It will be very difficult (if not impossible) to clarify the exact proportion.

Symptoms

A plethora of symptoms have been described in the past months, clearly indicating that COVID-19 is a complex disease, which in no way consists only of a respiratory infection. Many symptoms are unspecific so that the differential diagnosis encompasses a wide range of infections, respiratory and other diseases. However, different clusters can be distinguished in COVID-19. The most common symptom cluster encompasses the respiratory system: cough, sputum, shortness of breath, and fever. Other clusters encompass musculoskeletal symptoms (myalgia, joint pain, headache, and fatigue), enteric symptoms (abdominal pain, vomiting, and diarrhea); and less commonly, a mucocutaneous cluster. An excellent review on these extrapulmonary organ-specific pathophysiology, presentations and management considerations for patients with COVID-19 was recently published (Gupta 2020).

Fever, cough, shortness of breath

Symptoms occur in the majority of cases (for asymptomatic patients, see below). In early studies from China (Guan 2020, Zhou 2020), fever was the most common symptom, with a median maximum of 38.3 C; only a few 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 was cough, occurring in about two thirds of all patients. Among survivors of severe COVID-19 (Zhou 2020), median duration of fever was 12.0 days (8-13 days) and cough persisted for 19 days (IQR 12-23 days). According to a systemic review, including 148 articles comprising 24,410 adults with confirmed COVID-19 from 9 countries (Grant 2020), the most prevalent symptoms were fever (78%), cough (57%) and fatigue (31%).

Fever and cough do not distinguish between mild and severe cases nor do they predict the course of COVID-19 (Richardson 2020, Petrilli 2020). In contrast, shortness of breath has been identified as a strong predictor of severe disease in larger studies. In a cohort of 1,590 patients, dyspnea was associated

with an almost two-fold risk for critical disease (Liang 2020) and mortality (Chen 2020). Others found higher rates of shortness of breath, and temperature of > 39.0 in older patients compared with younger patients (Lian 2020). In the Wuhan study on patients with severe COVID-19, a multivariate analysis revealed that a respiratory rate of > 24 breaths per minute at admission was higher in non-survivors (63% versus 16%).

Over the last weeks, much cohort data from countries outside China have been published. However, almost all data applies to patients who were admitted to hospitals, indicating selection bias towards more severe and symptomatic patients.

- Among 20.133 patients in the UK who were admitted to 208 acute care hospitals in the UK between 6 February and 19 April 2020, the most common symptoms were cough (69%), fever (72%), and shortness of breath (71%), showing a high degree of overlap (Docherty 2020).
- Among 5700 patients who were admitted to any of 12 acute care hospitals in New York between March 1, 2020, and April 4, 2020, only 30,7% had fever of > 38C. A respiratory rate of > 24 breaths per minute at admission was found in 17.3% (Richardson 2020).
- Among the first 1000 patients presenting at the New York Presbyterian/Columbia University (Argenziano 2020), the most common presenting symptoms were cough (73%), fever (73%), and dyspnea (63%).

Musculoskeletal symptoms

The cluster of musculoskeletal symptoms encompasses myalgia, joint pain, headache, and fatigue. These are frequent symptoms, occurring each in 15-40% of patients (Argenziano 2020, Docherty 2020, Guan 2020). Although subjectively very disturbing and sometimes foremost in the perception of the patient, these symptoms tell us nothing about the severity of the clinical picture. However, they are frequently overlooked in clinical practice, and headache merits special attention.

According to a recent review (Bolay 2020), headache is observed in 11-34% of hospitalized COVID-19 patients, occurring in 6-10% as the presenting symptom. Significant features are moderate-severe, bilateral headache with pulsating or pressing quality in the temporo-parietal, forehead or periorbital region. The most striking features are sudden to gradual onset and poor response to common analgesics. Possible pathophysiological mechanisms include activation of peripheral trigeminal nerve endings by the SARS-CoV-2 directly or through the vasculopathy and/or increased circulating pro-inflammatory cytokines and hypoxia.

Gastrointestinal symptoms

Cell experiments have shown that SARS-CoV and SARS-CoV-2 are able to infect enterocytes (Lamers 2020). Active replication has been shown in both bats and human intestinal organoids (Zhou 2020). Fecal calprotectin as a reliable fecal biomarker allowing detection of intestinal inflammation in inflammatory bowel diseases and infectious colitis, was found in some patients, providing evidence that SARS-CoV-2 infection instigates an inflammatory response in the gut (Effenberger 2020). These findings explain why gastrointestinal symptoms are observed in a subset of patients and why viral RNA can be found in rectal swabs, even after nasopharyngeal testing has turned negative. In patients with diarrhea, viral RNA was detected at higher frequency in stool (Cheung 2020).

In the early Chinese studies, however, gastrointestinal symptoms were rarely seen. In a meta-analysis of 60 early 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. As with otolaryngeal symptoms, it remains unclear whether this difference reflects geographic variation or differential reporting. Among the first 393 consecutive patients who were admitted to two hospitals in New York City, diarrhea (24%), and nausea and vomiting (19%) were relatively frequent (Goyal 2020). Among 18.605 patients admitted to UK Hospitals, 29% of all patients complained of enteric symptoms on admission, mostly in association with respiratory symptoms; however, 4% of all patients described enteric symptoms alone (Docherty 2020).

It's not all criticial illness. Another study compared 92 critically ill patients with COVID-19–induced ARDS with 92 comparably ill patients with non–COVID-19 ARDS, using propensity score analysis. Patients with COVID-19 were more likely to develop gastrointestinal complications (74% vs 37%; $p < 0.001$). Specifically, patients with COVID-19 developed more transaminitis (55% vs 27%), severe ileus (48% vs 22%), and bowel ischemia (4% vs 0%). High expression of ACE 2 receptors along the epithelial lining of the gut that act as host-cell receptors for SARS-CoV-2 could explain this (El Moheb 2020).

Otolaryngeal symptoms (including anosmia)

Although upper respiratory tract symptoms such as rhinorrhea, nasal congestion, sneezing and sore throat are relatively unusual, it has become clear within a few weeks that anosmia and hyposmia are important signs of the disease (Luers 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 were not recorded well enough in the initial phase in China. There is now very good data from Europe: the largest study to date found that 1754/2013 patients (87%) reported loss of smell, whereas 1136 (56%) reported taste dysfunction. Most patients had loss of smell after other general and otolaryngologic symptoms (Lechien 2020). Mean duration of olfactory dysfunction was 8,4 days. Females seem to be more affected than males. The prevalence of self-reported smell and taste dysfunction was higher than previously reported and may be characterized by different clinical forms. Anosmia may not be related to nasal obstruction or inflammation. Of note, only two thirds of patients reporting olfactory symptoms and who had objective olfactory testing had abnormal results.

"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 SARS-CoV-2 positivity (anosmia: adjusted odds ratio 11, 95% CI: 5-24). Conversely, sore throat was independently associated with negativity (Yan 2020).

Among a total of 18,401 participants from the US and UK who reported potential symptoms on a smartphone app and who had undergone a SARS-CoV-2 test, the proportion of participants who reported loss of smell and taste was higher in those with a positive test result (65 vs 22%). A combination of symptoms, including anosmia, fatigue, persistent cough and loss of appetite was appropriate to identify individuals with COVID-19 (Menni 2020).

Post-mortem histological analysis of the olfactory epithelium in two COVID-19 patients showed prominent leukocytic infiltrates in the lamina propria and focal atrophy of the mucosa. However, it is unclear whether the observed inflammatory neuropathy is a result of direct viral damage or is mediated by damage to supporting non-neural cells (Kirschenbaum 2020). There is some evidence that sustentacular cells, which maintain the integrity of olfactory sensory neurons, express ACE2 and TMPRSS2. These cells represent a potential way in for SARS-CoV-2 in a neuronal sensory system that is in direct connection with the brain (Fodoulian 2020).

Among 49 confirmed COVID-19 patients with anosmia, there were no significant pathological changes in the paranasal sinuses on CT scans. Olfactory cleft and ethmoid sinuses appeared normal while in other sinuses, partial opacification was detected only in some cases (Naeini 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 the pathophysiological sequence remains unproven (Hendren 2020). Post-mortem examination by *in situ* hybridization suggested that the most likely localization of SARS-CoV-2 is not in the cardiomyocytes but in interstitial cells or macrophages invading the myocardial tissue (Lindner 2020). A second hypothesis to explain COVID-19-related myocardial injury centers on cytokine excess and/or antibody-mediated mechanisms. It has also been shown that the ACE2 receptor is widely expressed on endothelial cells and that direct SARS-CoV-2 infection of the endothelial cell is possible, leading to diffuse endothelial inflammation (Varga 2020). Post-mortem examination cases indicate a strong virus-induced vascular dysfunction (Menter 2020).

Clinically, COVID-19 can manifest with an acute cardiovascular syndrome (termed "ACovCS", for **a**cute *COVID*-19 **c**ardiovascular **s**yndrome). 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 non-obstructive 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). Among 2736 COVID-19 patients admitted to one of five hospitals in New York City who had troponin-I measured within 24 hours of admission, 985 (36%) patients had elevated troponin concentrations. After adjusting for disease severity and relevant clinical factors, even small amounts of myocardial injury (0,03-0,09 ng/mL) were significantly associated with death (Lala 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). For more information, see the chapter *Comorbidities*, page **Fehler! Textmarke nicht definiert.**.

Beside ACovCS, a wide array of cardiovascular manifestations is possible, including heart failure, cardiogenic shock, arrhythmia, and myocarditis. Among 100 consecutive patients diagnosed with COVID-19 infection undergoing complete echocardiographic evaluation within 24 hours of admission, only 32% had a normal echocardiogram at baseline. The most common cardiac pathology was right ventricular (RV) dilatation and dysfunction (observed in

39% of patients), followed by left ventricular (LV) diastolic dysfunction (16%) and LV systolic dysfunction (10%). In another case series of 54 patients with mild-to-moderate COVID-19 in Japan, relative bradycardia was also a common finding (Ikeuchi 2020).

Thrombosis, embolism

Coagulation abnormalities occur frequently in association with COVID-19, complicating clinical management. Numerous studies have reported on an incredibly high number of venous thromboembolism (VTE), especially in those with severe COVID-19. The initial coagulopathy of COVID-19 presents with prominent elevation of D-dimer and fibrin/fibrinogen degradation products, while abnormalities in prothrombin time, partial thromboplastin time, and platelet counts are relatively uncommon (excellent review: Connors 2020). Coagulation test screening, including the measurement of D-dimer and fibrinogen levels, is suggested.

But what are the mechanisms? Some studies have found pulmonary embolism with or without deep venous thrombosis, as well as presence of recent thrombi in prostatic venous plexus, in patients with no history of VTE, suggesting *de novo* coagulopathy in these patients with COVID-19. Others have highlighted changes consistent with thrombosis occurring within the pulmonary arterial circulation, in the absence of apparent embolism (nice review: Deshpande 2020). Some studies have indicated severe hypercoagulability rather than consumptive coagulopathy (Spiezia 2020) or an imbalance between coagulation and inflammation, resulting in a hypercoagulable state (review: Colling 2020).

According to a systematic review of 23 studies, among 7178 COVID-19 patients admitted to general wards and intensive care units (ICU), the pooled in-hospital incidence of pulmonary embolism (PE) or lung thrombosis was 147% and 23.4%, respectively (Roncon 2020).

Some of the key studies are listed here:

- In a single-center study from Amsterdam on 198 hospitalized cases, the cumulative incidences of VTE at 7 and 21 days were 16% and 42%. In 74 ICU patients, cumulative incidence was 59% at 21 days, despite thrombosis prophylaxis. The authors recommend performing screening compression ultrasound in the ICU every 5 days (Middeldorp 2020).
- Among 3334 consecutive patients admitted to 4 hospitals in New York City, a thrombotic event occurred in 16% (Bilaloglu 2020). Of these, 207 (6,2%) were venous (32% PE and 3,9% DVT) and 365 (11,1%) were arterial (1,6% ischemic stroke, 8,9% MI, and 1,0% systemic thromboembolism). All-

cause mortality was 24,5% and was higher in those with thrombotic events (43% vs 21%). D-dimer level at presentation was independently associated with thrombotic events.

- In a retrospective multi-center study, 103/1240 (8,3%) consecutive patients hospitalized for COVID-19 (patients directly admitted to an ICU were excluded) had evidence for PE. In a multivariate analysis, male gender, anticoagulation, elevated CRP, and time from symptom onset to hospitalization were associated with PE risk (Fauvel 2020).
- Autopsy findings from 12 patients, showing that 7/12 had deep vein thrombosis. Pulmonary embolism was the direct cause of death in four cases (Wichmann 2020).
- Acute pulmonary embolism (APE) can occur in mild-to moderate and is not limited to severe or critical COVID-19 (Gervaise 2020).
- Careful examination of the lungs from deceased COVID-19 patients with lungs from 7 patients who died from ARDS secondary to influenza A showed distinctive vascular features. COVID-19 lungs displayed severe endothelial injury associated with the presence of intracellular virus and disrupted cell membranes. Histologic analysis of pulmonary vessels showed widespread thrombosis with microangiopathy. Alveolar capillary microthrombi and the amount of vessel growth were 9 and almost 3 times as prevalent as in influenza, respectively (Ackermann 2020)
- Five cases of large-vessel stroke occurring in younger patients (age 33-49, 2 without any risk factors) (Oxley 2020).
- Five cases with profound hemodynamic instability due to the development of acute cor pulmonale, among them 4 younger than 65 years of age (Creel-Bulos 2020).

Empiric therapeutic anti-coagulation (AC) is now being employed in clinical practice in many centers. In the absence of contraindications it is recommended for all hospitalized patients (Piazza 2020) and will be evaluated in randomized clinical trials. To adjust for bias due to non-random allocation of potential covariates among COVID-19 patients, one study applied propensity score matching methods. Among > 3000 patients, propensity matching yielded 139 patients who received AC and 417 patients who did not receive treatment with balanced variables between the groups. Results suggest that AC alone is unlikely to be protective for COVID-19-related morbidity and mortality (Tremblay 2020).

There is also a quite controversial debate about a possible correlation between the use of ibuprofen and the increased risk of VTE development. Ac-

cording to a recent review (Arjomandi 2020), the causation between the effects of ibuprofen and VTE remains speculative. The role of ibuprofen on a vascular level remains unclear as well as whether ibuprofen is able to interact with SARS-CoV-2 mechanistically. However, the authors recommend careful considerations on avoiding high dosage of ibuprofen in subjects at particular risk of thromboembolic events.

Neurologic symptoms

Neuroinvasive propensity has been demonstrated as a common feature of human coronaviruses. Viral neuroinvasion may be achieved by several routes, including trans-synaptic transfer across infected neurons, entry via the olfactory nerve, infection of vascular endothelium, or leukocyte migration across the blood-brain barrier (reviews: Zubair 2020, Ellul 2020). 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). In a study of 4491 hospitalized COVID-19 patients in New York City, 606 (13,5%) developed a new neurologic disorder (Frontera 2020). The most common diagnoses were: toxic/metabolic encephalopathy (6,8%, temporary/reversible changes in mental status in the absence of focal neurologic deficits or primary structural brain disease, excluding patients in whom sedative or other drug effects or hypotension explained this), seizure (1,6%), stroke (1,9%), and hypoxic/ischemic injury (1,4%). Whether these more non-specific symptoms are manifestations of the disease itself remains to be seen. There are several observational series of specific neurological features such as Guillain–Barré syndrome (Toscano 2020), myasthenia gravis (Restivo 2020) or Miller Fisher Syndrome and polyneuritis cranialis (Gutierrez-Ortiz 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. Patients with COVID-19 might experience delirium, confusion, agitation, and altered consciousness, as well as symptoms of depression, anxiety, and insomnia (review: Rogers 2020). It remains unclear which of these features are due to critical illness–related encephalopathy, cytokines, or the effect or withdrawal of medication, and which features are specific to SARS-CoV-2 infection (Helms 2020). However, in a large retrospective cohort study comparing 1916 COVID-19 patients and 1,486 influenza patients (with emergency department visits or hospitalizations), there were 31 acute ischemic strokes with COVID-19, compared to 3

with influenza (Merkler 2020). After adjustment for age, sex, and race, the likelihood of stroke was almost 8-fold higher with COVID-19 (odds ratio, 7.6).

Of note, there is no clear evidence for CNS damage directly caused by SARS-CoV-2. In a single-center study on 52 patients, protein and albumin levels in cerebrospinal fluid (CSF) were increased in 38% and 23%, respectively (Lersy 2020). 40% of patients displayed an elevated albumin quotient suggesting impaired blood -liquor barrier function. An SARS-CoV-2 specific IgG oligoclonal band in CSF was found in five (11%) cases, suggesting an intrathecal synthesis of IgG, and 26 (55%) patients presented identical oligoclonal bands in serum and CSF. Only four (7%) patients harbored a positive SARS-CoV-2 RT-PCR in CSF. These data suggest that, although SARS-CoV-2 is able to replicate in neuronal cells *in vitro*, SARS-CoV-2 testing in CSF is not very relevant in the general population (Destras 2020). In a large post-mortem examination, SARS-CoV-2 could be detected in the brains of 21 (53%) of 40 examined patients but was not associated with the severity of neuropathological changes (Matschke 2020) which seemed to be mild, with pronounced neuroinflammatory changes in the brainstem being the most common finding. In another study, brain specimens obtained from 18 patients who died 0 to 32 days after the onset of symptoms showed only hypoxic changes and did not show encephalitis or other specific brain changes referable to the virus (Solomon 2020).

Dermatological symptoms

Numerous studies have reported on cutaneous manifestations seen in the context of COVID-19. The most prominent phenomenon, the so-called "COVID toes", are chilblain-like lesions which mainly occur at acral areas. [Chilblain: Frostbeule (de), engelure (fr), sabañón (es), gelone (it), frieira (pt), 冻疮 (cn)] These lesions can be painful (sometimes itchy, sometimes asymptomatic) and may represent the only symptom or late manifestations of SARS-CoV-2 infection. Of note, in most patients with "COVID toes", the disease is only mild to moderate. It is speculated that the lesions are caused by inflammation in the walls of blood vessels, or by small micro-clots in the blood. However, whether "COVID toes" represent a coagulation disorder or a hypersensitivity reaction is not yet known. Key studies:

- Two different patterns of acute acro-ischemic lesions can overlap (Fernandez-Nieto 2020). The chilblain-like pattern was present in 95 patients (72,0%). It is characterized by red to violet macules, plaques and nodules, usually at the distal aspects of toes and fingers. The erythema multiform-like pattern was present in 37 patients (28,0%).

- Five clinical cutaneous lesions are described (Galvan 2020): acral areas of erythema with vesicles or pustules (pseudo-chilblain) (19%), other vesicular eruptions (9%), urticarial lesions (19%), maculopapular eruptions (47%) and livedo or necrosis (6%). Vesicular eruptions appear early in the course of the disease (15% before other symptoms). The pseudo-chilblain pattern frequently appears late in the evolution of COVID-19 disease (59% after other symptoms).
- In a case series on 22 adult patients with varicella-like lesions (Marzano 2020), typical features were constant trunk involvement, usually scattered distribution and mild or absent pruritus, the latter being in line with most viral exanthems but not like true varicella. Lesions generally appeared 3 days after systemic symptoms and disappeared by day 8.
- Three cases of COVID-19 associated ulcers in the oral cavity, with pain, desquamative gingivitis, and blisters (Martin Carreras-Presas 2020).

Other case reports include digitate papulosquamous eruption (Sanchez 2020), petechial skin rash (Diaz-Guimaraens 2020, Quintana-Castanedo 2020). However, it should be kept in mind that not all rashes or cutaneous manifestations seen in patients with COVID-19 can be attributed to the virus. Co-infections or medical complications have to be considered. Newer studies reporting in negative PCR and serology have questioned a direct association between acral skin disease and COVID-19:

- Of 31 patients (mostly teenagers) who had recently developed chilblains, histopathologic analysis of skin biopsy specimens (22 patients) confirmed the diagnosis of chilblains and showed occasional lymphocytic or microthrombotic phenomena. In all patients, PCR and serology remained negative (Herman 2020).
- Among 40 young patients with chilblain lesions and with suspected SARS-CoV-2 infection, serology was positive in 12 (30%). All had negative PCR results at the time of presentation, suggesting that in young patients SARS-CoV-2 is completely suppressed before a humoral immune response is induced (Hubiche 2020).
- In a cohort series from Valencia following 20 patients aged 1 to 18 years with new-onset acral inflammatory lesions, all lacked systemic manifestations of COVID-19. Surprisingly, both PCR and serologic test results were negative for SARS-CoV-2 (Roca-Ginés 2020).

Comprehensive mucocutaneous examinations, analysis of other systemic clinical features or host characteristics, and histopathologic correlation, will be

vital to understanding the pathophysiologic mechanisms of what we are seeing on the skin (Review: Madigan 2020).

Kidneys

SARS-CoV-2 has an organotropism beyond the respiratory tract, including the kidneys and the liver. Researchers have quantified the SARS-CoV-2 viral load in precisely defined kidney compartments obtained with the use of tissue micro-dissection from 6 patients who underwent autopsy (Puelles 2020). Three of these 6 patients had a detectable SARS-CoV-2 viral load in all kidney compartments examined, with preferential targeting of glomerular cells. Renal tropism is a potential explanation of commonly reported clinical signs of kidney injury in patients with COVID-19, even in patients with SARS-CoV-2 infection who are not critically ill (Zhou 2020). Recent data indicate that renal involvement is more frequent than described in early studies (Gabarre 2020). Of the first 1000 patients presenting at the New York-Presbyterian/Columbia University, 236 were admitted or transferred to intensive care units (Argenziano 2020). Of these, 78,0% (184/236) developed acute kidney injury and 35,2% (83/236) needed dialysis. Concomitantly, 13,8% of all patients and 35,2% of patients in intensive care units required in-patient dialysis, leading to a shortage of equipment needed for dialysis and continuous renal replacement therapy.

In recent months, some case reports of collapsing glomerulopathy akin to those seen during the HIV epidemic have been published. All of these cases were in patients of African ethnicity (Velez 2020).

Liver

One of the largest studies, evaluating liver injury in 2273 SARS-CoV-2 positive patients, found that 45% had mild, 21% moderate, and 6,4% severe liver injury. In a multivariate analysis, severe acute liver injury was significantly associated with elevated inflammatory markers including ferritin and IL-6. Peak ALT was significantly associated with death or discharge to hospice (OR 1.14, p = 0,044), controlling for age, body mass index, diabetes, hypertension, intubation, and renal replacement therapy (Phipps 2020). In another meta-analysis of 9 studies with a total of 2115 patients, patients with COVID-19 with liver injury were at an increased risk of severity (OR 2.57) and mortality (1.66).

Ocular and atypical manifestations

Ocular manifestations are also common (Meduri 2020). 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). The retina can also be affected, as has been shown using optical coherence tomography (OCT), a non-invasive imaging technique that is useful for demonstrating subclinical retinal changes. Twelve adult patients showed hyper-reflective lesions at the level of the ganglion cell and inner plexiform layers more prominently at the papillomacular bundle in both eyes. Since their initial report, the authors have extended their findings to more than 150 patients, demonstrating an absence of blood flow within the retinal lesions of "many" patients (Marinho 2020). However, in another study of 25 patients with severe or critical disease in a study from Brazil, only three (12%) manifested convincing retinal changes (micro-hemorrhages, flame-shaped hemorrhage and nerve fiber layer infarcts). These retinal changes were likely secondary to clinical intercurrences or co-morbidities (Lani-Louzada 2020).

Other new and sometimes puzzling clinical presentations have emerged (and will emerge) 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).

Laboratory findings

The most evident laboratory findings in the first 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 362%, 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.

Table 2. Percentage of symptoms in first large cohort study from China (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
LDH ≥ 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

Inflammation

Parameters indicating inflammation such as elevated CRP and procalcitonin are very frequent findings. They have been proposed to be important risk factors for disease severity and mortality (Chen 2020). For example, in a multivariate analysis of a retrospective cohort of 1590 hospitalized subjects with COVID-19 throughout China, a procalcitonin > 0.5 ng/ml at admission had a HR for mortality of 8,7 (95% CI: 3,4-22,3). In 359 patients, CRP performed better than other parameters (age, neutrophil count, platelet count) in predicting adverse outcome. Admission serum CRP level was identified as a moderate discriminator of disease severity (Lu 2020). Of 5279 cases confirmed in a large medical center in New York, 52% of them admitted to hospital, a CRP > 200 was more strongly associated (odds ratio 5.1) with critical illness than age or comorbidities (Petrilli 2019).

Some studies have suggested that the dynamic change of interleukin-6 (IL-6) levels and other cytokines can be used as a marker in disease monitoring in patients with severe COVID-19 (Chi 2020, Zhang 2020). In a large study of 1484 patients, several cytokines were measured upon admission to the Mount Sinai Health System in New York (Del Valle 2020). Even when adjusting for disease severity, common laboratory inflammation markers, hypoxia and other vitals, demographics, and a range of comorbidities, IL-6 and TNF-α serum levels remained independent and significant predictors of disease severity and death. These findings were validated in a second cohort of 231 patients. The authors propose that serum IL-6 and TNF-α levels should be considered in the management and treatment of patients with COVID-19 to stratify prospective clinical trials, guide resource allocation and inform therapeutic options.

There is also one study suggesting that serum cortisol concentration seems to be a better independent predictor than other laboratory markers associated with COVID-19, such as CRP, D-dimer, and neutrophil to leukocyte ratio (Tan 2020).

Hematological: Lymphocytes, platelets, RDW

Lymphocytopenia and transient but severe T cell depletion is a well-known feature of SARS (He 2005). In COVID-19, lymphopenia is also among the most prominent hematological features. Lymphopenia may be predictive for progression (Ji 2020) and patients with severe COVID-19 present with lymphocytopenia of less than 1500/µl in almost 100% of cases (Guan 2020). It's not only the total lymphocyte count. There is growing evidence for a transient depletion of T cells. Especially the reduced CD4+ and CD8+ T cell counts upon admission were predictive of disease progression in a larger study (Zhang 2020). In another large study on COVID-19 patients, CD3+, CD4+ and CD8+ T cells as well as NK cells were significantly decreased in COVID-19 patients and related to the severity of the disease. According to the authors, CD8+ T cells and CD4+ T cell counts can be used as diagnostic markers of COVID-19 and predictors of disease severity (Jiang 2020). Beside T cells, B cells may also play a role. In 104 patients, a decrease in B cells was independently associated with prolonged viral RNA shedding (Hao 2020).

Another common hematological finding is low platelet counts that may have different causes (Review: Xu 2020). A meta-analysis of 24 studies revealed a weighted incidence of thrombocytopenia in COVID-19 patients of 12,4% (95% CI 7,9%–17,7%). The meta-analysis of binary outcomes (with and without thrombocytopenia) indicated an association between thrombocytopenia and a 3-fold enhanced risk of a composite outcome of ICU admission, progression to acute respiratory distress syndrome, and mortality (Zong 2020). Cases of

hemorrhagic manifestation and severe thrombocytopenia responding to immunoglobulins fairly quickly with a sustained response over weeks have been reported (Ahmed 2020).

Red blood cell distribution width (RDW) is another component of complete blood counts that quantifies the variation of individual red blood cell (RBC) volumes and has been shown to be associated with elevated risk for morbidity and mortality in a wide range of diseases. In a large cohort study including 1641 adults diagnosed with SARS-CoV-2 infection and admitted to 4 hospitals in Boston (Foy 2020), RDW was associated with mortality risk in Cox models (hazard ratio of 1.09 per 0,5% RDW increase and 2.01 for an RDW > 14,5% vs ≤ 14,5%).

However, there are also cohorts in which hematological parameters such as thrombocytes, neutrophil-to-lymphocyte ratio or D-dimers do not allow prediction of patient outcome (Pereyra 2020). These routine parameters, despite giving guidance on the overall health of the patient, might not always accurately indicate COVID-19-related complications.

Cardiac: Troponin

Given the cardiac involvement especially in severe cases (see above), it is not surprising that cardiac parameters are frequently elevated. A meta-analysis of 341 patients found that cardiac troponin I levels are significantly increased only in patients with severe COVID-19 (Lippi 2020). In 179 COVID-19 patients, cardiac troponin ≥ 0.05 ng/mL was predictive of mortality (Du 2020). Among 2736 COVID-19 patients admitted to one of five hospitals in New York City who had troponin-I measured within 24 hours of admission, 985 (36%) patients had elevated troponin concentrations. After adjusting for disease severity and relevant clinical factors, even small amounts of myocardial injury (0,03-0,09 ng/mL) were significantly associated with death (adjusted HR: 1,75, 95% CI 1,37-2,24) while greater amounts (> 0,09 ng/dL) were significantly associated with higher risk (adjusted HR 3,03, 95% CI 2,42-3,80). However, 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 has been recently published (Chapman 2020).

Coagulation: D-dimer, aPTT

Several studies have evaluated the coagulation parameter D-dimer in the progression of COVID-19. Among 3334 consecutive patients admitted to 4 hospitals at New York City, a thrombotic event occurred in 16,0%. D-dimer level at presentation was independently associated with thrombotic events, consistent with early coagulopathy (Bilaloglu 2020). 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 and many patients died from sepsis (Zhou 2020).

In a considerable proportion of patients, a prolonged aPTT can be found. Of 216 patients with SARS-CoV-2, this was the case in 44 (20%). Of these, 31/34 (91%) had positive lupus anticoagulant assays. As this is not associated with a bleeding tendency, it is recommended that prolonged aPTT should not be a barrier to the use of anti-coagulation therapies in the prevention and treatment of venous thrombosis (Bowles 2020). Another case series of 22 patients with acute respiratory failure present a severe hypercoagulability rather than consumptive coagulopathy. Fibrin formation and polymerization may predispose to thrombosis and correlate with a worse outcome (Spiezia 2020).

Lab findings as risk factor

It is not very surprising that patients with severe disease had more prominent laboratory abnormalities than those with non-severe disease. It remains unclear how a single parameter can be of clinical value as almost all studies were retrospective and uncontrolled. Moreover, the numbers of patients were low in many studies. However, there are some patterns which may be helpful in clinical practice. Lab risk factors are:

- Elevated CRP, procalcitonin, interleukin-6 and ferritin
- Lymphocytopenia, CD4 T cell and CD8 T cell depletion, leukocytosis
- Elevated D-dimer and troponin
- Elevated LDH

Clinical classification

There is no broadly accepted or valid clinical classification for COVID-19. The first larger 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 7483 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 > 65 years 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. Howev-

er, it will be important that these studies are carefully designed and carried out, especially to avoid bias and confounding.

Case fatality rates (CFR)

The country-specific crude case fatality rates (CFR), the percentage of COVID-19-associated deaths among confirmed SARS-CoV-2 infections, have been the subject of much speculation. There are still striking differences between countries. According to worldometer.com assessed on October 12, 2020, the crude CFR between the 100 most affected countries (in terms of absolute numbers) ranged from 0.05 (Singapore) to 10.2 (Mexico). Within the 10 most affected countries in Europe, the CFR range was between 0.8% (Czechia) and 10,2% (Italy).

Although it is well known that the CFR of a disease can be biased by detection, selection or reporting (Niforatos 2020), and although it became quickly clear that older age is a major risk factor for mortality (see below), many other factors contributing to regional differences throughout the world have been discussed in recent months. These factors include not only differences in the overall age structure of the general population of a country and co-residence patterns, but also co-morbidity burden, obesity prevalence and smoking habits as well as societal and social psychological factors. Others include heterogeneity in testing and reporting approaches, variations in health care system capacities and health care and even political regime. Different virus strains or even environmental factors such as air pollution have also been discussed, as well as potential differences in genetic variability or even "trained immunity" induced by certain live vaccines such as bacillus Calmette-Guérin (for references see Hoffmann C 2020).

We can probably exclude most of these speculations. SARS-CoV-2 is not deadlier in Italy (CFR 10,2%), United Kingdom (7,1%), or Sweden (6,0%), compared to Slovakia (0,3%), Israel (0,8%), India (1,5%) or USA (2,7%). Instead, there are three major factors that have to be taken into account:

- The age of the pandemic, especially of the population *which is first affected*. Data from the 20 most affected European countries and the USA and Canada show that the variance of crude CFR of COVID-19 is predominantly (80-96%) determined by the proportion of older individuals who are diagnosed with SARS-CoV-2 (Hoffmann C 2020). Of note, the age distribution of SARS-CoV-2 infections is still far from homogeneous. The proportion of individuals older than 70 years among confirmed SARS-CoV-2 cases still differs markedly between the countries, ranging from 5% to 40% (Figure 1).

Countries' testing policies (and capacities). The fewer people you test (all people, only symptomatic patients, only those with severe symptoms), the higher the mortality. In Germany, for example, testing systems and high lab capacities were established rapidly, within weeks in January (Stafford 2020).

- Stage of the epidemic. Some countries have experienced their first (or second) waves early while others lagged a few days or weeks behind. Death rates only reflect the infection rate of the previous 2 to 4 weeks.

There is no doubt that the marked variation of CFR across countries will diminish over time, for example, if less affected countries such as Korea or Singapore fail to protect their older age groups; or if countries with high rates at the beginning (such as Italy, Belgium or Sweden) start to implement broad testing in younger age groups. This process has already begun. In Belgium, for example, CFR peaked on May 11 with an appalling rate of 16,0%; it has now dropped to 6,3%. The CFR in the USA peaked on May 16 (6,1%) and is now less than half that. Germany started with a strikingly low CFR of 0,2% by the end of March (prompting much attention even in scientific papers), peaked on June 18 (4,7%) and is now (October 10) at 3,0%.

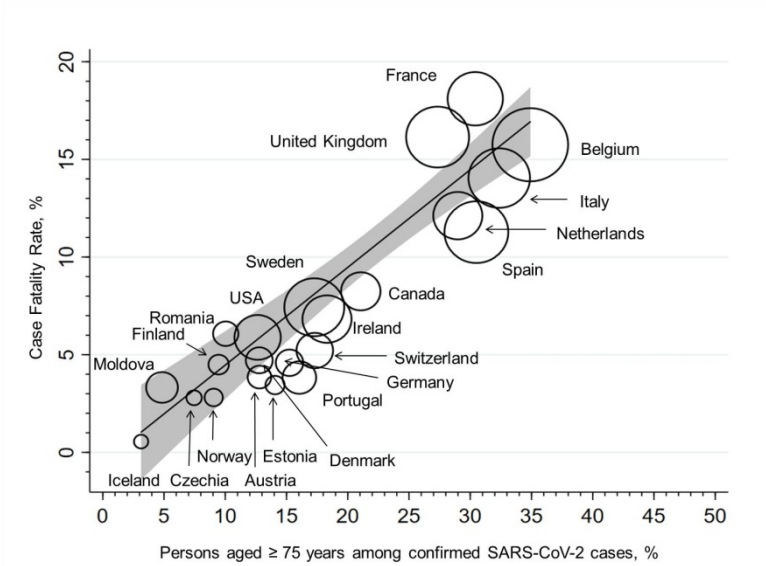

Figure 1. Association between case fatality rate (CFR) and the proportion of persons over 75 years of age among all confirmed SARS-CoV-2 cases (R2 = 0.8034, p < 0.0001). The circle sizes reflect the country-specific numbers of COVID-19 associated deaths per million habitants; the linear fit prediction plot with a 95% confidence interval was estimated by weighted linear regression (weight = total number of COVID-19 associated deaths).

CFR among health care workers, well-defined populations

In well-monitored populations in which under-reporting is unlikely or can be largely determined, the mortality rates may better reflect the "true" CFR of COVID-19. This applies to healthcare workers (HCW) but also to populations of "well-defined" (limited) outbreaks and in populations with available serology data. The low mortality rates in these populations are remarkable.

