Overview of information available to support the development of medical countermeasures and interventions against COVID-19

This document is conceived as a living document, to be updated on a weekly basis.

It is based on open-access publications (scientific journals and preprint databases, communications by WHO, health authorities and companies) in English language.

Please note that the present version has not been submitted to any peer-review process. Any comment / addition that can help improve the contents of this review will be most welcome.

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List of abbreviations

AAK1 AP2-associated protein kinase 1
ACE2 angiotensin-converting enzyme 2
ALB albumin
ALT alanine aminotransferase
AMPs Antimicrobial peptides
ARDS acute respiratory distress syndrome
AST aspartate aminotransferase
AT2 type II alveolar cells
CI confidence interval
CNS central nervous system
CoV coronavirus
CPK creatine phosphokinase
CRP C-reactive protein
CSF cerebrospinal fluid
CT computed tomography
ELISA enzyme-linked immunosorbent serologic assay
GISAID Global Initiative on Sharing All Influenza Data
HCW health care workers
IC50 half maximal inhibitory concentration
IFN interferon
ISG IFN-stimulated genes
mAb monoclonal antibody
MERS Middle East respiratory syndrome
MHV murine hepatitis virus
MOI multiplicity of infection
NAb neutralizing antibody
NCIP novel coronavirus-infected pneumonia
NK natural killer
N.R. not reported
PCR polymerase chain reaction
pfu plaque-forming unit
PRNT plaque-reduction neutralization test
R&D research and development
RBD receptor-binding domain
RT-PCR real-time polymerase chain reaction
SARS severe acute respiratory syndrome
S spike
WHO World Health Organization
Introduction
Coronaviruses are common human pathogens, causing generally-mild acute respiratory illnesses known as the common cold (Wu Eurosurv 2020, see below). Prior to December 2019 when clusters of pneumonia cases with unknown aetiology were detected in Wuhan, China, only two additional strains of coronaviruses had caused outbreaks of severe acute respiratory disease in humans: the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). On 9 January 2020, a novel coronavirus, 2019-nCoV (temporary name), was officially identified as the cause of an outbreak of viral pneumonia in Wuhan (https://www.who.int/china/news/detail/09-01-2020-who-statement-regarding-cluster-of-pneumonia-cases-in-wuhan-china). In the following weeks, the virus spread rapidly within China, and an increasing number of cases appeared in other countries. On January 30th 2020, the International Health Regulations (2005) Emergency Committee agreed that the outbreak meets the criteria for a Public Health Emergency of International Concern (https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov)). The disease was named COVID-19 by WHO on February 11 2020 (https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020), and the virus named SARS-CoV-2 by the International Committee on Virus Taxonomy on the same day (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses Nat Microbiol 2020, see below). Subsequently, a group of virologists in China suggested renaming SARS-CoV-2 as human coronavirus 2019 (HCoV-19), considering that such a name would distinguish the virus from SARS-CoV and keep it consistent with the WHO name of the disease it causes, COVID-19 (Jiang Lancet 2020, see below). Virus naming remains controversial (Voice from China Chin Med J 2020, see below). In the scientific literature, the virus remains referred to by these different names, even though Wu, Ho et al. (Lancet 2020, see below) suggested keeping SARS-CoV-2 as its name.


As of March 23 2020 09:00 CET, according to WHO, a total of 294 110 cases has been confirmed globally in 186 countries, including 12 944 deaths; 211 612 cases were confirmed outside China.

The virus
Coronaviruses
Coronaviruses (CoVs) are enveloped, positive-sense, single-stranded RNA viruses that belong to the subfamily Coronavirinae, family Coronaviridae, order Nidovirales. The virion has a nucleocapsid composed of genomic RNA and phosphorylated nucleocapsid (N) protein, which is buried inside phospholipid bilayers and covered by spike proteins (Li J Med Virol 2020, see below). The membrane (M) protein (a type III transmembrane glycoprotein) and the envelope (E) protein are located among the spike (S) proteins in the virus envelope. CoVs were given their name based on a characteristic crown-like appearance.

There are four genera of CoVs, namely, Alphacoronavirus (αCoV), Betacoronavirus (βCoV), Deltacoronavirus (δCoV), and Gammacoronavirus (γCoV) (Chan Em Micr Inf 2020, see below). Evolutionary analyses have shown that bats and rodents are the gene sources of most αCoVs and βCoVs, while avian species are the gene sources of most δCoVs and γCoVs. CoVs have repeatedly crossed species barriers and some have emerged as important human pathogens.

The genomic RNA is used as template to directly translate polyprotein (pp) 1a/1ab, which encodes non-structural proteins to form the replication-transcription complex (RTC) in a double-membrane vesicles (Chen J Med Vir 2020, see below). Subsequently, a nested set of subgenomic RNAs are synthesized by RTC in a manner of discontinuous transcription. The first ORFs (ORF1a/b), about two-third of the whole genome length, encode 16 non-structural
proteins (nsp1-16). Other ORFs on the one-third of the genome near the 3’-terminus encodes the main structural proteins: S, M, E, and N proteins. Besides these four main structural proteins, CoVs encode special structural and accessory proteins. All the structural and accessory proteins are translated from the subgenomic RNAs of CoVs.

SARS-CoV-2 is a betacoronavirus of bat origin

On January 3, 2020, the first complete genome of the novel β genus coronaviruses (2019-nCoVs, subsequently named SARS-CoV-2) was identified in samples of bronchoalveolar lavage fluid from a patient from Wuhan (http://weekly.chinacdc.cn/en/article/id/a3907201-f64f-4154-a19e-4253b453d10c and Wu Nature 2020, see below). A viral genome sequence was released via the community online resource virological.org on 10 January (Wuhan-Hu-1, GenBank accession number MN908947 (http://virological.org/t/novel-2019-coronavirus-genome/319). Additional sequences were rapidly obtained by other groups and complete genomes were submitted to GISAID (see for instance, Zhu New Engl J Med 2020 below).

SARS-CoV-2 falls into the genus betacoronavirus, which includes CoVs discovered in humans, bats, and other wild animals (SARS-CoV, bat SARS-like CoV, and others). As illustrated in Table 1 below, additional studies, based on subsequent virus isolates, confirmed that the virus is phylogenetically closest to bat SARS-like CoV (SL-ZC45 and SL-CoVZXC21).

<table>
<thead>
<tr>
<th>% homology with</th>
<th>SARS</th>
<th>MERS</th>
<th>bat SARS-like CoV*</th>
<th>BatCoV RaTG13</th>
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<tr>
<td>N.R.</td>
<td>N.R.</td>
<td>89.1%</td>
<td>N.R.</td>
<td>Wu Nature 2020, see below</td>
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<tr>
<td>79.0%</td>
<td>51.8%</td>
<td>87.6-87.7%</td>
<td>N.R.</td>
<td>Ren Chinese Med J 2020, see below</td>
</tr>
<tr>
<td>82%</td>
<td>N.R.</td>
<td>89%</td>
<td>N.R.</td>
<td>Jiang Em Micr Inf 2020, see below</td>
</tr>
<tr>
<td>82%</td>
<td>N.R.</td>
<td>89%</td>
<td>N.R.</td>
<td>Chan Em Micr Inf 2020, see below</td>
</tr>
<tr>
<td>79%</td>
<td>50%</td>
<td>88%</td>
<td>N.R.</td>
<td>Lu Lancet 2020, see below</td>
</tr>
<tr>
<td>&lt;80%</td>
<td>N.R.</td>
<td>N.R.</td>
<td>96.3%</td>
<td>Paraskevis Infect Genet Evol 2020, see below</td>
</tr>
<tr>
<td>79.7%</td>
<td>N.R.</td>
<td>N.R.</td>
<td>96.2%</td>
<td>Zhou Nature 2020, see below</td>
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* bat-SL-CoV-ZC45 and/or bat-SL-CoV-ZXC21

The observation that SARS-CoV-2 isolates have a single intact open reading frame gene 8 is a further indicator of bat-origin CoVs. In addition, although closely related to BatCoV RaTG13 sequence throughout the genome (sequence similarity 96.3%), SARS-CoV-2 shows discordant clustering with the Bat_SARS-like coronavirus sequences (Paraskevis Infect Genet Evol 2020, see below; Lu Lancet 2020, see below). Specifically, in the 5’-part spanning the first 11,498 nucleotides and the last 3’-part spanning 24,341-30,696 positions, SARS-CoV-2 and RaTG13 formed a single cluster with Bat_SARS-like coronavirus sequences, whereas in the middle region spanning the 3’-end of ORF1a, the ORF1b and almost half of the spike regions, SARS-CoV-2 and RaTG13 grouped in a separate distant lineage within the sarbecovirus branch. Consequently, the levels of genetic similarity between SARS-CoV-2 and RaTG13 suggest that the latter does not provide the exact variant that caused the outbreak in humans, but the hypothesis that SARS-CoV-2 has originated from bats is very likely.

Liu (Emerg Micr Inf 2020, see below) published a commentary addressing the speculations, rumours and conspiracy theories that SARS-CoV-2 is of laboratory origin. He showed the current lack of credible evidence to support the claim that SARS-CoV-2 originated from a laboratory-engineered CoV. And pointed out to the fact that evolution is stepwise and accrues mutations gradually over time, whereas synthetic constructs would typically use a known backbone and introduce logical or targeted changes instead of the randomly occurring mutations that are present in naturally isolated viruses such as bat CoV RaTG13. Along the same line, Hao (Emerg Microbes Inf 2020, see below) ruled out a published claim that SARS-CoV-2 would have a unique inserted sequence (1378 bp) located in the middle of its S
glycoprotein gene that had no match in other coronaviruses and that this unique sequence would be similar to some sequence in a common expression vector used in research laboratory.

**Genome structure**

Similar to other βCoVs, the SARS-CoV-2 genome contains two flanking untranslated regions and a single long open reading frame encoding a polyprotein (Chan Em Micr Inf 2020, see below). The SARS-CoV-2 genome is arranged in the order of 5’-replicase (orf1/ab)-structural proteins [S-E-M-N]-3’ and lacks the hemagglutinin-esterase gene which is characteristically found in lineage A β-CoVs, as illustrated in Figure 1.

The S glycoprotein is comprised of S1 and S2 subunits. The S1 subunit contains a signal peptide, followed by an N-terminal domain (NTD) and receptor-binding domain (RBD), while the S2 subunit contains conserved fusion peptide, heptad repeat 1 and 2, transmembrane domain, and cytoplasmic domain.