- In a large study of 3387 HCW from China infected with SARS-CoV-2, only 23 died, corresponding to a mortality rate of 0,68%. The median age was 55 years (range, 29 to 72), and 11 of the 23 deceased HCW had been reactivated from retirement (Zhan 2020). Current studies in the US have found similar mortality estimates of 0,3-0,6% (CDC 2020). Of the 27 HCW who 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.
- On the cruise ship Diamond Princess, as of May 31, the total number of infected reached 712, and 13 patients died from the disease leading to a CFR of 1,8% (Moriarty 2020). Of note, around 75% of the patients on the Diamond Princess were 60 years or older, many of them in their eighties. Projecting the Diamond Princess CFR onto the age structure of the general population, mortality would be in a range of 0,2-0,4%.
- According to an investigation of the shore-based USS Theodore Roosevelt outbreak, only 6/736 infected sailors were hospitalized, and one (a "senior listed member in his 40s") died during the study period (CFR 0.1%) (Alvarado 2020).
- Using population-based seroprevalences in Geneva (Switzerland) and after accounting for demography, the population-wide infection fatality rate (IFR) was 0,64% (0,38–0,98) (Perez-Saez 2020).

CFR compared to influenza

More time and data are needed before the COVID-19 pandemic can be accurately compared with past pandemics. But what makes SARS-CoV-2 different from pandemic influenza virus? It's not only that SARS-CoV-2 is a new pathogen and influenza is not and that the diseases differ clinically. The picture is more complex. It also depends on which flu season you are talking about – the influenza pandemic excess mortality ranged from extreme (1918) to mild (2009) over the past 100 years. Another key difference between SARS-CoV-2 and pandemic influenza is the age distribution of patients who are severely

ill. Mortality due to SARS-CoV-2 and SARS-CoV is strongly skewed towards people older than 70 years, very dissimilar to the 1918 and 2009 influenza pandemics.

Pooled estimates of all-cause mortality for 24 European countries for the period March–April 2020 showed that excess mortality of COVID-19 particularly affected ≥ 65-year-olds (91% of all excess deaths) and to a far lesser extent those 45–64 (8%) and 15–44-year-olds (1%) (Vestergaard 2020). The excess mortality of COVID-19 is markedly higher than for major influenza pandemics in the past. For example, the 2009 pandemic influenza A H1N1 globally led to 201.200 respiratory deaths (range 105.700-395.600) with an additional 83,300 cardiovascular deaths (Dawood 2012). This is by far lower than the deaths caused by COVID-19 to date. According to a recent review, the population risk of admission to the intensive care unit is five to six times higher in patients infected with SARS-CoV-2 than in those with the fairly mild 2009 influenza pandemic (Petersen 2020).

In New York City, a study analyzed standardized mortality ratios (SMR) of comparator pandemics and epidemics relative to the first 2020 wave of COVID-19 (Muscatello 2020). In older people, COVID-19 mortality until June 2020 was more than 10-fold higher than a severe influenza season, and more than 300-fold higher than the 2009–10 influenza pandemic. Compared to the catastrophic 1918–19 winter wave of the influenza pandemic, there are marked differences for different age groups. The 1918-19 influenza had a high mortality, especially in younger persons (5–15 years; ~25% of total deaths), possibly due to antibody-dependent enhancement and 'cytokine storms' in younger people but also due to some protective cross-immunity from previous influenza outbreaks among those older. Compared to COVID-19, the overall age-adjusted, all-age mortality rate of the influenza 1918-19 was 6.7 times higher. In younger people (< 45 years), the SMR was 42; that is, 42 times higher for influenza in 1918–19 than for COVID-19. However, in people older than 44 years of age, the SMR was 0,56; that is, 44% lower in 1918–19 than for COVID-19.

Modeling scenarios without appropriate mitigation measures, simulations predict incredibly high peaks in active cases and alarmingly high numbers of deaths far into the future. In Germany, for example, 32 million total infections would result in 730.000 deaths over the course of the epidemic, which would seem to occur only by the end of the summer 2021 under the assumption that no reliable treatment is available before then (Barbarossa 2020).

Older Age

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). The summarizing report from the Chinese CDC found a death rate of 2,3%, representing 1023 among 44.672 confirmed cases (Wu 2020). Mortality increased markedly in older people. In cases aged 70 to 79 years, CFR was 8,0% and cases in those aged 80 years older had a 14,8% CFR. There is now growing data from serology-informed estimates that the same is true for the infection fatality risk (IFR). After accounting for demography and age-specific seroprevalence, IFR was 0,0092% (95% CI 0,0042–0,016) for individuals aged 20–49 years, 0,14% (0,096–0,19) for those aged 50–64 years but 5,6% (4,3–7,4) for those aged 65 years and older (Perez-Saez 2020).

In recent months, these data have been confirmed by almost all studies published throughout the world. In almost all countries, age groups of 60 years or older contribute to more than 90% of all death cases.

- In a large registry analyzing the epidemic in the UK in 20.133 patients, the median age of the 5165 patients (26%) who died in hospital from COVID-19 was 80 years (Docherty 2020).
- Among 1591 patients admitted to ICU in Lombardy, Italy, older patients (> 63 years) had markedly higher mortality than younger patients (36% vs 15%). Of 362 patients older than 70 years of age, mortality was 41% (Grasselli 2020).
- According to the Italian National Institute of Health, an analysis of the first 2003 death cases, median age was 80,5 years. Only 17 (0,8%) were 49 years or younger, and 88% were older than 70 years (Livingston 2020).
- Detailed analysis of all-cause mortality at Italian hot spots showed that the deviation in all-cause deaths compared to previous years during epidemic peaks was largely driven by the increase in deaths among older people, especially in men (Piccininni 2020, Michelozzi 2020).
- In 5700 patients admitted to New York hospitals, there was a dramatic increase of mortality among older age groups, reaching 61% (122/199) in men and 48% (115/242) in women over 80 years of age (Richardson 2020).
- The median age of 10,021 adult COVID-19 patients admitted to 920 German hospitals was 72 years. Mortality was 53% in patients being mechanically ventilated (n = 1727), reaching 63% in patients aged 70–79 years and 72% in patients aged 80 years and older (Karagiannidis 2020).

- In an outbreak reported from King County, Washington, a total of 167 confirmed cases was observed in 101 residents (median age 83 years) of a long-term care facility, in 50 healthcare workers (HCW, median age 43 years), and 16 visitors. The case fatality rate was 33,7% among residents and 0% among HCW (McMichael 2020).

There is no doubt that older age is by far the most important risk factor for mortality. Countries failing to protect their elderly population for different reasons (such as Italy, Belgium or Sweden) are facing a higher CFR, while those without many older patients infected by SARS-CoV-2 (such as the Republic of Korea, Singapore, Australia) have markedly lower rates.

What are the reasons? Severe endothelial injury as seen in critically ill patients (Ackermann 2020) and endotheliopathy is an essential part of the pathological response to severe COVID-19, leading to respiratory failure, multi-organ dysfunction and thrombosis (Goshua 2020). Circulating endothelial cells are a marker of endothelial injury in severe COVID-19 (Guervilly 2020) and there is a direct and rapid cytotoxic effect of plasma collected from critically ill patients on vascular endothelial cells (Rauch 2020). It is therefore tempting to speculate that endothelial injury will be particularly harmful in older patients with atherosclerosis.

But maybe not all is due to arteriosclerosis. “Inflammaging”, a common denominator of age-associated frailty, may also contribute to the severe COVID-19 course in older people. One hypothesis is that pre-existing inflammatory cells, including senescent populations and adipocytes, create the inflammaging phenotype that amplifies subsequent inflammatory events. Nevertheless, high amounts of inflammation alone do not explain the devastating tissue destruction and it may be that age-associated changes in T cells have a role in the immunopathology (review: Akbar 2020). There is growing evidence that coordination of SARS-CoV-2 antigen-specific responses is disrupted in older individuals. Scarcity of naive T cells was also associated with ageing and poor disease outcomes (Rydyznski 2020).

Sex and ethnicity

A striking finding is the lower mortality in female patients, evident through almost all available data. In Italy, for example, male gender was an independent risk factor associated with mortality at ICU with a hazard ratio of 1,57 (Grasselli 2020). Using a health analytics platform covering 40% of all patients in England, COVID-19-related death was associated with being male, with a hazard ratio of 1,59 (95% CI 1,53–1,65) (Williamson 2020). The hitherto largest registry study with detailed data on demographics and other clinical factors

is shown in Table 3. There is some evidence that there are sex-specific differences in clinical characteristics and prognosis and that the presence of comorbidities is of less impact in females (Meng 2020). It has been speculated that the higher vulnerability in men is due to the presence of subclinical systemic inflammation, blunted immune system, down-regulation of ACE2 and accelerated biological aging (Bonafè 2020).

Table 3. Age and co-morbidities in a large registry study (Docherty 2020), providing multivariate analyses and hazard ratios.

	UK, n = 15.194
Hazard Ratio (95% CI)	**Death**
Age 50-59 vs < 50	2.63 (2.06-3.35)
Age 60-69 vs < 50	4.99 (3.99-6.25)
Age 70-79 vs < 50	8.51 (6.85-10.57)
Age > 80 vs < 50	11.09 (8.93-13.77)
Female	0.81 (0.75-0.86)
Chronic cardiac disease	1.16 (1.08-1.24)
Chronic pulmonary disease	1.17 (1.09-1.27)
Chronic kidney disease	1.28 (1.18-1.39)
Hypertension	
Diabetes	1.06 (0.99-1.14)
Obesity	1.33 (1.19-1.49)
Chronic neurological disorder	1.18 (1.06-1.29)
Dementia	1.40 (1.28-1.52)
Malignancy	1.13 (1.02-1.24)
Moderate/severe liver disease	1.51 (1.21-1.88)

An in-depth analysis performed on 137 COVID-19 patients found that male patients had higher plasma levels of innate immune cytokines such as IL-8 and IL-18 along with more robust induction of non-classical monocytes. A poor T cell response negatively correlated with patients' age and was associated with worse disease outcome in male patients, but not in female patients. Conversely, higher innate immune cytokines were associated with worse disease progression in female patients, but not in male patients (Takahashi 2020). Emerging knowledge on the basic biological pathways that underlie gender differences in immune responses needs to be incorporated into research efforts on SARS-CoV-2 pathogenesis and pathology to identify targets for therapeutic interventions aimed at enhancing antiviral immune function

and lung airway resilience while reducing pathogenic inflammation in COVID-19 (review: Bunders 2020).

Ethnic minorities may be disproportionately affected by the COVID-19 pandemic. Among the first 1.3 million lab-confirmed COVID-19 cases reported to CDC until May 30, 2020, 33% of persons were Hispanic (accounting for 18% of the US population), 22% (13%) were black, and 1,3% (0,7%) were non-Hispanic American Indian or Alaska Native (Stoke 2020). However, in a large cohort study on 5902 COVID-19 patients treated at a single academic medical center in New York, survival outcomes of non-Hispanic Black and Hispanic patients were at least as good as those of their non-Hispanic White counterparts when controlling for age, sex, and comorbidities (Kabarriti 2020). Several other US studies have also found no differences, after controlling for confounders such as age, gender, obesity, cardiopulmonary comorbidities, hypertension, and diabetes (McCarty 2020, Muñoz-Price 2020, Yehia 2020). There is some evidence indicating a longer wait to access care among black patients in the US, resulting in more severe illness on presentation to health care facilities (Price-Haywood 2020).

Obesity

Several studies have found obesity to be an important risk factor (Goyal 2020, Petrilli 2019). 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. Among 10.861 COVID -19 patients ad
Northwell Health system hospitals during March and April, underweight and obesity classes II and III were statistically associated with death (OR = 1,25-1,61). However, once mechanically ventilated, all patients regardless of BMI had similar odds of death (Kim 2020).

Obesity was also an important risk factor in France (Caussy 2020), Singapore and the US, especially in younger patients (Ong 2020, Anderson 2020). Of 3222 young adults (age 18 to 34 years) hospitalized for COVID-19 in the US, 684 (21%) required intensive care and 88 patients (2.7%) died. Morbid obesity and hypertension were associated with a greater risk of death or mechanical ventilation. Importantly, young adults aged 18 to 34 years with multiple risk factors (morbid obesity, hypertension, and diabetes) faced risks similar to 8862 middle-aged (age 35-64 years) adults without these conditions (Cunningham 2020). A recent review has described some hypotheses regarding the deleterious impact of obesity on the course of COVID-19 (Lockhart 2020), summarizing current knowledge on the underlying mechanisms. These are:

1. Increased inflammatory cytokines (potentiate the inflammatory response)
2. Reduction in adiponectin secretion (abundant in the pulmonary endothelium)
3. Increases in circulating complement components
4. Systemic insulin resistance (associated with endothelial dysfunction and with increased plasminogen activator inhibitor-1)
5. Ectopic lipid deposited in type 2 pneumocytes (predisposing to lung injury).

Co-morbidities

Besides older age and obesity, many risk factors for severe disease and mortality have been evaluated in the current pandemic.

Early studies from China found co-morbidities such as hypertension, cardiovascular disease and diabetes to be associated with severe disease and death (Guan 2020). Among 1590 hospitalized patients from mainland China, after adjusting for age and smoking status, COPD (hazard ratio, 2,7), diabetes (1,6), hypertension (1,6) and malignancy (3,5) were risk factors for reaching clinical endpoints (Guan 2020). Dozens of further studies have also addressed risk factors (Shi 2020, Zhou 2020). The risk scores that have been mainly proposed by Chinese researchers are so numerous that they cannot be discussed here. They were mainly derived from uncontrolled data and their clinical relevance remains limited. An interactive version of a relatively simple, so called "COVID-19 Inpatient Risk Calculator" (CIRC) evaluated in 787 patients admitted with mild-to-moderate disease between March 4 and April 24 in five US hospitals in Maryland and Washington (Garibaldi 2020), is available at https://rsconnect.biostat.jhsph.edu/covid_predict.

Smoking as a risk factor is under discussion, as well as COPD, kidney diseases and many others (see chapter *Comorbidities*, page **Fehler! Textmarke nicht definiert.**). Among 1150 adults admitted to two NYC hospitals with COVID-19 in March, older age, chronic cardiac disease (adjusted HR 1,76) and chronic pulmonary disease (2,94) were independently associated with in-hospital mortality (Cummings 2020).

The main problem of all studies published to date is that their uncontrolled data is subject to confounding and they do not prove causality. Even more importantly, the larger the numbers, the more imprecise the definition of a given comorbidity. What is a "chronic cardiac disease", a mild and well-controlled hypertension or a severe cardiomyopathy? The clinical manifestations and the relevance of a certain comorbidity may be very heterogeneous

(see chapter *Comorbidities*, page **Fehler! Textmarke nicht definiert.**). What is hypertension? In a huge study from 460 general practices in England, 4277 COVID-19 patients with hypertension were followed and 877 died within 28 days. Of note, there was no association between blood pressure (BP) control and COVID-19 diagnosis or hospitalization. Individuals with stage 1 uncontrolled BP had lower odds of COVID-19 death (OR 0,76, 95% CI 0,62-0,92) compared to patients with well-controlled BP. However, these patients were older, had more comorbidities and had been diagnosed with hypertension for longer, suggesting more advanced atherosclerosis and target organ damage (Sheppard 2020).

There is growing evidence that sociodemographic factors play a role. Many studies did not adjust for these factors. For example, in a large cohort of 3481 patients in Louisiana, US, public insurance (Medicare or Medicaid), residence in a low-income area, and obesity were associated with increased odds of hospital admission (Price-Haywood 2020). A careful investigation of the NYC epidemic revealed that the Bronx, which has the highest proportion of racial and ethnic minorities, the most persons living in poverty, and the lowest levels of educational attainment, had higher rates (almost two-fold) of hospitalization and death related to COVID-19 than the other 4 NYC boroughs Brooklyn, Manhattan, Queens and Staten Island (Wadhera 2020).

Taken together, large registry studies have found slightly elevated hazard ratios of mortality for multiple comorbidities (Table 3). It seems, however, that most patients with preexisting conditions are able to control and eradicate the virus. Co-morbidities play a major role in those who do not resolve and who fail to limit the disease to an upper respiratory tract infection and who develop pneumonia. Facing the devastation that COVID-19 can inflict not only on the lungs but on many organs, including blood vessels, the heart and kidneys (nice review: Wadman 2020), it seems plausible that a decreased cardiovascular and pulmonary capacity impact clinical outcome in these patients.

However, at this time, we can only speculate about the precise role of comorbidities and their mechanisms to contribute to disease severity.

Is there a higher susceptibility? In a large, population-based study from Italy, patients with COVID-19 had a higher baseline prevalence of cardiovascular conditions and diseases (hypertension, coronary heart disease, heart failure, and chronic kidney disease). The incidence was also increased in patients with previous hospitalizations for cardiovascular or non-cardiovascular diseases (Mancia 2020). A large UK study found some evidence of potential sociodemographic factors associated with a positive test, including deprivation, population density, ethnicity, and chronic kidney disease (de Lusignan 2020).

However, even these well perfomed studies cannot completely rule out the (probably strong) diagnostic suspicion bias. Patients with co-morbidities could be more likely to present for assessment and be selected for SARS-CoV-2 testing in accordance with guidelines. Given the high number of nosocomial outbreaks, they may also at higher risk for infection, just due to higher hospitalization rates.

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 *The First 8 Months*, page 453). So far, only assumptions can be made. The remarkable heterogeneity of disease patterns from a clinical, radiological, and histopathological point of view has led to the speculation that the idiosyncratic responses of individual patients may be in part related to underlying genetic variations. Many single nucleotide polymorphisms (SNPs) across a variety of genes (eg, ACE2, TMPRSS2, HLA, CD147, MIF, IFNG, IL6) have been implicated in the pathology and immunology of SARS-CoV-2 and other pathogenic coronaviruses (Ovsyannikova 2020).

The 'COVID-19 Host Genetics Initiative' brings together the human genetics community to generate, share, and analyze data to learn the genetic determinants of COVID-19 susceptibility, severity, and outcomes (CHGI 2020). It seems that regions on chromosome 3 are significantly associated with severe COVID-19 at the genome-wide level. The risk variant in this region confers an odds ratio for requiring hospitalization of 1,6 (95% confidence interval: 1,42-1,79).

The GenOMICC (Genetics Of Mortality In Critical Care) genome-wide association study (GWAS) in 2244 critically ill COVID-19 patients from 208 UK intensive care units has scanned each person's genes, which contain the instructions for every biological process, including how to fight a virus. Some genetic differences were identified (odds ratio of the tested risk alleles were 1,2-1,9) between patients with severe COVID-19 and the general population, revealing "robust genetic signals relating to key host antiviral defense mechanisms, and mediators of inflammatory organ damage" (Pairo-Castineira 2020).

Some further key studies are listed here:

- A large study identified a 3p21.31 gene cluster as a genetic susceptibility locus in patients with COVID-19 with respiratory failure and confirmed a potential involvement of the ABO blood-group system (Elinghaus 2020). A higher risk in blood group A was found compared to other blood groups

(odds ratio, 1,45; 95% CI, 1,20 to 1,75) and a protective effect in blood group O as compared with other blood groups (odds ratio, 0,65; 95% CI, 0,53 to 0,79)

- In a meta-analysis of 7 studies, comparing 7503 SARS-CoV-2 positive patients with 2.962.160 controls, SARS-CoV-2 positive individuals were more likely to have blood group A (pooled OR 1,23, 95% CI: 1,09–1,40) and less likely to have blood group O (pooled OR 0,77, 95% CI: 0,67–0,88) (Golinelli 2020).
- In another large study on 225.556 persons, O and Rh- blood groups were associated with a slightly lower risk for SARS-CoV-2 infection as well as severe COVID-19 illness or death (Ray 2020).
- Associations between ApoEe4 alleles and COVID-19 severity, using the UK Biobank data (Kuo 2020). ApoEe4e4 homozygotes were more likely to be COVID-19 test positives (odds ratio 2,31, 95% CI: 1,65-3,24) compared to e3e3 homozygotes. The ApoEe4e4 allele increased risks of severe COVID-19 infection, independent of pre-existing dementia, cardiovascular disease, and type 2 diabetes.
- 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, without underlying illnesses (Yousefzadegan 2020). However, there is also a case of a homozygous twin pair, displaying very different clinical courses, despite the same source and the same viral load at baseline (Lazzeroni 2020).
- Two families with rare germline variants in an innate immune-sensing gene, toll-like receptor 7 (TLR7), that leads to severe disease even in young males who inherit the mutated gene on a single copy of their X chromosome (van der Made 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. If you inhale large numbers of virus deeply, leading rapidly to a high amount of virus in the pulmonary system, this may be much worse than smearing a small amount of virus on your hand and, later, to your nose. In this latter case, the immune system in the upper respiratory tract may have much more time to limit further spread into the lungs and other organs. After an outbreak at a Swiss Army base, soldiers had to keep a distance of at least 2 m from each other at all times, and in situations where this could not be avoided (e.g., military training), they had to wear a surgical face mask. Of

the 354 soldiers infected prior to the implementation of social distancing, 30% fell ill from COVID-19. While no soldier in a group of 154 in which infections appeared after implementation of social distancing developed COVID-19 (Bielecki 2020).

Pre-existing SARS-CoV-2 S-reactive T cells may also play a role, contributing to the divergent manifestations of COVID-19. These cells represent cross-reactive clones, probably acquired during previous infections with endemic human coronaviruses (HCoVs). In healthy SARS-CoV-2-unexposed donors, they were found in 35% (Braun 2020). However, the clinical effect of these T cells and other immunological factors on clinical outcomes remains to be determined. There are hundreds of immunological papers focusing on the unresolved question why some patients develop severe disease, while others do not (review: Gutierrez 2020). It remains also to be seen whether T cells provide long-term protection from reinfection with SARS-CoV-2 and if there is a natural immunity, induced by cross-reactive T cells (Le Bert 2020, Mateus 2020).

Over the coming months, we will get a clearer view of 1) correlates of immunoprotection, such as virus-specific antibodies that limit disease and 2) correlates of immune dysregulation, such as cytokine over-production that may promote disease.

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 resulted in an insufficiency of health-care resources, thereby negatively affecting patient outcomes in Hubei, while this was not the case 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).

Finally, there may be differences between hospitals. In a US cohort of 2215 adults with COVID-19 who were admitted to ICUs at 65 sites, 784 (35.4%) died within 28 days. However, mortality showed a wide variation between hospitals (range, 6,6%-80,8%). One of the well-known factors associated with death was a hospital with fewer intensive care unit beds (Gupta 2020)! Patients ad-

mitted to hospitals with fewer than 50 ICU beds versus at least 100 ICU beds had a higher risk of death (OR 3,28; 95% CI: 2,16-4,99).

Reactivations, reinfections

Seasonal coronavirus protective immunity is not long-lasting (Edridge 2020). There are several reports of patients infected with SARS-CoV-2 who became positive again after negative PCR tests (Lan 2020, Xiao 2020, Yuan 2020). These reports have gained much attention, because this could indicate 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). 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.

However, in recent months several case reports of true (virologically proven: phylogenetically distinct strains) re-infections have been reported (To 2020, Gupta 2020, Van Elslande 2020). In most cases, the second episode was milder than the first. However, there is at least one case where the second infection was more severe, potentially due to immune enhancement, acquisition of a more pathogenic strain, or perhaps a greater in-oculum of infection as the second exposure was from within household contacts (Larson 2020). Up to now, however, these are anecdotal case reports.

Animal studies suggest that re-infection is unlikely (Chandrashekar 2020). Following initial viral clearance and on day 35 following initial viral infection, 9 rhesus macaques were re-challenged with the same doses of virus that were utilized for the primary infection. Very limited viral RNA was observed in BAL on day 1, with no viral RNA detected at subsequent timepoints. These data show that SARS-CoV-2 infection induced protective immunity against re-exposure in nonhuman primates. There is growing evidence for a long-lived and robust T cell immunity that is generated following natural SARS-CoV-2 infection (Neidleman 2020).

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 the main but not the only risk factor. Recently, a 106-year-old COVID-19 patient recently recovered in the UK. The precise mechanisms of how co-morbidities (and co-medications) may contribute to an increased risk for a severe disease course have to be elucidated. Genetic and immunological studies need 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.

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9. Long COVID-19

The term "Long COVID-19" covers a wide spectrum of symptoms that can occur or persist weeks and even months after an acute infection, not only after severe but also after initially mild courses. Primary symptoms are exhaustion and fatigue, but also dyspnea on exertion, headache and arthralgia, palpitations, concentration disorders and depressive symptoms in previously healthy individuals. The symptoms may fluctuate within hours or days. This chapter summarizes current knowledge (which is still limited, as of the end of January 2021).

"Long Haulers" have to be taken seriously

It was striking how much and how quickly the topic was put on the agenda from the beginning of the pandemic, mainly by patients themselves (Callard 2021). Stories of the so-called "long haulers" were published in social media but also in scientific journals. In early May, the report of the British infectious disease specialist Paul Garner in the BMJ about his seven-week "roller coaster of ill health, extreme emotions and utter exhaustion" gained much attention (Garner 2020). This was followed by many other case reports by medical professionals (Alwan 2020), including that of former UNAIDS Director Peter Piot (Draulens 2020).

Online surveys on persistent cognitive deficits also generated wide circulation (Hampshire 2020), and even if such observations strongly overestimate symptom prevalence, they do provide us with clues about symptoms and distress among long haulers (Goërtz 2020). Indeed, the distress appears to be considerable: among 114 long-haulers (including 32 physicians, almost all treated as outpatients), strong feelings of insecurity, stigmatization, and difficulty being taken seriously were evident (Ladds 2020). In particular, the experience of uncertainty and helplessness were described as stressful. Affected individuals demand that their primary care physician believe their symptoms and show empathy and understanding. Continuous support during recovery and rehabilitation seems to be crucial (Kingstone 2020).

Definition, categories

A generally accepted definition for the syndrome is lacking so far. None of the terms used is a good fit: whether "long", "chronic", "ongoing symptomatic", "post" or "post-acute COVID-19" - they are all incomplete. British experts recommend using the term "post-COVID syndrome" as of 12 weeks post-infection. But as no evidence exists of any particular physiological changes

(that predict chronicity) at 12 weeks, according to other experts it would be preferable to use the term long COVID-19 for symptoms of any duration beyond four weeks, as is strongly advocated by people with lived experience of this condition. Moreover, using the prefix "post" implies that acute infection and any active disease process are resolved, which is currently unknown (Sivan 2020). Different time windows of 3, 4, 12, or 24 weeks are discussed. Some authors suggest dividing post-acute manifestations into three categories, namely residual symptoms that persist after recovery from acute infection; organ dysfunction that persists after initial recovery; and new syndromes that develop after initially asymptomatic or mild infection (Amenta 2020). Still others have identified at least four or more distinct entities, including a post-intensive care syndrome (PICS), a post-viral fatigue syndrome, permanent organ damage, and "long-term COVID-19 syndrome" (NIHR 2020, Mahase 2020). To date, no concept has gained broad acceptance. In addition to the lack of definition and valid diagnostic tests, there are several pitfalls and problems that complicate the interpretation of the study data to date (see box).

Caveats and problems of studies of "long COVID-19"

- No universally accepted definition
- SARS-CoV-2 diagnosis inconsistent, not always confirmed (especially in early 2020)
- Different severity of acute illness (ICU, ward, outpatient)
- Different populations (age, culture), selection and recall bias possible
- Apps, online surveys: response bias!
- Many symptoms non-specific, difficult to objectify, baseline values not available
- Symptom prevalence: no comparison group
- Differentiation from other causes (underlying diseases, PICS, lockdown, emotional stress) difficult, psychosomatic overlap possible

Table 1. Important studies, most common symptoms, Follow-Up (FU) days

Reference, location	Patients, median age	n*	FU	Main symptoms at FU	Other findings at the last FU
Mandal (London)	Hospitalized (15% ICU), 60 years	384 (479)	54 (D)	"unchanged or detoriating": low sleep quality 34%, cough 26%, fatigue 20%, breathlessness 19%	89% symptomatic (at least one symptom)
Carfi (Rome)	Hospitalized (13% ICU), 57 years	143 (179)	60 (O)	Fatigue 53%, dyspnea 43%, joint pain 27%, chest pain 22%	87% symptomatic, worsened quality of life in 44%
Moreno-Pérez (Alicante)	Hospitalized (9% ICU), 62 years	277 (422)	77 (O)	Fatigue 35%, dyspnea 34%, cough 21%, anosmia-dysgeusia 21%, myalgias 20%, headache 18%, mnestic complaints 15%	Lymphopenia < 1500 20% CRP > 0.5mg/dL 12%, D-dimers > 0.5 mg/mL 25% Ferritin > 150 mg/L 41% Abnormal spirometry 9%
Garrigues (Paris)	Hospitalized (20% ICU), 63 years	120 (188)	111 (A)	Fatigue 55%, dyspnea 42%, loss of memory 34%, sleep disorders 31%, concentra-tion disorders 28%, hair loss 20%	32% not returned to work
Xiong (Wuhan)	Hospitalized (39% severe), 52 years	538 (891)	97 (D)	Alopecia 29%, fatigue 28%, sweating 24%, "postactivity polypnoea" 21%, sleep disorders 18%	50% symptomatic, sequelae more common in females
Petersen (Faeroe islands)	96% outpatient, 40 years	180 (187)	125 (O)	Loss of smell and taste 50%, fatigue 24% (if present during acute phase)	53% symptomatic, more persistence with increasing age
Huang (Wuhan)	Hospitalized (4% ICU), 57 years	1733 (2469)	186 (O)	Fatigue/muscle weakness 63%, pain or discomfort 27%, sleep difficulties 26%, anxiety or depression 23%, hair loss 22%	76% symptomatic, DLCO <80% than predicted 23%, at least one abnormal CT pattern 52%

* Number of patients surveyed (total number of patients), ICU = intensive care unit. DLCO = diffusion capacity for carbon monoxide. Median follow-up after onset of symptoms (O), hospital admission (A) or discharge (D).

Pathogenesis

The pathogenesis is certainly multifactorial. Persistent lung damage (as seen in SARS cases) is evident in many patients even after months, especially in severe COVID-19 cases (see below). However, this does not explain the whole syndrome. Speculations and theories about extrapulmonary manifestations involve chronic inflammation, immune dysregulation, sequelae of endotheliitis and coagulopathy, mitochondrial dysfunction - they may all be somewhat correct, and each apply to a subset of cases. Autonomic dysfunction probably also plays a role. It could explain part of the symptoms (dizziness, palpitations, orthostasis) and has been found in a small cohort (Dani 2020). In particular, the main symptom - fatigue, which is somewhat difficult to objectify, remains a puzzle. Overlaps and similarities with the so-called chronic fatigue syndrome (CFS/ME) have been postulated. Of note, a study that looked more closely at fatigue (Townsend 2020) found no association with the severity of COVID-19, but neither was it found with various laboratory markers (blood count, LDH, CRP, IL-6).

Clinical symptoms

Clinical symptoms and complaints are variable. Beside fatigue and muscle weakness, other common symptoms include shortness of breath, muscle and joint pain, palpitations, loss or alteration of taste and sense of smell, and hair loss. Cognitive problems with deficitis in concentration and memory ("foggy brain", etc.) are also frequently reported. In the largest studies of clinical symptoms, prevalence varied greatly (Table 1). This may be due to the different time of follow up but also due to very heterogeneous patient populations (severity of COVID-19, age, pre-existing co-morbidities, etc). The by far largest study to date has evaluated 1655 patients from Wuhan (Huang 2021). During acute COVID-19, all patients had been hospitalized (only 4% were treated on an ICU, but 68% had received oxygen). Fatigue and weakness six months later correlated strongly with acute severity of COVID-19, as did anxiety and depression, but also lung diffusion capacity (Huang 2021). Women were more frequently affected.

Persistent symptoms certainly occur even in mild courses. In one well-defined study, 180 (96% of the total) COVID-19 patients from the Faeroe Islands (a North Atlantic archipelago located about halfway between Norway and Iceland) from the first wave were followed: among the almost entirely non-hospitalized cohort, 53% reported persistence of at least one symptom after a mean of 125 days after symptoms onset, 33% reported one or two symptoms and 19% three or more symptoms (Petersen 2020). In the Wuhan

study, even of the 439 patients who had not required oxygen in the hospital, 81% reported at least one symptom after 6 months, including 66% fatigue and muscle weakness (Huang 2021). In another large study of 669 patients from Switzerland treated exclusively as outpatients, as many as 32% still had at least one symptom after 6 weeks (Nehme 2020). Long periods of disability are seen even in mild courses (see cases in the box). Of hospitalized patients, almost one-third are unable to work after 3 months (Garrigues 2020, Chopra 2020).

The often highly fluctuating course is striking: in a cross-sectional analysis of 70 "long haulers", the course of symptoms was intermittent in 43% of the cases, alternating symptom-free intervals of a few days or hours with sudden relapses, often worsening after physical or intellectual exercise (Salmon-Ceron 2020).

Studies with objectifiable tests

A selection of studies that focused on objectifiable tests and parameters is presented below. Again, it is important to consider different follow-up duration, but also the selection of the case population studied. In most studies, there was not only a correlation between the findings and the severity of acute COVID-19 but also a marked improvement over time. However, whether the remaining radiological or pulmonary diffusion abnormalities completely resolve needs to be investigated in future follow-up studies.

Pulmonary function

- Of 145 patients in a prospective study from Germany and Austria (75% hospitalized, 22% in ICU), 41% exhibited persistent symptoms (36% dyspnea) 100 days after COVID-19 onset. CT scans unveiled persistent lung pathologies in 63%, mainly consisting of bilateral ground-glass opacities, without radiological signs of pulmonary fibrosis. However, sequential follow-up evaluations at 60 and 100 days after COVID-19 onset demonstrated a vast improvement of both, symptoms and CT abnormalities over time. One-third displayed an impaired lung function, with a reduced diffusing capacity being the most prominent finding even more than 100 days after COVID-19 diagnosis (Sonnweber 2020).
- In the Wuhan cohort, a subgroup was tested for diffusion capacity for carbon monoxide (DLCO) at 6 months of follow-up. A low DLCO (< 80% of predicted) was found in 50% (48/86) and in 29% (66/228) in those

with and without mechanical ventilation during acute COVID-19, respectively (Huang 2021).

- Of 103 consecutively hospitalized patients from Norway (15 ICU cases), about half had exertional dyspnea at three months, a quarter had abnormal CT chest findings, and another quarter had reduced diffusion capacity (Lerum 2020). ICU admission was associated with pathologic CT findings but not with worsened pulmonary function.
- Improvement of CT findings in 99% in 124 patients from Nijmwegen, Netherlands (mostly with mild-moderate disease) after 13 weeks, but residual lesions were seen in 91% of discharged patients, as well as a correlation with diffusion capacity (van den Borst 2020). Cognitive dysfunction was seen in 36%.
- In 113 Swiss patients (66 with severe/critical COVID-19), there was a strong association between acute COVID-19 severity and diffusion capacity at 4 months, as well as between duration of mechanical ventilation and lung function (Guler 2021).

Cardiac MRT

- A total of 100 unselected patients (mean age 49 years, 67 outpatients, including 18 asymptomatic cases), received a cardiac MRI 71 (64-92) days after COVID-19 diagnosis (Puntmann 2020). In total, 36% reported persistent shortness of breath and general fatigue. Of note, MRI showed evidence of "cardiac involvement" in 78% and persistent myocardial inflammation in 60%. This was independent of preexisting disease, severity of COVID-19, or time of diagnosis.
- In contrast, among 145 young, otherwise healthy students with mild-moderate disease, only two students had an abnormal MRI 14 days after diagnosis (Starekova 2021).
- In 26 competitive athletes (14 asymptomatic, 12 mildly ill), a cardiac MRI was performed 11-53 days after quarantine (Rajpal 2020). Overall, 4/26 (15%) had CMR findings suggestive of myocarditis, and 8/26 (31%) had changes suggestive of previous myocardial injury.

Others

- Physical Fitness: 199 young Swiss recruits had undergone a baseline fitness test (3 months before a major COVID-19 outbreak in the company), including strength measurements and a progressive endurance run. Baseline fitness was compared with a second fitness test performed at a median of 45 days after SARS-CoV-2 diagnosis. Three

groups were formed: "symptomatic COVID-19" (n = 68, all mild-moderate), asymptomatic cases (n = 77), and symptom-free without evidence for infection (n = 54). The strength tests were comparable between the groups. However, there was a significant decrease in VO2 max in the symptomatic cases. Approximately 19% had a decrease in VO2 max of more than 10%, whereas none of the uninfected showed such a decrease (Crameri 2020).

Monitoring, treatment options

As early as August 2020, a preliminary guideline for the treatment of "long COVID-19" was published in the British Medical Journal (Greenhalgh 2020). After excluding serious ongoing complications or comorbidities, the recommendation was to manage patients "pragmatically and symptomatically with an emphasis on holistic support while avoiding over-investigation". It was noted that "many patients recover spontaneously (if slowly) with holistic support, rest, symptomatic treatment, and gradual increase in activity".

According to the authors, blood tests should "be ordered selectively and for specific clinical indications after a careful history and examination; the patient may not need any". In the largest and longest study to date from Wuhan, however, 35% of the patients showed a decreased glomerular filtration rate (GFR). Unexpectedly, 13% (107 of 822) of those who did not develop acute kidney injury during their hospital stay and presented with normal renal function, based on estimated GFR during the acute phase, exhibited a decline in eGFR (< 90 mL/min per 1,73 m^2) at 6 months of follow-up (Huang 2021). It seems therefore reasonable to monitor renal function at least once in long COVID-19 cases.

Fortunately, new onset diabetes mellitus and thrombosis were extremely rare in the Wuhan cohort study (Huang 2021). From our point of view, the control of blood glucose or D-dimers (as well as the use anticoagulation as suggested by some experts) does not seem to be necessary. This also applies to inflammatory parameters which can be slightly elevated in a considerable proportion of patients even after months (Moreno-Pérez 2021). These remain without consequences.

Case 1

51-year-old resident surgeon with mild-to-moderate COVID-19 (outpatient, no oxygen, but severe headache, nausea, no fever, 2-3 days of dyspnea). Unable to work (including quarantine) for 24 days. Reintegration to work (starting with 3 hrs) after another 35 days.

Current comment (after 90 days): "Still fluctuating days of strongest exhaustion, only very gradual recovery. I am glad to be able to get my work done. However, every day, I still have to sleep 1-3 hours after work. Improvement is slow. I was already jogging again, but still feelings of fatigue rapidly occuring, like 'plug out'".

Case 2

44-year-old psychiatrist with mild-to-moderate COVID-19 (outpatient, no oxygen). Beyond the two weeks of quarantine, there is dyspnea on exertion, severe exhaustion and cephalgia, concentration disorders, mild anxious-depressive symptoms (self-assessment) and anosmia/ageusia. Another 21 days of incapacity for work (total 35), followed by slow reintegration to work over a further 42 days.

Current comment (after 90 days): "Now just the second week of normal working hours. No longer headaches on all days. Fatigue still evident, severe on some days. Sense of smell and taste still not present. Sport is not to be thought of."

A light endurance training (including walking or Pilates, increasing intensity only very gradually) may be useful, as may relaxation techniques. Gradual reintegration into working life, as in the two case studies, is often helpful. Patience, empathy and a cautious (not too ambitious) goal-setting are required. Especially in severe cases, inpatient rehabilitation may be useful, preferably in multidisciplinary settings; some experiences have already been published (Puchner 2021, Brigham 2021).

Numerous clinical therapeutic trials of long COVID are ongoing or planned, including those with steroids, but also different drugs such as naltrexone, leronlimab, montelukast, or deupirfenidone. Up to now, no results have been published. In addition, many large prospective clinical trials will study long haulers prospectively and in a standardized manner in order to better understand the long-term effects on lung function, the cardiovascular system, hematologic parameters, renal function, and many other organ systems. For example, in the United Kingdom, the Post-Hospitalisation COVID-19 Study (PHOSP-COVID) will follow 10.000 patients for a year, analysing clinical factors such as blood tests and scans, and collecting data on biomarkers. Finally, existing cohort studies have also refocused and integrated COVID-19 aspects

in their study designs (Abbott 2020). These studies will have the advantage of having a control group (those who do not become infected).

Conclusion

A relevant number of people with COVID-19 are physically and psychologically impaired up to at least 6 months after diagnosis. Women appear to be more frequently affected. The studies to date are cause for concern; on the other hand, it must be kept in mind that retrospective studies are subject to numerous biases and tend to overestimate the disease burden. However, it has become evident that "long COVID-19" needs to gain even more attention in the current discussion. Those who now emphasize the collateral damage and costs of lockdown should also consider the consequences of long COVID-19 or the often very slow convalescence of many cases. Those who discuss potential long-term damage from vaccines should also consider the potential long-term damage from COVID-19. "Long haulers" need to be taken seriously. Given the high numbers of SARS-CoV-2 infections world-wide, all practicing physicians and outpatient clinics will be dealing with this syndrome in the future.

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10. Treatment

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Published 20 January 2021

Let's face it: after one year of intensive research, we have some steroids which have been shown to reduce mortality in patients with severe COVID-19 (see *Corticosteroids*, page 362); and we have one approved drug, remdesivir (Veklury®), which had a marginal benefit in a company-sponsored trial (Beigel 2020). There are also a few monoclonal antibodies, showing modest effects on viral load. And the JAK inhibitor baricitinib, in combination with remdesivir, in special patient populations. That's the COVID-19 treatment armamentarium as of January 2021.

So, the next pages will discuss many drugs that have so far shown NO effect. So why read this chapter? Because doctors need to know the state-of-the-art – even the 'state-of-the-non-art'. Doctors must know why substances have shown NO effect and why there may still be new, innovative and creative ideas; why the senior physician has been less enthusiastic about tocilizumab over the last few weeks and why the 89-year-old diabetic on Ward 1 still gets remdesivir and famotidine; and why the plasma therapy did not work in the 51-yr-old obese woman who died on Ward 2.

Hopefully, within a year or so, this chapter will be only ten pages. We only need one good drug (or, for that matter, five me-too-drugs). Only one drug that must not even be perfect but could become a game changer in this pandemic (perhaps even more so and even sooner than a vaccine) because it is good enough to prevent people from becoming seriously ill. One drug to downgrade SARS-CoV-2 to the rank of its stupid seasonal common cold siblings that nobody was really interested in in the last decades (except Christian Drosten, who would keep us all in lockdown indefinitely).

Research activity is immense. A brief look at ClinicalTrials.gov illustrates the 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). On October 14, these numbers increased to 3598, 1880 and 230. Unfortunately, many trials exclude those patients most in need: the elderly. A data query of ClinicalTrials.gov on June 8 revealed that 206/674 (31%) COVID-19 interventional trials had an upper age exclusion criterion. The median upper age exclusion was 75 years. Exclusion of older patients dramatically increases the risk of non-representative trial populations compared with their real-world counterparts (Abi Jaoude 2020).

Different therapeutic approaches are under evaluation: antiviral compounds that inhibit enzyme systems, those inhibiting the entry of SARS-CoV-2 into the cell and, finally, immune therapies, including convalescent plasma and monoclonal antibodies. Some immune modulators may enhance the immune system, others are supposed to reduce the cytokine storm and associated pulmonary damage that is seen in severe cases. In this chapter, we will discuss the most promising agents (those for which at least a bit of clinical data is available). We will not mention all compounds that may work in cell lines or that have been proposed from virtual screening models. We will also forget some.

On the following pages, the following agents will be discussed:

1.	**Inhibitors of viral RNA synthesis**	
	RdRp Inhibitors	Remdesivir, favipiravir, sofosbuvir
	Protease Inhibitors	Lopinavir/r
2.	**Other antiviral agents**	
	Various	APN1, Camostat, Umifenovir Hydroxy/chloroquine
3.	**Antibodies**	
	Monoclonal antibodies	Bamlanivimab, Etesevimab, Casirivimab/Imdevimab and others, other mAbs
	Convalescent plasma	
4.	**Immune modulators**	
	Corticosteroids	Dexamethasone, hydrocortisone
	Interferons	IFN-α2b, IFN-β
	JAK inhibitors	Baricitinib, ruxolitinib
	Cytokine blockers and anticomplement therapies	Anakinra, canakinumab, infliximab, mavrilimumab, tocilizumab, Siltuximab, sarilumab, vilobelimab
5..	**Various treatments** (with unknown or unproven mechanisms of action)	Acalabrutinib, ibrutinib, colchicine, famotidine, G-CSF, iloprost

Please enjoy reading the following pages. Most of the options are ineffective (in the end, page 374, we will make some brief recommendations).

1. Inhibitors of the viral RNA synthesis

SARS-CoV-2 is a single-stranded RNA betacoronavirus. Potential targets are some non-structural proteins such as protease, RNA-dependent RNA polymerase (RdRp) and helicase, as well as 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%, suggests that substances effective for SARS may also be effective for COVID-19.

RdRp inhibitors

Remdesivir (Veklury®)

Remdesivir (RDV) is a nucleotide analog and the prodrug of an adenosine C nucleoside which incorporates into nascent viral RNA chains, resulting in premature termination. It received an "Emergency Use Authorization" from the FDA in May and a so-called "conditional marketing" authorization from the EMA in July.

In vitro experiments have shown that remdesivir has broad anti-CoV activity by inhibiting RdRp in airway epithelial cell cultures, even at sub-micromolar concentrations. This RdRp inhibition works in rhesus macaques (Williamson 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). Resistance to remdesivir in SARS was generated in cell culture but was difficult to select and seemingly impaired viral fitness and virulence. However, there is a case report describing the occurrence of a mutation in the RdRp (D484Y) gene following failure of remdesivir (Martinot 2020). 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.

Safety was shown in the Ebola trial. In the Phase III studies on COVID-19, an initial dose of 200 mg was started on day 1, similar to the Ebola studies, followed by 100 mg for another 4-9 days. The key trials are listed here:

- Compassionate Use Program: this was a fragmentary cohort (Grein 2020) on some patients (only 53/61 patients were analyzed) with varying disease severity. Some improved, some didn't: random noise. We believe, for a number of reasons, that this case series published in the New England

Journal of Medicine is a cautionary tale for "science in a hurry", arousing false expectations. It might have been preferable to postpone the publication (Hoffmann 2020).

- NCT04257656: This multicentre RCT at ten hospitals in Hubei (Wang 2020) randomized a total of 237 patients with pneumonia, oxygen saturation of 94% or lower on room air and within 12 days of symptom onset to receive 10 days of single infusions or placebo. Clinical improvement was defined as the number of days to the point of a decline of two levels on a six-point clinical scale (from 1 = discharged to 6 = death). Patients were 65 years old (IQR 56–71), and many were co-treated with lopinavir (28%) and corticosteroids. The trial did not attain the predetermined sample size because the outbreak was brought under control in China. However, remdesivir was not associated with a difference in time to clinical improvement. Day 28 mortality was 14% versus 13%. Of note, the viral load decreased similarly in both groups. Some patients with remdesivir had dosing prematurely stopped due to adverse events (12% versus 5%, mainly gastrointestinal symptoms and increases of liver enzymes). The positive message from this trial is that time to recovery was "numerically" shorter in the remdesivir group, particularly in those treated within 10 days of symptom onset.
- SIMPLE 1: in this randomized, open-label RCT in 397 hospitalized patients with severe COVID-19 and not requiring IMV, clinical improvement at day 14 was 64% with 5 days and 54% with 10 days of remdesivir (Goldman 2020). After adjustment for (significant) baseline imbalances in disease severity, outcomes were similar. The most common adverse events were nausea (9%), worsening respiratory failure (8%), elevated ALT level (7%), and constipation (7%). Because the trial lacked a placebo control, it was not a test of efficacy for remdesivir. An expansion phase will enroll an additional 5600 (!) patients around the world.
- The second open-label SIMPLE trial, NCT04292730 (GS-US-540-5774), evaluated the efficacy of two remdesivir regimens compared to standard of care (SOC) in 584 hospitalized patients with moderate COVID-19, with respect to clinical status assessed by a 7-point ordinal scale on day 11. Clinical status distribution was significantly better for those randomized to a 5-day course of remdesivir compared with those randomized to SOC (Spinner 2020). According to the authors, however, this "difference was of uncertain clinical importance". The difference for those randomized to a 10-day course (median length of treatment, 6 days) compared with standard of care was not significant. By day 28, 9 patients had died: 2 (1%) and 3 (2%) in the 5-day and 10-day remdesivir groups, and 4 (2%) in the SOC group, respectively. Nausea (10% vs 3%), hypokalemia (6% vs 2%), and

headache (5% vs 3%) were more frequent among remdesivir-treated patients, compared with SOC.

- ACTT (Adaptive COVID-19 Treatment Trial): The conclusion of the final report for this double-blinded RCT that had randomized 1062 patients throughout the world, was remarkably short: remdesivir "was superior to placebo in shortening the time to recovery in adults who were hospitalized with COVID-19 and had evidence of lower respiratory tract infection" (Beigel 2020). Median recovery time was 10 versus 15 days. On an eight-category ordinal scale, patients who received remdesivir were more likely to improve at day 15. The benefit in recovery persisted when adjustment was made for glucocorticoid use. The Kaplan–Meier estimates of mortality were 6,7% with remdesivir and 11,9% with placebo by day 15. Serious adverse events were reported in 131 of the 532 patients who received remdesivir (24,6%) and in 163 of the 516 patients who received placebo (31,6%).
- WHO Solidarity Trial Consortium 2020: In SOLIDARITY, 11.330 adults (405 hospitals in 30 countries) were randomized, with 2750 allocated to remdesivir, 954 HCQ, 1411 lopinavir/r, 651 interferon plus lopinavir/r, 1412 only interferon, and 4088 no study drug. Kaplan-Meier 28-day mortality was 12%. No study drug definitely reduced mortality (in unventilated patients or any other subgroup of entry characteristics), initiation of ventilation or hospitalization duration. 301 of 2743 patients receiving remdesivir died as did 303 of 2708 receiving the control (WHO Solidarity 2020).

On 20 November, WHO issued a conditional recommendation against the use of remdesivir in hospitalized patients, regardless of disease severity, as there is currently no evidence that remdesivir improves survival and other outcomes in these patients (WHO Date).

What comes next? Several additional trials are ongoing, including combination therapies with other drugs such baricitinib (see below). Let's wait for the results, before we throw remdesivir out with the bathwater. According to a recent review, remdesivir (5 days) should be prioritized for hospitalized patients requiring low-flow supplemental oxygen as it appears that these patients derive the most benefit (Davis 2020). The data also support some benefit in hospitalized patients breathing ambient air (if there is adequate drug supply). Current data do NOT suggest benefit for those requiring high-flow oxygen or mechanical ventilation (non-invasive or invasive). It has become "clear that treatment with an antiviral drug alone is not likely to be sufficient for all patients" (Beigel 2020).

Of note, some new ideas on remdesivir as an inhalation therapy have been published (Contini 2020). Local instillation or aerosol in the first phase of infection, both in asymptomatic but nasopharyngeal swab positive patients, together with antiseptic-antiviral oral gargles and povidone-iodine eye drops for conjunctiva would attack the virus directly through the receptors to which it binds, significantly decreasing viral replication and the risk of severe COVID-19. Gilead is working on this (knowing that "early intravenous infusions" are not feasible).

Favipiravir

Favipiravir is another broad antiviral RdRp inhibitor that has been approved for influenza in Japan (but was never brought to market) and other countries. 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 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, followed by a maintenance dose of 1200-1800 mg QD. However, in 7 patients with severe COVID-19, the favipiravir trough concentration was much lower than that of healthy subjects in a previous clinical trial (Irie 2020). 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). Some encouraging preliminary results in 340 COVID-19 patients were reported from Wuhan and Shenzhen (Bryner 2020).

- A first open-label RCT posted on March 26 (Chen 2020) was conducted in China, comparing arbidol and favipiravir in 236 patients with pneumonia. Some improvement in the primary outcome (7-day clinical recovery rate) was found only in a subgroup). In the whole study population, no difference was seen.
- No effect of viral clearance was found in a RCT on 69 patients with asymptomatic to mild COVID-19 who were randomly assigned to early or late favipiravir therapy (same regimen starting day 1 or day 6). Viral clearance occurred within 6 days in 67% and 56%. Neither disease progression nor death occurred in any of the patients (Doi 2020).
- In the pilot stage of a Phase II/III clinical trial, 60 patients hospitalized with COVID-19 pneumonia were randomized to two different dosing groups or standard of care (Ivashchenko 2020). The proportion of patients

who achieved negative PCR on day 5 on both dosing regimens was twice as high as in the control group ($p < 0.05$).

In an RCT on 150 patients from India, the median time to the cessation of viral shedding was somewhat shorter (5 days versus 7 days) with favipiravir, compared to controls (Udwadia 2020).

Molnupiravir (MK-4482/EIDD-2801) is an orally-administered bioavailable prodrug of cytidine nucleoside analogue EIDD-1931. Originally developed for treatment of hepatitis C, some studies indicated potent activity of EIDD-1931 against SARS-CoV-2 in multiple cell types. Molnupiravir is able to mitigate SARS-CoV-2 infection and block transmission when therapeutically administered to ferrets (Cox 2020). The drug, initially developed as an inhibitor of influenza viruses, is currently in Phase II/III clinical trials (NCT04405570 and NCT04405739).

Other RdRp inhibitors: sofosbuvir, galidesivir

Some other RdRp inhibiting compounds have also been discussed. Sofosbuvir is a polymerase inhibitor which is also used as a direct-acting agent in hepatitis C. It is usually well tolerated. Modelling studies have shown that sofosbuvir could also inhibit RdRp by competing with physiological nucleotides for the RdRp active site (Elfiky 2020). Sofosbuvir could be combined with HCV PIs. The first randomized controlled trial in adult patients hospitalized with COVID-19 in Iran to evaluate the efficacy and safety of the two HCV drugs sofosbuvir and daclatasvir in combination with ribavirin (SDR) compared these drugs with standard of care (Abbaspour Kasgari 2020). Though there were trends in favor of the SDR arm for recovery and lower death rates, the trial was too small to make definite conclusions. In addition, there was an imbalance in the baseline characteristics between the arms.

Galidesivir is a nucleoside RNA polymerase inhibitor with broad-spectrum activity *in vitro* against more than 20 RNA viruses in nine different families, including coronaviruses and other viral families. A NIAID-funded, randomized, double-blind, placebo-controlled clinical trial to assess the safety, clinical impact and antiviral effects of galidesivir in patients with COVID-19 is underway. Of note, the drug also works against Zika: in the study presented here, galidesivir dosing in rhesus macaques was safe and offered post-exposure protection against Zika virus infection (Lim 2020).

Protease inhibitors (PIs)

A promising drug target is the viral main protease Mpro, which plays a key role in viral replication and transcription. Some HIV PIs have been extensively studied in COVID-19 patients.

Lopinavir

Lopinavir/r is thought to inhibit the 3-chymotrypsin-like protease of coronaviruses. To achieve appropriate plasma levels, it has to be boosted with another HIV PI called ritonavir (usually indicated by "/r": lopinavir/r). Due to some uncontrolled trials in SARS and MERS, lopinavir/r was widely used in the first months, despite the lack of any evidence. In an early retrospective study on 280 cases, early initiation of lopinavir/r and/or ribavirin showed some benefits (Wu 2020).

- The first open-label RCT in 199 adults hospitalized with severe COVID-19 did not find any clinical benefit beyond standard of care in patients receiving the drug 10 to 17 days after onset of illness (Cao 2020). There was no discernible effect on viral shedding.
- A Phase II, multi-center, open-label RCT from Hong Kong randomized 127 patients with mild-to-moderate COVID-19 (median 5 days from symptom onset) to receive lopinavir/r only or a triple combination consisting of lopinavir/r, ribavirin and interferon (Hung 2020). The results indicate that the triple combination can be beneficial when started early (see below, interferon). As there was no lopinavir/r-free control group, this trial does not prove lopinavir/r efficacy.
- After preliminary results were made public on June 29, 2020, we are now facing the full paper on the lopinavir/r arm in the RECOVERY trial: In 1616 patients admitted to hospital who were randomly allocated to receive lopinavir/r (3424 patients received usual care), lopinavir/r had no benefit. Overall, 374 (23%) patients allocated to lopinavir/r and 767 (22%) patients allocated to usual care died within 28 days. Results were consistent across all prespecified subgroups. No significant difference in time until discharge alive from hospital (median 11 days in both groups) or the proportion of patients discharged from hospital alive within 28 days was found. Although the lopinavir/r, dexamethasone, and hydroxychloroquine groups have now been stopped, the RECOVERY trial continues to study the effects of azithromycin, tocilizumab, convalescent plasma, and monoclonal antibodies.
- There was no effect in the SOLIDARITY trial of lopinavir/r (WHO Solidarity 2020)

At least two studies suggested that lopinavir/r pharmacokinetics in COVID-19 patients may differ from those seen in HIV-infected patients. In both studies, very high concentrations were observed, exceeding those in HIV-infected patients by 2-3 fold (Schoergenhofer 2020, Gregoire 2020). However, concentrations of protein-unbound lopinavir achieved by current HIV dosing is probably still too low for inhibiting SARS-CoV-2 replication. The EC_{50} for HIV is much lower than for SARS-CoV-2. It remains to be seen whether these levels will be sufficient for (earlier) treatment of mild cases or as post-exposure prophylaxis.

Other PIs

For another HIV PI, darunavir, there is no evidence from either cell experiments or clinical observations that the drug has any prophylactic effect (De Meyer 2020).

It is hoped that the recently published pharmacokinetic characterization of the crystal structure of the main protease SARS-CoV-2 may lead to the design of optimized protease inhibitors. 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 IC_{50} values ranging from 0.67 to 21.4 muM, among them two approved drugs, disulfiram and carmofur (a pyrimidine analog used as an antineoplastic agent) drugs (Jin 2020). Others are in development but still pre-clinical (Dai 2020).

2. Various antiviral agents

Most coronaviruses attach to cellular receptors via their spike (S) protein. Within a few weeks after the discovery of SARS-CoV-2, several groups elucidated the entry of the virus 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 ACE2 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 chapter *Clinical Presentation*, page 279).

Human recombinant soluble ACE2 (APN01)

HrsACE2 is a therapeutic candidate that neutralizes infection by acting as a decoy. It may act by binding the viral spike protein (thereby neutralizing

SARS-CoV-2) and by interfering with the renin–angiotensin system. APN01 has been shown to be safe and well-tolerated in a total of 89 healthy volunteers and patients with pulmonary arterial hypertension (PAH) and ARDS in previously completed Phase I and Phase II clinical trials. It is developed by APEIRON, a privately-held European biotech company based in Vienna, Austria. There is a report of an Austrian case of a 45-year-old woman with severe COVID-19 who was treated with hrsACE2. The virus disappeared rapidly from the serum and the patient became afebrile within hours (Zoufaly 2020). Several Phase II/III studies of hrsACE2 are ongoing.

Camostat (Foipan®)

In addition to binding to ACE2, 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, approved in Japan for the treatment of chronic pancreatitis (trade name Foipan®), may block the cellular entry of SARS-CoV-2 (Hoffmann 2020). Clinical data are pending. Some trials are ongoing, mostly in mild-to-moderate disease.

Umifenovir

Umifenovir (Arbidol®) is a broad-spectrum antiviral drug 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). 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 in the combination group, SARS-CoV-2 nasopharyngeal specimens became negative in 75%, compared to 35% 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. Another retrospective study on 45 patients from a non-intensive care unit in Jinyintan, China failed to show any clinical benefit (Lian 2020). There is a preliminary report of a randomized study indicating a weaker effect of umifenovir compared to favipiravir (Chen 2020).

Oseltamivir

Oseltamivir (Tamiflu®) is a neuraminidase inhibitor that is 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 immediately after the onset of symptoms may be crucial. Oseltamivir is best indicated for accompanying influenza co-infection, 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.

Hydroxychloroquine (HCQ) and chloroquine (CQ)

HCQ is an anti-inflammatory agent approved for certain autoimmune diseases and for malaria. The story of HCQ in the current pandemic is a warning example of how medicine shouldn't work. Some lab experiments, a mad French doctor, bad uncontrolled studies, many rumors and hopes, reports without any evidence and an enthusiastic tweet that this had "a real chance to be one of the biggest game changers in the history of medicine" - hundreds of thousands people received an ineffective (and potential dangerous) drug. Moreover, many turned away from clinical trials of other therapies that would have required them to give up HCQ treatments. In some countries, the HCQ frenzy prompted serious delays in trial enrolment, muddled efforts to interpret data and endangered clinical research (Ledford 2020). Some countries stockpiled CQ and HCQ, resulting in a shortage of these medications for those that need them for approved clinical indications. Only a few months later, we are now facing an overwhelming amount of data strongly arguing against any use of both HCQ and CQ. So please, let's forget it. Completely. But let us learn from the bad HQC story which should never happen again (Kim 2020, Ledford 2020).

No clinical benefit from Hydroxychloroquine (HCQ)

- In an observational study from New York City (Geleris 2020) of 1376 hospitalized patients, 811 received HCQ (60% received also azithromycin, A). After adjusting for several confounders, there was no significant association between HCQ use and intubation or death.
- Another retrospective cohort of 1438 patients from 25 hospitals in the New York metropolitan region (Rosenberg 2020), there were no significant differences in mortality for patients receiving HCQ + Azithromycin (A), HCQ alone, or A alone. Cardiac arrest was significantly more likely seen with HCQ + A (adjusted OR 2.13).
- A randomized, Phase IIb trial in Brazil on severe COVID-19 patients was terminated early (Borba 2020). By day 13, 6/40 patients (15%) in the low-dose CQ group had

died, compared with 16/41 (39%) in the high-dose group. Viral RNA was detected in 78% and 76%, respectively.

- In a study of 251 patients receiving HCQ plus A, extreme new QTc prolongation to > 500 ms, a risk marker for torsades, occurred in 23% (Chorin 2020).
- In 150 patients with mainly persistent mild to moderate COVID-19, conversion to negative PCR by day 28 was similar between HCQ and SOC (Tang 2020). Adverse events were recorded more frequently with HCQ (30% vs 9%, mainly diarrhea).
- Symptomatic, non-hospitalized adults with lab-confirmed or probable COVID-19 and high-risk exposure were randomized within 4 days of symptom onset to HCQ or placebo. Among 423 patients, change in symptom severity over 14 days did not differ. At 14 days, 24% receiving HCQ had ongoing symptoms compared with 30% receiving placebo (p = 0.21). Adverse events occurred in 43% versus 22% (Skipper 2020).
- HCQ does not work as prophylaxis. In 821 asymptomatic participants randomized to receive HCQ or placebo within 4 days of exposure, incidence of confirmed SARS-CoV-2 was 12% with CQ and 14% with placebo. Side effects were more common (40% vs 17%) (Boulware 2020).
- No, HCQ does not work as prophylaxis, even in HCW. This double-blind, placebo-controlled RCT included 132 health care workers and was terminated early. There was no significant difference in PCR-confirmed SARS-CoV-2 incidence between HCQ and placebo (Abella 2020).
- And finally, the RECOVERY Collaborative Group discovered that among 1561 hospitalized patients, those who received HCQ did not have a lower incidence of death at 28 days than the 3155 who received usual care (27% vs 25%).

3. Monoclonal Antibodies and Convalescent Plasma

The development of highly successful monoclonal antibody-based therapies for cancer and immune disorders has created a wealth of expertise and manufacturing capabilities. As long as all other therapies fail or have only modest effects, monoclonal antibodies are the hope for the near future. There is no doubt that antibodies with high and broad neutralizing capacity, many of them directed to the receptor binding domain (RBD) of SARS-CoV-2, are promising candidates for prophylactic and therapeutic treatment. On the other hand, these antibodies will have to go through all phases of clinical trial testing programs, which will take time. Safety and tolerability, in particular, is an important issue. The production of larger quantities is also likely to cause problems. Finally, there is the issue that mAbs are complex and expensive to produce, leaving people from poor countries locked out (Ledford 2020). Moroever, now that vaccines, cheaper and easier to administer, are being deployed—with priority for the most vulnerable populations—the question is what role remains for monoclonals in the first place (Cohen 2020). One potential drawback is that these antibodies could undermine the effectiveness of vaccines. According to some experts, they might be important for the

elderly and other people with compromised immune systems who do not have vigorous responses to vaccines.

However, the 'COVID-19 antibodysphere' (Amgen, AstraZeneca, Vir, Regeneron, Lilly, Adagio) is still very active, building partnerships. Several mAbs entered clinical trials in the summer of 2020. Trials include treatment of patients with SARS-CoV-2 infection with varying degrees of illness to block disease progression. Given the long half-life of most mAbs (approximately 3 weeks for IgG1), a single infusion should be sufficient. In November 2020, the FDA issued emergency use authorizations (EUA) for the investigational monoclonal antibody combination casirivimab plus imdevimab (REGN-CoV-2) and for bamlanivimab (from Lilly).

Casirivimab plus Imdevimab (REGN-COV2)

The antibodies given to Trump. Casivirimab (REGN10933) binds at the top of the RBD, extensively overlapping the binding site for ACE2, while the epitope for imdevimab (REGN10987) is located on the side of the RBD, away from the REGN10933 epitope, and has little to no overlap with the ACE2 binding site. Proof of principle was shown in in a cell model, using vesicular stomatitis virus pseudoparticles expressing the SARS-CoV-2 spike protein. Simultaneous treatment with both mAbs precluded the appearance of escape mutants (Baum 2020, Hansen 2020). Thus, this cocktail called REGN-COV2 did not rapidly select for mutants, presumably because escape would require the unlikely occurrence of simultaneous viral mutation at two distinct genetic sites, so as to ablate binding and neutralization by both antibodies in the cocktail.

On 21 November, the FDA issued an emergency use authorization for both mAbs to be administered together for the treatment of mild-to-moderate COVID-19 in patients 12 years of age or older (weighing at least 40 kilograms) and who are at high risk for progressing to severe COVID-19 (65 years of age or older or certain chronic medical conditions). Neither antibody is authorized for patients hospitalized due to COVID-19 or who require oxygen therapy due to COVID-19. Regeneron will distribute REGN-COV2 in the US and Roche is responsible for distribution outside the US.

Clinical data is still limited:

- An interim analysis of an ongoing Phase I–III trial randomly assigning 275 non-hospitalized patients to receive placebo, 2,4g or 8,0g of REGN-COV2 (Weinreich 2020). The least-squares mean difference (REGN-COV2 dose groups vs. placebo group) in the time-weighted average Δ in viral load from day 1 through day 7 was minus 0,56 $\log_{10}$ copies/mL among patients who were serum antibody–negative at baseline and minus 0,41 $\log_{10}$ cop-

ies/mL in the overall trial population. But did this translate into a clinical benefit? Maybe. At least one medical attended visit was necessary in 3% vs 6% (placebo) overall and in 6% vs 15% (placebo) in serum antibody-negative at baseline. Both doses were well-tolerated. Infusion reactions and severe adverse events were balanced across all groups, no deaths occurred.

Bamlanivimab, Etezevimab

Bamlanivimab (LY-CoV555, BAM) from Lilly is a neutralizing IgG1 monoclonal antibody (mAb) directed against the spike protein of SARS-CoV-2. On 9 November, the FDA issued an emergency use authorization (EUA) for the **treatment of mild to moderate** COVID-19 in patients who are 12 years of age and older weighing at least 40 kilograms, and who are at high risk for progressing to severe COVID-19 and/or hospitalization. These are elderly patients but also with a BMI ≥ 35, chronic kidney disease, diabetes, immunosuppressive status, cardiovascular disease and others (depending on age). Of note, BAM is **not authorized** for patients who are hospitalized due to COVID-19 or who require oxygen therapy due to COVID-19.

As with REGN-2, clinical data is still limited with BAM:

- The interim analysis of an ongoing Phase II study (BLAZE-1) in 452 patients with mild to moderate COVID-19 showed some clinical benefit (Chen P 2020). Those who received a single dose BAM (three different dosages) had fewer hospitalizations (1,6% versus 6,3%) and a lower symptom burden than those who received placebo, with the most pronounced effects observed in high-risk cohorts.
- The final data set of BLAZE-1 in 577 outpatients (Gottlieb 2020) revealed that there was no significant difference in change in viral load with 3 different doses of BAM compared with placebo. However, treatment with a combination of BAM and another mAb (etesevimab) significantly decreased SARS-CoV-2 viral load by -0.57 log at day 11 compared with placebo. Further ongoing clinical trials will focus on assessing the clinical benefit.
- In another RCT involving 314 hospitalized patients, BAM did not demonstrate efficacy among hospitalized patients who had COVID-19 without end organ failure. The trial was stopped (ACTIV 2020).

Other mAbs, some key papers:

- The first report of a human monoclonal antibody that neutralizes SARS-CoV-2 (Wang 2020). **47D11** binds a conserved epitope on the spike RBD

explaining its ability to cross-neutralize SARS-CoV and SARS-CoV-2, using a mechanism that is independent of receptor-binding inhibition. This antibody could be useful for development of antigen detection tests and serological assays targeting SARS-CoV-2.

- From 60 convalescent patients, 14 potent neutralizing antibodies were identified by high-throughput single B cell RNA-sequencing (Cao 2020). The most potent one, **BD-368-2**, exhibited an IC_{50} of 15 ng/mL against SARS-CoV-2, displaying strong therapeutic efficacy in mice. The epitope overlaps with the ACE2 binding site.
- Several mAbs from ten convalescent COVID-19 patients. The most interesting mAb, named **4A8**, exhibited high neutralization potency but did not bind the RBD (like most other mAbs). Cryo-EM revealed that the epitope of 4A8 seems to be the N terminal domain (NTD) of the S protein (Chi 2020).
- Isolation and characterization of 206 RBD-specific monoclonal antibodies derived from single B cells of eight SARS-CoV-2 infected individuals. Some antibodies showed potent anti-SARS-CoV-2 neutralization activity that correlates with their competitive capacity with ACE2 for RBD binding (Ju 2020).
- **CR3022** tightly binds the RBD and neutralizes SARS-CoV-2 (Huo 2020). The highly conserved, structure-stabilising epitope is inaccessible in the prefusion Spike, suggesting that CR3022 binding facilitates conversion to the fusion-incompetent post-fusion state. The mechanism of neutralisation is new and was not seen for coronaviruses.
- **H014** neutralizes SARS-CoV-2 and SARS-CoV pseudoviruses as well as authentic SARS-CoV-2 at nanomolar level by engaging the S receptor binding domain. In the hACE2 mouse model, H014 prevented pulmonary pathology. H014 seems to prevent attachment of SARS-CoV-2 to its host cell receptors (Lv 2020).
- Four human neutralizing monoclonal antibodies were isolated from a convalescent patient. **B38** and **H4** blocked the binding between the virus S protein RBD and the cellular receptor ACE2. A competition assay indicates their different epitopes on the RBD. In a mouse model, both antibodies reduced viral titers in infected lungs. The RBD-B38 complex structure revealed that most residues on the epitope overlap with the RBD-ACE2 binding interface, explaining the blocking effect and neutralizing capacity (Wu 2020).
- Of a total of 178 S1 and RBD binding human monoclonal antibodies from the memory B cells of 11 recently recovered patients, the best one, **414-1**,

showed neutralizing IC_{50} at 1.75 nM (Wan J 2020). Epitope mapping revealed that the antibodies bound to 3 different RBD epitopes, and epitope B antibody 553-15 could substantially enhance neutralizing abilities of most other neutralizing antibodies.