Remarkably, orf3b encodes a completely novel short protein. Furthermore, new orf8 likely encodes a secreted protein with an alpha-helix, following with a beta-sheet(s) containing six strands.

![Figure 1 Genome organization of the SARS-CoV-2 genome compared to other betacoronaviruses (from Chan 2020)](https://www.biorxiv.org/content/10.1101/2020.02.21.959817v1)

Of note, a manuscript by Tran Thi Nhu Thao on Biorxiv described a reverse-genetics platform for SARS-CoV-2, consisting of a yeast-based synthetic genomics platform. Viral subgenomic fragments were generated using viral isolates, cloned viral DNA, clinical samples, or synthetic DNA, and reassembled in one step in Saccharomyces cerevisiae using transformation associated recombination (TAR) cloning to maintain the genome as a yeast artificial chromosome (YAC). T7-RNA polymerase has been used to generate infectious RNA, which was then used to rescue viable virus. Based on this platform the authors have been able to engineer and resurrect chemically-synthetized clones of the recent epidemic SARS-CoV-2 in only a week after receipt of the synthetic DNA fragments.
Sequence diversity among isolates

Virus isolates from five patients with severe pneumonia (hospitalized from December 18 to December 29, 2019 at Jin Yin-tan hospital in Wuhan) revealed 99.8-99.9% nucleotide identities (Ren Chinese Med J 2020, see below). Zhou (Nature 2020, see below) also reported more than 99.9% identity among isolates obtained from 7 patients at the beginning of the outbreak. Lu (Lancet 2020, see below) reported 10 genome sequences of SARS-CoV-2 obtained from nine patients exhibiting more than 99.98% sequence identity. Most recently, Ceraolo (J MedVir 2020, see below) analyzed 56 genomes of SARS-CoV-2 and confirmed high sequence similarity (>99%). Of note, at least two hyper-variable genomic hotspots were detected, one of which is responsible for a Serine/Leucine variation in the viral ORF8-encoded protein. Another study conducted on 32 genomes of strains sampled from China, Thailand, and USA between 24 December 2019 and 23 January 2020 suggested increasing tree-like signals from 0 to 8.2%, 18.2%, and 25.4%) overtime, which may be indicative of increasing genetic diversity of SARS-CoV-2 in human hosts (Li, Wang et al. J Med Vir 2020, see below).

Following the analysis of 54 gene sequences, Wen (J Infect 2020, see below) noted the hyper-variable genomic hotspot to be established in the SARS-CoV-2 population at the nucleotide but not the amino acid level, suggesting that there have been no beneficial mutations acquired. Of note, nsp1, nsp3, and nsp15 of ORF1ab and gene S were found to carry significantly more mutations than other genes.

Subsequently, Wang (J Med Vir 2020, see below) reported on the analysis of 95 full-length genomic sequences of SARS-CoV-2 strains from NCBI and GISAID databases. The homology among all viral strains was generally high, among them 99.99% (99.91%-100%) at the nucleotide level, 99.99% (99.79%-100%) at the amino acid level. Although overall variation in ORF regions is low, 13 variation sites in 1a, 1b, S, 3a, M, 8, and N regions were identified, among which positions nt28144 in ORF 8 and nt8782 in ORF 1a showed mutation rate of 30.53% (29/95) and 29.47% (28/95) respectively.

While the number of sequences deposited to GISAID increases rapidly (949 sequences by March 23 10:30 CET; see https://www.gisaid.org/epiflu-applications/next-betacov-app/), continued monitoring of the virus sequence diversity among the newest isolates will be important to ensure.

The website of the China National Center for Bioinformation (https://bigd.big.ac.cn/ncov?lang=en), available in Chinese and English, constitutes another useful resource on SARS-CoV-2 sequences. Moreover, a new resource of interest, described by Cleemput (Bioinform 2020, see below) is the Genome Detective Coronavirus Typing Tool, available at https://www.genomedetective.com/app/typingtool/cov, which can accurately identify SARS-CoV-2 sequences isolated in China and around the world. The tool can accept up to 2 000 sequences per submission and the analysis of a new whole genome sequence will take approximately one minute. The tool has been tested and validated with hundreds of whole genomes from ten coronavirus species. The tool also allows tracking of new viral mutations as the outbreak expands globally.

Following metatranscriptome sequencing for the bronchoalveolar lavage fluid of SARS-CoV-2 patients, Shen (Clin Inf Dis 2020, see below) presented data suggesting that SARS-CoV-2 evolves in vivo after infection. The median number of intra-host variants was 1-4 in SARS-CoV-2 infected patients, ranging between 0 and 51 in different samples. The distribution of variants on genes was similar to those observed in the population data (110 sequences). However, very few intra-host variants were observed in the population as polymorphism, implying either a bottleneck or purifying selection involved in the transmission of the virus, or a consequence of the limited diversity represented in the current polymorphism data.

Phylogenetics

Phylogenetic and likelihood-mapping analyses of 12 genome sequences of the virus with known sampling date (24 December 2019 and 13 January 2020) and geographic location (primarily Wuhan city, Hubei Province, China) suggested...
a potentially large ‘first generation’ human-to-human virus transmission. Li, Zai et al. (J Med Virol 2020, see below) estimated that SARS-CoV-2 likely originated in Wuhan on 9 November 2019 (95% credible interval: 25 September 2019 and 19 December 2019). Li, Wang et al. (J Med Vir 2020, see below) confirmed the recent and rapid human-to-human transmission, with estimates of virus emergence ranging from 15 October to 10 November 2019 or 16 November to 22 December 2019 depending on the calculation method.

Paraskevis (Infect Genet Evol 2020, see below) described the lack of a mosaic relationship of SARS-CoV-2 to the closely related sarbecoviruses, indicating the lack of a recombination event in the emergence of SARS-CoV-2. Hence, SARS-CoV-2 likely emerged from the accumulation of mutations responding to altered selective pressures or from the infidelity of RNA polymerase perpetuated as replication-neutral mutations (Fahmi Infect Genet Evol. 2020, see below).

Patino-Galindo (manuscript on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.02.15.950568v2) suggested a two-hit scenario in the emergence of the SARS-CoV-2 virus whereby the virus ancestors in bats first acquired genetic characteristics of SARS by incorporation of a SARS-like RBD through recombination before 2009, and subsequently, those recombinants underwent convergent evolution.

Gu (on BioRxiv https://www.biorxiv.org/content/10.1101/2020.02.15.950568v2) reported that the amino acid usage pattern of SARS-CoV-2 was generally found similar to bat and human SARSr-CoVs. He also found greater synonymous codon usage distance between SARS-CoV-2 and its phylogenetic relatives on S and M genes, suggesting these two genes of SARS-CoV-2 are subjected to different evolutionary pressures.

Based on an analysis of the 4 structural genes, Kandeel (J MedVir 2020, see below) further reported that SARS-CoV-2 prefers pyrimidine rich codons to purines. Most high-frequency codons were found to end with A or T, while the low frequency and rare codons were ending with G or C. SARS-CoV-2 structural proteins showed 5-20 lower ENC values, compared with SARS, bat SARS and MERS-CoVs. This implies higher codon bias and higher gene expression efficiency of SARS-CoV-2 structural proteins. SARS-CoV-2 encoded the highest number of over biased and negatively biased codons. Pangolin Beta-CoV showed little differences with SARS-CoV-2 ENC values, compared with SARS, bat SARS and MERS CoV.

A manuscript by Zhang (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.25.20027953v1.full.pdf) provides a new hypothesis to explain the initial spread of the disease. Based on the analysis of 97 virus sequences, the authors were able to propose a classification of current SARS-CoV-2 isolates into two main types, with three sources of transmission, namely Type IA, Type IB, and Type II. Among them, Type IA corresponds to the earliest transmission source, which did not occur in the Huanan Market, indicating that the original transmission source was not from the Huanan Market. Type II comes from the Huanan Market. As most samples detected belong to Type II, it is speculated that a Type II virus is the major outbreak source. By analysing the three genomic sites distinguishing Type I and Type II strains, it was found that the synonymous changes at two of the three sites confer higher protein translational efficiencies to Type II strains. The authors speculate that this observation may be related to higher transmissibility of Type II strains.

Tang (preprint on National Science Review: https://academic.oup.com/nss/advance-article/doi/10.1093/nsr/nwaa036/5775463?searchresult=1) presented new data on the origin and evolution of SARS-CoV-2. Although the authors found only 4% variability in genomic nucleotides between SARS-CoV-2 and the bat SARSr-CoV RaTG13, the difference at neutral sites was 17%, suggesting the divergence between the two viruses is much larger than previously estimated. The report also suggests that new variations in functional sites in the receptor-binding domain (RBD) of the spike seen in SARS-CoV-2 and viruses from pangolin SARSr-CoVs are likely caused by mutations and natural selection besides recombination. Based on the analyses of 103 SARS-CoV-2 genomes, the authors confirmed the publication by Zhang (see above) indicating that these viruses evolved into two major types.
These 2 types were here designated L and S, with the L type (~70%) being more prevalent than the S type (~30%), and the S type representing the ancestral version. Of note, both types of virus were detected outside China.

Yi (Clin Inf Dis 2020, see below) used a different approach to the analysis of 84 sequences in GISAID to provide evidence for genetic recombination underlying the evolution of the virus.

While comparing ORF1ab polyprotein with other βCoVs, Cárdenas-Conejo (J Med Vir 2020, see below) found a 42 amino acid signature that is only present in SARS-CoV-2. Members from clade 2 of sarbecoviruses have traces of this signature. The amino acid signature located in the acidic-domain of papain-like protein of SARS-CoV2 and bat-SL-RatG13 guided the authors to suggest that SARS-CoV-2 probably emerged by genetic drift from bat-SL-CoV-RaTG13.

Sequence homology of the S gene
The S gene of SARS-CoV-2 appears highly divergent to other CoVs, with less than 75% nucleotide sequence identity to all previously described SARS-CoVs, except a 93.1% nucleotide identity to RaTG13 (Zhou Nature 2020, see below). The S genes of SARS-CoV-2 and RaTG13 S gene are longer than other SARS-CoVs. The major differences in SARS-CoV-2 are three short insertions in the N-terminal domain, and 4/5 key residues changes in the receptor-binding motif, in comparison with SARS-CoV.