- Isolation and characterization of two ultra-potent SARS-CoV-2 human neutralizing antibodies (**S2E12** and S**2M11**) that were identified among almost 800 screened Abs isolated from 12 COVID-19 patients (Tortorici 2020). Both nAbs protected hamsters against SARS-CoV-2 challenge.
- Using a high-throughput rapid system for antibody discovery, more than 1000 mAbs were isolated from 3 convalescent donors by memory B cell selection using SARS-CoV-2 S or RBD recombinant proteins. Of note, only a small fraction was neutralizing, highlighting the value of deep mining of responses to access the most potent Abs. RBD-nAbs that directly compete with ACE2 are clearly the most preferred for prophylactic and therapeutic applications, and as reagents to define nAb epitopes for vaccine. With these nABs, Syrian hamsters were protected from weight loss. However, animals that received higher doses also showed body weight loss, possibly indicating antibody-mediated enhanced disease (Rogers 2020).
- Antibodies from convalescent patients had low levels of somatic hypermutation. Electron microscopy studies illustrate that the SARS-CoV-2 spike protein contains multiple distinct antigenic sites. In total, 19 neutralizing antibodies were identified that target a diverse range of antigenic sites on the S protein, of which two showed picomolar (very strong!) neutralizing activities (Brouwer 2020).
- Isolation of 61 SARS-CoV-2-neutralizing mAbs from 5 hospitalized patients, among which are 19 mAbs that potently neutralized the authentic SARS-CoV-2 *in vitro*, 9 of which exhibited exquisite potency, with 50% virus inhibitory concentrations of 0,7 to 9 ng/mL (Liu 2020).

Antibody fragments, nanobodies

- Antibody domains and fragments such as VH (heavy chain variable domain, 15 kDa) are attractive antibody formats for candidate therapeutics. They may have better tissue penetration compared to full-sized antibodies. One of those VHs**,** ab8, in an Fc (human IgG1, crystallizable fragment) fusion format, showed potent neutralization activity and specificity against SARS-CoV-2 both *in vitro* and in mice and hamsters, possibly enhanced by its relatively small size (Li 2020).

- An early inhalation of nanobodies - a future treatment? VHH antibodies or nanobodies (Nbs) are minimal, monomeric antigen-binding domains derived from camelid single-chain antibodies. Unlike IgG antibodies, Nbs are small, highly soluble and stable, readily bioengineered into bi/multivalent forms, and are amenable to low-cost, efficient microbial production. They can also be administered by inhalation, making their use against respiratory viruses very appealing. This study discovered several Nbs (Xiang 2020) with picomolar to femtomolar affinities that inhibit viral infection at sub-ng/ml concentration and determined a structure of one of the most potent in complex with RBD. Multivalent Nb constructs achieved ultrahigh neutralization potency and may prevent mutational escape. While the research is still preliminary, it is hoped that Nbs might someday be the key ingredient in an antiviral drug that could be easily delivered via nasal spray.
- The ultrapotent Nb6 binds Spike in a fully inactive conformation with its receptor binding domains (RBDs) locked into their inaccessible down-state, incapable of binding ACE2. Affinity maturation and structure-guided design of multi-valency yielded a trivalent nanobody, mNb6-tri, with femtomolar affinity for Spike and picomolar neutralization of SARS-CoV-2 infection (Schoof 2020).

Convalescent plasma (passive immunization)

Human convalescent plasma (CP) could be a rapidly available option for prevention and treatment of COVID-19 disease when there are sufficient numbers of people who have recovered and can donate immunoglobulin-containing serum (Casadevall 2020). Passive immune therapy appears to be relatively safe. However, an unintended consequence of receiving CP may be that recipients won't develop their own immunity, putting them at risk for re-infection. Other issues that have to be addressed in clinical practice (Kupferschmidt 2020) are plasma supply (regulatory conderations; logistical work flow may become a challenge) and rare but relevant risks (transfusion-related acute lung injury, in which transferred antibodies damage pulmonary blood vessels, or transfusion-associated circulatory overload). Fortunately, antibodies that are found in CP are very stable. Pathogen inactivation (using psoralen and UV light) did not impair the stability and neutralizing capacity of SARS-CoV-2-specific antibodies that was also preserved at 100% when the plasma was shock frozen at –30°C after pathogen-inactivation or stored as liquid plasma for up to 9 days (Tonn 2020).

The major caveat of CP is consistency (concentration differs). In plasma from 149 patients collected on average 39 days after the onset of symptoms, neu-

tralizing titers were extremely variable. Most plasmas did not contain high levels of neutralizing activity (Robbiani 2020). Pre-screening of CP may be necessary for selecting donors with high levels of neutralizing activity for infusion into patients with COVID-19 (Bradfute 2020). There seems to be a correlation between serum neutralizing capacity and disease severity, suggesting that the collection of CP should be restricted to those with moderate to severe symptoms (Chen 2020). Others have suggested more detailed selection criteria: 28 days after the onset of symptoms with a disease presentation of fever lasting longer than 3 days or a body temperature exceeding 38,5°C. Selection based on these criteria can ensure a high likelihood of achieving sufficiently high titers (Li 2020).

On March 26, the FDA approved the use of plasma from recovered patients to treat people who are critically ill with COVID-19 (Tanne 2020). This was a remarkable decision, because at that time data was still scarce. Now, there is growing evidence that high titer CP may have some benefit:

- The first RCT was published in June (Li 2020). Unfortunately, the study was terminated prematurely (no more patients could be recruited in China) and underpowered. Of 103 patients who were randomized, clinical improvement (on a 6-point disease severity scale) occurred within 28 days in 52% vs 43%. There was no significant difference in 28-day mortality (16% vs 24%). Of note, CP treatment was associated with a negative conversion rate of viral PCR at 72 hours in 87% of the CP group versus 38% (OR, 11,39). Main take-homes: CP is not a silver bullet and antiviral efficacy does not necessarily lead to better survival.
- The second RCT came from India (Agarwal 2020). This open-label RCT investigated the effectiveness of CP in adults with moderate COVID-19, assigning 235 patients to two doses of 200 mL CP and 229 patients to a control arm. Progression to severe disease or all-cause mortality at 28 days occurred in 44 (19%) and 41 (18%). Moreover, CP treatment did not show anti-inflammatory properties and there was no difference between patients with and without neutralizing antibodies at baseline. The main limitation was that the antibody titers in CP before transfusion were not measured because validated, reliable commercial tests were not available when the trial started.
- Another RCT on 338 hospitalized adult patients with severe COVID-19 pneumonia from Argentina did not find any difference in clinical status or overall mortality or in prespecified subgroups (Simonovich 2020).
- So it may depend on the patients – and on the level of antibody titers. Among 3082 patients hospitalized with COVID-19, the efficacy was moder-

ated by mechanical ventilation status (Joyner 2020). In patients who were not receiving mechanical ventilation, transfusion of plasma with higher antibody levels was associated with a lower risk of death than transfusion of CP with lower antibody levels.

- In an RCT on early administration of high titer CP to 160 mildly ill older adults, severe respiratory disease developed in 16% who received CP and in 31% who received placebo (Lipster 2020).
- CP may be also very helpful in patients with humoral deficiency induced by anti-CD20 monoclonal antibodies such as rituximab. In 17 consecutive patients with profound B cell lymphopenia and prolonged COVID-19 symptoms, all but one patient experienced an improvement of clinical symptoms within 2 days.

4. Immunomodulators

While antiviral drugs are most likely to prevent mild COVID-19 cases from becoming severe, adjuvant strategies will be needed, particularly 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. 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 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. Numerous 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. Other immunomodulatory and other approaches in clinical testing include bevacizumab, brilacidin, cyclosporin, fedratinib, fingolimod, lenadilomide and thalidomide, sildenafil, teicoplanin and many more. However, convincing clinical data is pending for most strategies.

Corticosteroids

Corticosteroids are thus far the only drugs which provide a survival benefit in patients with severe COVID-19. During the first months of the pandemic, according to current WHO guidelines, steroids were controversially discussed and were not recommended outside clinical trials. With a press release on June 16, 2020 reporting the results of the UK-based RECOVERY trial, the treatment of COVID-19 underwent a major change. In the dexamethasone group, the incidence of death was lower than that in the usual care group among patients receiving invasive mechanical ventilation. The RECOVERY results had a huge impact on other RCTs around the world. The therapeutic value of corticosteroids has now been shown in numerous studies:

- RECOVERY: In this open-label trial (comparing a range of treatments), hospitalized patients were randomized to receive oral or intravenous dexa (at a dose of 6 mg once daily) for up to 10 days or to receive usual care alone. Overall, 482 patients (22,9%) in the dexa group and 1110 patients (25,7%) in the usual care group died within 28 days (age-adjusted rate ratio, 0,83). The death rate was lower among patients receiving invasive mechanical ventilation (29,3% vs. 41,4%) and among those receiving oxygen without invasive mechanical ventilation (23,3% vs. 26,2%) but not among those who were receiving no respiratory support (17,8% vs. 14,0%).
- REMAP-CAP (different countries): In this Bayesian RCT, 384 patients were randomized to fixed-dose (n = 137), shock-dependent (n = 146), and no (n = 101) hydrocortisone. Treatment with a 7-day fixed-dose course or shock-dependent dosing of hydrocortisone, compared with no hydrocortisone, resulted in 93% and 80% probabilities of superiority, respectively, with regard to the odds of improvement in organ support free days within 21 days. However, due to the premature halt of the trial, no treatment strategy met pre-specified criteria for statistical superiority, precluding definitive conclusions.
- CoDEX (Brazil). A multicenter, open-label RCT in 299 COVID-19 patients (350 planned) with moderate-to-severe ARDS (Tomazini 2020). Twenty mg of dexamethasone intravenously daily for 5 days, 10 mg of dexamethasone daily for 5 days or until ICU discharge, plus standard of care (n = 151) or standard of care alone (n = 148). Patients randomized to the dexamethasone group had a mean 6,6 ventilator-free days during the first 28 days vs 4,0 ventilator-free days in the standard of care group (difference, 2,26; 95% CI, 0,2-4,38; p = 0,04). There was no significant difference in the prespecified secondary outcomes of all-cause mortality at 28 days, ICU-free

days during the first 28 days, mechanical ventilation duration at 28 days, or the 6-point ordinal scale at 15 days.

- CAPE COD: Multicenter double-blinded RCT, in 149 (290 planned) critically-ill patients admitted to the intensive care unit (ICU) for COVID-19–related acute respiratory failure (Dequin 2020). The primary outcome, treatment failure on day 21, occurred in 32 of 76 patients (42,1%) in the hydrocortisone group compared with 37 of 73 (50,7%) in the placebo group (p = 0,29).
- A prospective WHO meta-analysis that pooled data from 7 randomized clinical trials that evaluated the efficacy of corticosteroids in 1703 critically ill patients with COVID-19. The fixed-effect summary odds ratios for the association with mortality were 0,64 (95% CI: 0.50-0.82; p < 0,001) for dexamethasone compared with usual care or placebo, 0,69 (95% CI: 0,43-1,12; p = 0,13) for hydrocortisone and 0.91 (95% CI: 0,29-2.87; p = 0,87) for methylprednisolone, respectively. There was no suggestion of an increased risk of serious adverse events.
- Another study with 206 patients suggested that the effect of corticosteroids on viral shedding may be in a dose-response manner. High-dose (80 mg/d) but not low-dose corticosteroids (40 mg/d) delayed viral shedding of patients with COVID-19 (Li 2020).
- Treatments for respiratory disease, specifically inhaled corticosteroids (ICSs) do not have a protective effect. In 148,557 persons with COPD and 818,490 persons with asthma who were given relevant respiratory medications in the 4 months before the index date (March 1), people with COPD who were prescribed ICSs were at increased risk of COVID-19-related death compared with those prescribed LABA–LAMA combinations (adjusted HR 1,39) (Schultze 2020). Compared with those prescribed short acting beta agonists only, people with asthma who were prescribed high-dose ICS were at an increased risk of death (1,55, 1,10-2,18]), whereas those given a low or medium dose were not. Sensitivity analyses showed that the apparent harmful association could be explained by relatively small health differences between people prescribed ICS and those not prescribed ICS.

Conclusions: WHO suggests NOT to use corticosteroids in the treatment of patients with non-severe COVID-19. The WHO recommends systemic corticosteroids for the treatment of patients with severe and critical COVID-19 (strong recommendation, based on moderate certainty evidence). However, the WHO panel noted that the oxygen saturation threshold of 90% to define severe COVID-19 was arbitrary and should be interpreted cautiously when used for determining which patients should be offered systemic corticoster-

oids. For example, clinicians must use their judgement to determine whether a low oxygen saturation is a sign of severity or is normal for a given patient suffering from chronic lung disease. Similarly, a saturation above 90–94% on room air may be abnormal if the clinician suspects that this number is on a downward trend.

Interferons

The interferon (IFN) response constitutes the major first line of defense against viruses. This complex host defense strategy can, with accurate understanding of its biology, be translated into safe and effective antiviral therapies. In a recent comprehensive review, the recent progress in our understanding of both type I and type III IFN-mediated innate antiviral responses against human coronaviruses is described (Park 2020).

IFN may work on COVID-19 when given early. Several clinical trials are currently evaluating synthetic interferons given before or soon after infection, in order to tame the virus before it causes serious disease (brief overview: Wadman 2020). *In vitro* observations shed light on antiviral activity of IFN-β1a against SARS-CoV-2 when administered after the infection of cells, highlighting its possible efficacy in an early therapeutic setting (Clementi 2020). In patients with coronaviruses such as MERS, however, 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).

- A Phase II, multicentre, open-label RCT from Hong Kong randomized 127 patients with mild-to-moderate COVID-19 (median 5 days from symptom onset) to receive lopinavir/r only or a triple combination consisting of lopinavir/r, ribavirin and interferon (Hung 2020). This trial indicates that the triple combination can be beneficial when started early. Combination therapy was given only in patients with less than 7 days from symptom onset and consisted of lopinavir/r, ribavirin (400 mg BID), and interferon beta-1b (1-3 doses of 8 Mio IE per week). Combination therapy led to a significantly shorter median time to negative results in nasopharyngeal swab (7 versus 12 days, p = 0,001) and other specimens. Clinical improvement was significantly better, with a shorter time to complete alleviation of symptoms and a shorter hospital stay. Of note, all differences were driven by the 76 patients who started treatment less than 7 days after onset of symptoms. In these patients, it seems that interferon made the difference. Up to now, this is the only larger RCT showing a virological response of a specific drug regimen.

- A retrospective multicenter cohort study of 446 COVID-19 patients, taking "advantage of drug stock disparities" between two medical centers in Hubei. Early administration ≤ 5 days after admission of IFN-α2b was associated with reduced in-hospital mortality in comparison with no admission of IFN-α2b, whereas late administration of IFN-α2b was associated with increased mortality (Wang 2020).
- In the WHO Solidarity Trial conducted at 405 hospitals in 30 countries, 2063 were randomly assigned to interferon (including 651 to interferon plus lopinavir), and 4088 to no trial drug. There was no efficacy of subcutaneous interferon alone or with lopinavir/r (WHO Solidarity).
- SNG001 is a formulation of recombinant interferon beta for inhaled delivery by nebulizer that is in development for the treatment of virus-induced lower respiratory tract illnesses. In this pilot trial, patients randomly assigned to SNG001 (n = 48) had greater odds of improvement versus placebo and more rapid recovery (Monk 2020). This corroborates findings from *in vitro* studies and animal models showing that the interferon pathway is crucial.

JAK inhibitors

Several inflammatory cytokines that correlate with adverse clinical outcomes in COVID-19 employ a distinct intracellular signalling pathway mediated by Janus kinases (JAKs). JAK-STAT signalling may be an excellent therapeutic target (Luo 2020).

Baricitinib (Olumiant®) is a 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. In rhesus macaques, viral shedding measured from nasal and throat swabs, bronchoalveolar lavages and tissues was NOT reduced with baricitinib. However, animals treated with baricitinib showed reduced inflammation (Hoang 2020).

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 to the fact that baricitinib causes lymphocytopenia, neutropenia and viral reactivation (Praveen 2020) as well as pancreatitis (Cerda-Contreras 2020). There is also a dose-dependent association with arterial and venous thromboembolic events (Jorgensen 2020). It is possible that

the pro-thrombotic tendencies could exacerbate a hypercoagulable state, underscoring the importance of restricting the use of baricitinib to clinical trials.

On December 28, 2020, baricitinib has been granted an FDA Emergency Use Authorization (EUA) for treatment of confirmed or suspected COVID-19 in hospitalized patients ≥ 2 years old who require supplemental oxygen, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO); the EUA requires that baricitinib be used in combination with remdesivir. The EUA is based on these findings:

- In a large RCT of 1033 hospitalized adults with COVID-19, baricitinib plus remdesivir was superior to remdesivir alone in reducing recovery time and accelerating improvement in clinical status among those receiving high-flow oxygen or non-invasive ventilation. The 216 patients receiving high-flow oxygen or non-invasive ventilation at enrollment had a time to recovery of 10 days with combination treatment (n = 103) and 18 days with control (n = 103). The 28-day mortality was 5,1% and 7,8%, respectively.
- One observational study provides some evidence for a synergistic effect of baricitinib and corticosteroids (Rodriguez-Garcia 2020). Patients with moderate to severe SARS-CoV-2 pneumonia received lopinavir/r and HCQ plus either corticosteroids (controls, n = 50) or corticosteroids and baricitinib (n = 62). In the controls, a higher proportion of patients required supplemental oxygen both at discharge (62% vs 26%) and 1 month later (28% vs 13%).

Ruxolitinib (Jakavi®) is a JAK inhibitor manufactured by Incyte. It is used for myelofibrosis, polycythemia vera (PCV) and certain chronic graft versus host diseases in patients following a bone marrow transplant. As many of the elevated cytokines signal through Janus kinase (JAK)1/JAK2, inhibition of these pathways with ruxolitinib has the potential to mitigate the COVID-19-associated cytokine storm and reduce mortality.

- A small placebo-controlled Phase II RCT on 43 patients with severe COVID-19, ruxolitinib was not associated with significantly accelerated clinical improvement, although ruxolitinib recipients had a numerically faster clinical improvement (Cao 2020).
- In a retrospective study, 12/14 patients achieved significant reduction of the "COVID-19 Inflammation Score" with sustained clinical improvement in 11/14 patients (La Rosée 2020). Treatment was safe with some signals of efficacy to prevent or overcome multi-organ failure. A Phase II RCT has been initiated (NCT04338958).

- Another non-randomized study suggested a clinical benefit from ruxolitinib in 32 patients with severe COVID-19 pneumonia, compared to a control group (D'Alessio).

Cytokine blockers and anticomplement therapies

The hypothesis that quelling the cytokine storm with anti-inflammatory therapies directed at reducing interleukin-6 (IL-6), IL-1, or even tumor necrosis factor TNF alpha might be beneficial has led to several ongoing trials. It is suggestive that interleukin blocking strategies might improve the hyperinflammatory state seen in severe COVID-19. A recent review on this strategy, however, was less enthusiastic and urged caution (Remy 2020). Past attempts to block the cytokine storm associated with other microbial infections and with sepsis have not been successful and, in some cases, have worsened outcomes. Moreover, there is concern that suppressing the innate and adaptive immune system to address increased cytokine concentrations, could enable unfettered viral replication, suppress adaptive immunity, and delay recovery processes. There is growing recognition that potent immunosuppressive mechanisms are also prevalent in such patients. Following, we will briefly discuss the evidence on cytokine blockers.

Anakinra (Kineret®) is an FDA-approved treatment for rheumatoid arthritis and neonatal onset multisystem inflammatory disease. It is a recombinant human IL-1 receptor antagonist that prevents the binding of IL-1 and blocks signal transduction. Anakinra is thought to abrogate the dysfunctional immune response in hyperinflammatory COVID-19 and is currently being investigated in almost 20 clinical trials. Some case series have reported on encouraging results and anakinra is considered to be included as an option in the RECOVERY trial.

- A study from Paris, comparing 52 "consecutive" patients treated with anakinra with 44 historical patients. Admission to the ICU for invasive mechanical ventilation or death occurred in 25% of patients in the anakinra group and 73% of patients in the historical group. The treatment effect of anakinra remained significant in the multivariate analysis (Hayem 2020). According to the authors, their study was "not perfect from a statistical point of view..."
- Of 120 patients with hyperinflammation (33% on mechanical ventilation), 65 were treated with anakinra and methylprednisolone and 55 were untreated historical controls. At 28 days, mortality was 14% in treated patients and 36% in controls (p = 0,005). Unadjusted and adjusted risk of

death was significantly lower for treated patients com-pared to controls (Bozzi 2020).

- An RCT from France, however, was stopped early following the recommendation of the data and safety monitoring board, after the recruitment of 116 patients: anakinra did not improve outcomes in patients with mild-to-moderate COVID-19 pneumonia (CORIMUNO 2020). Some experts still argue that a true test of anakinra would be in patients with more severe COVID-19, or with evidence of IL-1-mediated hyperinflammation (Cavalli 2020).

Canakinumab (Illaris®) is human monoclonal antibody against IL-1β, approved for the treatment of juvenile rheumatoid arthritis and other chronic autoinflammatory syndromes. In a pilot trial, 10 patients with hyperinflammation (defined as CRP ≥ 50 mg/L) and respiratory failure showed a rapid improvement in serum inflammatory biomarkers and an improvement in oxygenation (Ucciferri 2020). There are other uncontrolled studies on 83 patients (Landi 2020) and on 17 patients (Katia 2020), suggesting clinical benefits. However, RCTs are pending.

Infliximab (Remicade®) is a chimeric monoclonal anti-TNF antibody, approved to treat a number of autoimmune diseases, including Crohn's disease, ulcerative colitis, rheumatoid arthritis and psoriasis. As a major component of deteriorating lung function in patients with COVID-19 is capillary leak, a result of inflammation driven by key inflammatory cytokines such as TNF, making TNF-blocking agents an attractive strategy (Robinson 2020). Administration of anti-TNF to patients for treatment of autoimmune disease leads to reductions in all of these key inflammatory cytokines. A small case series of seven patients who were treated with a single infusion of IFX (5 mg/kg body weight) has been reported (Stallmach 2020).

Mavrilimumab is an anti-granulocyte–macrophage colony-stimulating factor (GM-CSF) receptor-α monoclonal antibody. GM-CSF is an immunoregulatory cytokine with a pivotal role in initiation and perpetuation of inflammatory diseases (Mehta 2020). In small uncontrolled pilot trial on 13 patients, mavrilimumab treatment was associated with improved clinical outcomes compared with standard of care in non-mechanically ventilated patients with severe COVID-19 pneumonia and systemic hyperinflammation. Treatment was well tolerated (De Luca 2020).

Tocilizumab (TCZ, RoActemra® or Actemra®) is a monoclonal antibody that targets the interleukin-6 receptor. It is used for rheumatic arthritis and has a good safety profile. The initial dose should be 4-8 mg/kg, with the recommended dosage being 400 mg (infusion over more than 1 hour). Of note, the

current level of evidence supporting the use of TCZ is weak, and many guidelines recommend against the use of TCZ except in the context of a clinical trial. TCZ continues to be tested in the RECOVERY trial while results are still pending.

- A large multicenter cohort included 3924 critically ill patients admitted to ICU at 68 hospitals across the US (Gupta 2020). The risk of in-hospital death was lower with TCZ (29% versus 41%). However, TCZ patients were younger and had fewer comorbidities. According to the authors, the findings "may be susceptible to unmeasured confounding, and further research from randomized clinical trials is needed".
- COVACTA: On July 29, Hoffmann-La Roche announced disappointing results from its much-anticipated Phase III COVACTA trial. TCZ did not improve patient mortality, although patients spent roughly a week less in hospital compared with those given placebo. However, it may be too early to quit this strategy (Furlow 2020). Cautious interpretation of COVACTA is needed, in view of the study's broad patient selection criteria.
- EMPACTA: In 249 hospitalized patients with COVID-19 pneumonia who were not receiving mechanical ventilation, tocilizumab did not improve survival, but it reduced the likelihood of progression to the composite outcome of mechanical ventilation or death. Death from any cause by day 28 occurred in 10,4% of the patients in the tocilizumab group and 8,6% of those in the placebo group (Salama 2020).
- CLORIMUNO: In this RCT that included 130 patients with moderate-to-severe pneumonia, tocilizumab did not reduce the WHO Scale scores at day 4. The proportion of patients with non-invasive ventilation, intubation, or death at day 14 was 36% with usual care and 24% with tocilizumab. No difference in mortality over 28 days was found (Hermine 2020).
- BACC Bay Trial: In this double-blind, placebo-controlled RCT in 243 moderately ill hospitalized patients (BACC Bay Trial), TCZ was not effective for preventing intubation or death (Stone 2020).
- In an open label RCT in 126 patients hospitalized with COVID-19 pneumonia, the rate of the primary clinical endpoint (clinical worsening) was not significantly different between the control group and the TCZ group (Salvarani 2020). The proportion of patients discharged within 14 and 30 days was the same.
- An open-label RCT from Brazil (Veiga 2020) among patients who were receiving supplemental oxygen or mechanical ventilation was stopped early, after 129 patients had been enrolled, because of an increased number of

deaths at 15 days in the TCZ group, compared to standard of care (17% vs 3%).

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. 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).

Sarilumab (Kevzara®) is another recombinant human IL-6 receptor antagonist. An open-label study of sarilumab in severe COVID-19 pneumonia with hyperinflammation. Sarilumab 400 mg was administered intravenously in addition to standard of care to 28 patients and results were compared with 28 contemporary matched patients treated with standard of care alone. At day 28, 61% of patients treated with sarilumab experienced clinical improvement and 7% died. These findings were not significantly different from the comparison group. However, sarilumab was associated with faster recovery in a subset of patients showing minor lung consolidation at baseline (Della-Torre 2020).

Vilobelimab is an anaphylatoxin and complement protein C5a blocking monoclonal antibody. In an open-label, randomized Phase II trial (part of the PANAMO trial), 30 patients with severe COVID-19 were randomly assigned 1:1 to receive vilobelimab (up to seven doses of 800 mg intravenously) or best supportive care only (control group). At day 5 after randomization, the primary endpoint of mean relative change in the ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air (PaO2/FiO2) was not significantly different between groups. Kaplan-Meier estimates of mortality by 28 days were 13% (95% CI 0–31) for the vilobelimab group and 27% (4–49) for the control group. The frequency of serious adverse events was similar between groups and no deaths were considered related to treatment assignment. According to the authors, the secondary outcome results support the investigation of vilobelimab in a Phase III trial using 28-day mortality as the primary endpoint. Pharmacokinetic and pharmacodynamic data, including C5a, have not yet been published (Campbell 2020). Investigators using the other C5 complement pathway inhibitors eculizumab and ravulizumab have significantly increased their dose and dosing frequency in

the acute setting of COVID-19 compared with the doses approved for use in atypical hemolytic uremic syndrome.

Other treatments for COVID-19 (with unknown or unproven mechanisms of action)

Acalabrutinib and ibrutinib

Acalabrutinib and ibrutinib are bruton tyrosine kinase inhibitors, used for CLL and lymphoma treatment. *Ex vivo* analysis revealed significantly elevated BTK activity (BTK regulates macrophage signalling and activation), as evidenced by autophosphorylation, and increased IL-6 production in blood monocytes from patients with severe COVID-19 compared with blood monocytes from healthy volunteers. In a pilot study, 19 patients with severe COVID-19 received the BTK inhibitor acalabrutinib (Roschewski 2020). Within 10-14 days, oxygenation improved "in a majority of patients", often within 1-3 days, and inflammation markers and lymphopenia normalized quickly in most patients. At the end of acalabrutinib treatment, 8/11 (72.7%) patients in the supplemental oxygen cohort had been discharged on room air. These results suggest that targeting excessive host inflammation with a BTK inhibitor can be a therapeutic strategy. A confirmatory RCT is underway. Some reports have speculated about a protective effect of ibrutinib, another BTK inhibitor (Thibaud 2020).

ASS/Aspirin

Aspirin may help (a little bit). In a retrospective, observational cohort study of 412 adult patients admitted with COVID-19 to multiple US hospitals between March and July, 98 (24%) received aspirin within 24 hours of admission or 7 days prior to admission. Aspirin use had a crude association with less mechanical ventilation (36% vs. 48%, p = 0,03) and ICU admission (39% vs. 51%, p = 0,04), but no crude association with in-hospital mortality (26% vs. 23%, p = 0,51). After adjusting for 8 confounding variables, aspirin use was independently associated with decreased risk of mechanical ventilation (adjusted HR 0,56, 95% CI: 0,37-0,85, p = 0,007), ICU admission (adjusted HR 0,57, 95% CI: 0,38-0,85, p = 0,005), and in-hospital mortality (adjusted HR 0,53, 95% CI: 0,31-0,90, p = 0,02). According to the authors, a sufficiently powered randomized controlled trial is needed. The RECOVERY trial includes ASS as an option.

Colchicine

Colchicine is one of the oldest known drugs which has been used for over 2000 years as a remedy for acute gout flares. Given its anti-inflammatory and anti-viral properties, it is also being tested in COVID-19 patients. In a prospective, open-label RCT from Greece, 105 hospitalized patients were randomized to either standard of care (SOC) or colchicine plus SOC (Deftereos 2020). Participants who received colchicine had statistically "significantly improved time to clinical deterioration". However, there were no significant differences in biomarkers and the observed difference was based on a narrow margin of clinical significance; according to the authors their observations "should be considered hypothesis generating" and "be interpreted with caution". In a retrospective cohort there was some evidence on clinical benefit (Brunetti 2020). Colchicine has been included as an early immunomodulation therapy in the RECOVERY trial.

Famotidine

Famotidine is a histamine-2 receptor antagonist that suppresses gastric acid production. It has an excellent safety profile. Initially it was thought to inhibit the 3-chymotrypsin-like protease (3CLpro), but it seems to act rather as an immune modulator, via its antagonism or inverse-agonism of histamine signaling. While results of the randomized clinical trial on the benefits of intravenous famotidine in treating COVID-19 (NCT04370262) are eagerly awaited, we can only speculate on the potential mechanisms of action of this drug (Singh 2020).

- In a retrospective study on 1620 patients, 84 (5.1%) received different doses of famotidine within 24 hours of hospital admission (Freedberg 2020). After adjusting for baseline patient characteristics, use of famotidine remained independently associated with risk for death or intubation (adjusted hazard ratio 0.42, 95% CI 0.21-0.85) and this remained unchanged after careful propensity score matching to further balance the covariables. Of note, there was no protective effect of PPIs. Plasma ferritin values during hospitalization were lower with famotidine, indicating that the drug blocks viral replication and reduces the cytokine storm.
- A second propensity-matched observational study included 878 consecutive COVID-19-positive patients admitted to Hartford hospital, a tertiary care hospital in Connecticut, USA (Mather 2020). In total, 83 (9.5%) patients received famotidine. These patients were somewhat younger (63.5 vs 67.5 years) but did not differ with respect to baseline demographics or pre-existing comorbidities. Use of famotidine was associated with a de-

creased risk of in-hospital mortality (odds ratio 0.37, 95% CI 0.16-0.86) and combined death or intubation (odds ratio 0.47, 95% CI 0.23-0.96). Patients receiving famotidine displayed lower levels of serum markers for severe disease including CRP, procalcitonin and ferritin levels. Logistic regression analysis demonstrated that famotidine was an independent predictor of both lower mortality and combined death/intubation.

Fluvoxamine

Fluvoxamine (a potent agonist of the sigma-1 receptor (σ1R)), is an antidepressant which functions pharmacologically as a selective serotonin reuptake inhibitor. In a small RCT that included 152 adult outpatients with COVID-19 and symptom onset within 7 days, clinical deterioration occurred in 0 patients treated with fluvoxamine vs 6 (8,3%) patients treated with placebo over 15 days (Lenze 2020). The authors acknowledge the limitations of their study: a small number of endpoint events, which makes the findings fragile. The potential advantages of fluvoxamine for outpatient treatment of COVID-19 would include its safety, widespread availability, low cost, and oral administration. Note that fluvoxamine can cause drug-drug interactions. Eagerly awaiting data from larger trials.

G-CSF

G-CSF may be helpful in some patients (Cheng 2020). In an open-label trial at 3 Chinese centers, 200 patients with lymphopenia and no comorbidities were randomized to standard of care or to 3 doses of recombinant human G-CSF (5 µg/kg, subcutaneously at days 0-2). Time to clinical improvement was similar between groups. However, the proportion of patients progressing to ARDS, sepsis, or septic shock was lower in the rhG-CSF group (2% vs 15%). Mortality was also lower (2% vs 10%).

Iloprost

Iloprost is a prostacyclin receptor agonist that promotes vasodilation of circulatory beds with minimal impact on hemodynamic parameters. It is licensed for the treatment of pulmonary arterial hypertension and is widely used for the management of peripheral vascular disease and digital vasculopathy, including digital ulcers and critical digital ischemia in systemic sclerosis. There is a case series of three morbidly obese patients with severe COVID-19 and systemic microvasculopathy who obviously benefitted from its use (Moezinia 2020).

Ivermectin

Ivermectin, an inexpensive, over-the-counter medicine, is widely used as a preventative against COVID-19 in many South & Latin American countries. However, the evidence that ivermectin protects from COVID-19 is scant. This group from Bangladesh conducted a randomized, double-blind, placebo-controlled trial of oral ivermectin alone or in combination with doxycycline compared with placebo among 72 hospitalized patients. Virological clearance was earlier in the 5-day ivermectin treatment arm vs the placebo group (9,7 days vs 12,7 days; p = 0,02); but not in the ivermectin + doxycycline arm (11,5 days; p = 0,27). There were no severe adverse drug events recorded in the study. According to the authors, "larger trials will be needed to confirm these preliminary findings".

Other treatments with no effects

Azithromycin

Azithromycin as a macrolide antibiotic has probably no effect against SARS-CoV-2 (see the many studies above, testing it in combination with HCQ). In a large RCT conducted at 57 centers in Brazil, 214 patients who needed oxygen supplementation of more than 4 L/min flow, high-flow nasal cannula, or mechanical ventilation (non-invasive or invasive) were assigned to the azithromycin group and 183 to the control group. Azithromycin had no effect (Furtado 2020).

Leflunomide

Leflunomide (Arava®) is an approved antagonist of dihydroorotate dehydrogenase, has some antiviral and anti-inflammatory effects and has been widely used to treat patients with autoimmune diseases. In a small RCT from Wuhan on 50 COVID-19 patients with prolonged PCR positivity, no benefit in terms of the duration of viral shedding was observed with the combined treatment of leflunomide and IFN α-2a vs IFN α-2a alone (Wang 2020).

N-acetylcysteine

N-acetylcysteine had no effect, even at high-doses (De Alencar 2020). In an RCT from Brazil of 135 patients with severe COVID-19, 16 patients (24%) in the placebo group were submitted to endotracheal intubation and mechanical ventilation, compared to 14 patients (21%) in the NAC group (p = 0.675). No difference was observed on secondary endpoints.

Outlook and Recommendations

It is hoped that at least some of the options given in this overview will show positive results over time. It is also important, though, that despite the immense pressure, the basic principles of drug development and research including repurposing are not abandoned. Time is needed.

The aim of the COVID Reference textbook is to scan the literature, not to write guidelines. However, after reviewing the studies published until January 20, 2021, presented above, we would recommend reviewing the following treatment options, considering the severity of the disease:

Outpatient, mild-to-moderate (no risk factors)

- Do NOTHING, except down-talking the patient. And make sure that he or she (and their households) stays home

Outpatient, mild-to-moderate (with risk factors)

- Do NOT use dexamethasone (could be harmful) or remdesivir (daily infusions not feasible)
- Do NOT use hydroxychloroquine, chloroquine, tocilizumab, convalescent plasma or lopinavir (not efficient, plus side effects)
- Consider ASS, colchicine, vitamin D, famotidine (potential harm seems to be limited)
- In high risk patients: consider monoclonal antibodies
- Interferon may work, if given early (optimal usage and administration is unclear)

Hospital, severe

- Use dexamethasone (only a few days)
- Use remdesivir (5 days) as soon as possible (no benefit in those requiring high-flow oxygen or mechanical ventilation)
- Consider cytokine blocking agents and/or baricitinib if available

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11. Severe COVID-19

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Last revised: 1 November 2020

Definition and classification

In a small percentage of patients, COVID-19 will take a severe course. As seen in the chapter *Clinical Presentation* (page 298), there was no broadly accepted clinical definition for severe COVID-19 at the beginning of the pandemic. According to a brief and practical definition by IDSA (Infectious Diseases Society of America), severe COVID is defined by "SpO2 ≤94% on room air, including patients on supplemental oxygen" and critical COVID is "mechanical ventilation and ECMO" (Bhimraj). In this chapter, all symptomatic cases not mild or moderate (i.e. severe and critical) are termed as severe.

Features, course and outcome

The course of the disease as well as the outcome have changed during the pandemic. Most studies refer to the early pandemic phase and cover severely affected regions and countries. The results vary greatly from country to country and depend on the timing as well. The first data from China revealed shocking numbers at a moment when local epidemics were taking off in Europe (Wu 2020). The spectrum of disease was classified as mild in 81% of cases. In total, 14% were classified as severe, and 5% were critical cases. The case-fatality rate was 14.8% in patients aged ≥ 80 years and 8.0% in patients aged 70-79 years. In a large single-center case study on 344 severe and critically ill patients admitted to Tongji hospital in **China** from January 25 through February 25, 2020, 133 (38.7%) patients died at a median of 15 days (Wang 2020). Besides older age, hypertension and COPD were more common in non-survivors but not diabetes. No difference was seen between patients with or without ACE inhibitors.

An observational study on 10,021 adult patients with a confirmed COVID-19 diagnosis, who were admitted to 920 hospitals in **Germany** between 26 February and 19 April 2020 revealed a huge death toll. The median age was 72 years and 1,727 patients (17%) needed mechanical ventilation. Patients on mechanical ventilation had more co-morbidities than patients without mechanical ventilation. Morbidity and mortality were particularly high in older patients, with a considerably lower mortality among patients younger than 60 years

(Karagiannidis 2020). Mortality was 52% (906/1,727) in patients being mechanically ventilated, with lower rates reaching 63% of patients aged 70–79 years and 72% of patients aged 80 years and older (Table 1). In-hospital mortality in ventilated patients who were also treated with dialysis was particularly high at 73% (342 of 469), and 71% (84 of 119) of patients on extracorporeal membrane oxygenation (ECMO) died.

Table 1. In house mortality in patients with/without ventilation, percentage of absolute numbers

	Without ventilation	With ventilation (all types)
18-59 years	0.7% (n = 2474)	27.7% (n = 422)
60-69 years	5.4% (n = 1239)	45.5% (n = 382)
70-79 years	14.6% (n = 1623)	62.6% (n = 535)
≥ 80 years	33.8% (n = 2958)	72.2% (n = 388)

High mortality rates were also seen in other countries. During the early phase of the pandemic, chances of surviving an ICU stay in **Lombardia, Italy**, were only 50% (Grasselli 2020). In a large cohort study of 3988 critically ill patients, most required invasive mechanical ventilation, and mortality rate was high. In the subgroup of the first 1715 patients, 915 patients died in the hospital for an overall hospital mortality of 53.4%.

The mortality in patients requiring mechanical ventilation was equally large in the **New York City** Area at the beginning of the pandemic (Richardson 2020). A case series from New York included 5700 COVID-19 patients admitted to 12 hospitals between March 1 and April 4, 2020. Median age was 63 years (IQR 52-75), the most common co-morbidities were hypertension (57%), obesity (42%), and diabetes (34%). At triage, 31% of patients were febrile, 17% had a respiratory rate greater than 24 breaths/minute, and 28% received supplemental oxygen. Of 2634 patients with an available outcome, 14% (median age 68 years, IQR 56-78, 33% female) were treated in ICU, 12% received invasive mechanical ventilation and 21% died. Mortality for those requiring mechanical ventilation was 88.1%.

In another study in **New York City** among 1,150 adults who were admitted to two NYC hospitals with COVID-19 in March, 257 (22%) were critically ill (Cummings 2020). The median age of patients was 62 years (IQR 51-72), 67% were men and 82% patients had at least one chronic illness. As of the end of April, 101 (39%) patients had died and 94 (37%) remained hospitalized. 203 (79%) patients received invasive mechanical ventilation for a median of 18 days, 66% received vasopressors and 31% received renal replacement therapy.

In a multivariate Cox model, older age, chronic cardiac disease (adjusted HR 1.76) and chronic pulmonary disease (2.94) were independently associated with in-hospital mortality. This was also seen for higher concentrations of interleukin-6 and D-dimer, highlighting the role of systemic inflammation and endothelial-vascular damage in the development of organ dysfunction.

COVID-19 characteristics may vary considerably by location. In a **United States** cohort of 2215 adults who were admitted to ICUs at 65 sites, 784 (35.4%) died within 28 days (Gupta 2020). However, mortality showed an extremely wide variation among hospitals, ranging from 6.6% to 80.8%. Factors associated with death included older age, male sex, obesity, coronary artery disease, cancer, acute organ dysfunction, and, importantly, admission to a hospital with fewer intensive care unit beds. Of note, patients admitted to hospitals with fewer than 50 ICU beds versus at least 100 ICU beds had a higher risk of death (OR 3.28; 95% CI, 2.16-4.99).

Another large prospective observational study in the **United Kingdom** presented clinical data from 20,133 patients, admitted to (or diagnosed in) 208 acute care hospitals in the UK until April 19 (Docherty 2020). Median age was 73 years (interquartile range 58-82) and 60% were men. Co-morbidities were common, namely chronic cardiac disease (31%), diabetes (21%) and non-asthmatic chronic pulmonary disease (18%). Overall, 41% of patients were discharged alive, 26% died, and 34% continued to receive care. 17% required admission to high dependency or intensive care units; of these, 28% were discharged alive, 32% died, and 41% continued to receive care. Of those receiving mechanical ventilation, 17% were discharged alive, 37% died, and 46% remained in hospital. Increasing age, male sex, and co-morbidities including chronic cardiac disease, non-asthmatic chronic pulmonary disease, chronic kidney disease, liver disease and obesity were associated with higher mortality in hospital.

Spotlight: The situation in a German COVID-19 hospital

The *Klinik Mühldorf am Inn* Hospital was designated as a COVID-19 clinic on March 16, 2020, in order to keep other facilities free for emergencies and elective care. From that day, a total of 276 SARS-CoV-2 positive and 730 suspected cases were treated there. The largest number of symptomatic patients was admitted at the end of March, and the highest number of simultaneously treated SARS-CoV-2 positive patients was 100 patients on April 6, 2020. In total, 18.5% of these in-patients received intensive care during their hospital stay. The peak of intensive care patients was highest on April 10, 2020 with 17 patients. Due to timely preparation, no triage decisions about withholding ventilation treatments had to be made. All COVID-19 patients who had to be

treated in the hospital until July 15th, 2020, and who were in need of mechanical ventilation received it. A total of 51 COVID-19 patients required intensive care treatment (18.5% of all COVID-19 in-patients) and 37 patients (13.4%) were ventilated during their intensive care stay. Seven patients were directly intubated and invasively ventilated without a non-invasive ventilation (NIV) attempt after administration of oxygen through a nasal cannula or mask alone. In total, 9/37 patients did not wish to be intubated. In 16 patients, a prone positioning was carried out, including one patient under NIV.

Management and mechanical ventilation

The cardinal COVID-19 symptom leading to intensive care admission is hypoxemic respiratory failure with tachypnea (> 30/min). Initially, in order to protect staff from aerosols as much as possible, intubation and invasive mechanical ventilation was preferred over non-invasive ventilation (NIV) and nasal high-flow (HFNC).

Likewise, due to lack of knowledge and experience, recommendations on how to deal with these patients were not homogeneous, and ARDS ventilation was the preferred technique (Griffiths 2019). According to the ARDS recommendations, patients should be ventilated with a tidal volume (VT) of < 6ml/kg standardized body weight, a peak pressure of < 30 cmH2O and a PEEP based on the ARDS network table.

In one study, these ventilator settings were used except for the lower PEEP/higher FiO2 table. The driving pressure should not exceed 15 mbar. In addition, prone positioning was recommended in case of a P_aO_2/F_iO_2 < 150 for more than 16 hours (Ziehr 2020).

Quickly it became obvious that acute respiratory distress syndrome (ARDS) in COVID-19 is not the same as ARDS. COVID-19 in patients with ARDS – CARDs – appears to include an important vascular insult that potentially mandates a different treatment approach than customarily used for ARDS. It may be helpful to categorize patients as having either type L or H phenotype and accept that different ventilatory approaches are needed, depending on the underlying physiology (Marini 2020). In type L (low lung elastance, high compliance, low response to PEEP), infiltrates are often limited in extent and initially characterized by a ground-glass pattern on CT that signifies interstitial rather than alveolar edema. Many patients do not appear overtly dyspneic and may stabilize at this stage without deterioration. Others may transit to a clinical picture more characteristic of typical ARDS: Type H shows extensive CT consolidations, high elastance (low compliance) and high PEEP response. Clearly, types L and H are the conceptual extremes of a spectrum that includes intermediate stages.

Factors and characteristics to develop one type over the other have been identified: severity of the initial infection, the patient's immune response, the patient's physical fitness and comorbidities, the response of the hypoxemia to the ventilation, and the time between first symptoms and hospital admission (Gattinoni 2020). L type patients remain stable before improvement or deterioration. In the latter case the patients develop H type pneumonia (Pfeifer 2020). According to this theory, a ventilation strategy starting with respiratory support with high flow oxygen has been recommended (Gattinoni 2020).

To adequately assess oxygenation, the oxygen content (CaO_2) in the blood is helpul, as it describes the actual oxygen supply (DO_2) better than the oxygen partial pressure (pO_2), particularly when combined with the cardiac output (CO):

$DO_2 = CaO_2 \times CO$ and $CaO_2 = Hb \times SaO_2 \times 1.4$

With a CaO_2 limit of 10 g/100 ml blood, and an appropriate cardiac output, i.e., absence of cardiac failure, a lower O_2 saturation (hypoxemia) can be tolerated in the blood before a critical oxygen shortage in the tissue (hypoxia) develops.

Therefore, rather than strictly focusing on pO_2 values as represented by the oxygenation index P_aO_2/F_iO_2 of < 150, it is more reasonable to consider the overall clinical picture while setting individual target values before intubation. Attempting high-flow oxygen and non-invasive ventilation in patients with type L pneumonia is recommended. Intubation should only be performed if there is significant clinical deterioration (Lyons 2020, Pfeifer 2020).

Special situations in severe COVID-19

Prone positioning

Prone position (PP) has become a therapeutic option, even in awake, non-intubated patients, during spontaneous and assisted breathing (Telias 2020). In one study, among 50 patients, the median SpO2 at triage was 80%. After supplemental oxygen was given to patients on room air it was 84%. After 5 minutes of proning was added, SpO2 improved to 94% (Caputo 2020). Whether PP prevents intubation is not known yet.

In a prospective before-after study in Aix-en-Provence, France among 24 awake, non-intubated, spontaneously breathing patients with COVID-19 and hypoxemic acute respiratory failure requiring oxygen supplementation, the effect of PP was only moderate. 63% were able to tolerate PP for more than 3 hours. Oxygenation increased in only 25% and was not sustained in half of

those after resupination. However, prone sessions were short, partly because of limited patient tolerance (Elharrar 2020).

In a small single-center cohort study, use of the prone position for 25 awake, spontaneously breathing patients with COVID-19 was associated with improved oxygenation. In addition, patients with an SpO2 of 95% or greater after 1 hour of the prone position had a lower rate of intubation. Unfortunately, there was no control group and the sample size was very small. Ongoing clinical trials of prone positioning in non–mechanically ventilated patients (NCT04383613, NCT04359797) will hopefully help clarify the role of this simple, low-cost approach for patients with acute hypoxemic respiratory failure (Thompson 2020).

Extracorporal Membrane Oxygenation (ECMO)

Since the beginning of the pandemic, extracorporeal lung replacement procedures such as ECMO have been recommended with caution and only in selected patients with severe and persistant hypoxemia (P_aO_2/F_iO_2 < 80), with minor comorbidities and with full usage of all other measures, such as relaxation and recruiting maneuvers (Smereka 2020).

In a single center narrative study regarding ECMO, support for 27 patients with COVID-19 was described (Kon 2020). At the time of the paper submission, survival was 96.3% (one death) in over 350 days of total ECMO support. Thirteen patients (48.1%) remained on ECMO support, while 13 patients (48.1%) were successfully decannulated. Seven patients (25.9%) were discharged from the hospital while six patients (22.2%) remained in the hospital, of which four were on (unmodified) room air. The authors conclude that the judicious use of ECMO support may be clinically beneficial.

Tracheostomy

During the pandemic, an old problem in a new situation arose: When to perform tracheostomy (and how) in COVID-19 patients? In a review of the current evidence and misconceptions that predispose to uncontrolled variation in tracheostomy among COVID-19 patients, the authors conclude that decisions on tracheostomy must be personalized; that some patients may be awake but cannot yet be extubated (favoring tracheostomy); while others may have immediate, severe hypoxemia when lying supine or with any period of apnea (favoring deferral) (Tay 2020, Schultz 2020). Meanwhile, detailed consensus guidance has been published, including on important issues such as timing of tracheostomy (delayed until at least day 10 of mechanical ventilation and considered only when patients are showing signs of clinical im-

provement), optimal setting (hierarchic approach to operative location, enhanced PPE), optimal procedure and management after tracheostomy (McGrath 2020).

Lung Transplantation

As in other terminal lung diseases, lung transplantation (LTX) can be a potential therapeutic option. Of course, the indication needs to be considered especially careful. In an editorial published in August 2020, the authors list ten considerations that they believe should be carefully weighed when assessing a patient with COVID-19-associated ARDS regarding potential candidacy for lung transplantation (< 65 years, only single-organ dysfunction, sufficient time for lung recovery, radiological evidence of irreversible lung disease, such as severe bullous destruction or established fibrosis, etc) (Cypel 2020).

Up to now, only case reports have been published. After 52 days of critical COVID-19, ECMO and several complications, a comprehensive interdisciplinary discussion on the direction of treatment resulted in a consensus that the lungs of the otherwise healthy 44-year-old woman from Klagenfurt, Austria had no potential for recovery. On day 58, a suitable donor organ became available, and a sequential bilateral lung transplant was performed. At day 144, the patient remained well. Despite the success of this case, the authors emphasize that lung transplantation is an option for only a small proportion of patients (Lang 2020).

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12. Co-morbidities

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Published 20 January 2021

Hundreds of articles have been published over the last twelve months, making well-meaning attempts to determine whether patients with different co-morbidities are more susceptible for SARS-CoV-2 infection or at higher risk for severe disease. This deluge of scientific publications has resulted in worldwide uncertainty. For a number of reasons, many studies must be interpreted with extreme caution.

First, in many articles, the number of patients with specific co-morbidities is low. Small sample sizes preclude accurate comparison of COVID-19 risk between these patients and the general population. They may also overestimate mortality, especially if the observations were made in-hospital (reporting bias). Moreover, the clinical manifestation and the relevance of a condition may be heterogeneous. Is the hypertension treated or untreated? What is the stage of the COPD, only mild or very severe with low blood oxygen levels? Is the "cancer" cured, untreated or actively being treated? Are we talking about a seminoma cured by surgical orchiectomy years ago or about palliative care for pancreatic cancer? What is a "former" smoker: someone who decided to quit 20 years ago after a few months puffing during adolescence or someone with 40 package-years who stopped the day before his lung transplantation? Does "HIV" mean a well controlled infection while on long-lasting, successful antiretroviral therapy or an untreated case of AIDS? Unfortunately, many researchers tend to combine these cases, in order to get larger numbers and to get their paper published.

Second, there are numerous confounding factors to consider. In some case series, only symptomatic patients are described, in others only those who were hospitalized (and who have *per se* a higher risk for severe disease). In some countries, every patient with SARS-CoV-2 infection will be hospitalized, in others only those with risk factors or with severe COVID-19. Testing policies vary widely between countries. The control group (with or without co-morbidities) is not always well-defined. Samples may not be representative, risk factors not correctly taken into account. Sometimes, there is incomplete information about age distribution, ethnicity, co-morbidities, smoking, drug use and gender (there is some evidence that, in female patients, co-morbidities have no or less impact on the course of the disease, compared to

males (Meng 2020)). All these issues present important limitations and only a few studies have addressed all of them.

Third, co-morbidity papers have led to an information overload. Yes, virtually every medical discipline and every specialist has to cope with the current pandemic. And yes, everybody has to be alert these days, psychiatrists as well as esthetic surgeons. Hundreds of guidelines or position papers have been published, trying to thoughtfully balance fear of COVID-19 against the dire consequences of not treating other diseases than COVID-19 in an effective or timely manner – and all this in the absence of data. On May 15, a PubMed search yielded 530 guidelines or considerations about specific diseases in the context of COVID-19, among them those for grade IV glioma (Bernhardt 2020, bottom line: do not delay treatment), but also for dysphonia and voice rehabilitation (Mattei 2020: can be postponed), infantile hemangiomas (Frieden 2020: use telehealth), ocular allergy (Leonardi 2020: very controversial), high resolution anoscopy (Mistrangelo 2020: also controversial), migraine management (Szperka 2020: use telehealth) and breast reconstruction (Salgarello 2020: defer "whenever possible"), to name just a few. These recommendations are usually not helpful. They apply for a few weeks, during acute health crisis scenarios as seen in overwhelmed health care systems in Wuhan, Bergamo, Madrid or New York. In other cities or even a few weeks later, proposed algorithms are already outdated. Nobody needs a 60-page recommendation, concluding that "clinical judgment and decision making should be exercised on a case-by-case basis".

However, some important papers have been published over the last months, a couple of them with very helpful data, supporting the management of patients with co-morbidities. In the following, we will briefly go through these.

Hypertension and cardiovascular co-morbidities

From the beginning of the pandemic, hypertension and/or cardiovascular disease (CVD) have been identified as potential risk factors for severe disease and death (Table 1). However, all studies were retrospective, included only hospitalized patients and did not distinguish between uncontrolled and controlled hypertension or used different definitions for CVD. Multivariate analyses adjusting for confounders were performed in only a few studies. Moreover, different outcomes and patient groups were analyzed. According to some experts, current data do not necessarily imply a causal relationship between hypertension and severity of COVID-19. There is no study that demonstrates the independent predictive value of hypertension. It is "unclear whether uncontrolled blood pressure is a risk factor for acquiring COVID-19, or whether controlled blood pressure among patients with hypertension is or is not less

of a risk factor" (Schiffrin 2020). The same applies to CVD, with the difference that the numbers here are even lower.

From a mechanistic point of view, however, it seems plausible that patients with underlying cardiovascular diseases and pre-existing damage to blood vessels such as artherosclerosis may face higher risks for severe diseases. During recent weeks, it has become clear that SARS-CoV-2 may directly or indirectly attack the heart, kidney and blood vessels. Various cardiac manifestations of COVID-19 do occur contemporarily in many patients (see chapter *Clinical Presentation*, page 279). Infection may lead to cardiac muscle damage, blood vessel constriction and to elevated levels of inflammation-inducing cytokines. These direct and indirect adverse effects of the virus may be especially deleterious in those with already established heart disease. During the next months, we will learn more about the role and contributions of arteriosclerosis in the pathogenesis of COVID-19.

Table 1. Hypertension in larger cohort studies, prevalence and outcome

Study	Setting	Hypertension present?	Multivariate, hazard or odds ratio (95% CI) for endpoint
Wang 2020	344 ICU pts, Tongji, China	Survivors vs Non-Survivors: 34 vs 52%	Not done
Grasselli 2020	521 ICU pts, 72 hospitals in Italy	Discharge from ICU vs death at ICU: 40 vs 63%	Not done
Guan 2020	1099 hospitalized pts, 522 hospitals in China	Non-severe disease vs severe: 13 vs 24%	Not done
Zhou 2020	191 hospitalized pts from Jinyintan and Wuhan	Survivors vs Non-Survivors: 23 vs 48%	Not done
Shi 2020	487 hospitalized pts in Zhejing Province	Non-severe disease at admission vs severe: 17 vs 53%	OR 2,7 (1,3-5,6) for severe disease at admission
Guan 2020	1590 hospitalized pts, 575 hospitals in China	Non-severe vs severe courses: 13 vs 33%	HR 1,6 (1,1-2,3) for severe course (ICU, IMV, death)
Goyal 2020	393 hospitalized pts, 2 hospitals in New York	No IMV vs IMV during stay: 48 vs 54%	Not done

IMV invasive mechanical ventilation, ICU intensive care units

Treatment of hypertension during the pandemic

There has hardly been a topic that has kept doctors and their patients as busy as the question of whether antihypertensive drugs such as ACE inhibitors (ACEIs) or angiotensin-receptor blockers (ARBs) can cause harm to patients. The uncontrolled observations of increased mortality risk in patients with hypertension, CVD (see above) and diabetes raised concerns. These conditions share underlying renin-angiotensin-aldosterone system pathophysiology that may be clinically insightful. In particular, activity of the angiotensin-converting enzyme 2 (ACE2) is dysregulated (increased) in cardiovascular disease (Vaduganathan 2020). As SARS-CoV-2 cell entry depends on ACE2 (Hoffmann 2020), increased ACE2 levels may increase the virulence of the virus within the lung and heart.

There is a large study that examined the association between pre-infection blood pressure (BP) control and COVID-19 outcomes using data from 460 general practices in England (Sheppard 2020). Eligible patients were adults with hypertension who were diagnosed with COVID-19. A total of 4277 patients (9,4%) were diagnosed with COVID-19 and 877 died within 28 days. There was no association between BP control and COVID-19 diagnosis or hospitalization. Of note, individuals with stage 1 uncontrolled BP had lower odds of COVID-19 death (OR 0,76, 95%CI: 0,62-0,92) compared to patients with well-controlled BP. However, these patients were older, had more co-morbidities and had been diagnosed with hypertension for longer, suggesting more advanced atherosclerosis and target organ damage.

ACEIs or ARBs may alter ACE2, and variation in ACE2 expression may in part be responsible for disease virulence. However, the first substantial study to examine the association between plasma ACE2 concentrations and the use of ACEIs/ARBs did not support this hypothesis: in two large cohorts from the pre-COVID-19 era, plasma concentrations of ACE2 were markedly higher in men than in women, but not with ACEI/ARB use (Sama 2020). A recent review of 12 animal studies and 12 human studies overwhelmingly implies that administration of both drug classes does not increase ACE2 expression (Sriram 2020).

However, some concerns on deleterious effects continue and some media sources and even scientific papers have called for the discontinuation of these drugs. This is remarkable as almost no clinical study showed any evidence of harm.

- Among 2573 COVID-19 patients with hypertension from New York City, there were no differences in the likelihood for severe COVID-19 for different classes of antihypertensive medications – ACE inhibitors, ARBs, beta

blockers, calcium channel blockers, and thiazide diuretics (Reynolds 2020).

- Comparing 6272 Italian cases (positive for SARS-CoV-2) to 30,759 controls (matched for sex, age, and municipality of residence), no evidence was found that ACE inhibitors or ARBs modify susceptibility to COVID-19 (Mancia 2020). The results applied to both sexes as well as to younger and older persons.
- In a retrospective study from Denmark (one of the countries with the best epidemiological data) of 4480 COVID-19 patients, prior ACEI/ARB use, compared with no use, was not significantly associated with mortality. In a nested case-control study of a cohort of 494.170 patients with hypertension, use of ACEI/ARB, compared with use of other antihypertensive medications, was not significantly associated with COVID-19 diagnosis (Fosbøl 2020).
- In a multicenter cohort study following more than 1,3 million patients with hypertension from the USA and Spain, no clear association of increased risk of COVID-19 diagnosis, hospital admission, or subsequent complications was seen with the outpatient use of ACEI or ARB. Furthermore, the marginal difference between ACEIs and ARBs does not warrant class switching (Morales 2020).
- Does discontinuation compared with continuation of ACEIs or ARBs change anything? No. In an open label RCT that included 659 patients from Brazil who were hospitalized with mild to moderate COVID-19 who were taking ACEIs or ARBs before hospital admission, the mean number of days alive and out of the hospital for those assigned to discontinue versus continue these medications was 21,9 vs 22,9, respectively (Lopes 2020).

In conclusion, ACE inhibitors and/or ARBs should not be discontinued. Several other RCT plan to evaluate ACEIs and ARBs for treatment of COVID-19 (Mackey 2020). According to a brief review, adjuvant treatment and continuation of pre-existing statin therapy could improve the clinical course of patients with COVID-19, either by their immunomodulatory action or by preventing cardiovascular damage (Castiglion 2020). In a retrospective study on 13.981 patients in Hubei Province, China, the use of statins was independently associated with lower all-cause mortality (5,2% vs 9,4%). However, an observational cohort study using data from Danish nationwide registries, 843/4842 (17%) COVID-19 patients redeemed a prescription of statins in the 6 months prior to COVID-19 diagnosis. Recent statin exposure was not associated with an increased or decreased risk of all-cause mortality or severe infection (Butt

2020). Randomized controlled trials involving statin treatment for COVID-19 are needed.

Treatment of coronary heart disease during the pandemic

Pre-existing cardiovascular disease is linked with higher morbidity and mortality in patients with COVID-19, whereas COVID-19 itself can induce myocardial injury, arrhythmia, acute coronary syndrome and venous thromboembolism (nice review: Nishiga 2020). Myocardial injury, evidenced by elevated cardiac biomarkers, was recognized among early cases and myocardial infarction (STEMI or NSTEMI) and may represent the first clinical manifestation of COVID-19. Of note, a culprit lesion is often not identifiable by coronary angiography. In a study of 28 patients with STEMI, this was the case in 39% (Stefanini 2020). According to the authors, a dedicated diagnostic pathway should be delineated for COVID-19 patients with STEMI, aimed at minimizing procedural risks and healthcare providers' risk of infection. There are already preliminary reports on a significant decline of 32% in the number of percutaneous coronary interventions for acute coronary syndromes (Piccolo 2020). Other authors have suggested that, in settings with limited resources to protect the work force, fibrinolytic therapies may be preferred over primary percutaneous coronary interventions (Daniels 2020).

In a meta-analysis, outcomes of 50.123 patients from 10 studies were assessed. Data showed that acute and timely medical care of these patients had been maintained during the pandemic in most countries. Consequently, despite a significant reduction in overall admission rates of patients with STEMI during the COVID-19 pandemic, there was no significant difference in hospital mortality compared with patients with STEMI admitted before the outbreak (Rattka).

Of note, several studies have found a spectacular drop in admissions for STEMI during the peak of the epidemic. In France a steep decline of 25% was found for both acute (< 24hrs) and late presentation (> 24 hrs) STEMI (Rangé 2020). Similar observations have been made in Italy (De Filippo 2020) and the US (Solomon 2020). Possible explanations for this phenomenon may be patients' fear of coming to the hospital or disturbing busy caregivers, especially in the case of mild STEMI clinical presentation. Other hypothetical reasons are reduced air pollution, better adherence to treatment, limited physical activity or absence of occupational stress during lockdown. However, there is some evidence that the lower incidence does not reflect a true decline but just one more collateral damage of the pandemic. For example, Italian researchers have found a 58% increase of out-of-hospital cardiac arrests in March 2020 compared to the same period in 2019 (Baldi 2020). In New York,

this increase seemed to be even more pronounced (Lai 2020). Others have observed an increased observed/expected mortality ratio during the early COVID-19 period indicating that patients try to avoid hospitalization (Gluckman 2020).

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Diabetes mellitus

Diabetes mellitus is a chronic inflammatory condition characterized by several macrovascular and microvascular abnormalities. As with hypertension and CVD, many of the above cited studies have also revealed that diabetic patients were overrepresented among the most severely ill patients with COVID-19 and those succumbing to the disease. Among the 23,698 in-hospital COVID-19-

related deaths during the first months in the UK, a third occurred in people with diabetes: 7,434 (31.4%) in people with type 2 diabetes, 364 (1.5%) in those with type 1 diabetes (Barron 2020).

Current data suggest that diabetes in patients with COVID-19 is associated with a two-fold increase in mortality as well as severity of COVID-19, as compared to non-diabetics. In a meta-analysis of 33 studies and 16.003 patients (Kumar 2020), diabetes was found to be significantly associated with mortality from COVID-19 with a pooled odds ratio of 1.90 (95% CI: 1,37-2,64). Diabetes was also associated with severe COVID-19 and a pooled odds ratio of 2.75 (95% CI: 2,09-3,62). The pooled prevalence of diabetes in patients with COVID-19 was 9,8% (95% CI: 8,7%-10,9%). However, it is too early to say whether diabetes is acting as an independent factor responsible for COVID severity and mortality or if it is just a confounding factor.

A large retrospective study on the impact of type 2 diabetes (T2D) carefully analyzed 7337 cases of COVID-19 in Hubei Province, China, among them 952 with pre-existing T2D (Zhu 2020). The authors found that subjects with T2D required more medical interventions and had a significantly higher mortality (7,8% versus 2,7%; adjusted hazard ratio, 1,49) and multiple organ injury than non-diabetic individuals. Of note, well-controlled blood glucose was associated with markedly lower mortality (in-hospital death rate 1,1% versus 11,0%) compared to individuals with poorly controlled blood glucose. Similar results were found in a large UK cohort (Holman 2020).

A recent review has made some suggestions on the possible pathophysiological mechanisms of the relationship between diabetes and COVID-19, and its management (Hussain 2020). Rigorous glucose monitoring and careful consideration of drug interactions might attenuate worsening of symptoms and adverse outcomes. In a retrospective cohort study of 1213 hospitalized individuals with COVID-19 and pre-existing T2D, metformin use was significantly associated with a higher incidence of acidosis, particularly in cases with severe COVID-19, but not with 28-day COVID-19-related mortality (Cheng 2020).

Some treatment strategies for COVID-19 such as steroids and lopinavir/r bear a risk for hyperglycemia. On the other hand, hydroxychloroquine may improve glycemic control in decompensated, treatment-refractory patients with diabetes (Gerstein 2002, Rekedal 2010). However, it remains unclear which COVID-19 treatment strategy works best and if treatment of diabetic patients has to be different from those without diabetes. It is also unclear whether specific diabetes drugs such as DPP4 inhibitors increase or decrease the susceptibility or severity of SARS-CoV-2 infection.

Isolated reports of new-onset diabetes in COVID-19 cases have also led to the hypothesis that SARS-CoV-2 is directly cytotoxic to pancreatic islet β cells. However, a careful investigation (Coate 2020) suggested that the interaction of diabetes and SARS-CoV-2 is mediated by systemic inflammation and/or metabolic changes in other organs such as liver, muscle or adipose tissue (and not by a direct infection of β cells in the pancreas).

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COPD and smoking

Chronic Obstructive Pulmonary Disease (COPD) is a common and preventable dysfunction of the lung associated with limitation in airflow. It is a complex disease associated with abnormalities of the airway and/or alveoli which is predominantly caused by exposure to noxious gases and particulates over a

long period. A meta-analysis of 15 studies, including a total of 2473 confirmed COVID-19 cases showed that COPD patients were at a higher risk of more severe disease (calculated RR 1,88) and with 60% higher mortality (Alqahtani 2020). Unfortunately, the numbers in this review were very small and only 58 (2,3%) had COPD.

A meta-analysis of 5 early studies comprising 1399 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). In a more recent review, current smokers were 1.45 times more likely to have severe complications compared to former and never smokers. Current smokers also had a higher mortality rate (Alqahtani 2020).

Ever-smoking increased pulmonary ACE2 expression by 25% (Cai 2020). The significant smoking effect on ACE2 pulmonary expression may suggest an increased risk for viral binding and entry of SARS-CoV-2 into the lungs of smokers. Cigarette smoke triggers an increase in ACE2 positive cells by driving secretory cell expansion (Smith 2020). The overabundance of ACE2 in the lungs of smokers may partially explain a higher vulnerability of smokers.

However, it's not that easy – both quitting smoking and finding clinical correlations to the above cell experiments. Within a surveillance center primary care sentinel network, multivariate logistic regression models were used to identify risk factors for positive SARS-CoV-2 tests (Lusignan 2020). Of note, active smoking was associated with decreased odds (yes, decreased: adjusted OR 0,49, 95% CI; 0,34–0,71). According to the authors, their findings should not be used to conclude that smoking prevents SARS-CoV-2 infection, or to encourage ongoing smoking. Several explanations are given, such as selection bias (smokers are more likely to have a cough, more frequent testing could increase the proportion of smokers with negative results). Active smoking might also affect RT-PCR test sensitivity.

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HIV infection

HIV infection is of particular interest in the current crisis. First, many patients take antiretroviral therapies that are thought to have some effect against SARS-CoV-2. Second, HIV serves as a model of cellular immune deficiency. Third, and by far the most important point, the collateral damage caused by COVID-19 in the HIV population may be much higher than that of COVID-19 itself.

Preliminary data suggest no elevated incidence of COVID-19. In 5700 patients from New York, only 43 (0,8%) were found to be HIV-positive (Richardson 2020). In Barcelona, the standardized incidence rate was lower in persons living with HIV (PLWH) than in the general population (Inciarte 2020). Given the fact that HIV+ patients may be at higher risk for other infectious diseases such as STDs, these percentages were so low that some experts have already speculated on potential "protective" factors (i.e., antiviral therapies or immune activation). Moreover, a defective cellular immunity could paradoxically be protective for severe cytokine dysregulation, preventing the cytokine storm seen in severe COVID-19 cases. Appropriately powered and designed studies are still needed to draw conclusions on the effect of COVID-19.

In our own retrospective analysis of 33 confirmed SARS-CoV-2 infections between March 11 and April 17 in 12 participating German HIV centers, no excess morbidity or mortality was revealed (Haerter 2020). However, a multicenter cohort study which evaluated risk factors for morbidity and mortality of COVID-19 in PLWH infected with SARS-CoV-2 in three countries, patients with severe COVID-19 had a lower current CD4 T cell count and a lower CD4 T cell nadir, compared with patients with mild-to-moderate COVID-19 (Hoffmann 2020). In a multivariate analysis, the only factor associated with risk for severe COVID-19 was a current CD4+ T cell count of < 350/µl (adjusted odds

ratio 2,85, 95% CI: 1,26-6,44, p = 0,01). The only factor associ-ated with mortality was a low CD4 T cell nadir. In a large population study from South Africa, HIV was independently associated with increased COVID-19 mortality, showing an adjusted hazard ratio for mortality of 2,14 for HIV (95% CI: 1,70-2,70) (Boulle 2020). Among 286 HIV-infected patients who were included by US healthcare providers, mortality rates were higher in patients with low CD4 counts (< 200 cells/mm³) (Dandachi 2020). In a large study from the UK (Bhaskaran 2020), PLWH had higher risk of COVID-19 death than those without HIV after adjusting for age and sex: hazard ratio (HR) 2,90 (95% CI: 1,96–4,30; p < 0,0001).

There is still an ongoing debate about potential effects of antiretroviral therapies against SARS-CoV-2. For lopinavir/r (and darunavir/r), there is now strong evidence that they don't work (see *Treatment* chapter, page 331). An ART regimen should not be changed to include a PI to prevent or treat COVID-19 (EACS 2020, US 2020). Tenofovir alafenamide (TAF) has some chemical similarities to remdesivir and has been shown to bind to SARS-CoV-2 RNA polymerase (RdRp) with high binding energies, and has been suggested as a potential treatment for COVID-19 (Elfiky 2020). In Spain, a large randomized Phase III placebo-controlled study (EPICOS, NCT04334928) compares the use of tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC), hydroxychloroquine or the combination of both versus placebo as prophylaxis for COVID-19 in healthcare workers. In combined cohorts from Milan, Madrid and Germany, there was no evidence that any specific antiretroviral drug (such as tenofovir or PIs) affected COVID-19 susceptibility or severity (Hoffmann 2020). Most patients, however, received TAF and not TDF for which preliminary data from Spain suggest a beneficial effect. Of 77.590 HIV+ persons receiving ART in Spain, 236 were diagnosed with COVID-19, 151 were hospitalized, 15 were admitted to the ICU, and 20 died (Del Amo 2020). The risk for COVID-19 hospitalization was higher among patients receiving TAF/FTC and ABC/3TC, compared to those receiving TDF/FTC. However, residual confounding by co-morbid conditions cannot be completely excluded. In a small group from France, attack rates were not lower with TDF/FTC in PrEP users (Charre 2020).