At the level of amino acids, the S glycoprotein of SARS-CoV-2 was found to have 76.3% identity and 87.3% similarity with the S glycoprotein of SARS-CoV (Baruah J Med Virol 2020, see below).

The S2 subunit of SARS-CoV-2 was found highly conserved, sharing 99% sequence identity with those of the two bat SARS-like CoVs (SL-CoV ZXC21 and ZC45) and human SARS-CoV (Chan Em Microf Inf 2020, see below). This observation suggests that broad spectrum antiviral peptides against S2 may be considered as therapeutic candidates.

The S1 subunit of SARS-CoV-2 shares around 70% identity to that of the two bat SARS-like CoVs and human SARS-CoV. The core domain of the receptor binding domain (RBD) (excluding the external subdomain) is highly conserved, but the external subdomain of the SARS-CoV-2 RBD (which is responsible for the direct interaction with the host receptor) shares only 40% amino acid identity with other SARS-related coronaviruses. Of note, homology modelling in another study revealed that SARS-CoV-2 has a similar RBD structure to that of SARS-CoV, despite amino acid variation at some key residues (Lu Lancet 2020, see below). Moreover, several critical residues in SARS-CoV-2 RBD (particularly Gln493) provide favourable interactions with human ACE2, consistent with SARS-CoV-2’s capacity for human cell infection (Wan J Virol 2020, see below). Several other critical residues in SARS-CoV-2 RBD (particularly Asn501) are compatible with, but not ideal for, binding human ACE2.

Structure of S and interactions with the ACE2 receptor
Jaimes (manuscript on ArXiv: https://arxiv.org/ftp/arxiv/papers/2002/2002.06196.pdf) performed structural modelling of the SARS-CoV-2 S glycoprotein. The data provided support for a similar receptor utilization between SARS-CoV-2 and SARS-CoV, despite a relatively low amino acid similarity in the receptor binding module. Compared to SARS-CoV, an extended structural loop containing basic amino acids was identified at the interface of the receptor binding (S1) and fusion (S2) domains, which was predicted to be proteolytically-sensitive. Jaimes suggested this loop confers fusion activation and entry properties more in line with other coronaviruses.

Wrapp (Science 2020, see below) disclosed the 3.5 Å-resolution cryo-EM structure of the SARS-CoV-2 S trimer in the prefusion conformation. The predominant state of the trimer has one of the three receptor-binding domains (RBDs) rotated up in a receptor-accessible conformation. He also showed biophysical and structural evidence that SARS-CoV-2 S binds ACE2 with higher affinity than SARS-CoV S. Additionally he tested several published SARS-CoV RBD-specific monoclonal antibodies and found that no appreciable binding to SARS-CoV-2 S, confirming previous conclusions from sequence analyses that antibody cross-reactivity may be limited between the two virus RBDs.
Yan (Science 2020, see below) presented the cryo-EM structures of full-length human ACE2, in the presence of a neutral amino acid transporter B0AT1, with or without the receptor binding domain (RBD) of the surface spike glycoprotein (S protein) of SARS-CoV-2, both at an overall resolution of 2.9 Å, with a local resolution of 3.5 Å at the ACE2-RBD interface. The ACE2-B0AT1 complex is assembled as a dimer of heterodimers, with the Collectrin-like domain (CLD) of ACE2 mediating homo-dimerization (see Figure 2). The RBD is recognized by the extracellular peptidase domain (PD) of ACE2 mainly through polar residues.

Figure 2 Interactions between SARS-CoV-2-RBD and ACE2 (from Yan Science 2020).

Letko (Nature Microb 2020, see below) confirmed previous observations in terms of receptor usage of the virus, and suggests that SARS-CoV-2 is capable of using human ACE2 as efficiently, if not more, as SARS-CoV, which may help to explain the human-to-human transmissibility. The experiments were based on the use of pseudotypes and investigated the mechanism of entry of a whole set of lineage B βCoVs.

Ibrahim (J Inf 2020, see below) developed predictions of the COVID-19 spike binding site to the cell-surface receptor (Glucose Regulated Protein 78 (GRP78)). The study revealed that binding is more favourable between regions III (C391-C525) and IV (C480-C488) of the spike protein model and GRP78. Region IV was found the main driving force for GRP78 binding with the predicted binding affinity of -9.8 kcal/mol. These nine residues could be used to develop therapeutics specific against COVID-19.

Of note, Xia (Cell Mol Immunol 2020, see below) published a report on the fusion mechanism of SARS-CoV-2 and fusion inhibitors targeting HR1 domain in S protein.
Other SARS-CoV-2 genes
A manuscript by Alam (https://www.biorxiv.org/content/10.1101/2020.02.17.952895v1.full.pdf) shows the conservation of the E gene, differing between SARS and SARS-CoV2 with a difference of single amino acid substitution and a single amino acid insertion present in SARS but absent from SARS-CoV-2. The authors recommend diagnosis based on this protein.

Immune responses to SARS-CoV-2 infection
Up to now, there has been no report describing immune responses to SARS-CoV-2 infection. However, data are available to characterize both innate and adaptive immune responses to SARS-CoV and MERS-CoV (see for instance Li J Med Virol 2020 below). Such knowledge can be expected to facilitate vaccine development as well as specific immunotherapy against COVID-19.

In addition, data pertaining to immunity against other coronaviruses may be very relevant to the understanding of immune responses to and pathogenesis of SARS-CoV-2. For instance, Wang (Virol Sin 2018, see below) found antibodies against bat SARS-related coronavirus in people living near caves inhabited by bats in China. A recent report also described serology testing against common human CoV strains in a prospective study of 200 subjects evaluated for respiratory infections in the U.S. (Gorse J Med Vir 2020, see below). Interestingly, a publication by Chan (J Clin Virol 2009, see below) presented the seroprevalence of HCoV HKU1 according to age, showing steadily increasing seroprevalence in childhood and early adulthood, from 0% in the < 10 years age group to a plateau of 21.6% in the 31-40 years age group in Hong Kong.

First observations in COVID-19 patients
Thevarajan (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.20.20025841v1) reported the kinetics of the immune response in relation to clinical and virological features of a patient with mild-to-moderate COVID-19 requiring hospitalisation. Increased antibody-secreting cells, follicular T-helper cells, activated CD4+ and CD8+ T-cells and IgM/IgG SARS-CoV-2-binding antibodies (immunofluorescence assay using SARS-CoV-2-infected Vero cells) were detected in blood, prior to symptomatic recovery. These immunological changes persisted for at least 7 days following full resolution of symptoms. Of note, the authors detected reduced frequencies of CD16+CD14+ monocytes in peripheral blood at day 7-9, which might indicate efflux of CD16+CD14+ monocytes from blood to the site of infection. Low levels of activated HLA-DR+CD3-CD56+ NK cells were found in both the COVID-19 patient and healthy controls.

A study of the dynamic changes of lymphocyte subsets and cytokines profiles of 40 COVID-19 patients has been reported by Liu (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.16.20023671v1). Significant decreases in the counts of T cells, especially CD8+ T cells, were observed as well as increases in IL-6, IL-10, IL-2 and IFN-γ levels in the peripheral blood in the severe cases compared to those in the mild cases. T cell counts and cytokine levels in severe COVID-19 patients who survived the disease gradually recovered at later time points to levels that were comparable to those of the mild cases.

A manuscript by Liao (not peer-reviewed, on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.23.20026690v1) characterized the lung immune microenvironment with the bronchoalveolar lavage fluid (BALF) from 3 severe and 3 mild COVID-19 patients. The data show that monocyte-derived FCN1+ macrophages, but not FABP4+ alveolar macrophages that represent a predominant macrophage subset in BALF from patients with mild diseases, overwhelm in the severely damaged lungs from patients with acute respiratory distress syndrome (ARDS). These cells are highly inflammatory and enormous chemokine producers implicated in cytokine storm. Furthermore, the formation of tissue resident, highly expanded

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clonal CD8+ T cells in the lung microenvironment of mild symptom patients suggests a robust adaptive immune response.

**Antigens and epitopes**

**Epitope predictions**

While *in vitro* as well as *in vivo* data are still lacking to characterize adaptive immune responses to SARS-CoV-2, immune-informatics approaches targeting identification of T and B cell epitopes of SARS-CoV-2 have been described by several authors.

Baruah (J Med Virol 2020, see below) for instance, predicted five CTL epitopes, three sequential B cell epitopes and five discontinuous B cell epitopes in the S glycoprotein, as illustrated by Table 2 below. Simulations suggested that the CTL epitopes bind MHC class I peptide-binding grooves via multiple contacts, with continuous hydrogen bonds and salt bridge anchors, supporting their potential in generating immune responses. Of note, the study found only one overlapping CTL epitope between MERS-CoV and SARS-CoV-2 with one gap and one mismatch (Y-LQPRTFLL/YKLQPLTFLL), and no comparable epitopes with SARS-CoV.

**Table 2 Epitopes predicted in SARS-CoV-2 S glycoprotein (from Baruah J Med Virol 2020)**

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Epitope score ANN/SVM</th>
<th>Antigenicity (score)</th>
<th>HLA (%Rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TYLQYRTFLL</td>
<td>0.83/0.64</td>
<td>Y (0.45)</td>
<td>HLA-A*02:01 (0.01)</td>
</tr>
<tr>
<td>GYVFASTEK</td>
<td>0.58/0.98</td>
<td>Y (0.71)</td>
<td>HLA-A*03:01 (0.00)</td>
</tr>
<tr>
<td>EPVLEKGVKL</td>
<td>0.73/0.61</td>
<td>Y (1.23)</td>
<td>HLA-B*07:02 (0.28)</td>
</tr>
<tr>
<td>VVQNQAQL</td>
<td>0.77/0.78</td>
<td>Y (0.47)</td>
<td>HLA-B*07:02 (0.78)</td>
</tr>
<tr>
<td>WTAGAAAYY</td>
<td>0.82/0.54</td>
<td>Y (0.63)</td>
<td>HLA-B*15:01 (0.37)</td>
</tr>
</tbody>
</table>

**B sequential epitopes**

<table>
<thead>
<tr>
<th>Epitope:Position</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVNLTTRTQLPPAYTN</td>
<td>0.74</td>
</tr>
<tr>
<td>CVNLTTRTQLPPAYTN</td>
<td>Y (1.38)</td>
</tr>
<tr>
<td>N (0.92)</td>
<td></td>
</tr>
<tr>
<td>NVTWFHAIHVSTNGT</td>
<td>0.55</td>
</tr>
<tr>
<td>NVTWFHAIHVSTNGT</td>
<td>Y (0.84)</td>
</tr>
<tr>
<td>N (0.30)</td>
<td></td>
</tr>
<tr>
<td>SFSTFKCVGVSPKDLN</td>
<td>0.69</td>
</tr>
<tr>
<td>SFSTFKCVGVSPKDLN</td>
<td>Y (1.06)</td>
</tr>
<tr>
<td>N (0.16)</td>
<td></td>
</tr>
</tbody>
</table>

Kumar (manuscript on Preprints: [https://www.preprints.org/manuscript/202002.0071/v1](https://www.preprints.org/manuscript/202002.0071/v1)) predicted 8 B cell epitopes in the S protein based on the antigenicity score by using Vaxigen 2.0 (Table 3), some of which displayed overlap with those predicted by Baruah in Table 2.

**Table 3**, some of which displayed overlap with those predicted by Baruah in Table 2.

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Table 3: B-cell epitopes present on surface predicted by Kumar

<table>
<thead>
<tr>
<th>Number</th>
<th>Epitope sequence</th>
<th>Vaxigen score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VLPLVSSQCVNLTRTQLPPAYTN</td>
<td>1.0555</td>
</tr>
<tr>
<td>2</td>
<td>RSSVHLSTQD</td>
<td>0.5404</td>
</tr>
<tr>
<td>3</td>
<td>VTVFHAIVHSGTNGTFRFDN</td>
<td>0.5485</td>
</tr>
<tr>
<td>4</td>
<td>VYFASTEKSNII</td>
<td>0.7795</td>
</tr>
<tr>
<td>5</td>
<td>GTTLDKTSQSLIUVNNATNVKVC</td>
<td>0.4494</td>
</tr>
<tr>
<td>6</td>
<td>YHHNNK5W5M5E5R5V5Y5S5Q5VS5Q5P5FL5</td>
<td>0.2569</td>
</tr>
<tr>
<td>7</td>
<td>IYSKHTPIN</td>
<td>0.9013</td>
</tr>
<tr>
<td>8</td>
<td>DLPQGFSALEPLYDLPLIGNITRFQTLLAH R5YLTPG5OSSSSGWTAGAAAYLYNK5N5G5TIT DAVDCA5LD5PLE5T5K5C5TL5KSFT5VE5K5G5I5Y5Q5TS5 FR5Q5PT5ES5VR5FPN5IT5NL5CP5GE</td>
<td>0.6329</td>
</tr>
</tbody>
</table>

Predictions of CD4 and CD8 epitopes were also reported by various teams, reaching quite different conclusions (Kumar on Preprints: https://www.preprints.org/manuscript/202002.0071/v1; or Bojin on Preprints: https://www.preprints.org/manuscript/202002.0102/v1). The approach selected by Ahmed (Vir 2020, see below) focused on one side on S and N epitopes conserved across isolates and T cell epitopes offering broad coverage.

Fast (on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.02.19.955484v1) reported the use of various computational tools from structural biology and machine learning to identify SARS-CoV-2 epitopes based on viral protein antigen presentation and antibody binding properties. The study identified two potential neutralizing B-cell epitopes near the spike protein RBD (positions 440-460 and 494-506) and a whole set of potential MHC I and II epitopes (see Table 4).

Table 4. Top potential T cell epitopes for key 2019-nCoV proteins*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Position</th>
<th>MHC-I Cov.</th>
<th>MHC-II Cov.</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>SYGFQPTN5GQ5PY 494</td>
<td>Yes</td>
<td>52%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>SQ51IAY5MS5L5G5A5EN 689</td>
<td>Yes</td>
<td>74%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>IPT5N5TISVT5E5IL5P 714</td>
<td>Yes</td>
<td>70%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>AXAY5Y55G5L55Q5PR5T5FL 262</td>
<td>Yes</td>
<td>65%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>AP5H5V55F5L5H55V55Y55PA 1056</td>
<td>Yes</td>
<td>65%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td>ORF1ab</td>
<td>DGEV5IT5P5D5L5K5L5TTLS 1547</td>
<td>Yes</td>
<td>83%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>EV5RT5IK5F5T5V5D5IN5IN 1564</td>
<td>Yes</td>
<td>78%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>IN5L5V5Q5M5AP5IS5AM5VR 2368</td>
<td>Yes</td>
<td>70%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>NPT5FH5L5D5GE5V5IT5FD 1540</td>
<td>Yes</td>
<td>74%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>VAA5IF5Y55L5F5P5V55H55MS 2476</td>
<td>Yes</td>
<td>65%</td>
<td>Yes</td>
<td>90%</td>
</tr>
<tr>
<td>M</td>
<td>ISAS5F5RL5F5AR55T5SM5NS 97</td>
<td>Yes</td>
<td>65%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td>N</td>
<td>AT5K5AY5N5V5Q5A5F5G5R5G 264</td>
<td>Yes</td>
<td>74%</td>
<td>Yes</td>
<td>100%</td>
</tr>
<tr>
<td>E</td>
<td>V5K5PS5Y55V5Y55S5R55K5N5LN 52</td>
<td>Yes</td>
<td>100%</td>
<td>Yes</td>
<td>?</td>
</tr>
</tbody>
</table>

* Epitopes were ranked based on their likely coverage of presentation by MHC-I and MHC-II alleles. S protein 494-508 is highly ranked based on MHC presentation and is also one of the predicted top B-cell epitopes, localized near the S protein receptor binding domain. MHC-I coverage is calculated by the 9mer with the highest MHC-I coverage for each epitope (highlighted in orange). All candidates are likely to be presented by both MHC-I and MHC-II. A question mark (?) under the antibody column indicates that one or more SARS homolog of this peptide is a known B-cell epitope.

Additional epitope predictions were also reported by Bhattacharya (J Med Virol 2020, see below).

Grifoni (Cell Host & Micr 2020: https://marlin-prod.literatumonline.com/pb-assets/journals/research/cell-host-microbe/PDFs/CHOM_2264_S50.pdf) identified multiple specific regions in SARS-CoV2 that have high homology to the SARS-CoV virus. Parallel bioinformatic predictions identified a priori potential B and T cell epitopes for SARS-CoV-2. The authors suggested that independent identification of the same regions using two approaches reflects the high probability that these regions are promising targets for immune recognition of SARS-CoV-2.
Cross-reactivity
Based on structure analyses, Tian (Em Inf Dis 2020, see below) predicted potent binding of COVID-19 S protein by SARS-specific human monoclonal antibody CR3022. However, the hypothesis remains to be supported by in vitro experiments.

Using MLV-based pseudotypes neutralization assays, Walls (Cell 2020, see below) investigated the ability of plasma from four mice immunized with a stabilized SARS-CoV S to inhibit SARS-CoV-2 S- and SARS-CoV S-mediated entry into target cells. All sera tested completely inhibited transduction of SARS-CoV S-MLV and reduced SARS-CoV-2 S-MLV transduction to \( \sim 10\% \) of control in Vero E6 cells. The elicitation of a heterotypic response blocking SARS-CoV-2 S-mediated entry into host cells concurred with the sequence and structural conservation of SARS-CoV-2 S and SARS-CoV S along with their comparable glycans shields and suggested that immunity against one virus of the sarbecovirus subgenus can potentially provide protection against related viruses.

Antibody response
By using an ELISA based assay using the recombinant viral nucleocapsid, Guo (Clin Inf Dis 2020, see below) examined the host humoral response against SARS-CoV-2 including IgA, IgM and IgG responses. A total of 208 plasma samples were collected from 82 confirmed and 58 probable cases. The diagnostic value of IgM was evaluated in this cohort. The median duration of IgM and IgA antibody detection were 5 days (IQR 3-6), while IgG was detected on 14 days (IQR 10-18) after symptom onset, with a positive rate of 85.4%, 92.7% and 77.9% respectively. In confirmed and probable cases, the positive rates of IgM antibodies were 75.6% and 93.1%, respectively. The detection efficiency by IgM ELISA was higher than that of qPCR method after 5.5 days of symptom onset. The positive detection rate was significantly increased (98.6%) when combined IgM ELISA assay with PCR for each patient compare with a single qPCR test (51.9%).

Clinical disease
Initial observations in Wuhan
In December, 2019, a series of pneumonia cases of unknown cause emerged in Wuhan, Hubei, China, with clinical presentations greatly resembling viral pneumonia (http://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/). By Jan 2, 2020, 41 admitted hospital patients had been identified as having laboratory-confirmed COVID-19 (Huang Lancet 2020, see below). Most of the infected patients were men (30 [73%] of 41); less than half had underlying diseases (13 [32%]), including diabetes (eight [20%]), hypertension (six [15%]), and cardiovascular disease (six [15%]). Median age was 49.0 years (IQR 41.0-58.0). 27 (66%) of 41 patients had been exposed to Huanan seafood market. One family cluster was found. Common symptoms at onset of illness were fever (40 [98%] of 41 patients), cough (31 [76%]), and myalgia or fatigue (18 [44%]); less common symptoms were sputum production (11 [28%] of 39), headache (three [8%] of 38), haemoptysis (two [5%] of 39), and diarrhoea (one [3%] of 38). Dyspnoea developed in 22 (55%) of 40 patients (median time from illness onset to dyspnoea 8-0 days [IQR 5.0-13.0]). 26 (63%) of 41 patients had lymphopenia. All 41 patients had pneumonia with abnormal findings on chest CT. Complications included acute respiratory distress syndrome (12 [29%]), RNAemia (six [15%]), acute cardiac injury (five [12%]) and secondary infection (four [10%]). 13 (32%) patients were admitted to an ICU and six (15%) died. Compared with non-ICU patients, ICU patients had higher plasma levels of IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFα.