The most serious concern regarding HIV, however, is the collateral damage induced by COVID-19. In Western countries, there exist few reports of HIV+ patients having problems in gaining access to their HIV medications or having trouble taking them due to COVID-19 or the plans to manage it (Sanchez 2020). In contrast, disruption to delivery of health care in sub-Saharan African settings could well lead to adverse consequences beyond those from COVID-19 itself. Lockdown, transport restrictions and fear of coronavirus in-

fection have already led to a dramatic drop in HIV and TB patients collecting medication in several African countries (Adepoju 2020). Using five different existing mathematical models of HIV epidemiology and intervention programs in sub-Saharan Africa, investigations have already estimated the impact of different disruptions to HIV prevention and treatment services. Predicted average relative excess in HIV-related deaths and new HIV infections (caused by unsuppressed HIV RNA during treatment interruptions) per year over 2020-2024 in countries in sub-Saharan Africa that would result from 3 months of disruption of HIV-specific services, were 1,20-1,27 for death and 1,02-1,33 for new infections, respectively. A 6-month interruption of ART would result in over 500.000 excess HIV deaths in sub-Saharan Africa (range of estimates 471.000 – 673.000). Disrupted services could also reverse gains made in preventing mother-to-child transmission. According to WHO, there is a clear need for urgent efforts to ensure HIV service continuity and preventing treatment interruptions due to COVID-19 restrictions in sub-Saharan Africa.

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Immunosuppression (other than HIV)

Immunosuppression may bear a higher risk for SARS-CoV-2 infection and severe COVID-19. But the story is not that simple. Neither is it clear what immunosuppression actually means, nor are the available data sufficient to draw any conclusion. We just don't know enough. Nevertheless, some authors are trumpeting the news that there is an increased risk. A bad example? A systematic review and meta-analysis on 8 studies and 4007 patients came to the conclusion that "immunosuppression and immunodeficiency were associated with increased risk of severe COVID-19 disease, although the statistical differences were not significant" (Gao 2020). The authors also state that "in response to the COVID-19 pandemic, special preventive and protective measures should be provided." There is null evidence for this impressive statement. The total number of patients with immunosuppression in the study was 39 (without HIV: 11!), with 6/8 studies describing less than 4 patients with different modalities of immunosuppression.

Despite the large absence of data, numerous viewpoints and guidelines have been published on how to manage immunosuppressed patients that may be more susceptible to acquire COVID-19 infection and develop severe courses. There are recommendations for intranasal corticosteroids in allergic rhinitis

(Bousquet 2020), immunosuppressants for psoriasis and other cutaneous diseases (Conforti 2020, Torres 2020), rheumatic diseases (Favalli 2020, Figueroa-Parra 2020) or inflammatory bowel diseases (Kennedy 2020, Pasha 2020). The bottom line of these heroic attempts to balance the risk of immune-modifying drugs with the risk associated with active disease: what is generally needed, has to be done (or to be continued). Exposure prophylaxis is important.

However, several studies have indeed found evidence for deleterious effects of glucocorticoids, indicating that these drugs should be given with particular caution these days.

- In 600 COVID-19 patients with rheumatic diseases from 40 countries, multivariate-adjusted models revealed a prednisone dose ≥ 10 mg/day to be associated with higher odds of hospitalization. There was no risk with conventional disease-modifying anti-rheumatic drugs (DMARD) alone or in combination with biologics and Janus kinase (JAK) inhibitors (https://doi.org/10.1136/annrheumdis-2020-217871).
- In 525 patients with inflammatory bowel disease (IBD) from 33 countries (Brenner 2020), risk factors for severe COVID-19 included systemic corticosteroids (adjusted odds ratio 6,9, 95% CI: 2,3-20,5), and sulfasalazine or 5-aminosalicylate use (aOR 3,1). TNF antagonist treatment was not associated with severe COVID-19.
- In 86 patients with IBD and symptomatic COVID-19, among them 62 receiving biologics or JAK inhibitors, hospitalization rates were higher in patients treated with oral glucocorticoids, hydroxychloroquine and methotrexate but not with JAK inhibitors (Haberman 2020).
- In a large French database, including patients with inflammatory rheumatic and musculoskeletal diseases (iRMD), of 694 adults, 438 (63%) developed mild (not hospitalized), 169 (24%) moderate (hospitalized non-ICU) and 87 (13%) severe (ICU/deceased) disease. In multivariable imputed analyses, the variables associated with severe infection were age, male gender, hypertension and higher BMI. Use of corticosteroids (OR = 1,97), mycophenolate mofetil (OR = 6,6) and rituximab (OR = 4,21) were also risk factors.

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Cancer

Providing continuous and safe care for cancer patients is challenging in this pandemic. Oncologic patients may be vulnerable to infection because of their underlying illness and often immunosuppressed status and may be at increased risk of developing severe complications from the virus. On the other hand, the COVID-19 triage and management may stretch an already fragile system and potentially leave uncovered some vital activities, such as treatment administration or surgeries. It is well established that suboptimal tim-

ing and delayed oncologic treatment may lead to disease progression, leading to worse survival outcomes. There are several recommendations to minimizing exposure of oncology patients to COVID-19 without compromising oncological outcome: radiation for breast cancer (Coles 2020), hematopoietic cell transplant (Dholaria 2020) and leukemia treatment (Zeidan 2020).

What is known about risk factors, besides general risk factors such as age, male gender and other co-morbidities?

- Compared to 519 statistically matched patients without cancer, 232 patients from Wuhan were more likely to have severe COVID-19 (64% vs 32%). An advanced tumour stage was a risk factor (odds ratio 2,60, 95% CI: 1,05–6,43) (Tian 2020).
- A systematic review of all studies until June 3 indicated that patients with hematological malignancies, especially those diagnosed recently (and likely those with myeloid malignancies), were at increased risk of death with COVID-19 compared to the general population. The evidence that this risk is higher than for those with solid malignancies was conflicting (El-Sharkawi 2020).
- Patients with Chronic Lymphatic Leukemia (CLL) seem to be at particular high risk of death. Of 198 CLL patients diagnosed with symptomatic COVID-19, 39% were treatment-naïve ("watch and wait") while 61% received at least one CLL therapy. At 16 days, the overall CFR was 33%, while another 25% were still in hospital (Mato 2020).
- In a retrospective study from Italy, including 536 patients with a diagnosis of a hematological malignancy, 198 (37%) had died. Progressive disease status, diagnosis of acute myeloid leukemia, indolent or aggressive NHL were associated with worse overall survival (Passamonti 2020).
- In a large cohort study of 928 cancer patients with COVID-19 from the USA, Canada, and Spain, most prevalent malignancies were breast (21%) and prostate (16%). In total 121 (13%) patients had died. Independent risk factors were an ECOG status of 2 or higher and "active" cancer (Kuderer 2020).
- SARS-CoV-2 viral load in nasopharyngeal swab specimens of 100 patients with cancer who were admitted to three New York City hospitals predicted outcome. The authors also found that patients with hematologic malignancies had higher median viral loads than patients without cancer (Westblade 2020).

Does anti-neoplastic treatment lead to increased risk of complications?

- Among a total of 309 patients, cytotoxic chemotherapy administered within 35 days of a COVID-19 diagnosis was not significantly associated with a severe or critical COVID-19 event. However, patients with active hematologic or lung malignancies, lymphopenia, or baseline neutropenia had worse COVID-19 outcomes.
- Among 423 cases of symptomatic COVID-19 patients, 40% were hospitalized and 12% died within 30 days. Age older than 65 years and treatment with immune checkpoint inhibitors were predictors for hospitalization and severe disease, whereas receipt of chemotherapy and major surgery were not (Robilotti 2020).
- In a systemic review and meta-analysis of 34 adult and 5 pediatric studies (3377 patients) from Asia, Europe, and North America (14 of 34 adult studies included only hospitalized patients), adult patients with hematologic malignancy and COVID-19 found a 34% risk of death (Vijenthira 2020), whereas pediatric patients had a 4% risk of death. Patients on systemic anticancer therapy had a similar risk of death to patients on no treatment.
- Among 77 patients with SARS-CoV-2 who were recipients of cellular therapy (Allo, 35; Auto, 37; CAR T, 5; median time from cellular therapy, 782 days), overall survival at 30 days was 78% (Shah 2020). Mortality was largely driven by patients with active malignancy, especially relapsed leukemia, in whom the goals of care were affected by COVID-19 severity. Many patients were able to recover from COVID-19 and mount an antibody response.

All these studies are not controlled. A myriad of potential factors may lead to a difference in COVID-19 outcomes and risk for patients with malignancies, compared to the rest of the population (nice review: El-Sharkawi 2020). These include patient behavior (exposure to the virus?), healthcare professional behavior (i.e., testing patients with a history of cancer for COVID-19 more frequently?), biological differences but also several confounders (more comorbidities, older age in cancer patients). Continued analysis of the data is required to attain further understanding of the risk factors for cancer patients in this pandemic.

Finally, it's not only treatment, it's also diagnosis. Diagnostic delays may lead to an increase in the numbers of avoidable cancers (Maringe 2020). During the pandemic, a large cross-sectional study in the US has observed significant declines in several cancer types, ranging from 24,7% for pancreatic cancer to 51,8% for breast cancer, indicating that a delay in diagnosis will likely lead to presentation at more advanced stages and poorer clinical outcomes (Kaufman 2020).

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Transplantation

During a health crisis such as the COVID pandemic, it is crucial to carefully balance cost and benefits in performing organ transplantation (Andrea 2020). There is no doubt that the current situation has deeply affected organ donation and that this represents an important collateral damage of the pandemic. All Eurotransplant countries have implemented preventive screenings policies for potential organ donors. For detailed information on the national policy, please visit https://www.eurotransplant.org/2020/04/07/covid-19-and-organ-donation/. Preliminary data indicate a significant reduction in transplantation rates even in regions where COVID-19 cases are low, suggesting a global and nationwide effect beyond the local COVID-19 infection prevalence (Loupy 2020). During March and April, the overall reduction in deceased donor transplantations since the COVID-19 outbreak was 91% in France and 51% in the USA, respectively. In both France and the USA, this reduction was mostly driven by kidney transplantation, but a substantial effect was also seen for heart, lung, and liver transplants, all of which provide meaningful improvement in survival probability. In Germany, however, compared with the previous year, the cumulative numbers of deceased organ donors and transplants showed no significant reduction (Qu 2020).

Solid organ transplant recipients are generally at higher risk for complications of respiratory viral infections (in particular influenza), due to their chronic immunosuppressive regimen, and this may hold particularly true for SARS-CoV-2 infection. The first cohort of COVID-19 in transplant recipients from the US indeed indicated that transplant recipients appear to have more severe outcomes (Pereira 2020). Some key studies:

Liver: In the largest cohort, 16/100 patients died from COVID-19. Of note, mortality was observed only in patients aged 60 years or older (16/73) and was more common in males than in females (Belli 2020). Although not statistically significant, more patients who were transplanted at least 2 years earlier died than did those who received their transplant within the past 2 years (18% vs 5%). A systematic search on June 15 revealed 223 liver transplant recipients with COVID-19 in 15 studies (Fraser 2020). The case fatality rate was 19,3%. Dyspnea on presentation, diabetes mellitus, and age 60 years or older were significantly associated with increased mortality ($p = 0.01$) with a trend to a higher mortality rate observed in those with hypertension and those re-

ceiving corticosteroids at the time of COVID-19 diagnosis. However, in a multicenter cohort study, comparing 151 adult liver transplant recipients from 18 countries with 627 patients who had not undergone liver transplantation, liver transplantation did not significantly increase the risk of death in patients with SARS-CoV-2 infection (Webb 2020).

Kidney: In a single center with 36 kidney transplant recipients, 10/36 died (Akalin 2020). Patients appear to have less fever as an initial symptom, lower CD4 and CD8 T cell counts and more rapid clinical progression.

Heart: In a case series of 28 patients who had received a heart transplant in a large academic center in New York, 22 patients (79%) were hospitalized. At the end of the follow-up, 4 remained hospitalized and 7 (25%) had died (Latif 2020). In Germany, mortality was also high, and 7/21 patients died (Rivinius 2020).

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Renal diseases

The OpenSAFELY project analysed factors associated with COVID-19 deaths in 17 million patients. The picture that arose differs significantly from initial reports. For example, hypertension is not an independent risk factor for COVID-19 death, but renal disease very much is. Dialysis (aHR 3,69), organ transplantation (aHR 3,53) and CKD (aHR 2,52 for patients with eGFR < 30 mL/min/1.73 m^2) represent three of the four co-morbidities associated with the highest mortality risk from COVID-19. The risk associated with CKD Stages 4 and 5 was higher than the risk associated with diabetes mellitus (aHR range 1,31–1,95, depending upon glycemic control) or chronic heart disease (aHR 1,17). These findings define essential action points, among which is advocating the inclusion of CKD patients in clinical trials when testing the efficacy of drugs and vaccines to prevent severe COVID-19 (ERA 2020).

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Other co-morbidities

Ultimately, the current situation might lead to substantial changes in how research and medicine are practiced in the future. The SARS-CoV-2 pandemic has created major dilemmas in almost all areas of health care. Scheduled operations, numerous types of treatment and appointments have been cancelled world-wide or postponed to priorities hospital beds and care for those who are seriously ill with COVID-19. Throughout the world, health systems had to consider rapidly changing responses while relying on inadequate information. In some settings such as HIV or TB infection, oncology or solid organ transplantation, these collateral damages may have been even greater than the damage caused by COVID-19 itself. Treatment interruptions, disrupted drug supply chains and consequent shortages will likely exacerbate this issue. During the next months, we will learn more and provide more information on the consequences of this crisis on various diseases.

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13. Pediatrics

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Acknowledgements: Without the skillful help of **Andrea Groth** (Helios Klinikum Krefeld), the preparation of this manuscript would not have been possible. We thank cand. med. **Lars Dinkelbach** (Heinrich Heine Universität Düsseldorf) for critically reading the manuscript.

Last revised: 1 November 2020

SARS-CoV-2 infection in children

Children are less susceptible to SARS-COV-2 infection, have a lower seroprevalence and a less severe COVID-19 disease course than adults (Castagnoli 2020, Viner 2020, Merckx 2020, Zimmermann 2020, Parri 2020, Ludvigsson 2020). In this regard, COVID is strikingly different from other virus-induced respiratory diseases, which can be fatal in children (e.g. RSV in infants). The CoV-2 pandemic causes a large collateral damage to children because they are taken out of their normal social environment (kindergartens, schools etc.), and because of parents' resistance to seek medical care despite need e.g. for vaccination (Bramer 2020) or even if their children are having an emergency (Lazzerini 2020).

Common 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 (COV) 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 (Hufert 2020): α-Coronaviruses HCoV-229e (discovered in 1966), HCoV-NL63 (in 2004); β-Coronaviruses HCoV-OC43 (in 1967)-HKU1 (in 2005), -OC43; MERS-CoV (in 2012), SARS-CoV (in 2003) and SARS-CoV-2 that originally derive from bats (NL63, 229e, SARS-CoV), dromedary camels (229e, MERS-CoV), cattle (OC43) and 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 cyclical pattern with seasonal outbreaks between December and May or March to November in the southern hemisphere.

Single-strand RNA coronaviruses are capable of 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

On 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 2143 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.. 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). In Germany, 9657 children and adolescents with COVID-19 were reported up to May

4th, 2020; only 128 were admitted to 66 hospitals, only one child died (Armann 2020).

The European Surveillance System (TESSy) collects data from EU/EEA countries and the UK on laboratory-confirmed cases of COVID-19. Out of 576,024 laboratory confirmed COVID-19 cases 0,7% were 0-4 years, 0,6% 5-9 years, 0,9% 10-14 years (https://covid19-surveillance-report.ecdc.europa.eu). The multicentre cohort study (82 participating health-care institutions across 25 European countries), Paediatric Tuberculosis research Network (ptbnet) confirmed that COVID-19 is generally a mild disease in children. Of 582 children and adolescents (median age 5.0 years, 25% with pre-existing conditions) with PCR-confirmed SARS-CoV-2 infection, 363 (62%) were admitted to hospital and 48 (8%) required ICU admission. Significant risk factors for requiring ICU admission in multivariate analyses were being younger than 1 month (odds ratio 5.1), male sex (2.1) and pre-existing medical conditions (3.3). Four children died (Götzinger 2020).

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. Children often have an asymptomatic or less severe COVID-19 disease course than adults (Zimmermann 2020, Parri 2020). Among a total of 100 children with SARS-CoV-2 from Italy, 21% were asymptomatic, 58% had mild disease, 19% had moderate disease, 1% had severe disease, and 1% were in critical condition (Parri 2020).

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 (see below, pathophysiology, 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). Even in the severely immunosuppressed and in children with significant cardiac and pulmonary comorbidities COVID-19 can be overcome (Dinkelbach 2020).

In The European Surveillance System (TESSy) deaths among children aged below 15 years are rare, 4 out of 44,695 (0.009%) were reported. The rate of hospitalization was higher in children under the age of five especially in infants compared to persons aged 5-29. However, it is believed that the threshold for admission is lower in young children. A severe course requiring ad-

mission to ICU seems not to be more likely in younger children. The likelihood of being hospitalised was higher when children had an underlying condition, and a severe course was rare (https://covid19-surveillance-report.ecdc.europa.eu).

In a cross-sectional study including 48 children with COVID-19 (median age 13 years; admitted to 46 North American pediatric ICUs between March 14 and April 3, 2020), forty patients (83%) had significant pre-existing co-morbidities and 18 (38%) required invasive ventilation. Targeted therapies were used in 28 patients (61%, mainly HCQ). Two patients (4%) died and 15 (31%) were still hospitalized, with 3 still requiring ventilatory support and 1 receiving ECMO (Shekerdemian 2020). In an observational retrospective cohort study that included 177 children and young adults with clinical symptoms and laboratory confirmed SARS-CoV-2 infection treated between March 15 and April 30, 2020 at the Children's National Hospital in Washington, 44 were hospitalized and 9 were critically ill. Of these, 6/9 were adolescents and young adults > 15 years of age. Although asthma was the most prevalent underlying condition overall, it was not more common among patients with severe disease (DeBiasi 2020).

Although the natural course of COVID-19 is uneventful in most pediatric patients, a very small percentage can develop a potentially fatal severe hyperinflammatory state 2-4 weeks after acute infection with SARS-CoV-2 (Riphagen 2020). This hyperinflammatory state is termed as pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 (PIMS-TS) (or synonym Multisystem Inflammatory Syndrome in Children (MIS-C). Of the 570 MIS-C cases reported to the CDC by July 2020, 10 patients had died (1.8%) and 364 (63.9%) patients required treatment in an intensive care unit. Obesity was the most commonly reported underlying medical condition (Godfred-Cato 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 but age-dependent differences in ACE2 receptor expression may explain why outcomes differ in children versus adults (Bunyavanich 2020).

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-19 is unclear. SARS-CoV-2 infection of cardiac tissue can be a major contributor to fatal myocarditis (Dolhnikoff 2020, Prieto 2020).

An explanation for the milder disease course in children could be age-related differences in innate or adaptive immune responses to CoV-2 between adults and children. In the innate immune response to any virus, Type I (IFN α, IFN β) and type III (IFN Ω) interferons are the most important cytokines. In 659 patients (1 month to 99 years old) with life-threatening COVID-19 pneumonia, inborn defects in the type 1 IFN signaling were found in 23 unrelated patients (Zhang 2020). Moreover, neutralizing auto-antibodies to type I/III IFN were found in 101/987 patients with life-threatening COVID-10 pneumonia (Bastard 2020). These findings show that inborn defects in the IFN I/II pathway or auto-antibodies to IFN I/III may predispose to life-threatening COVID-19. Based on influenza animal models it has been proposed that BCG vaccination (for tuberculosis prevention, 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). A search of the BCG World Atlas and correlation with data of COVID-19 cases and death per country found that countries without universal policies of BCG vaccination (Italy, the Netherlands, USA) have been more severely affected compared to countries with universal and long-standing BCG policies and that BCG vaccination also reduced the number of reported COVID-19 cases in a country (Miyasaka 2020, Hauer 2020). Recent data from a large population-based study did not show decreased infection rates in Israeli adults aged 35 to 41

years who were BCG-vaccinated in childhood as compared to non-BCG-vaccinated. Data on the effect of BCG vaccination on COVID-19 disease severity are unavailable (Hamiel 2020).

In the adaptive response to any virus, 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. Regarding humoral immunity, IgG maternal antibodies are actively transferred to the child via placenta and/or IgA via breast milk. They 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. As shown in SARS CoV-1 it is likely that in SARS-CoV-2 a newly infected child will mount a significant humoral response with neutralizing IgM (within days) and IgG antibodies (within 1-3 weeks) to one of the immunodominant epitopes, e.g. the crown-like spike proteins giving the coronaviruses their name. Infections with non-SARS COV are very common in children (see above); however, to what extent previous infections with non-SARS coronaviruses may have led to protective cross-reactive antibodies is unclear.

Data regarding IgG and IgM seroprevalence and quality of the immune response in children are lacking. No human re-infections with CoV-2 have been demonstrated yet but overall it is not clear whether children mount a durable memory immune response to CoV-2. In summary, differences in the immune system such as more efficient innate and adaptive immunity to COV-2 (associated with better thymic function), cross-reactive immunity to common cold coronaviruses and differences in the ACE2 receptor expression as well as better overall health may be factors leading to a better COVID-19 outcome in children (Consiglio 2020).

Transmission

Studies on the risk of acquiring SARS-CoV-2 infection in children in comparison to adults have shown contradicting results (Mehta 2020, Gudbjartsson 2020, Bi 2020). The exact role that children play in the transmission of SARS-CoV-2 is not yet fully understood. Population based studies performed so far indicate that children might not play a major factor in the spreading of COVID-19 (Gudbjartsson 2020).

Vertical 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. 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, Chen 2020). Among the 24 infants born to women with COVID-19, 15 (62.5%) had detectable IgG and 6 (25.0%) had detectable IgM; nucleic acid test results were all negative. Among 11 infants tested at birth, all had detectable IgG and 5 had detectable IgM. IgG titers with positive IgM declined more slowly than those without (Gao 2020). In the PRIORITY study (n = 263), adverse outcomes, including preterm birth, NICU admission, and respiratory disease, did not differ between infants born to mothers testing positive for SARS-CoV-2 (n = 184) and those born to mothers testing negative (n = 79), suggesting that infants born to mothers infected with SARS-CoV-2 generally do well in the first 6-8 weeks after birth (Flaherman 2020).

Transmission of COVID-19 appears unlikely to occur if correct hygiene precautions are undertaken. In 1481 deliveries at three hospitals in New York City, 116 (8%) mothers tested positive for SARS-CoV-2; 120 neonates were identified and none were positive for SARS-CoV-2 (Salvatore 2020).

In another study from New York, 101 newborns of SARS-CoV-2 infected mothers no transmission was observed despite sleeping in the same room and breastfeeding (Dumitriu 2020). Initially it was thought that CoV-2 is not vertically transmitted, but in a more recent analysis of 31 mothers with SARS-CoV-2, SARS-CoV-2 genome was detected in one umbilical cord blood, two at-term placentas, one vaginal mucosa and one breast-milk specimen. Three cases of vertical transmission of SARS-CoV-2 have been documented (Fenizia 2020).

In a UK national population-based cohort study on SARS-CoV-2 infected pregnant women, twelve (5%) of 265 infants subsequently tested positive for SARS-CoV-2 RNA, six of them within the first 12 hours after birth (Knight 2020). Postpartum acquisition appears to be the most common mode of infection; in a recent review only 4/1141 neonates born to SARS-CoV-2 infected mothers were thought to have a congenital infection (Dhir 2020).

Horizontal transmission

Culture-competent SARS-CoV-2 has been grown from the nasopharynx of symptomatic neonates, children, and adolescents: 12 (52%) of 23 symptomatic SARS-CoV-2–infected children, the youngest being 7 days old. SARS-CoV-2 viral load and shedding patterns of culture-competent virus in the 12 symp-

tomatic children resembled those in adults. Systematic measurements of SARS-CoV-2 viral load measurements in children are lacking. Therefore, transmission of SARS-CoV-2 from children is plausible (L'Huillier 2020). SARS-CoV-2 in children is transmitted through family contacts and mainly through respiratory droplets (Garazzino 2020). In a study from France, child-to-child and child-to-adult transmission seems to be uncommon (Danis 2019). Prolonged exposure to high concentrations of aerosols may facilitate transmission (She 2020).

SARS-CoV-2 may theoretically 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 for as long as 2-4 weeks) (Cai 2020). However, direct evidence of a fecal-to-oral transmission has not yet been documented.

Onward transmission from children to others is low (Viner 2020, Merckx 2020). In a study from Milan, Itlay, in 83 children and 131 adults hospitalized and symptomatic in regard to COVID-19, adults were retrospectively more likely to be CoV-2 positive, asymptomatic carriers as compared to children (9% vs 1%) (Milani 2020).

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 chain reaction RT-PCR on respiratory secretions. 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.

In some patients, SARS-CoV-2 RNA is negative in respiratory samples while stool samples are still positive indicating that a viral gastrointestinal infection can last even after viral clearance in the respiratory tract. (Xiao 2020). Fecal testing may thus be of value in diagnosing COVID-19 in these patients.

As in other viral infections, a CoV-2 IgM and IgG seroconversion will appear in days (IgM) to 1-3 weeks (IgG) after infection and may or may not indicate protective immunity (still to be determined). Interestingly, asymptomatic

seroconversion has been hypothesized in a very small series of health workers (mean age 40 years) exposed to a child with COVID-19 in a pediatric dialysis unit (Hains 2020).

Serology may be useful in patients with clinical symptoms highly suggestive of SARS-CoV-2 who are RNA negative, i.e in children with pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 (PIMS-TS). If serology indicates protective immunity, this will be extremely important from a public health perspective, e.g. it will allow for strategic staffing in medical care and for the assessment of CoV-2 epidemiology (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: Acute infection

Children and adolescents

In a 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 in less than 10% of the children (Lu 2020). In the cohort 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). In a Korean case series of children with COVID-19, 20 children (22%) were asymptomatic during the entire observation period. Among 71 symptomatic cases, only 6 (9%) were diagnosed at the time of symptom onset while 47 children (66%) had unrecognized symptoms before diagnosis and 18 (25%) developed symptoms after diagnosis. Fifty-one percent had "mild" disease, 22% "moderate" disease and 2% "severe" disease. No patient required intensive care (Han 2020). A larger UK series reports on 651 children and young people aged less than 19 years. Median age was 4.6 years, 35% (225/651) were under 12 months old. 18% (116/632) of children were admitted to critical care. Six patients died in hospital, all of whom had profound comorbidity (Swann 2020).

A recent comprehensive systematic review analysed 131 studies in 7780 pediatric COVID-19 patients across 26 different countries (Hoang 2020). In this review 19,3% of the patients were asymptomatic, the most common symptoms were fever (59%), cough (55,9%), rhinorrhea (20%) and myalgia/fatigue (18,7%). The need for intensive care treatment was low (3,3%).

In 52 hospitalized children from London, UK, renal dysfunction was frequent especially in those with pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2. 24 (46%) had elevated serum creatinine, and 15 (29%) met the diagnostic criteria for acute kidney injury (Stewart 2020).

In a case series of 4 children with PIMS-TS (see below) from London, UK, neurological symptoms were described (encephalopathy, headaches, brainstem and cerebellar signs, muscle weakness, reduced reflexes) with signal changes in the splenium of the corpus callosum on neuroimaging and required intensive care admission for the treatment of COVID-19 pediatric multisystem inflammatory syndrome (Abdel-Mannan 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 radiological signs of pneumonia. In one child disseminated intravascular coagulation was described but eventually all children had stable vital signs three weeks after the infection (Zeng L 2020). In a second cohort, 9 infants aged 1 month to 9 months were described without any severe complications (Wei 2020). Whether there are long-term complications of COVID-19 in these newborns and infants is unclear at this stage of the pandemic.

Pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 (PIMS-TS) (or synonym Multisystem Inflammatory Syndrome in Children (MIS-C) or Kawasaki-like Disease

While most children with COVID-19 have a very mild disease, in April 2020 clinicians from the UK, France, Italy, Spain and the US reported on children with a severe inflammatory syndrome with Kawasaki-like features, some of whom had tested positive for CoV-2, while others not. Prior to this, Jones had described the case of a six-month-old baby girl with fever, rash and swelling characteristic of a rare pediatric inflammatory condition, Kawasaki disease (Jones 2020).

Eight patients from the UK and 10 patients from Bergamo in Italy with features of Kawasaki disease were published including one death in a 14-year-old boy in the UK during the SARS-CoV-2 epidemic (Riphagen 2020, Verdoni 2020). Some children presented with vasculitic skin rash (Schneider 2020). In Bergamo, the region with the highest infection rate in Italy, a 30-fold increased incidence of Kawasaki disease has been reported following the SARS-CoV-2 epidemic (Verdoni 2020). Of 21 children and adolescents from London, UK (19 with recent SARS-CoV-2 infection), 12 (57%) presented with Kawasaki disease shock syndrome, 16 (76%) with myocarditis, 17 (81%) required intensive care support. All had noticeable gastrointestinal symptoms and high levels of inflammatory markers, received intravenous immunoglobulin and 10 (48%) corticosteroids; the outcome was favourable in all (Toubiana 2020).

In the UK, 78 of the PIMS-TS cases reported 36 (46%) were invasively ventilated, 28 (36%) had evidence of coronary artery abnormalities, three children needed ECMO and two children died (Davies 2020).

In another study from the UK, 50% of the 58 "PIMS-TS" cases developed shock and required inotropic support or fluid resuscitation; 22% met diagnostic cri-

teria for Kawasaki disease; and 14% had coronary artery dilatation or aneurysms (Whittaker 2020).

In a US MIS-C study on 186 patients 131 (70%) were positive for SARS-CoV-2 by RT-PCR or antibody testing. Detailed analysis of clinical manifestation revealed the gastrointestinal system (92%), cardiovascular (80%), hematologic (76%), mucocutaneous (74%), and respiratory involvement (70%). In total, 148 patients (80%) received intensive care, 37 (20%) received mechanical ventilation, and 4 (2%) died. Coronary-artery aneurysms were documented in 15 patients (8%), and Kawasaki disease–like features were documented in 74 (40%) (Feldstein 2020). In the largest cohort to date,

570 US MIS-C patients were reported as of July 29. A total of 203 (35.6%) of the patients had a typical MIS-C clinical course (shock, cardiac dysfunction, abdominal pain, and markedly elevated inflammatory markers) and almost all had positive SARS-CoV-2 test results (Class 1). The remaining 367 (64.4%) of MIS-C patients (Class 2 and 3) had manifestations that appeared to overlap with acute COVID-19 or had features of Kawasaki disease. 364/570 patients (63.9%) required care in an intensive care unit. Ten patients (1.8%) died. Approximately two thirds of the children had no pre-existing underlying medical conditions (Godfred-Cato 2020).

In summary, the pathophysiological overlap between COVID-19-associated inflammation and Kawasaki disease is not yet clear, their features are summarized in Table 2. The main pathophysiological differences appear to be an IL17A-driven inflammation in Kawasaki disease (KD) and a stronger endothel activation in coronary artery involvement in MIS-C. In both, MIS-C and KD autoantibodies may play an important role and MIS-C patients show distinct CD4 subset abnormalities. (Consiglio 2020).

Table 2. Features of Kawasaki Disease and pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2

	Kawasaki (Hedrich 2017, ECDC 2020) (previously called mucocutaneous lymph -node syndrome)	**PIMS-TS** (pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2 or **MIS-C** (multisystem inflammatory syndrome in children) (Verdoni 2020; Riphagen 2020, https://covid19-surveillance-report.ecdc.europa.eu/) **"Kawasaki-like disease"**
Epidemiology	Incidence 5–19/100,000 annually < 5 years of age (EU, US), in north-east Asia higher; seasonal increase in winter/spring, geographic wave-like spread of illness during epidemics (Rowley 2018)	Incidence unknown 230 suspected cases temporally associated with COVID-19 reported to ECDC by May 15th (EU/EEA, UK). More common in afro-caribbean descent, obesity? (Riphagen 2020)
Age, sex	>90% < 5 years of age, more males	5-15 years of age, sex distribution unclear
Etiology	Unknown, hypothesis: infection with common pathogens, e.g. bacteria, fungi and viruses which cause immune-mediated damage (Dietz 2017) (Jordan-Villegas 2010, Kim 2012, Turnier 2015). Genetic factors (increased frequency in Asia and among family members of an index case)	Unknown, no working hypothesis yet. Hyperinflammation/shock associates with immune response to SARS-CoV-2. In CoV-1 antibody-dependent enhancement (ADE): presence of antibodies can be detrimental, enable the virus to spread (demonstrated in SARS-CoV)
Case definition	fever ≥5 days, combined with at least 4 of the 5 following items 1.Bilateral bulbar conjunctival injection 2. Oral mucous membrane changes, including injected or fissured lips, injected pharynx, or strawberry tongue 3. Peripheral extremity changes, including erythema of palms or soles, edema of hands or feet (acute phase) or periungual desquamation (convalescent phase) 4. Polymorphous rash	1. Persistent fever, inflammation (neutrophilia, elevated CRP and lymphopenia) and single or multi-organ dysfunction (shock, cardiac, respiratory, renal, gastrointestinal or neurological disorder) with other additional clinical, laboratory or imagining and ECG features. Children fulfilling full or partial criteria for Kawasaki Disease may be included 2. Exclusion of any other microbial cause, including bacterial sepsis, staphylococcal or streptococcal shock syndromes, infections associated with myocarditis such as enterovirus 3. SARS-CoV-2 PCR testing positive or negative (Royal College of Paediatrics and Child Health)

Table 2. Features of Kawasaki Disease and pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2

	5. Cervical lymphadenopathy (McCrindle 2017) Children suspected of having KD who do not fulfill diagnostic criteria may have incomplete or atypical KD (Cimaz 2009)	
CoV-2 status in most cases	CoV-2 Ag (PCR); Abs (Elisa) negative	CoV-2 Ag (PCR) negative and Abs (Elisa) positive
Typical Lab	Marked Elevation of acute-phase reactants (eg, C-reactive protein [CRP] or erythrocyte sedimentation rate [ESR]) Thrombocytosis (generally after day 7 of illness Leukocytosis, left-shift (increased immature neutrophils)	Marked elevation of acute phase reactants CRP, ESR Thrombocytopenia Leucopenia Lymphopenia Hyperferritinemia Elevated myocarditis markers Troponin, pro-BNP
Acute Complications	Kawasaki disease shock syndrome (KSSS) (rare), features of macrophage activation syndrom, MAS (rare), coronary artery abnormalities, mitral regurgitation, prolonged myocardial dysfunction, disseminated intravascular coagulation (Kanegaye 2009) Gastrointestinal complications (Ileitis, vomiting, abdominal pain) rare	Shock (common), features of macrophage activation syndrome (common), myocardial involvement evidenced by markedly elevated cardiac enzymes (common), myocardial infarction, aneurysms, disseminated intravascular coagulation Gastrointestinal complications (Ileitis, vomiting, abdominal pain) are very common
Long term Complications	Artery abnormalities (aneurysms of mid-sized arteries, giant coronary artery aneurysms CAAs)	Aneurysms
Management	High-dose intravenous immunoglobulin (IVIG) (2g/kg) first-line treatment; effective in reducing the risk of coronary	So far, most patients published were treated with high dose IVIG, glucocorticoids, ASS (Verdoni 2020, Riphagen 2020, Ahmed 2020) IVIG resistance requiring adjunctive steroid

Table 2. Features of Kawasaki Disease and pediatric inflammatory multisystem syndrome temporarily associated with SARS-CoV-2

	artery disease when administered within 10 days of onset of fever. In addition, acetylsalicylic acid, glucocorticoids and anti-TNF monoclonal antibodies have been used	treatment is common (Verdoni 2020,) Management on the pediatric intensive care unit is often necessary: progression to vasoplegic shock is common Hemodynamic support, treatment with noradrenaline and milrinone, mechanical ventilation is often required (Riphagen 2020)
Prognosis	Self-limited vasculitis lasting for an average of 12 days without therapy. Without timely treatment, CAAs, and in particular aneurysms, can occur in up to 25% of children	Overall prognosis not yet clear More severe course than KD Potentially fatal in individual cases

Management

National guidelines and guidance documents have been published from different medical societies in China, North America, Italy, UK and Germany (https://rcpch.ac.uk; Venturini 2020, Chiotos 2020, Liu 2020; https://www.rcpch.ac.uk/key-topics/covid-19; https://dgpi.de/stellungnahme-medikamentoese-behandlung-von-kindern-mit-covid-19/)

Infection control in the medical setting

Early identification of COVID-19 and quarantine of contacts is imperative. In the in- and out-patient 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. 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 is protected by non-pharmacological interventions (wearing FFP-2 masks, gowns, etc.) and maintains distance and is cohorted themselves (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 the nursing of the child.