Incubation period
Among the first 425 patients with confirmed COVID-19-pneumonia, the mean incubation period was 5.2 days (95% confidence interval, 4.1 to 7.0), with the 95th percentile of the distribution at 12.5 days (Li New Engl J Med 2020, see below). This observation was confirmed by other datasets as illustrated in Table 5 below.
Table 5 Incubation period as reported by different studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Incubation period and 95% confidence interval</th>
<th>Other information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li NewEngl J Med 2020</td>
<td>5.2 days (95% CI, 4.1 to 7.0)</td>
<td>95th percentile of the distribution ranging from 2 to 11 days</td>
</tr>
<tr>
<td>Liu (<a href="https://www.biorxiv.org/content/10.1101/2020.01.25.919787v1.full">https://www.biorxiv.org/content/10.1101/2020.01.25.919787v1.full</a>)</td>
<td>4.8 days (±2.6)</td>
<td>95th percentile of the distribution ranging from 2.1 to 11.1 days (2.5th to 97.5th percentile)</td>
</tr>
<tr>
<td>Backer (<a href="https://www.medrxiv.org/content/10.1101/2020.01.27.20018986v2">https://www.medrxiv.org/content/10.1101/2020.01.27.20018986v2</a>)</td>
<td>6.4 days (95% CI, 5.6 - 7.7)</td>
<td>Median of 3 days; ranging from 0 to 24 days</td>
</tr>
<tr>
<td>Guan (<a href="https://www.medrxiv.org/content/10.1101/2020.02.06.20020974v1.full.pdf">https://www.medrxiv.org/content/10.1101/2020.02.06.20020974v1.full.pdf</a>)</td>
<td></td>
<td>Median 4 days (interquartile range 3-5 days)</td>
</tr>
<tr>
<td>Xu BMI 2020</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lauer (Ann Intern Med 2020, see below) re-assessed the incubation period, using a compilation of 181 published cases with identifiable exposure and symptom onset windows. A median incubation period of 5.1 days (95% CI, 4.5 to 5.8 days) was found, with 97.5% of those who develop symptoms doing so within 11.5 days (CI, 8.2 to 15.6 days) of infection. 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. Whether this risk is acceptable will depend on the underlying risk of infection and consequences of missed cases.

Similar results were obtained by Linton (J Clin Med 2020 see below), who found the incubation period to falls within the range of 2–14 days with 95% confidence and to have a mean of around 5 days. Based on the 95th percentile estimate of the incubation period, she recommended that the length of quarantine should be at least 14 days.

Interestingly, based on reports collected in China, Han (manuscript on MedRxiv: [https://www.medrxiv.org/content/10.1101/2020.02.24.20027474v1](https://www.medrxiv.org/content/10.1101/2020.02.24.20027474v1)) found that the incubation periods of groups of individuals with age>=40 years and age<40 years demonstrated a statistically significant difference. The former group had a longer incubation period and a larger variance than the latter.

**Description of clinical disease**

**Clinical disease in China**

A large number of reports provide descriptions of the clinical signs associated with COVID-19 in Wuhan and other cities in China. The disease ranges from mild infection to severe acute respiratory infection. Table 6 illustrates the signs and symptoms detected in a selection of early reports describing the disease as observed in hospitalized patients.

**Table 6 Clinical presentation in different cohorts of patients with COVID-19 pneumonia (frequency of reported symptoms)**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Chen Lancet 2020 (n=99*)</th>
<th>Song Radiol 2020 (n=51)</th>
<th>Chang JAMA 2020 (n=13)</th>
<th>Guan NEJM 2020 (n=1099**)</th>
<th>Wang JAMA 2020 (n=138)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>83%</td>
<td>96%</td>
<td>92.3%</td>
<td>88.7%</td>
<td>98.6%</td>
</tr>
<tr>
<td>Cough</td>
<td>82%</td>
<td>47%</td>
<td>46.3%</td>
<td>67.8%</td>
<td>59.4%</td>
</tr>
<tr>
<td>Shortness of breath (dyspnoea)</td>
<td>31%</td>
<td>31%</td>
<td>23.1%</td>
<td>14.9%</td>
<td>34.8%</td>
</tr>
<tr>
<td>Muscle ache (myalgia)</td>
<td>11%</td>
<td>31%</td>
<td>23.1%</td>
<td>38.1%</td>
<td>69.6%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>9%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>8%</td>
<td>16%</td>
<td>23.1%</td>
<td>13.6%</td>
<td></td>
</tr>
<tr>
<td>Sore throat</td>
<td>5%</td>
<td></td>
<td></td>
<td></td>
<td>13.9%</td>
</tr>
<tr>
<td>Rhinorrhea</td>
<td>4%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest pain</td>
<td>2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>2%</td>
<td>10%</td>
<td></td>
<td>3.8%</td>
<td>10.1%</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>1%</td>
<td></td>
<td></td>
<td>5%</td>
<td>10.1%</td>
</tr>
<tr>
<td>Acute respiratory distress syndrome</td>
<td>17%</td>
<td></td>
<td></td>
<td></td>
<td>3.4%</td>
</tr>
</tbody>
</table>

* Among the 99 patients, 76% patients received antiviral treatment, including oseltamivir (75 mg every 12 h, orally), ganciclovir (0.25 g every 12 h, intravenously), and lopinavir and ritonavir tablets (500 mg twice daily, orally). The duration of antiviral treatment was 3-14 days (median 3 days)
Additional data were also made available in the reports listed below:

- Zhang (Virol Sin 2020, see below) described 2 cases of COVID-19 in Wuhan
- Huang (Trav Med Inf Dis 2020, see below) described 34 cases in Wuhan
- Chen (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.16.20023903v1) described 21 patients with COVID-19
- Li (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.11.20022053v1.full.pdf) described 17 patients outside Wuhan
- Cai (on MedRXiv: https://www.medrxiv.org/content/10.1101/2020.02.17.20024018v1) described 298 confirmed cases in the Third People's Hospital of Shenzhen, from January 11, 2020 to February 6, 2020
- Zhang (Allergy 2020, see below) described 140 patients in Wuhan
- Liu (Chin Med J 2020, see below) described 78 patients in Wuhan
- Wang (https://www.medrxiv.org/content/10.1101/2020.02.21.20026112v2.full.pdf) used the publicly released data for 1212 patients to described the characteristics of COVID-19 in Henan, China
- Sun (Lancet 2020, https://www.thelancet.com/journals/landig/article/PIIS2589-7500(20)30026-1/fulltext) used crowdsourced data from social media sources to monitor the COVID-19 outbreak; 507 patients reported from Jan 13, to Jan 31, 2020, including 364 from mainland China and 143 from outside of China were studied
- Yang (Lancet 2020, see below) described 52 critically ill patients
- Liu (on MedRxiv https://www.medrxiv.org/content/10.1101/2020.02.17.20024166v3) described 109 patients, including 53 severe disease cases.
- Xu (BMJ 2020, see below) described 62 hospitalized patients with confirmed infection in seven hospitals in Zhejiang province.
- Wu (Clin Inf Dis 2020, see below) described 80 patients in Jiangsu Province.
- Zhou (Lancet 2020, see below) provided details on 191 patients with laboratory-confirmed disease in Wuhan.
- Qian (QJM 2020, see below) described 91 hospitalized patients with COVID-19 in Zhejiang.

A metaanalysis by Sun (J Med Vir 2020, see below) covered ten of these studies1, including a total number of 50 466 patients. It confirmed that fever and cough are the most common symptoms in patients with SARS-CoV-2 infection, and that a vast majority of these patients (96.6%) have abnormal chest CT examination. The incidence of fever was estimated at 89.1%, the incidence of cough 72.2%, and the incidence of muscle soreness or fatigue 42.5%. In this analysis, the incidence of acute respiratory distress syndrome (ARDS) reached 14.8%. Diarrhoea, haemoptysis, headache, sore throat, shock, and other symptoms were reported to occur only in a small number of patients.

Of note, Sun (JMedVir 2020, see below) reported a definition of fever as temperature ≥ 37.3°C. He did not provide details on the method of temperature recording (e.g. axillary, forehead or sublingual). This definition was indeed reported for instance by Song (Radiol 2020). By contrast, Guan (NEJM 2020, see below) mentioned a definition of fever as an axillary temperature of 37.5°C or higher. Such discrepancies can be expected to result in some variability across hospitals with regard to the detection of this symptom.

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1 Huang Lancet 2020; Wang JAMA 2020 ; Chen Lancet 2020; Guan NEJM 2020 ; Chen Zhonghua Jie He He Hu Xi Za Zhi.; Sun, Lancet 2020; Yang medRxiv 2020 (manuscript subsequently withdrawn); Li medRxiv 2020; The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, Chinese Center for Disease Control and Prevention. Zhong Hua Liu Xing Bing Xue Za Zhi 2020; Xu BMJ 2020.
Another metaanalysis by Li (J Med Vir 2020, see below), including a somewhat different set of ten studies found the main clinical symptoms of COVID-19 patients to be fever (88.5%), cough (68.6%), myalgia or fatigue (35.8%), expectoration (28.2%), and dyspnœa (21.9%). In addition to common respiratory symptoms, the symptoms of headache or dizziness (12.1%), diarrhoea (4.8%), nausea, and vomiting (3.9%) were also obvious in some patients.

A third metaanalysis by Rodriguez-Morales (Trav Med Inf Dis 2020, see below) found that in 656 patients, fever (88.7%, 95%CI 84.5-92.9%), cough (57.6%, 40.8-74.4%) and dyspnea (45.6%, 10.9-80.4%) were the most prevalent manifestations. Among the patients, 20.3% (95%CI 10.0-30.6%) required intensive care unit (ICU), 32.8% presented with ARDS (95%CI 13.7-51.8), 6.2% (95%CI 3.1-9.3) with shock. Some 13.9% (95%CI 6.2-21.5%) of hospitalized patients had fatal outcome.

Hu (Eur Heart J, see below) presented a COVID-19 case with fulminant myocarditis with cardiogenic shock. This clinical presentation is considered to be rare.

Of note, while expression of the ACE2 receptor in kidney and bladder had suggested the possibility of renal involvement in COVID-19, Wang (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.19.20025288v1) analysed data from 116 hospitalized patients, and concluded that acute renal impairment was uncommon in COVID-19, and that there was no aggravation of chronic renal failure observed in this cohort.

Clinical disease outside China

Descriptions of cases that occurred outside China are also available. For instance:

- Ki (Epidemiol Health 2020) described the early cases identified in Korea,
- Holshue the first case in the United States of America (USA) (New Engl J Med 2020, see below), and Harcourt (Emerg Infect Dis 2020, see below) the virus isolation from this patients and its characterization,
- Arentz (JAMA 2020, see below) 21 critically Ill patients with COVID-19 in Washington State, USA
- Bastola (Lancet Inf Dis 2020, see below) and Shrestha (J Travel Med 2020, see below) the first case in Nepal,
- Bernard Stoecklin the first cases in France (Eurosurv 2020, see below),
- Silverstein (Lancet 2020, see below) the first case in Canada,
- Van Cuong the first case in Vietnam (Lancet Inf Dis 2020, see below)
- Huang (J Micr Imm Inf 2020, see below) described 2 cases in Taiwan
- Lillie (J Inf 2020, see below) described 2 cases in the UK with person to person transmission
- Cheng (J Formos Med Assoc 2020, see below) described the first case in Taiwan
- Young (JAMA 2020, see below) described the case series of the first 18 patients with PCR-confirmed SARS-CoV-2 infection at 4 hospitals in Singapore from January 23 to February 3, 2020
- Marchand-Senecal (Clin Inf Dis 2020, see below) described the first case in Canada.
- The COVID-19 National Emergency Response Center (Osong Public Health Res Perspect 2020, see below) presented 28 cases in South Korea.

Spiteri (Euro Surv 2020: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.9.2000178) described the first cases detected in Europe, excluding cases reported in the United Kingdom (UK), as at 21 February 2020. The analysis included both sporadic cases among travellers from China (14 cases) and cases who acquired infection due to subsequent local transmission in Europe (21 cases). The clinical presentation observed in the cases in

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Paper version: dd. 23 MAR 2020
Europe is that of an acute respiratory infection. However, of the 31 cases with information on symptoms, 20 cases presented with fever and nine cases presented only with fever and no other symptoms.

Clinical imaging

A number of reports provide a detailed description of chest computed tomography (CT) scan findings of patients with COVID-19 pneumonia. For instance:

- Kong Radiol 2020 on [https://pubs.rsna.org/doi/full/10.1148/ryct.2020200028](https://pubs.rsna.org/doi/full/10.1148/ryct.2020200028);
- Li Radiol 2020 on [https://pubs.rsna.org/doi/full/10.1148/ryct.2020200026](https://pubs.rsna.org/doi/full/10.1148/ryct.2020200026);
- Ng Radiol 2020 on [https://pubs.rsna.org/doi/full/10.1148/ryct.2020200034](https://pubs.rsna.org/doi/full/10.1148/ryct.2020200034);
- Song Radiol 2020, see below
- Chung Radiol 2020, see below
- Bernheim Radiol 2020, see below
- Yoon Korean J Radiol 2020, see below
- Xu Eur J Nucl Med Mol Imaging 2020, see below
- Yang (J Inf 2020, see below) presented clinical imaging data from 149 RT-PCR confirmed positive patients in three tertiary hospitals of Wenzhou.
- Shi Lancet Inf Dis 2020, see below
- Xu (J Inf 2020, see below) described data from 50 patients, including mild type, common, severe and critically severe cases.
- Albarello (Int J Infect Dis 2020, see below) presented the CT findings in 2 cases in Italy.
- Li and Xia (AJR Am J Roentgenol 2020, see below) described 51 cases in Wuhan.
- Zhou (AJR Am J Roentgenol 2020, see below) described CT findings in 62 patients in Wuhan.
- Xiong (Invest Radiol 2020, see below) described 42 cases in Wuhan including cases with progressive disease features.
- Zhu (J Inf 2020, see below) described 6 cases in Guangzhou.
- Li (Ped Radiol 2020, see below) presented CT findings from 5 children at a large tertiary-care hospital in China with positive RT-PCR for COVID-19.
- Xia (Pediatr Pulmonol 2020, see below) described 20 paediatric patients, of which 6 presented with unilateral pulmonary lesions (6/20, 30%), 10 with bilateral pulmonary lesions (10/20, 50%), and 4 showed no abnormality on chest CT (4/20, 20%).

Guan (NEJM 2020, see below) found that on admission ground-glass opacity (see Figure 3) was the typical radiological finding on chest CT (50.00%, in a dataset of 1 099 patients with laboratory-confirmed disease). The typical radiological imaging of COVID-19 pneumonia demonstrated destruction of the pulmonary parenchyma including interstitial inflammation and extensive consolidation, similar to SARS (Pan Radiol 2020, see below). However, some patients with COVID-19 pneumonia consistently demonstrated no hypoxemia or respiratory distress during the course of hospitalization. A study in 21 patients recovering from COVID-19 pneumonia (without severe respiratory distress during the disease course) showed that lung abnormalities on chest CT showed greatest severity approximately 10 days after initial onset of symptoms. Dai (Can Assoc Radiol 2020, see below) also discussed the difference between COVID-19 and other lung diseases.
Figure 3 CT lung imaging from a 41-year-old woman who tested positive for COVID-19. This 3-D reconstruction shows multifocal ground glass opacities without consolidation (from https://www.itnonline.com/content/radiologists-describe-coronavirus-ct-imaging-features).

Zhang (Int Care Med 2020, see below) observed white “Septal Lines” in a 75-year-old male confirmed with severe COVID-19 pneumonia, suggesting that cellulosic exudation occurred at the surface of lung lobes.

Salehi (AJR Am J Roentgenol 2020, see below) published a systematic review of imaging findings in 919 patients. The authors found the characteristic patterns and distribution of CT manifestations: ground glass opacification (GGO) (88.0%), bilateral involvement (87.5%), peripheral distribution (76.0%), and multilobar (more than one lobe) involvement (78.8%) (Table 7). Isolated GGO or a combination of GGO and consolidative opacities were some of the most common CT findings. Other CT findings included interlobular septal thickening, bronchiectasis, pleural thickening, and subpleural involvement, with various rates across the studies. Pleural effusion, pericardial effusion, lymphadenopathy, cavitation, CT halo sign, and pneumothorax were less common or rare.

Table 7 Common Patterns and Distribution on Initial CT Images of 919 Patients With COVID-19 (from Salehi AJR Am J Roentgenol 2020)

<table>
<thead>
<tr>
<th>Imaging Finding</th>
<th>No. of Studies</th>
<th>No. (%) of Reported Cases/ Total No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral involvement</td>
<td>12</td>
<td>435/497 (87.5)</td>
</tr>
<tr>
<td>Peripheral distribution</td>
<td>12</td>
<td>92/121 (76.0)</td>
</tr>
<tr>
<td>Posterior involvement</td>
<td>1</td>
<td>41/51 (80.4)</td>
</tr>
<tr>
<td>Multilobar involvement</td>
<td>5</td>
<td>108/137 (78.8)</td>
</tr>
<tr>
<td>Ground-glass opacification</td>
<td>22</td>
<td>346/393 (88.0)</td>
</tr>
<tr>
<td>Consolidation</td>
<td>10</td>
<td>65/204 (31.8)</td>
</tr>
</tbody>
</table>

Qin (Eur J Nucl Med Mol Imaging 2020, see below) described for the first time the 18F-FDG PET/CT findings of four patients with COVID-19. The data confirmed previous observations of peripheral ground-glass opacities and/or lung consolidations (in more than two pulmonary lobes). Lung lesions were characterized by a high 18F-FDG uptake and there was evidence of lymph node involvement. Conversely, disseminated disease was absent, a finding suggesting that COVID-19 has pulmonary tropism.
Following the evaluation of 80 patients, Wu (Invest Radiol 2020, see below) suggested significant correlations between the degree of pulmonary inflammation and the main clinical symptoms and laboratory results. Similarly, Zhao (AJR Am J Roentgenol 2020, see below) investigated the relationship between chest CT findings and the clinical condition of 101 patients with COVID-19 pneumonia in Hunan, China, and found that architectural distortion, traction bronchiectasis, and CT involvement score aided in the evaluation of the severity and extent of the disease.

Based on a retrospective analysis of 27 consecutive patients, Yuan (PLoS One 2020, see below) found that a simple CT scoring method was able to predict mortality.

Chest CT has thus acquired a pivotal role for the diagnosis and assessment of lung involvement in COVID-19, and CT protocols are used to estimate the pulmonary damage. Unfortunately, CT scanning is not available in all emergency departments. Lung ultrasound is a surface imaging technique greatly developed in the last decades and strongly recommended for acute respiratory failure. Poggiali (Radiol 2020, see below) presented preliminary data from 12 patients suggesting the feasibility of using bedside ultrasound for the early diagnosis of COVID-19 pneumonia. A recommendation for more studies on this topic was also made by Soldati (J Ultrasound Med 2020, see below), who presented data from 2 additional cases.

**Laboratory finding & biomarkers**

A number of reports present the laboratory observations associated with COVID-19. Various studies addressed the search for a prognostic marker of severe infection, while others focused on understanding pathological mechanisms.

**Virus load**

A number of reports described the virus load in COVID-19 patients. For instance, a study in 12 patients in China showed that the viral load of SARS-CoV-2 detected from patient respiratory tracts was positively linked to lung disease severity (Liu Sci China Life Sci 2020, see below). Kim (J Kor Med Sci 2020, see below) presented the kinetics of the viral load in 2 patients in Korea with mild and moderate disease who received lopinavir/ritonavir therapy.

Pan (Lancet Inf Dis 2020, see below) reported virus load data from different types of clinical specimens collected from 82 infected individuals. The data can be summarized as follows:

- In 2 patients monitored daily, the viral loads in throat swab and sputum samples peaked at around 5-6 days after symptom onset, ranging from around $10^4$ to $10^7$ copies per mL during this time.
- In individuals at different stages of infection, viral loads ranged from 641 copies per mL to $1.34 \times 10^{11}$ copies per mL, with a median of $7.99 \times 10^4$ in throat samples and $7.52 \times 10^5$ in sputum samples.
- A sputum sample collected on day 8 post-onset from a patient who died had a very high viral load ($1.34 \times 10^{11}$ copies per mL).
- Notably, two individuals, who were under active surveillance because of a history of exposure to infected patients showed positive results on RT-PCR a day before onset, suggesting that infected individuals can be infectious before they become symptomatic.
- From 17 confirmed cases with available data (representing days 0–13 after onset), stool samples from nine (53%; days 0–11 after onset) were positive on RT-PCR analysis, but with lower viral loads than respiratory samples.

Another study by Chen, Lan et al. (Em Micr Inf 2020, see below) found detectable SARS-CoV-2 RNA was in the blood of 6 of 57 patients. Importantly, all of these 6 patients progressed to severe symptom stage, indicating a strong correlation of serum viral RNA with disease severity ($p$-value = 0.0001).

In the cohort of 191 patients with laboratory-confirmed disease described by Zhou (Lancet 2020, https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30566-3/fulltext), duration of viral shedding
ranged between 8 and 37 days. The median duration of viral shedding was 20.0 days (IQR 17.0–24.0) in survivors, but continued until death in fatal cases.