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 strict hygiene as much as possible in this mother-child setting.

During peak phases of the COVID-19 pandemic, 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.

Infection control outside the medical setting

Some of the interventions to control the COVID pandemic have caused significant damage to children and adolescents. The description of their impact is beyond the scope of this artivcel and reviewed elsewhere.[6]

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 is unclear in COVID-19. 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,

[6] Recommendations regarding attendance to kindergartens and schools have been published (Cohen 2020).

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 decided, children should be included into clinical trials (https://www.clinicaltrialsregister.eu) if at all possible. However, there are 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 3)).

A panel of pediatric infectious diseases physicians and pharmacists from North American institutions published an initial guidance on the use of antivirals for children with SARS. It is advised to limit antiviral therapy to children in whom the possibility for benefit outweighs the risk of toxicity and remdesvir is the preferred agent (Chiotos 2020).

Inhibitors of viral RNA synthesis

Remdesivir is available as 150 mg vials. Child dosing is

- < 40 kg: 5 mg/kg iv loading dose, then 2,5 mg/kg iv 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 metabolites 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 3. 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). 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 end point in a controlled clinical trial. Despite the fact that there is long experience with LPV/r in HIV, **it is not advised to use it in children with COVID-**

19 as it does not appear to be effective at all (see *Treatment* chapter, page 331

Inhibitors of viral entry

Hydroxychloroquine (HCQ, Quensyl®), Chloroquine (CQ, Resochin junior®, Resochin®) The experience among pediatricians with HCQ/CQ (except pediatriciancs working with malaria) is very limited. Authorities in the US are now warning about a widespread use of HCQ/CQ in COVID-19 (https://mailchi.mp/clintox/aact-acmt-aapcc-joint-statement). **It is not advised to use HCQ or CQ in children with COVID as neither drug appears to be effective at all (see *Treatment* chapter, page 331).**

Immunomodulatory drug treatment

The rational for immunomodulation in COVID-19 adult patients comes from a high expression of pro-inflammatory cytokines (Interleukin-1 (IL-1) and interleukin-6 (IL-6)), 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. In children, the proinflammatory cytokines TNF and IL-6 do not appear to be central in CoV-2 induced hyperinflammation (Consiglio 2020). **Blocking IL-1 or IL-6** can be successful in children with (auto) inflammatory disease (reviewed in Niehues 2019), but 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 they are resistant to other therapies, anakinra may be an option as IL-1 seems to play a role in endothelial activation.**

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 iv 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. Initially, the use of corticosteroids in children and adults with CoV-induced ARDS was controversial (Lee 2004, Arabi 2018, Russell 2020). Only in severe and critically ill children the use of dexamethasone appears justified in children. In adults, the use of steroids in severe COVID-19 is clearly beneficial although the corticosteroid-induced decrease of antiviral immunity (e.g. to eliminate CoV-2 viruses) might be theoretically disadvantageous. Data supporting the use of steroids in children with CoV-induced ARDS are lacking.

Only in severe and critically ill children might the use of dexamethasone appear justified.

Most patients with pediatric inflammatory multisystem syndrome associated with SARS-CoV-2 (PIMS-TS) published so far were treated with high dose IVIG and methylprednisolone (Verdoni 2020, Riphagen 2020). In these patients, features of macrophage activation syndrome and IVIG resistance were common, requiring adjunctive steroid treatment (Verdoni 2020). Clearly, any child severly affected by CoV-2 will need steroids at some stage.

Tocilizumab (Roactemra®) is available in 80/200/400 mg vials (20 mg/ml). Dosing is

- < 30 kg: 12 mg/kg iv QD, sometimes repeated after 8 hrs
- ≥ 30 kg: 8mg/kg iv QD iv (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 sc QD daily as long as hyperinflammation persists. Thereafter, dose reduction by 10-30% per day. Higher dosage (> 4mg/kg-10mg/kg; max 400mg/d) may be necessary in patients with PIMS-TS. 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

There are no systematic data on the use of convalescent plasma in children yet, but in a child with acute lymphoblastic leukemia and a young adult with a SCID (Severe Combined Immunodeficiency) phenotype and a high CoV-2 viral load, administration of convalescent plasma resulted in complete viral suppression (Shankar 2020, unpublished observation). 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 generally not 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 I/III 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).

PIMS / MIS-C

Based on the information published so far, most patients were treated with high dose intravenous Immunglobulin (see Table 2) and corticosteroids (Verdoni 2020). More data are needed to determine the optimal treatment strategies for patients with MIS-C.

DOI: Tim Niehues has received authorship fees from uptodate.com (Wellesley, Massachusetts, USA) and reimbursement of travel expenses during consultancy work for the European Medicines Agency (EMA), steering committees of the PENTA Paediatric European Network for Treatment of AIDS (Padua, Italy), the Juvenile Inflammatory Cohort (JIR) (Lausanne, Switzerland), and, until 2017, the FIND-ID Initiative (supported by the Plasma Protein Therapeutics Association [PPTA] [Brussels, Belgium]).

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14. The First Eight Months

Bernd Sebastian Kamps

Dezember - March

Sunday, 1 December

According to a retrospective study published in The Lancet on 24 January 2020[7], the earliest laboratory confirmed case of COVID-19 in Wuhan was in a man whose symptoms began on 1 December 2019. No epidemiological link could be found with other early cases. None of his family became ill.

Thursday, 12 December

In **Wuhan**, health officials start investigating a cluster of 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.

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 Mar-

[7] Huang, Chaolin et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China January 24, 2020 https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30183-5/fulltext#%20

ket. 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 about the outbreak.

Thursday, 1 January

The Huanan Seafood Wholesale Market is shut down.

Friday, 3 January

While examining bronchoalveolar lavage fluid collected from hospital patients between 24 and 29 December, Chinese scientists at the National Institute of Viral Disease Control and Prevention ruled out the infection with 26 common respiratory viruses, determined the genetic sequence of a novel β-genus coronaviruses (naming it '2019-nCoV') and identified three distinct strains.[8]

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 issues an alert 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/

[8] Notes from the Field: An Outbreak of NCIP (2019-nCoV) Infection in China — Wuhan, Hubei Province, 2019–2020, China CDC Weekly, 2020, 2(5): 79-80 http://weekly.chinacdc.cn/en/article/id/e3c63ca9-dedb-4fb6-9c1c-d057adb77b57

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. Two coronavirus have also been responsible for human outbreaks of SARS and MERS. 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.

Friday, 10 January

The gene sequencing data of the new virus was posted on Virological.org by researchers from Fudan University, Shanghai. A further three sequences were posted to the Global Initiative on Sharing All Influenza Data (GISAID) portal.

On 10 January 2020, Li Wenliang, coronavirus whistleblower, started having symptoms of a dry cough. Two days later, Wenliang started having a fever and was admitted to the hospital on 14 January 2020. His parents also contracted the coronavirus and were admitted to the hospital with him. Wenliang tested negative several times until finally testing positive for the coronavirus on 30 January 2020.

Sunday, 12 January

Using the genetic sequence of the new coronavirus made available to WHO, laboratories in different countries start producing specific **diagnostic PCR tests**.

The Chinese government reports that there is no clear evidence that the virus passes easily from person to person.

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, and South Korea report cases over the following 10 days.

Tuesday, 14 January

WHO tweeted that "preliminary investigations conducted by the Chinese authorities have found no clear evidence of human-to-human transmission of the novel coronavirus (2019-nCoV) identified in Wuhan, China". On the same day, WHO's Maria Van Kerkhove said that there had been "limited human-to-human transmission" of the coronavirus, mainly small clusters in families, adding that "it is very clear right now that we have no sustained human-to-human transmission"[9]

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.

After two medical staff were infected in Guangdong, the investigation team from China's National Health Commission confirmed for the first time that the coronavirus can be transmitted between humans. [10]

Wednesday, 22 January 2020

A WHO China office field mission to Wuhan issued a statement saying that there was evidence of human-to-human transmission in Wuhan, but more investigation was needed to understand the full extent of transmission.[11]

9 WHO says new China coronavirus could spread, warns hospitals worldwide". Reuters. 14 January 2020.

10 https://www.theguardian.com/world/2020/jan/20/coronavirus-spreads-to-beijing-as-china-confirms-new-cases

11 https://www.who.int/china/news/detail/22-01-2020-field-visit-wuhan-china-jan-2020

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.

The WHO IHR (2005) Emergency Committee convened on 22-23 Janaury acknowledged that human-to-human transmission was occurring with a preliminary R0 estimate of 1.4-2.5 and that 25% of confirmed cases were reported to be severe. However, the Committee felt that transmission was limited and there was "no evidence" of the virus spreading at community level outside of China. Since the members could not reach a consensus, the committee decided that it was still too early to declare a Public Health Emergency of International Concern (PHEIC) and agreed to reconvene in approximately ten days' time. [12]

A scientific preprint from the Wuhan institute of Virology, later published in *Nature,* announced that a bat virus with 96% similarity had been sequenced in a Yunnan cave in 2013. The sequence is posted the next day on public databases.[13] It is confirmed that the novel coronavirus uses this same entry receptor as SARS-CoV.

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.

The first confirmed evidence of human-to-human transmission outside of China was documented by the WHO in Vietnam.[14]

France reported its first three confirmed imported cases, the first occurrences in the EU.[15]

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:

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[12] https://www.who.int/news-room/detail/23-01-2020-statement-on-the-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov)

13 Zhou, Peng et al. "A pneumonia outbreak associated with a new coronavirus of probable bat origin". Nature. 579 (7798): 270–273 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7095418/

14 "Novel Coronavirus (2019-nCoV) SITUATION REPORT - 4" WHO 24 January 2020.

15 "Coronavirus : un troisième cas d'infection confirmé en France". Le Monde.fr (in French). 24 January 2020.

https://doi.org/10.1056/NEJMoa2001017). They describe sensitive assays to detect viral RNA in clinical specimens.

Huang et al. publish on *The Lancet* the **clinical features** of 41 patients (full-text: doi.org/10.1016/S0140-6736(20)30185-9). The report indicated the risk of contagious infection without forewarning signs during the incubation period and suggested a "pandemic potential" for the new virus.

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.

Monday, 27 January

In Germany**,** the first cluster of infections with person to person transmission from asymptomatic patients in Europe **was reported.** The source of infection was an individual from Shanghai visiting a company in Bavaria[16]. She developed symptoms on the way back to China. Contacts at the company were tested and transmission was confirmed to asymptomatic contact**s** but also to people who had no direct contact with the index patient. Authors state that "The fact that asymptomatic persons are potential sources of 2019-nCoV infection may warrant a reassessment of transmission dynamics of the current outbreak."[17]

Tuesday, 28 January

WHO DG Dr. Tedros Adhanom Ghebreyesus met China President Xi Jinping in Beijing. They shared the latest information on the outbreak and reiterated their commitment to bring it under control. The WHO delegation highly appreciated the actions China has implemented in response to the outbreak, its

[16] Böhmer MM, Buchholz U, Cormann VM: **Investigation of a COVID-19 outbreak in Germany resulting from a single travel-associated primary case: a case series.** Published online May 15, 2020. Full-text: https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30314-5/fulltext

[17] 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

speed in identifying the virus and openness to sharing information with WHO and other countries.[18]

Thursday, 30 January

On the advice of the IHR Emergency Committee, WHO DG declared a Public Health Emergency of International Concern and advised "all countries should be prepared for containment, including active surveillance, early detection, isolation and case management, contact tracing and prevention of onward spread of 2019-nCoV infection, and to share full data with WHO." WHO had received reports of 83 cases in 18 countries outside China and that there had been evidence of human-to-human transmission in 3 countries.

China reports 7,711 cases and 170 deaths. The virus has now spread to all Chinese provinces.

Giuseppe Conte, Italy's Prime Minister, confirms the first two COVID-19 imported cases in Italy.

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.

[18] https://www.who.int/news-room/detail/28-01-2020-who-china-leaders-discuss-next-steps-in-battle-against-coronavirus-outbreak

Friday, 7 February

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

Saturday, 8 February

The French Health Minister confirmed that a cluster of 5 COVID-19 cases were detected in a ski resort in the French Alps. The index patient was a UK citizen who had traveled to Singapore on 20-23 January and then spent four days (24-28 January) in a chalet in Contamines-Montjoie, in Haute-Savoie. He tested positive upon return to England. Four contacts in the same chalet tested positive, including a 9-year old boy who was attending a local school. None of the child's contacts in school or at home became infected.

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)** and that the new virus will be called SARS-CoV-2.

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.

Thursday, 13 February

China changed the COVID-19 case definition to include clinical (radiological) diagnosis of patients without confirmatory test. As a result, Hubei reported 14,840 newly confirmed cases, nearly 10 times more than the previous day, while deaths more than doubled to 242. WHO indicated that for consistency it would report only the number of laboratory-confirmed cases.[19]

[19] https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200213-sitrep-24-covid-19.pdf

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 tested positive for SARS-CoV-2 and was admitted to the intensive care unit (ICU) in **Codogno** Hospital (Lodi, Lombardy, Italy). The symptomatic patient had visited the hospital the day before but was not tested as he did not meet the suspected case epidemiological criteria (no link with China). His wife, 5 hospital staff, 3 patients and several contacts of the index patients also tested positive to the COVID-19. Over the next 24 hours, the number of reported cases would increase to 36, many without links to the Codogno patient or previously identified positive cases. A first COVID-19 death in a 78-year-old man was also reported. It is the beginning of the Italian epidemic. jamanetwork.com/journals/jama/fullarticle/2763188

Saturday, 22 February

South Korea reports a sudden spike of 20 new cases of coronavirus infection, raising concerns about a potential "super spreader" who has already infected 14 people in a church in the south-eastern city of Daegu.

Sunday, 23 February

Italy confirms 73 new cases, bringing the total to 152, and a third death, making Italy the third country in the world by number of cases, after China and South Korea. A "red zone" area around Codogno is created, isolating 11 municipal areas. Schools are closed.

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 WHO 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.

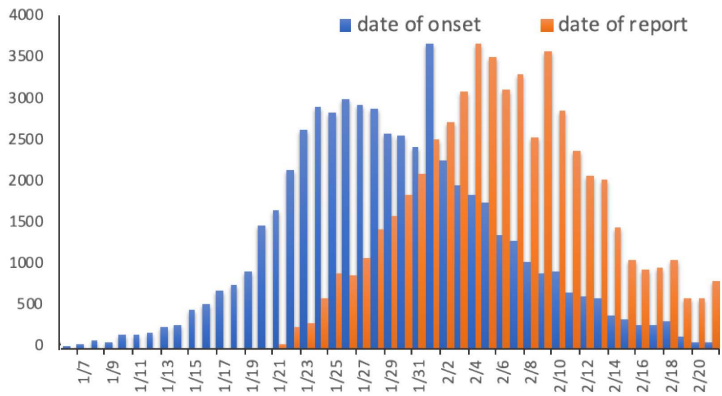


Figure 1. COVID-19 cases in China, January/February 2020. Epidemic curves by symptom onset and date of report on 20 February 2020 for laboratory confirmed COVID-19 cases for all of China. Modified from *Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19)*. 16-24 February 2020. 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."

P.S. On 28 March, The Guardian would ask why this person failed the biggest test of his life.

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.

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

A president on Twitter: "So last year 37,000 Americans died from the common Flu. It averages between 27,000 and 70,000 per year. Nothing is shut down, life & the economy go on. At this moment there are 546 confirmed cases of CoronaVirus, with 22 deaths. Think about that!" (The Guardian)

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

With more than 118,000 COVID-19 cases in 114 countries and 4,291 deaths, WHO DG 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."

P.S. Five weeks later, The Guardian would still ask, "How did Britain get its coronavirus response so wrong?"

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, going to 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, avoiding 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!

Sunday, 22 March

Byung-Chul Han publishes *La emergencia viral y el mundo de mañana* (El País): "Asian countries are managing this crisis better than the West. While there you work with data and masks, here you react late and borders are opened."

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

America First: 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 and the Boston Globe ask who might have blood on their hands in the current pandemic. The evolution of the US epidemic is being described as 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%).

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.

April

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.

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.

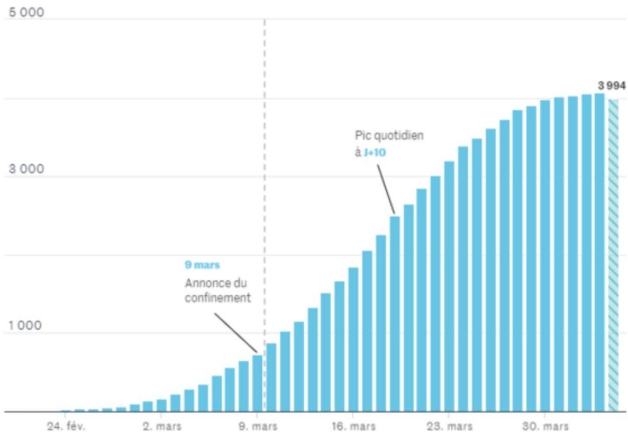


Figure 2. Patients treated in intensive care units in Italy. For the first time since the beginning of the epidemic, the number decreases on 4 April.
Souce: Le Monde

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.

The Guardian publishes a well-documented timeline: "Coronavirus: 100 days that changed the world."

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.

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?

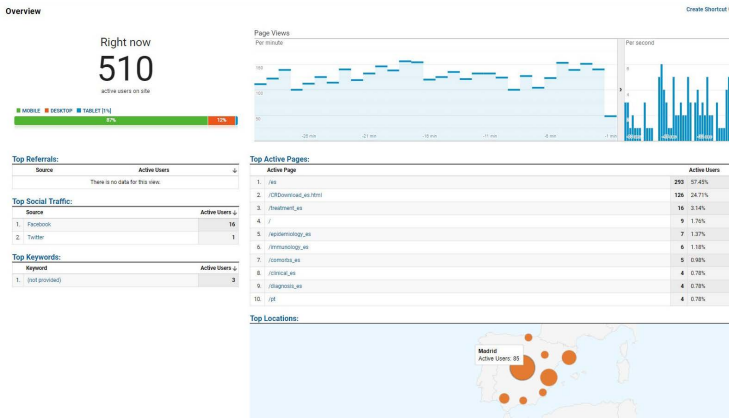


Figure 3. Google Analytics data for www.CovidReference.com on 10 April. At one moment, more than 500 people, mostly from Spain, were visiting the website simultaneously.

Saturday, 11 April

More than **400 of 700 long-term care facilities** (*EHPAD* in French, ***E**tablisse-ment 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

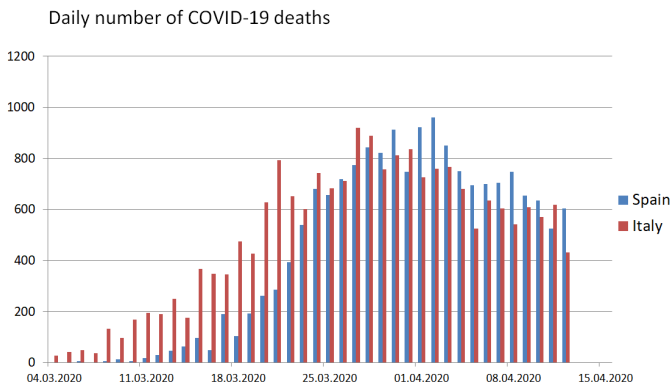


Figure 4. Daily number of COVID-19 deaths in Italy (red) and Spain (blue).

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.

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 roadmap for the coming weeks and months:

- 1 May: All stores, shopping malls and hairdressers reopen (see also the April 3 entry, page 469).
- 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 eurozone, 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!

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 is. Among the 1760 sailors, 1,046 (59%) are positive for SARS-CoV-2, 500 (28%)

present symptoms, 24 (1.3%) sailors are hospitalized, 8 on oxygen therapy and one in intensive care.

Saturday, 18 April

Chancellor Angela Merkel makes a television speech, her first in over 14 years in office. She describes the coronavirus crisis "as the greatest challenge since the Second World War" and exhorts the Germans: "It is serious. Take it seriously."

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 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

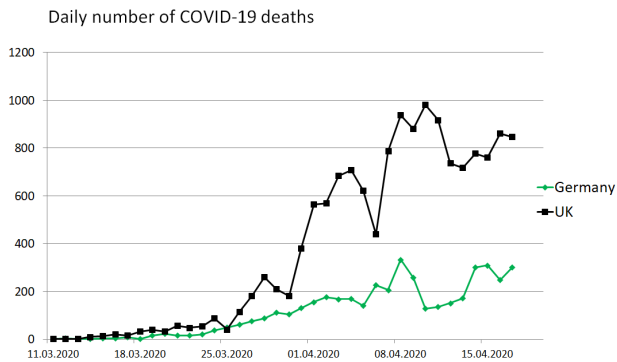


Figure 5. Daily number of COVID-19 deaths in Germany (green) and the United Kingdom (black).

Air traffic in Europe has plummeted more than 95% as nicely shown by this YouTube video by The Guardian: https://www.youtube.com/watch?v=lOVP2o3c4Gw

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 US dollars (-34 euros). After nearly two months of continu-

ous 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.

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.

Tuesday, 21 April

The Spanish newspaper El País publishes an intelligible overview of the battle between SARS-CoV-2 and the human body: "Así es la lucha entre el sistema inmune y el coronavirus."¡Fantástico!

Cancer Research UK reports that every week, 2,300 people with cancer symptoms are no longer examined. Screening examinations for breast and uterine cancer of over 200,000 women per week have been cancelled. According to The British Heart Foundation, 50 percent fewer people suspected of having a heart attack attended hospital emergency rooms in March. A 50% drop would be "equivalent to approximately 5000 of the expected people every month, or more than 1100 people every week, with possible heart attack symptoms not being seen in emergency departments." Will we discover a hidden epidemic of COVID-19-related morbidity and mortality with millions of people dying not from coronavirus, but from other, actually treatable diseases?

Thursday, 23 April

Pandemic hilarity, as a president known for his poor science record stammers speculations about "injecting" "disinfectant" to cure COVID-19.

Sunday, 26 April

The city of Wuhan announces that all remaining COVID-19 cases have been discharged from the hospitals.

Monday, 27 April

Are genes determining coronavirus symptoms? After studying 2,633 identical and fraternal twins who were diagnosed with COVID-19, a group from King's College London reports that COVID-19 symptoms appear to be 50% genetic

(fever, diarrhea, delirium and loss of taste and smell)[20]. It is as yet unclear whether and to what extent reported deaths of identical twins can be attributed to genetic factors.

[20] Williams FMK et al. **Self-reported symptoms of covid-19 including symptoms most predictive of SARS-CoV-2 infection, are heritable.** MedRxiv 27 April (accessed 8 May 2020). Abstract: https://www.medrxiv.org/content/10.1101/2020.04.22.20072124v2

May

Friday, 1 May

A new SARS-CoV-2 test could be able to identify virus carriers before they are infectious, according to a report by The Guardian. The blood-based test would be able to detect the virus's presence as early as 24 hours after infection – before people show symptoms and several days before a carrier is considered capable of spreading it to other people.

Sunday 3 May

Roche gets US Food and Drug Administration emergency use approval for its antibody test, **Elecsys** Anti-SARS-CoV-2, which has a specificity rate of about 99.8% and a sensitivity rate of 100%.

Monday, 4 May

Italy is cautiously easing lockdown measures. People can go jogging but may not go to the beach; they may surf but now swim; and they can visit 6th grade relatives, but not friends, lovers or mistresses.

A French hospital that retested old samples from pneumonia patients discovers that it treated a man with the coronavirus as early as 27 December, a month before the French government confirmed its first cases.

Researchers from Bonn University, Germany, report a sero-epidemiological study of 919 people from Gangelt, a small German town which was exposed to a super-spreading event (carnival festivities). 15.5% were infected, with an estimated infection fatality rate of 0.36%. 22% of infected individuals were asymptomatic.

Tuesday, 5 May

Neil Ferguson, epidemiologist at the Imperial College, resigns his post as member of the British government's Scientific Advisory Group for Emergiences (SAGE) over an "error of judgement". A newspaper had reported that he did not respect the rules of confinement (which he himself had contributed to establishing!) by receiving at least twice a 38-year-old woman at his home.

Anthony Fauci, the director of the United States National Institute of Allergy and Infectious Diseases, says that there is no scientific evidence to back the

theory that the coronavirus was made in a Chinese laboratory or leaked from a laboratory after being brought in from the wild (CGTN).

Wednesday, 6 May

The official COVID-19 death toll in the UK exceeds 30,000.

Thursday, 7 May

According to data released by the US Department of Labor, more than 33 million Americans have filed for initial jobless claims. This corresponds roughly to 21% of the March labor force.

Only 15 countries in the world have not officially reported a case of COVID-19 to WHO, namely: North Korea, Turkmenistan, Kiribati, Marshall Islands, Micronesia, Samoa, Salomon Island, Tonga, Tuvalu, Vanuatu, Cook Island, Nauru, Niue, Palau and Lesotho. (We know North Korea is cheating, and Turkmenistan and Lesotho cannot deny for long... It's a true pandemic!)

According to figures by the Office of National Statistics, black people are more than four times more likely to die from COVID-19 than white people.

Friday, 8 May 2020

After pipedreams (German: Hirngespinste; French: élucubrations; Italian: visioni; Spanish: fantasías) about hydroxychloroqine and injecting desinfectants, today is the day where COVID-19 will "go away without vaccine". The sad developments of the coronavirus pandemic have now accumulated sufficient evidence that the individual doesn't believe himself what he is saying. The carefully timed and well-orchestrated ungrammatical utterings just obey one supreme life mission: continue staying in the news. Alas, there is an even more tragic aspect to the drama: Why on Earth do the world's media insist on talking about this individual? Why can't we read the news without seeing his face every single day? Why couldn't we simply *totschweigen* him? (Totschweigen is a superbly descriptive German verb: 1. tot *dead*; 2. schweigen *to be silent*; 3. totschweigen *make someone dead silent* – English: to hush up; French: passer sous silence; Italian: fare come se non esistesse; Portuguese: não falar em alguém.)

Today, we make a funereal promise: we'll never talk about the individual again, not even on the day he dies.

Sunday, 10 May

Italians are looking on aghast at the UK's coronavirus response, says The Guardian. Is it really no accident that Britain and America are the world's biggest coronavirus losers?

Everything you always wanted to know about false negatives and false positives* (*but were afraid to ask) is now summarized in 10 steps to understand COVID-19 antibodies. The colors will help you memorize true and false negatives and positives.

Spain's best newspaper El País publishes '*ccu ccg ccg gca – The 12 letters that changed the world*.' (If you read Spanish, take a look.)

Monday, 11 May

France eases lockdown restrictions among a sense of incertainty. The newspaper Le Monde reports that according to official figures 8,674 new positive tests for SARS-CoV-2 were registered between May 1 and 9. Epidemiologist Daniel Lévy-Bruhl, head of the respiratory infections unit of Santé Publique France (Public Health France) estimates that the real figures are probably twice or three times as high (3,000 to 4,000 new infections each day) – despite barrier gestures, social distancing and general confinement.

Tuesday, 12 May

The MMWR publish a report about a high SARS-CoV-2 attack rate following exposure at a choir practice.

Wednesday, 13 May

There is evidence that **China** is **censoring COVID Reference**. Google Analytics data of two dozen websites, both medical (Amedeo, Free Medical Journals, FreeBooks4Doctors) and non-medical (TheWordBrain, Ear2Memory, GigaSardinian, GigaMartinique, SardoXSardi, Polish Yiddish and ItalianWithElisa, among others) show that by number of visitors, China was always among the *Top 10 countries*, generating between 3.3% and 14.8% of website traffic (see https://covidreference.com/censorship).

Not so with COVID Reference. Six weeks after the launch of COVID Reference, China is 27th, after Paraguay, accounting for 0.39% of global traffic. Is someone standing on the data line between COVID Reference and China (Figure 6)?

Figure 6. Google Analytics data for www.CovidReference.com on 13 May. Six weeks after the launch of COVID Reference, China is 27th, after Paraguay and right before the Netherlands and Russia.

Friday, 15 May

In a memorable blog entry for the British Medical Journal, Paul Garner, professor of infectious diseases at Liverpool School of Tropical Medicine, discusses his COVID-19 experience as having "been through a roller coaster of ill health, extreme emotions, and utter exhaustion".

A video experiment using black light and a fluorescent substance demonstrates how quickly germs can be spread in environments such as restaurant buffets and cruise ships: www.youtube.com/watch?v=kGQEuuv9R6E.

Saturday, 16 May

A new highly transmissible and potentially deadly virus is detected in Germany: **SADS**, Severe Acute Dementia Syndrome. The new syndrome manifests as an irrepressible desire to ignore the danger of COVID-19. In several German cities, an improbable alliance takes to the streets – left- and right-wing extremists, antisemites, conspiracy theorists and anti-vaxxers –, claiming the right to live and to die without social distancing and face masks. The German Government immediately informs WHO.

Monday, 18 May

Merkel and Macron announce a 500,000 million euro aid plan for the reconstruction of Europe (El País).

Moderna announces that its experimental vaccine mRNA-1273 has generated antibodies in eight healthy volunteers ages 18 to 55. The levels of neutralizing antibodies matched or exceeded the levels found in patients who had recovered from SARS-CoV-2 infection (The Guardian).

Wednesday, 20 May

After an outbreak of coronavirus, Chinese authorities seal off the city of Shulan, a city of 700,000 close to Russian border, imposing measures similar to those used in Wuhan (The Guardian).

Google and Apple release their Exposure Notification System to notify users of coronavirus exposure: https://www.google.com/covid19/exposurenotifications.

We discover a website which shows where infected people in Hong Kong are at all times: https://chp-dashboard.geodata.gov.hk/covid-19/en.html (Figure 7). There is no doubt that the tighter you control the infected, the less restriction you have to impose on the uninfected. In Europe, strict measures such as those adopted in Hong Kong and South Korea are currently not compatible with existing legislation about privacy.

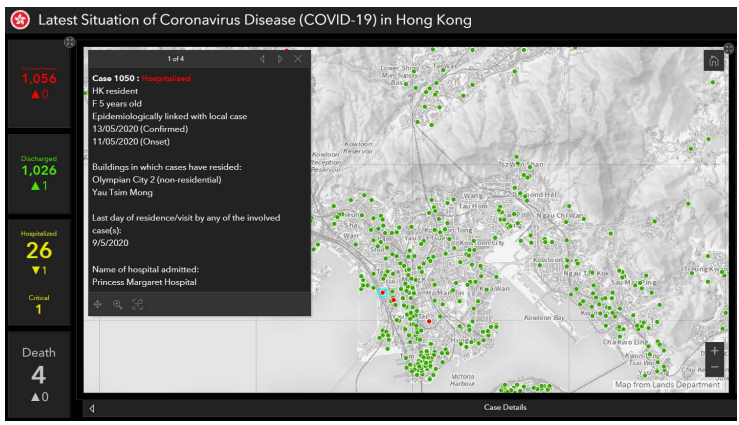


Figure 7. Screenshot of the "Latest Situation of Coronavirus Disease (COVID-19) in Hong Kong", https://chp-dashboard.geodata.gov.hk/covid-19/en.html.

Thursday, 21 May

The Centers for Disease Control and Prevention (CDC) informs that rats rely on the food and waste generated by restaurants and other commercial establishments, the closures of which have led to food shortage among rodents, especially in dense commercial areas. CDC warns of unusual or aggressive rodent behavior.

Will SARS-CoV-2 seal the fate of the Airbus A380? Air France chooses to end the operations of the aircraft, judged to be too expensive, too polluting and not profitable enough (Le Monde).

Friday, 22 May

Zhu et al. publish *Safety, Tolerability, and Immunogenicity of a Recombinant Adenovirus type-5 Vectored COVID-19 Vaccine*.

Fafi-Kremer 2020 et al. pre-publish *Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France*, reporting that neutralizing antibodies against SARS-CoV-2 were detected in virtually all hospital staff (n=160) sampled from 13 days after the onset of COVID-19 symptoms (see also Le Monde).

Saturday, 23 May

In Lower Saxony, Germany, 50 people are in quarantine after an outbreak in a restaurant (Der Spiegel).

In Frankfurt, Germany, authorities report more than 40 people infected with SARS-CoV-2 after a religious service (Der Spiegel).

Wednesday, 27 May

Colombian designers prepare cardboard hospital beds that double as coffins (The Guardian).

Andrzej Krauze publishes a cartoon on the fallout from the COVID-19 pandemic.

Sunday, 31 May

More than 50 million people across the US could go hungry without help from food banks or other aid (Feeding America).

June

Wednesday, 3 June

In the hope of saving its tourist industry, Italy reopens its borders.

Tuesday, 4 June

The Lancet *makes one of the biggest retractions in modern history* (The Guardian).

Friday, 5 June

The chief investigators of the RECOVERY trial report that there is no clinical benefit from use of hydroxychloroquine in hospitalised patients with COVID-19.

Saturday, 6 June

The Guardian reports that nearly 600 US health workers have died of COVID-19.

Sunday, 7 June

Three super-spreading events in an office, a restaurant and a bus show how easily SARS-CoV-2 can be spread over distances of more than 1 meter. The feature by El País is worth taking a look, even if you don't understand Spanish: https://elpais.com/ciencia/2020-06-06/radiografia-de-tres-brotes-asi-se-contagiaron-y-asi-podemos-evitarlo.html.

Monday, 8 June

Attending a sporting event, concert or play? Attending a wedding or a funeral? Stopping routinely wearing a face covering? Attending a church or other religious service? Hugging or shaking hands when greeting a friend? Going out with someone you don't know well? When asked by The New York Times when they would expect to resume these activities of daily life, 42% to 64% of epidemiologists and infectious disease specialists answered they would prefer waiting a year before doing it again. The enquiry by Margot Sanger-Katz, Claire Cain Miller and Quoctrung Bui: When 511 epidemiologists expect to fly, hug and do 18 other everyday activities again.

It becomes increasingly clear that not all patients recover fully from SARS-CoV-2 infection. See 'It feels endless': four women struggling to recover from Covid-19. (If you read Spanish, check also Los últimos de la UCI).

Dozens of new infections reported in Kabukicho, a district of more than 4,000 bars, restaurants and commercial sex establishments in Tokyo.

Tuesday, 9 June

New Zealand returns back to pre-COVID-19 life.

In Brazil, "poverty, poor access to health services and overcrowding all play a part in a disproportionate number of deaths", reports The Guardian. Coronavirus death rates expose Brazil's deep racial inequalities.

Wednesday,V 10 June

The Guardian publishes an analysis of the Surgisphere scandal (the retracted paper about hydroxychloroquine trial).

NIAID Director Anthony Fauci says the coronavirus pandemic is far from over.

The OECD says Britain will top the developing world's recession league table.

British theatre might go out of business.

Thursday, 11 June

India, Mexico, Russia, Iran and Pakistan decide to end lockdowns.

Neil Ferguson, a former scientific adviser to the British government, says earlier restrictions could have halved the death toll.

If you read Spanish: *Las mascarillas, claves para evitar una segunda oleada de la pandemia* (El País).