Cell counts

A manuscript by Liu based on the monitoring of 61 patients suggests the **neutrophil/lymphocyte ratio** as a predictive marker of severe illness. This biomarker proved superior to the MuLBSTA score that had been suggested before for COVID-19 patients monitoring. [https://www.medrxiv.org/content/10.1101/2020.02.10.20021584v1.full.pdf](https://www.medrxiv.org/content/10.1101/2020.02.10.20021584v1.full.pdf). A subsequent report from data in 40 patients confirmed this conclusion [https://www.medrxiv.org/content/10.1101/2020.02.16.20023671v1](https://www.medrxiv.org/content/10.1101/2020.02.16.20023671v1).

Chen (on MedRxiv [https://www.medrxiv.org/content/10.1101/2020.02.16.20023903v1](https://www.medrxiv.org/content/10.1101/2020.02.16.20023903v1)) reported significantly lower **lymphocyte counts** in severe cases (7000 million/L) than moderate cases (11000 million/L). Absolute number of total T lymphocytes, CD4+T cells and CD8+T cells decreased in nearly all the patients, and were significantly lower in severe cases (332.5, 185.6 and 124.3 million/L) than moderate cases (676.5, 359.2 and 272.0 million/L). The expressions of IFN-γ by CD4+T cells tended to be lower in severe cases (14.6%) than moderate cases (23.6%).

A study by Zheng (manuscript on MedRxiv: [https://www.medrxiv.org/content/10.1101/2020.02.19.20024885v1](https://www.medrxiv.org/content/10.1101/2020.02.19.20024885v1)) investigated differences in laboratory parameters between 103 COVID-19 and 22 non-COVID-19 pneumonia cases. The lymphocyte subsets counts were found to exhibit a significant negative correlation with biochemical indices relating to organ injury in the COVID-19 infected patients.

Similarly, Zeng (manuscript on MedRxiv: [https://www.medrxiv.org/content/10.1101/2020.03.08.20031229v1.full.pdf](https://www.medrxiv.org/content/10.1101/2020.03.08.20031229v1.full.pdf)) described a phenomenon of lymphocyte depletion (PLD) observed in 100% severe or critical cases (ICU). As the disease progressed and clinical status deteriorated, levels of lymphocytes were found progressively decreased before death.

Based on the observation that **eosinopenia** is frequently observed in COVID-19 patients (79% in SARS-CoV-2 positive patients vs. 36% in SARS-CoV-2 negative patients, Li (on MedRxiv: [https://www.medrxiv.org/content/10.1101/2020.02.13.20022830v1](https://www.medrxiv.org/content/10.1101/2020.02.13.20022830v1)) suggested an alternative, simple, approach to facilitate triage of patients. The approach led to a diagnosis sensitivity and specificity of 79% and 64%, respectively. Zhang (Allergy 2020, see below) also reported eosinopenia in most patients, but the frequency of the observation (52.9%) does not support the diagnostic value of this marker.

Qin (Clin Inf Dis 2020, see below) described 452 patients who underwent laboratory examinations on admission. Similar to previous reports, the authors reported that severe cases tend to have lower lymphocytes counts, higher leukocytes counts and neutrophil-lymphocyte-ratio (NLR), as well as lower percentages of monocytes, eosinophils, and basophils. Most of severe cases demonstrated elevated levels of infection-related biomarkers and inflammatory cytokines. Lymphocyte subsets were analyzed in 44 patients with COVID-19 on admission. The total number of B cells, T cells and NK cells were significantly decreased in patients with COVID-19, and particularly in severe cases. The percentage of naive helper T cells (CD3+CD4+CD45RA+) increased and memory helper T cells (CD3+CD4+CD45RO+) were found decreased in severe cases.

Biochemistry

Elevated C-reactive protein (CRP) is an important feature of COVID-19 (Zhang Lancet Resp Med 2020, see below). A study in 12 patients (Liu Sci China Life Sci 2020, see below) found blood biochemistry indexes, albumin (ALB), CRP, lactate dehydrogenase (LDH), may be predictors of disease severity. Similarly, Liu (Chin Med J 2020, see below) found CRP to be significantly elevated in a progression group compared to another group of patients with improvement/stabilization (38.9 [14.3, 64.8] vs. 10.6 [1.9, 33.1] mg/L, U = 1.315, P = 0.024). Albumin was significantly
lower in the progression group than in the improvement/stabilization group (36.62 ± 6.60 vs. 41.27 ± 4.55 g/L, U = 2.843, P = 0.006).

A manuscript by Fan (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.26.20026971v1.full.pdf) describes a cohort of 148 patients, of which (50.7%) showed abnormal liver function at admission, characterized by increased ALT, AST, GGT, AKP.

Alanine aminotransferase, LDH levels, high-sensitivity CRP and ferritin were significantly higher in severe cases (41.4 U/L, 567.2 U/L, 135.2 mg/L and 1734.4 ug/L) than moderate cases (17.6 U/L, 234.4 U/L, 51.4 mg/L and 880.2 ug/L) (Chen (on MedRxiv https://www.medrxiv.org/content/10.1101/2020.02.16.20023903v1)). IL-2R, TNF-α and IL-10 concentrations on admission were significantly higher in severe cases (1202.4 pg/mL, 10.9 pg/mL and 10.9 pg/mL) than moderate cases (441.7 pg/mL, 7.5 pg/mL and 6.6 pg/mL).

Moreover, the angiotensin II level in the plasma sample from COVID-19 patients was markedly elevated and linearly associated to viral load and lung injury (Liu Sci China Life Sci 2020, see below).

A metaanalysis by Lippi (Clin Chim Acta 2020, see below) showed that increased procalcitonin values are associated with a nearly 5-fold higher risk of severe SARS-CoV-2 infection (OR, 4.76; 95% CI, 2.74-8.29). The heterogeneity among the different studies was found to be modest (i.e., 34%). As the synthesis of this biomarker is inhibited by INF-y, whose concentration is expected to increase during viral infections, the authors speculate that increased procalcitonin could reflect bacterial superinfection in severe disease cases. However, more investigations are still needed to identify the origin of the biomarker.

Another metaanalysis by Lippi (Prog Cardiovasc Dis 2020, see below) assessed cardiac troponin I (cTnI) in patients with COVID-19. Although the heterogeneity was considerably high, the values of cTnI were found to be significantly increased in patients with severe disease than in those without (SMD, 25.6 ng/L; 95% CI, 6.8–44.5 ng/L).

**Coagulation parameters**

Tang (J Thromb Haemost 2020, see below) described the coagulation data of 183 consecutive patients with confirmed COVID-19 pneumonia. The non-survivors revealed significantly higher D-dimer and fibrin degradation product (FDP) levels, longer prothrombin time and activated partial thromboplastin time compared to survivors on admission (P<0.05). 71.4% of non-survivors and 0.6% survivors met the criteria of disseminated intravascular coagulation during their hospital stay.

Zhou (Lancet 2020, https://www.thelancet.com/journals/lancet/article/PII/S0140-6736(20)30566-3/fulltext) found increasing odds of in-hospital death associated with D-dimer levels greater than 1.0 μg/L (18.42, 2.64–128.55; p=0.0033) on admission. Gao (J Med Vir 2020: https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25770) found that IL-6 and D-Dimer were closely related to the occurrence of severe COVID-19 in adult patients, and their combined detection had the highest specificity and sensitivity for early prediction of the severity of disease. In this study in 43 patients, the specificity of predicting the severity of COVID-19 during IL-6 and D-Dimer tandem testing was up to 93.3%, while the sensitivity of such testing reached 96.4%.

A metaanalysis by Lippi (Clin Chim Acta 2020, see below) included 1779 COVID-19 patients, of whom 399 (22.4%) had severe disease. The pooled analysis revealed that platelet count was significantly lower in patients with more severe COVID-19 (WMD -31×109/L; 95% CI, from -35 to -29×109/L). A subgroup analysis comparing patients by survival, found an even lower platelet count observed with mortality (WMD, -48×109/L; 95% CI, -57 to -39×109/L). In the four studies which reported data on rate of thrombocytopenia (n=1427), a low platelet count was associated with over five-fold enhanced risk of severe COVID-19 (OR, 5.1; 95% CI, 1.8-14.6).
Time from illness onset to death

In an analysis of published data, Linton (J Clin Med 2020, see below) found a median time delay of 13 days from illness onset to death (17 days with right truncation).

Case fatality rate

Case fatality rate in China

Early data from China yielded an estimated mortality of the COVID-19 of approximately 2.84%, based on 1 975 infections and 56 deaths reported in 26 days since the first official announcement of the epidemic (Wang J Med Virol 2020, see below). Data available by March 23 10:00 CET currently point towards a slightly higher value (81 454 confirmed cases and 3 153 deaths in China, corresponding to 3.87% (Johns Hopkins University dashboard at https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6).

Obviously, this type of estimate has to be taken with caution. As indicated by Kobayashi (J Clin Med 2020, see below), the observed dataset of reported cases represents only a proportion of all infected individuals and there can be a substantial number of asymptomatic and mildly infected individuals who are never diagnosed. Several authors suggested that the number of reported cases of the disease, in China as well as in other countries, is likely to be underestimated (see for instance De Salazar on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.13.20022707v1). Battegay (Swiss Med Wkly 2020, see below), like Kobayashi (J Clin Med 2020, see below), or Baud (Lancet Inf Dis 2020, see below) also pointed to the fact that diagnosis of COVID-19 infection will precede recovery or death by days to weeks and that the number of deaths should therefore be compared to the past case counts. Lack of a standardized case definition also affects estimates of case fatality rates (see Case definition below).

A publication by Ji, Ma et al. (Lancet 2020, see below) also highlights the difference in mortality rates between Hubei and other Chinese provinces. The author postulates that this difference is likely to be related to the rapid escalation in the number of infections around the epicentre of the outbreak, which has resulted in an insufficiency of health-care resources, thereby negatively affecting patient outcomes in Hubei, while this has not yet been the situation for the other parts of China.

A similar observation was made by Mizumoto (Em Inf Dis 2020, see below), who estimated the time-delay adjusted risk for death from COVID-19 as of February 28, 2020 in China. The estimates of the risk for death in Wuhan reached values as high as 12% in the epicenter of the epidemic and ≈1% in other, more mildly affected areas. Comparable results were obtained by Wilson (Em Inf Dis 2020, see below), who reported case-fatality risks, when adjusted for a 13-day lag time from reporting to death, of 3.5% in China and 0.8% in China, excluding Hubei Province.