Friday, 12 June

Beijing reimposes lockdown measures after a new COVID-19 outbreak around the wholesale market of Xinfadi (北京新发地水果批发市场).

Northwestern Memorial Hospital in Chicago announces that a young woman in her 20s whose lungs were destroyed by COVID-19 received a double lung transplant.

If you read French: Coronavirus – au cœur de la bataille immunitaire contre le virus.

Saturday, 13 June

What have Venice, Amsterdam and Barcelona in common? Before the COVID-19 pandemic they were overrun by tourists. Tourism certainly contributes to the wealth of these cities, but the vast majority of the populations – all those who are not directly or indirectly employed in mass tourism – receive no

benefits from millions of people transiting their neighborhood. The weekend of 13/14 June, just before the reopening of the Schengen area (see 15 June entry), is therefore a unique opportunity for people in hundreds of small and big charming cities throughout Europe. They enjoy the place where they live with those who were born there or chose to live there – like 10, 20 or 30 years ago, before the beginning of the tourist pandemic.

According to figures from the British Office for National Statistics (ONS), people living in more deprived areas are twice as likely to die from coronavirus (ONS | The Guardian).

Most Europeans now trust their leaders generally a little less than when the crisis began.

Malta's abortion taboo leaves women in despair.

Sunday, 14 June

Lancet editor Richard Horton describes the management of the outbreak as 'the greatest science policy failure of a generation'.

Immunologist Scott Canna and rheumatologist Rachel Tattersall publish a 23-minute audio about cytokine storms.

A study by Ben Etheridge and Lisa Spantig shows that one third of women suffered from lockdown loneliness.

Thailand, Malaysia, Vietnam... some countries managed to keep COVID at bay.

When should we send children back to school? Here is what 132 epidemiologists would be inclined to do.

Monday, 15 June

Mauro Giacca of King's College London: "Covid-19 can result in complete disruption of the lung architecture."

With a few exceptions, all borders in the European Schengen area are open again for free travel of European citizens. The Balearic Islands open to 11,000 German tourists.

Every stairway a marathon? There is no standard therapy for patients who have survived a severe corona infection. For many survivors, the way back to a normal life begins in rehabilitation clinics. If you read German, read this.

Tuesday, 16 June

Results from the RECOVERY trial: Dexamethasone is the first life-saving coronavirus drug (Study | The Guardian).

After hundreds of infections at the Xinfadi market, the Chinese authorities close all schools and call on residents to avoid "non-essential" travel outside of the city. Around thirty residential areas surrounding the market are quarantined. Companies are encouraged to favor teleworking and people can no longer, except in cases of force majeure, leave the capital. Around 67% of domestic flights are canceled. Libraries, museums, art galleries and parks can only operate at 30% of their capacity. Restaurants can no longer accommodate groups. Beijing begins screening tens of thousands of inhabitants, bringing its daily testing capacity to more than 90,000 people.

The U.S. Food and Drug Administration revokes its emergency use authorization for hydroxychloroquine sulfate and chloroquine phosphate to treat COVID-19.

Coronavirus cases rise in US prisons.

Wednesday, 17 June

Investigations from Nanjing show that turbulence from a toilet bowl can create a large plume that is potentially infectious to a bathroom's next visitor (Paper | The New York Times).

After two women recently arrived from Britain were infected with COVID-19 and allowed to leave quarantine without being tested, New Zealand puts COVID-19 quarantine in the hands of the military.

Thursday, 18 June

The end of tourism? Christopher de Bellaigue publishes an insightful Guardian *long read* about the devastated global tourism industry. One key paragraph: "Tourism is an unusual industry in that the assets it monetizes – a view, a reef, a cathedral – do not belong to it. The world's dominant cruise companies (...) pay little towards the upkeep of the public goods they live off. By incorporating themselves in overseas tax havens with benign environmental and labor laws – respectively Panama, Liberia and Bermuda – cruising's big three, which account for three-quarters of the industry, get to enjoy low taxes and avoid much irksome regulation, while polluting the air and sea, eroding coastlines and pouring tens of millions of people into picturesque ports of call that often cannot cope with them."

Eric Rubin and Lindsey Baden discuss SARS-CoV-2 transmission in a 20-minute audio by the New England Journal of Medicine.

A 13-day-old baby becomes one of the UK's youngest victims.

Antibodies may fade quickly in asymptomatic people (Nature | The New York Times).

Again, meat processing plants are proving to be ideal transmission settings. In the German town of Gütersloh, North Rhine-Westphalia, 657 employees test positive for SARS-CoV-2.

Richard Horton publishes *The COVID-19 Catastrophe: What's Gone Wrong and How to Stop It Happening Again*. "The book returns again and again to the catastrophe in both the United Kingdom and the United States. It is haunted by the question: how did two of the richest, most powerful and most scientifically advanced countries in the world get it so wrong, and cause such ongoing pain for their citizens?" (Nature)

Friday, 19 June

Beijing residents react with frustration and anxiety after finding almost 200 new cases of coronavirus.

A study by the Italian Istituto Superiore di Sanità detects SARS-CoV-2 RNA in wastewater samples collected in Milan and Turin on 18 December 2019.

Investigations from the University of Sussex describe society as regressing back to the 1950s for many women (The Guardian).

UK abandons developing its own contact-tracing app and switches to the alternative design by Google and Apple.

Three experts exchange their views on the risks of travelling by plane.

Alexandra Villarreal describes a new American way of life: some Americans return to bars, dining and beaches, others shy away, concerned that the virus is still raging.

Sunday, 20 June

Spain plunges into the so-called new normal after 98 days of COVID-19 state-of-alarm.

The coronavirus outbreak in the German meat processing plant *Tönnies* near Gütersloh continues. By midday, 1,029 employees test positive and 2,098 negative for SARS-CoV-2. Nineteen people, almost all employees of Tönnies, are being treated for COVID-19. Six of them are in intensive care, two patients are ventilated (DIE ZEIT).

Those who might be tempted to attend a political rally should read the summary of COVID Reference's *Transmission* chapter:

1. It appears that a high percentage (as high as 80%?) of secondary transmissions could be caused by a small fraction of infectious individuals (as low as 10%?; Endo 2020); if this is the case, then the more people are grouped together, the higher the probability that a **superspreader** is part of the group.
2. It also appears that aerosol transmission might play an important role in SARS-CoV-2 transmission (Prather 2020); if this is the case, then building a wall around this same group of people and putting a ceiling above them further enhances the probability of SARS-CoV-2 infection.
3. It finally appears that shouting and speaking loudly emits thousands of oral fluid droplets per second which could linger in the air for minutes (Anfinrud 2020, Stadnytskyi 2020, Chao 2020, Asadi 2019); if this is the case, then creating noise (machines, music) around people grouped in a closed environment would create the perfect setting for a superspreader event.

Stay away from mass gatherings.

Week 26

This week has seen important local outbreaks. The recurring patterns: family celebrations (Melbourne, Berlin, Lagos) and people living (Malaga, Lisbon), working (Gütersloh, Tokyo, Huesca) or playing (Adria Tennis Tour) close together. The next outbreaks are anticipated in Liverpool, Naples (football celebrations) and some Italian cities (movida).

On 24 June, the US established a new national SARS-CoV-2 record. In Texas, the number of deaths is expected to increase about two to three weeks from now.

Sunday, 21 June

The number of infections in the Gütersloh (Germany) meat-processing plant exceeds one thousand. Nearly 7,000 employees are quarantined. After repeated outbreaks in the meat industry, The Guardian publishes *Why you should go animal-free: 18 arguments for eating meat debunked.*

The Spanish authorities increase the purchase of flu vaccines. Immunizations will start as soon as possible and priority will be given to health personnel.

Monday, 22 June

France reopens schools, colleges, kindergartens, cinemas, game rooms and small sports.

In India, 25 luxury hotels are to be transformed into COVID-19 care centers.

Injectable dexamethasone is more difficult to manufacture than tablets, and could soon run out.

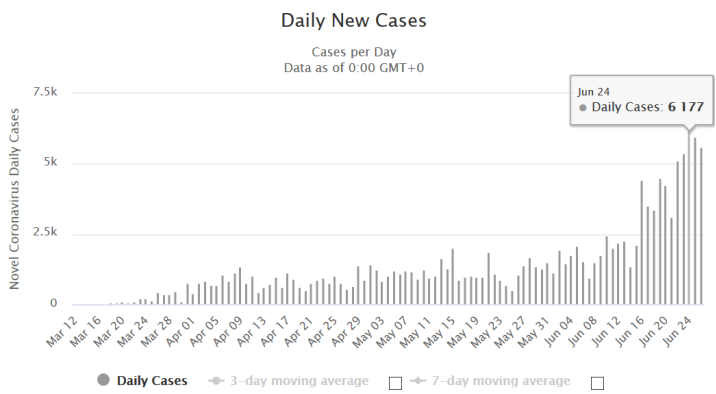
The New York Times publishes *Lessons on Coronavirus Testing From the Adult Film Industry.*

Wednesday, 24 June

More than 1,500 workers have tested positive in Gütersloh, Germany. The abattoir cooling systems may have contributed to spreading aerosol droplets laden with coronavirus. The authorities order a lockdown for 640,000 people.

In the US, more than 38,000 cases are detected, a record since the start of the coronavirus epidemic. The states that lifted containment measures, mainly governed by Republicans, are the most affected.

Daily New Cases in Texas



Source: https://www.worldometers.info/coronavirus/usa/texas

Income emerges as a major predictor of coronavirus infections, along with race.

Tennis player Novak Djokovic tests positive for COVID-19 amid Adria Tour fiasco (dixit Le Monde: Adria *Cluster* Tour).

The Guardian publishes *The coronavirus backlash: how the pandemic is destroying women's rights.*

Thursday, 25 June

In young children, SARS-CoV-2 infection is largely asymptomatic or accompanied by few symptoms. Now, two pre-published studies by Fontanet et al. from the Institut Pasteur, Paris, also suggest lower infection rates in a French primary school (6 to 11-years-old) when compared to a high school in Crépy-en-Valois, a small town 60 km northeast of Paris. The studies show that 38% of high school students had antibodies against SARS-CoV-2, but only 8.8% of primary school students in the same town (see following table).

A study of residents in the Alpine ski resort of Ischgl find that 42% have antibodies for the virus.

More than 80 people test positive in an outbreak at a Red Cross center in Malaga.

Tokyo detects new outbreaks of coronavirus in offices, with 55 new cases, its biggest rebound in a month and a half.

	High school students*		Children in primary school**	
Pupils	240	92 (38.3%)	510	45 (8.8%)
Parents	211	24 (11.4%)	641	n.n. (12%)
Close family	127***	13 (10.2%)	119	
Teachers	53	23 (43.4%)	42	3 (7%)
Staff	27	16 (59.3%)	28	1 (3.6%)
Others	3	3 (100%)		
Total	661	171 (25.9%)	1 340	

* Cluster of COVID-19 in northern France, By Fontanet A, et al.*
** Press report (Le Monde), incomplete data
*** Siblings

Sokolowska et al. publish *Immunology of COVID-19: Mechanisms, Clinical Outcome, Diagnostics and Perspectives - A Report of the European Academy of Allergy and Clinical Immunology (EAACI)*

The Guardian publishes *On different planets: how Germany tackled the pandemic, and Britain flailed.*

The New York Times publishes *How the Virus Won*, analyzing travel patterns, hidden infections and genetic data to show how the epidemic spun out of control.

Liverpool wins Premier League. At the title party, thousands gather on the streets without face masks. Rallies on UK beaches and at street parties in London.

Friday, 26 June

The Challenges of Safe Reopening – NEJM audio Interview (17:33) with Eric Rubin, Lindsey Baden and Stephen Morrissey.

The Guardian publishes *I'm a viral immunologist. Here's what antibody tests for Covid-19 tell us.*

The New York Time publishes *How the Coronavirus Short-Circuits the Immune System* and *Can Covid Damage the Brain?*

Saturday, 27 June

The European Union is preparing to restrict most US residents from visiting the region.

If you read Spanish, read *Más de 100 días arrastrando el coronavirus |by* Isabel Valdés.

If you read French, read *Qu'est-ce que le « R0 », le taux de reproduction du virus ?* by Gary Dagorn.

If you read Portuguese, read *Durante a Gripe Espanhola, houve uma Liga Anti-Máscara. E tudo piorou*.

Week 27

This week witnesses an important resurgence of SARS-CoV-2 infections in the US and India. Meanwhile, Europe which has more or less successfully managed the first wave, is holding its breath: will the economically all-important tourist season smoothly go ahead or will it be grounded by a second COVID wave? For now, smaller outbreaks (Gütersloh, Leicester, Lleida) are being kept under control. In this context, the opening of closed space where strangers can meet (bars, brothels and restaurants) may not be a good idea.

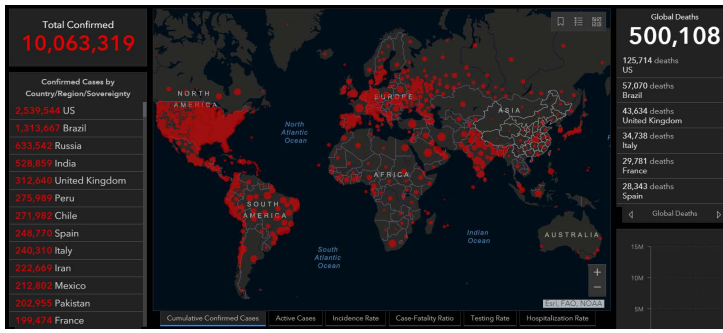
In the meantime, the EU opens its borders to 15 countries, car rental companies expect to lose up to 80%, Gilead imposes a price of about 350 euros per dose for its (weak) anti-SARS-CoV-2 drug, China starts testing a vaccine on military personnel, and asymptomatic spread continues – why shouldn't it.

Astonishingly, the question of using face masks continues to be debated. While you can probably do without them in low-prevalence areas such as most parts of Southern Italy, you are well-advised to wear them in the US. A

British journalist stated that not wearing face masks in epidemiological hotspots is like driving drunk. Imagine how people feel who are governed by drunkards.

Sunday, 28 June

Ten million official cases and 500,000 COVID-19 deaths.



Source: Johns Hopkins Coronavirus Resource Center.

Monday, 29 June

Chinese CanSino Biologics receives the green light to use a recombinant novel coronavirus vaccine (Ad5-nCoV) within the military.

Tuesday, 30 June

Anthony Fauci warns that a "general anti-science, anti-authority, anti-vaccine feeling" is likely to thwart vaccination efforts (The Guardian).

India has more than 450,000 confirmed cases, making it the world's fourth-worst-hit country. Major cities such as Delhi and Mumbai are particularly badly affected (Nature).

China cuts off more than 400,000 people in Anxin county to tackle a small COVID-19 cluster (The Guardian).

The new poor in Italy? Only a small percentage of companies have received promised lockdown help (The Guardian).

The English city of Leicester is in local confinement again after 866 new cases are diagnosed in two weeks.

The pharmaceutical company Gilead imposes a price of about 350 euros per dose for its (weak) anti-SARS-CoV-2 drug.

The New England Journal and The Lancet publish three articles (one | two | three) and a comment about Multisystem Inflammatory Syndrome in Children (MIS-C).

July

Wednesday, 1 July

The New York Times publishes an update on super-spreaders.

Outbreak in Melbourne, Australia. The authorities confine 300,000 people in 30 neighborhoods for a month.

The EU publishes a list of 15 countries from where people should be allowed into the Union. Visitors from the US to remain banned from entering the EU because of the country's rising infection rate.

We discover this YouTube video by Tang and al. visualizing airflow patterns associated with common, everyday respiratory activities. In this case, talking illustrates rapidly changing airflow patterns exchanged between talkers.

The US buys up the world stock of remdesivir.

Testing finds cases at US meat-processing plants but officials refuse to release the information (The Guardian).

According to an article by Science, only 50% of Americans plan to get a COVID-19 vaccine.

Thursday, 2 July

California rolls back the reopening of bars, restaurants and indoor venues (The Guardian).

Anthony S. Fauci and H. Clifford Lane publish *Four Decades of HIV/AIDS — Much Accomplished, Much to Do*.

Nicholas Kristof publishes *Refusing to Wear a Mask Is Like Driving Drunk*.

Friday, 3 July

Cheng et al. publish *How to Safely Reopen Colleges and Universities During COVID-19: Experiences From Taiwan*.

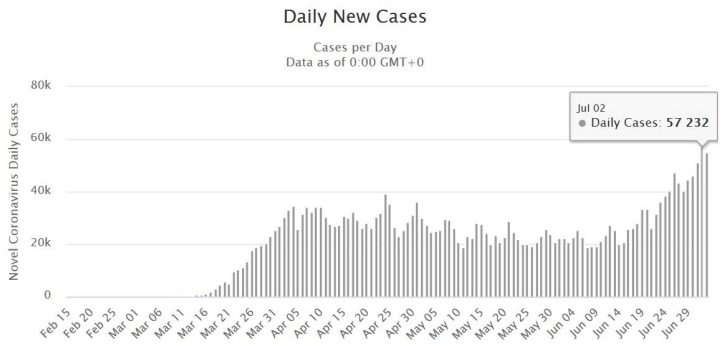
The Guardian describes the new emergency in Los Angeles.

Saturday, 4 July

The HIV drug lopinavir/ritonavir fails to reduce mortality in an interim analysis of the Solidarity trial. WHO discontinues both the lopinavir/ritonavir and the hydroxychloroquine treatment arms for COVID-19 (who.int).

After SARS-CoV-2 outbreaks in fruit companies, a nursing home, a neighborhood community and a hostel for homeless people, Catalonia imposes a lockdown on 200,000 people around Lleida.

The epidemic is taking off in the US:



Source: https://www.worldometers.info/coronavirus/country/us/

Adam Gabbatt publishes *Fourth of July celebrations increase risk of 'superspreader' events.*

Jesse Wegman publishes *Seriously, Just Wear Your Mask.*

Michelle Cottle publishes *Florida, America's Pandemic Playground.*

Pubs reopen in England.

Week 28

Week 28 will be recorded as a watershed in the perception of SARS-CoV-2 transmission risk: yes, the virus is transmitted by fat droplets, and yes, it is also transmitted tiny aerosol particles. If this shift is proven to be right, SARS-CoV-2 may go down in history as the virus that unified the almost century-old dichotomy of droplets vs. aerosol transmission. The merit goes to Lidia Morawska and Donald K. Milton, supported by 237 scientists (see also the comment in The Guardian and in The New York Times). In the next days, we will publish an update of our *Transmission* chapter.

Paterson et al. publish a worrisome article about the neurological complications of COVID-19.

Second waves are leading to partial lockdowns in Australia, Spain, Serbia and Israel while Catalonia and the Balearic Islands order wearing face masks even when the required 1.5-metre social distancing can be observed.

The first wave continues in the US. People in Mexico border towns try to stop Americans from crossing.

Sunday, 5 July

Is it time to address airborne transmission of SARS-CoV-2? It may be high time, say Lidia Morawska and Donald K Milton, supported by other 237 scientists. See also *WHO underplaying risk of airborne spread of Covid-19* (The Guardian), *239 Experts With One Big Claim: The Coronavirus Is Airborne* and *Airborne Coronavirus: What You Should Do Now* (The New York Times).

Spain puts part of Galicia back into lockdown.

Monday, 6 July

Find out how Anthony Fauci, Elizabeth Connick, Paul A. Volberding, Linda Bell, Barry Bloom and David Satcher deal with COVID-19 risks in their everyday lives.

Tuesday, 7 July

If you read Spanish, read "La enigmática mutación del coronavirus que ahora domina el planeta" (El País).

Wednesday, 8 July

COVID-19 fears: People in Mexico border towns try to stop Americans from crossing (The Guardian).

Paterson et al. publish *The emerging spectrum of COVID-19 neurology: clinical, radiological and laboratory findings.* See also the article published in The Guardian.

Violence at Belgrade protest over renewed lockdown measures

Churches at risk: SARS-CoV-2 infiltrates Sunday services, church meetings and youth camps. More than 650 cases have been linked to reopened religious facilities.

Second COVID-19 wave in Israel.

Thursday, 9 July

WHO update information about SARS-CoV-2 transmission (WHO 20200709): "There have been reported outbreaks of COVID-19 in some closed settings, such as restaurants, nightclubs, places of worship or places of work where people may be shouting, talking, or singing. In these outbreaks, aerosol transmission, particularly in these indoor locations where there are crowded

and inadequately ventilated spaces where infected persons spend long periods of time with others, cannot be ruled out."

Five million Melbourne residents are locked down again (read also this article).

Catalonia orders wearing face masks even when the required 1.5-metre social distancing can be observed. The fine for not observing the new rules: 100 euros. The Balearic islands is set to follow Catalonia's lead soon.

The Tokyo authorities pay nightclubs as well as host and hostess bars thousands of dollars if they close for more than 10 days.

Indonesia announces a new cluster of more than 1,000 cases at a military training center in West Java.

Friday, 10 July

Guardian live (10 July): Bogotá to re-enter strict lockdown.

The Guardian Global report (10 July).

Rats torment New York alfresco diners.

Scotland asserts separateness from England.

If you read Spanish, read *El mapa de los brotes de coronavirus: el 40% tiene su origen en encuentros familiares*.

Saturday, 11 July

The Guardian: Coronavirus live + Global report.

New outbreak in Spain in L'Hospitalet, the second biggest city in the Barcelona metropolitan area (3.2 million people; El País).

Over 40 Florida hospitals max out their intensive care unit capacity (The Guardian).

Rapid serological tests are now available in French pharmacies. The test requires taking a drop of blood by pricking the skin, usually at the fingertip, then putting it in contact with a reagent. The result appears in a few minutes (Le Monde).

The NY Times publishes *'I Couldn't Do Anything': The Virus and an E.R. Doctor's Suicide.*

If you read Spanish, read the Fauci interview *"La cuestión es que todo el mundo debería llevar mascarilla"* (El País).

Is the governor of the hard-hit Lombardy region (almost 50% of all Italian cases) opening the dance for the second wave in his country? In a bold (suicidal?) move he allows discos to reopen open-air discos. The Repubblica

newspaper reports that people "filled the slopes of the main Milanese discos without wearing personal protective equipment and without respecting the social distancing." The countdown has begun.

Week 29

This week, the publication of detailed results of a phase 1, dose-escalation, open-label trial (14 July) reminded us that the race for a vaccine is gaining momentum. More encouraging results from competitor researchers are expected within days.

Meanwhile, the pandemic is gaining momentum, too, with sad records recorded from all over the world. A new area of concern is Europe, where a second wave may be building up (18 July). In contrast to what happened in March, local epidemics seem now to be fueled by the infection of younger people. Wearing face masks may soon be required in many European countries (16 July).

In the US, daily new SARS-CoV-2 cases are on track to go beyond 100,000. As Rudolf Virchow, the great 19th century father of pathological anatomy, liked to say: "An epidemic is a social phenomenon that has some medical aspects." (Cited by Bernard Henri-Lévy in *Ce virus qui rend fou*, Grasset, June 2020)

Sunday, 12 July

Fourteen renowned doctors (Antoine Pelissolo, Jimmy Mohamed Philippe Amouyel, Francis Berenbaum, Eric Caumes, Robert Cohen, Anne-Claude Crémieux, Gilbert Deray, Vianney Descroix, Philippe Juvin, Axel Kahn, Karine Lacombe, Bruno Megarbane and Christine Rouzioux) demand **"the wearing of a mandatory mask in all enclosed public places"** in order to prevent a second COVID-19 wave (Le Parisien, Le Monde).

In **Sydney**, **thousands of pub-goers** have been asked to self-isolate for two weeks after a hotel staff member and three other people became the latest cases in an emerging coronavirus cluster (The Guardian).

Will COVID-19 help to **cure over-tourism** in the future? Many cities around the world are searching for a new balance. Reflections about the current situation in Paris (Le Monde, Édition abonnés).

If you read Spanish, read *Los delirios mortales del rey Donald*, by Paul Krugman, and *Jornaleros de la pandemia*, by Guillermo Abril.

Monday, 13 July

California, 40 million people, return to the closure of all indoor operations for restaurants wineries, movie theaters and family entertainment, zoos, museums and cardrooms bars. The state is one of the main SARS-CoV-2 foci in the United States (more than 300,000 cases, 7,000 deaths).

A study examining data for 355 Dutch municipalities finds evidence of a positive relationship between **air pollution** and Covid-19 cases, hospital admissions and deaths (Cole MA, Ozgen C, Strobl E (PDF); The Guardian).

The Guardian: 30-year-old dies after attending **'Covid party'** in Texas | 'I think I made a mistake, I thought this was a hoax, but it's not.' See also the video by Jane Appleby.

Do **men without a mask** look tough? (The Guardian)

Returning **German tourists as superspreaders**? The CEO of the World Medical Association Frank Ulrich Montgomery proposes a two-week quarantine for holidaymakers returning from the Mallorca island (audio in German) after hundreds of drunken tourists celebrate in a pre-COVID atmosphere.

No re-opening of discos in France as the French Council of State estimates that the prolonged closing of the night clubs is not "disproportionate" (Le Monde).

Tuesday, 14 July

Jackson et al. publish a preliminary report about 45 healthy adults, 18 to 55 years of age, who received two vaccinations, 28 days apart, with **mRNA-1273** in a dose of 25 μg, 100 μg, or 250 μg. Read also the editorial by Editorial by Penny M. Heaton: *The Covid-19 Vaccine-Development Multiverse* and the audio interview *Covid-19 Vaccine Development*, by Rubin, Baden and Morrissey.

Israel, Uzbekistan, Melbourne, California – certain states, areas and cities enter **new lockdowns**. Le Monde updates a non-exhaustive list of new pandemic hotspots, classified by number of inhabitants concerned and by country.

Jeneen Interlandi publishes *Why We're Losing the Battle With Covid-19*. (The New York Times)

Michelle Goldberg publishes *In Some Countries, Normal Life Is Back. Not Here.* (The New York Times)

Twitter comment on **British tourists in Spain**: "Parts of Spain in lockdown, the elderly shut away in care homes, we all wear masks in the street, but in Magaluf the antisocial and irresponsible Brits do whatever they please. It's shameful." (The Guardian, text and video)

Wednesday, 15 July

If you read Spanish, read *Una sanitaria en L'Hospitalet de Llobregat: "El ambulatorio roza el colapso, peor que en abril"*. (El País).

Matthew J. Belanger, Michael A. Hill, Angeliki M. Angelidi, Maria Dalamaga, James R. Sowers, and Christos S. Mantzoros publish *Covid-19 and Disparities in Nutrition and Obesity*. (The New England Journal of Medicine)

Renee N. Salas, James M. Shultz, and Caren G. Solomon publish *The Climate Crisis and Covid-19 — A Major Threat to the Pandemic Response*. (The New England Journal of Medicine)

Thursday, 16 July

The French government decides that **wearing mask will be compulsory in closed public places** from next week. They describe the situation as "problematic" in Mayenne, "worrying" in New Aquitaine, and increasing number of cases in Paris and in Finistère. (Le Monde)

In Spain, 40% of recent outbreaks might have been associated with family events ("...a wedding in Tudela, a celebration of San Juan in a neighborhood of Castellón, a meal with friends in Alcanar (Tarragona)." (El País).

In a response to the paper by Jackson et al. (see 14 July), British researchers working on another Covid-19 vaccine at the University of Oxford spread the word that their vaccine, too, triggers two types of immune response: the production of antibodies – proteins that can bind to the virus, preventing it from entering cells and flagging it to immune cells – but it also seems to result in the production of "killer" T cells – immune cells that attack infected human cells. (The Guardian)

Danielle Renwick publishes *How quickly will there be a vaccine? And what if people refuse to get it?* (The Guardian)

Merlin Chowkwanyun and Adolph L. Reed publish *Racial Health Disparities and Covid-19 — Caution and Context*. (The New England Journal of Medicine)

If you read Spanish, read Miguel Ángel Criado: *Más de la mitad de los españoles ingresados por coronavirus han desarrollado problemas neurológicos* (El País)

Friday, 17 July

Israel returns to partial lockdown. All indoor gatherings of 10 or more people are banned. Restaurants return to takeaways and deliveries only. During the weekend, all shops, hairdressers and attractions are closed. All gyms and fitness studios are closed at all times.

Saturday, 18 July

Spain seems on the brink of a second COVID-19 wave. In the last 7 days, the country had 10 times more new cases than a month ago (El País). **Four million residents of Barcelona** and 12 municipalities around the city have been **urged to stay at home**. The regional Government announces that the restrictions also include the reduction of capacity in bars and restaurants and closure of nightlife venues, cultural activities and gyms, and a ban on gatherings of more than 10 people from Saturday.

In **France**, which already announced plans to make mask wearing mandatory in enclosed public spaces, authorities reported a sharp rise in the infection rate in Brittany. According to data released on Friday, the disease's reproduction rate in Brittany has risen from 0.92 to 2.62 between 10-14 July.

Infections in **India** pass one million.

Tom McCarthy publishes *'The virus doesn't care about excuses': US faces terrifying autumn as Covid-19 surges* (The Guardian).

Week 30

This week may be recalled as the timid beginning of the second European COVID-19 wave. At the beginning of the week, bars in Barcelona were ordered to limit the number of clients. On Saturday, Norway and the UK imposed a 10 (UK: 14) day quarantine on all people coming back from Spain, mostly holidaymakers, and Barcelona ordered the closure of discos, dance halls, etc. All over the continent, outbreaks are linked to seasonal farm laborers, family meetings and night life. 2020 tourism was severely affected by the continent-wide spring lockdowns. It is now doubtful that the holiday season will continue to summer's end.

The daily new cases in Australia:

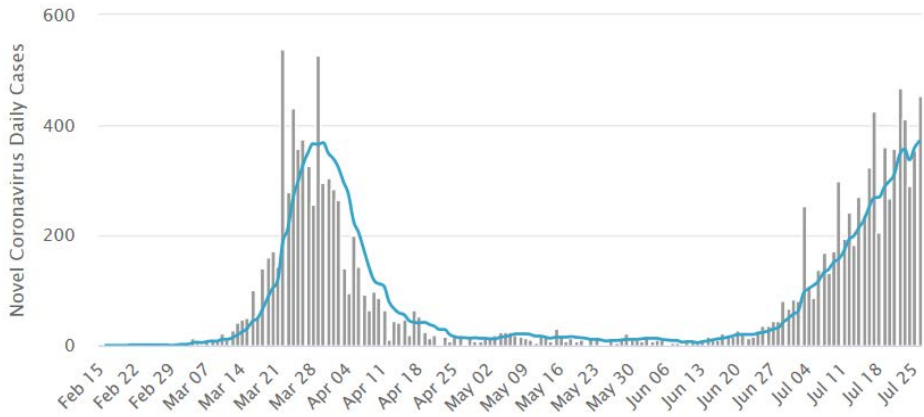


Figure 30.1. Daily new cases in Australia (blue line: 7-day monthly average).

Monday, 20 July

This is vaccine day. Andrew Pollard and colleagues report their phase 1/2 randomized trial of a chimpanzee adenovirus-vectored vaccine (ChAdOx1 nCoV-19); and Wei Chen and colleagues report results from a randomized phase 2 trial of an Ad5-vectored COVID-19 vaccine. Read also the comment by Naor Bar-Zeev and William John Moss.

In Sao Paulo, 900 health professionals will participate in a phase 3 trial of a vaccine developed by the Chinese Sinovac Biotech laboratory. In total, the vaccine will be offered to 9,000 volunteers in six Brazilian states.

In France, the wearing of a mask becomes compulsory in closed places which are open to the public.

In Barcelona, the capacity in bars is limited to 50%. Visits to nursing homes are prohibited.

Tuesday, 21 July

Historic pact of the European Union to overcome the COVID-19 crisis: for the first time in its history, the EU member states will borrow money to finance an extraordinary economic stimulus with 390,000 million in grants and 360,000 million in credits, sending a strong message that they will continue to stay together. Presidents in the east and in the west will have taken notice (see also The Guardian).

Indian authorities claim that SARS-CoV-2 antibody testing of people living in the Delhi region showed that 23.5% had antibodies against the virus. Samples

from 21,387 people were examined. This percentage would be 50 times higher than the officially reported figures. Delhi, with a population of 29 million, has reported only 123,747 infections.

Jennifer Steinhauer and Thomas Gibbons-Neff explain how American military officials are trying to contain the spread of the SARS-Cov-2 in its ranks (The New York Times).

See also the feature by The Guardian: How coronavirus is reshaping Europe's tourism hotspots. An opportunity to rethink their business model?

Barcelona reduces the capacity of its beaches (El País).

Wednesday, 22 July

Belgium is recording a significant increase in Covid-19 cases. During the period July 12-18, the number of new infections rose 89% with an average of 184 cases diagnosed per day, up from 98 the week before. Most cases are among people between 20 and 59 years old who were infected during parties or gatherings.

On the eve of a four-day long weekend in Japan, the governor of Tokyo calls on her constituents to stay at home, as the number of new daily cases of Covid-19 is sharply increasing in the region. As Covid-19 infections appear to be spreading widely, the Japanese capital is on its maximum alert level.

In Spain, 40% of people newly infected with SARS-CoV-2 are under 40 years of age and most do not know where they have been infected.

Thursday, 23 July

The Spanish newspaper El País sounds the alarm: The virus rebounds in Spain: data from 10 communities show more infections and more hospitalizations.

In the U.S., SARS-CoV2 testing laboratories struggle to find the chemicals and plastic pieces they need to carry out coronavirus tests (The New York Times).

Lazaro Gamio, *Sarah Mervosh* and *Keith Collins* show *Where the Virus Is Sending People to Hospitals.*

Friday, 24 July

Authorities order the closure of nightlife (discos, dance halls, etc.) in Catalonia for at least 15 days. The hours of activity in casinos and game rooms are limited until midnight (El País + El País).

Norway reinstates mandatory 10-day quarantine for travelers coming back from Spain.

The U.K. makes wearing masks compulsory in stores.

The New York Times and El País ask "Who will receive the first COVID vaccines?"

Lauren Leatherby publishes How the U.S. compares With the world's worst coronavirus hot spots.

Saturday, 25 July

Catalonia exceeds 50 hospitalized daily, 10 times more than the figures reported by the Ministry of Health (El País).

In Belgium, wearing masks is now compulsory on markets, in shopping streets, in hotels, cafes and restaurants (except at the table).

With immediate effect, the UK re-quarantines travelers from Spain. Those who come back home must isolate themselves for 14 days. This measure will affect Spain's tourism industry. But not only Spain is suffering.

If you read Spanish, read El coronavirus ha repuntado en 30 provincias: el mapa con la situación de los contagios en cada una | En el último mes han aumentado los casos y las hospitalizaciones en media España (El País).

Sunday, 26 July

A *tsunami of fake news* hurts Latin America's effort to fight SARS-CoV-2. A report by Tom Phillips in São Paulo, David Agren in Mexico City, Dan Collyns in Lima and Uki Goñi in Buenos Aires (The Guardian).

A surge in COVID-19 cases has forced a hospital in rural Texas to set up "death panels" to decide which patients it can save and which ones will be sent home to die. By Michael Sainato.

Victoria, Australia, reports a national record of 10 Covid-19 deaths.

North Korea reports the first COVID-19 case (...) and declares a state of emergency (The Guardian).

How Hawaii avoided a coronavirus spike, but severely damaged its economy. Lauren Aratani explains.

If you read Spanish, read this: Un verano con virus: qué hacer | Viajar con amigos o ir a visitar a la familia unos días entraña riesgos. ¿Se comparte el salón? ¿Y el coche? ¿Se puede ligar? Los expertos explican cómo minimizar la exposición.

The true number of excess deaths due to COVID-19 is probably more than 50% higher than the officially reported data. See the analysis by El País.

Monday, 27 July

If you understand German, meet Dr Camilla Rothe (6 minutes) who detected the first SARS-CoV-2 positive patient in Germany at the end of January. Within days, it became clear that asymptomatic transmission would play an important role in the pandemic. In the video interview, Dr Rothe looks back - and forward.

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