Nevertheless, according to the large retrospective study reported by the Novel Coronavirus Pneumonia Emergency Response Epidemiology Team (Zhonghua Liu Xing Bing Xue Za Zhi 2020, see below; and Wu JAMA 2020, see below), based on the 72 314 reports received through February 11 2020 by the Chinese Centre for Disease Control and Prevention in mainland China, 1023 deaths were observed out of a total of 44 672 confirmed cases, corresponding to a case-fatality rate of 2.3%. This analysis also showed that the case-fatality rate is largely influenced by the age of the patients (Table 8).
Table 8 Patients, deaths, and case fatality rates, as well as observed time and mortality for n=44,672 confirmed COVID-19 cases in Mainland China as of February 11, 2020 (from The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team Zhonghua Liu Xing Bing Xue Za Zhi 2020).

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Confirmed Cases, N (%)</th>
<th>Deaths, N (%)</th>
<th>Case Fatality Rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>44,672</td>
<td>1,023</td>
<td>2.3</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–9</td>
<td>416 (0.9)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10–19</td>
<td>549 (1.2)</td>
<td>1 (0.1)</td>
<td>0.2</td>
</tr>
<tr>
<td>20–29</td>
<td>3,619 (8.1)</td>
<td>7 (0.7)</td>
<td>0.2</td>
</tr>
<tr>
<td>30–39</td>
<td>7,500 (17.0)</td>
<td>18 (1.8)</td>
<td>0.2</td>
</tr>
<tr>
<td>40–49</td>
<td>8,571 (19.2)</td>
<td>38 (3.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>50–59</td>
<td>10,008 (22.4)</td>
<td>130 (12.7)</td>
<td>1.3</td>
</tr>
<tr>
<td>60–69</td>
<td>8,583 (19.2)</td>
<td>309 (30.2)</td>
<td>3.6</td>
</tr>
<tr>
<td>70–79</td>
<td>3,918 (8.8)</td>
<td>312 (30.5)</td>
<td>8.0</td>
</tr>
<tr>
<td>≥80</td>
<td>1,408 (3.2)</td>
<td>206 (20.3)</td>
<td>14.8</td>
</tr>
</tbody>
</table>

A study by Wu (Nat Med 2020: [https://www.nature.com/articles/s41591-020-0822-7](https://www.nature.com/articles/s41591-020-0822-7)) provided somewhat lower estimates of the case fatality rate in Wuhan, of 0.3% (0.1–0.7%), 0.5% (0.3–0.8%) and 2.6% (1.7–3.9%) for those aged <30 years, 30–59 years and >59 years, respectively.

Using a different approach, and based on early data, Wu (Eurosurv 2020, see below) also estimated the risk of fatality among hospitalised cases at 14% (95% confidence interval: 3.9-32%). This estimate of the hospital fatality risk remained fairly stable over the 10-day period since the first death was announced on 11 January. Subsequently, Leung (Rev Med Vir 2020, see below) calculated that as of 2 February 2020, over 17 000 cases were confirmed in China, with a hospital fatality rate of 2.1%; in Hubei province, the hospital fatality rate reached 3.1%, significantly above the rest of China (Figure 4).

![Figure 4 Trends of hospital fatality rates in Hubei province and the rest of China with 95% CI (from Leung J Med Vir 2020)](image)

**Case fatality rate outside China**

Wilson (on MedRxiv: [https://www.medrxiv.org/content/10.1101/2020.02.15.20023499v1](https://www.medrxiv.org/content/10.1101/2020.02.15.20023499v1)) considered symptomatic cases outside of China (countries/settings with 20+ cases) and the proportion who are in intensive care units (4.0%, 14/349 on 13 February 2020). Given what is known about case fatality rates for intensive care unit patients with severe...
respiratory conditions from a meta-analysis, he estimated a case fatality rate of 1.37% (95%CI: 0.57% to 3.22%) for COVID-19 cases outside of China.

Wilson (Em Inf Dis 2020, see below) also reported a case fatality estimate in 82 countries, territories, and areas reaching 4.2%; and on a cruise ship 0.6%.

Porcheddu (J Infect Dev Ctries 2020, see below) noted that the case fatality rate in China and Italy are identical at 2.3% (estimate based on the first 888 cases confirmed in Italy). Livingston (JAMA 2020, see below) provided the case fatality rate per age group in Italy. It was found to increase with age, up to 22.7% in subjects 90 years of age and older.

**Special populations**

**Older men, cancer and immunocompromised patients**
In the study reported by Chen (Lancet 2020, see below), the disease was found more likely to affect older males with comorbidities. The impact of gender and comorbidities is also described in the section Human to human transmission below.

Liang (Lancet Oncol 2020, see below) described a retrospective analysis of cancer patients among 1590 COVID-19 cases. Eighteen (1%; 95% CI 0.61–1.65) of 1590 COVID-19 cases had a history of cancer, which seems to be higher than the incidence of cancer in the overall Chinese population (285.83 [0.29%] per 100,000 people, according to 2015 cancer epidemiology statistics). Lung cancer was the most frequent type (5/18 patients). Patients with cancer were observed to have a higher risk of severe events (a composite endpoint defined as the percentage of patients being admitted to the intensive care unit requiring invasive ventilation, or death) compared with patients without cancer (seven [39%] of 18 patients vs 124 [8%] of 1572 patients; Fisher’s exact p=0.0003).

A manuscript by Ma depicted 37 cases of disease in a cohort of 230 haemodialysis patients in Wuhan (https://www.medrxiv.org/content/10.1101/2020.02.24.20027201v2.full.pdf). Despite the death of 6 patients with COVID-19 vs. 1 without COVID-19 during the study, the symptoms reported for most of the patients were mild, and there were no cases admitted to ICU.

Zhu (J Med Vir 2020, see below) reported on a unique severe case involving co-infection of SARS-CoV-2 and HIV. Unfortunately, the publication does not provide details as to the time of HIV diagnosis.

Infections in transplant and other immunocompromised patients, or extremes of the body-mass index (BMI) are yet to be reported (Tang J Inf 2020, see below).

**Children**

**Paediatric data from China**
The first confirmed paediatric case of SARS-CoV-2 infection is said to have been reported in Shenzhen on January 20 (Cao J Formos Med Assoc 2020, see below). By January 30, there were 28 children (1 month to 17 years) with confirmed infection in China (Shen World J Pediatr 2020, see below). The clinical features appeared variable. Several patients displayed no obvious clinical symptoms at diagnosis, and they were found by screening because of close contacts with confirmed patients; and further chest imaging suggested pneumonia. Several gradually presented with fever, fatigue, dry cough, accompanied by other upper respiratory symptoms including nasal congestion, runny nose, and seldom gastrointestinal symptoms such as nausea, vomiting and diarrhoea. Laboratory examination in paediatric patients showed that blood routine was often normal, and C-reactive protein was normal or transiently elevated. Lung imaging examination revealed mild increase of lung markings or ground-glass opacity or pneumonia. Most paediatric patients had mild symptoms, without fever or pneumonia. They had good prognosis and recovered within 1-2 weeks after disease onset. Only a few patients had lower respiratory tract infections. No severe cases or deaths have been reported in the paediatric population up to now.
With the progression of the outbreak, the first infant case was reported from Xiaogan, Hubei province. This was a 3-month-old female infant who had fever for one day and as discharged uneventfully 2 weeks later (Cao J Formos Med Assoc 2020, see below). A subsequent retrospective study described 9 cases in children (7 females/2 males) aged 1 to 11 months (Wei JAMA 2020, see below). Four patients were reported to have fever, 2 had mild upper respiratory tract symptoms, 1 had no symptoms but tested positive for COVID-19 in a designated screening because of exposure to infected family members, and 2 had no information on symptoms available. None of the 9 infants required intensive care or mechanical ventilation or had any severe complications.

Liu (NEJM 2020, see below) retrospectively reported 6 paediatric cases treated in Wuhan hospitals in January 2020. One of the 6 children was admitted to the paediatric intensive care unit. All the patients recovered after hospitalization for a median of 7.5 days (range, 5 to 13).

Xia (Pedriatril Pulmonol 2020, see below) presented the clinical, laboratory, and chest CT features of 20 paediatric inpatients with COVID-19 in Wuhan. Fever (12/20, 60%) and cough (13/20, 65%) were the most common symptoms. Procalcitonin elevation was found frequently (16/20, 80%).

A case in a 55 day-old infant was reported in detail by Cui (J Infect Dis 2020, see below). The patient initially presented with mild dry cough and no fevers. However, symptoms became gradually worse from day 7 to day 11 of illness, and symptomatic support was strengthened. This case highlighted that infants with COVID-19 can also present with multiple organ damage and rapid disease changes.

The retrospective Chinese study involving COVID-19 cases reported through February 11, 2020, and corresponding to 44672 confirmed cases, 549 cases were identified in the 10-19 years age group (1%) and 416 cases among children less than 10 years (1%) (Wu JAMA 2020, see below).

Lu (NEJM 2020, see below) tested 1391 children from January 28 through February 26, 2020 in Wuhan, of whom a total of 171 (12.3%) were confirmed to have SARS-CoV-2 infection. The median age of the infected children was 6.7 years. Fever was detected in 41.5%. Other common signs and symptoms included cough and pharyngeal erythema. A total of 27 patients (15.8%) did not have any symptoms of infection or radiologic features of pneumonia. A total of 12 patients had radiologic features of pneumonia but did not have any symptoms of infection. During the course of hospitalization, 3 patients required intensive care support and invasive mechanical ventilation; all had coexisting conditions (hydronephrosis, leukemia, and intussusception). Lymphopenia was present in 6 patients (3.5%). The most common radiologic finding was bilateral ground-glass opacity (32.7%). As of March 8, 2020, there was one death: a 10-month-old child with intussusception had multiorgan failure and died 4 weeks after admission.

Another large paediatric cohort in China was reported by Dong (Pediatrics 2020, see below). There were 731 (34.1%) laboratory-confirmed cases and 1412 (65.9%) suspected cases. The median age of all patients was 7 years (interquartile range: 2-13). Over 90% of all patients were asymptomatic, mild, or moderate cases.

While the disease seems to have a milder course in children than adults, a manuscript by Qifang Bi on MedRxiv (https://www.medrxiv.org/content/10.1101/2020.03.03.20028423v1) suggests that children are at a similar risk of infection as the general population. This conclusion was driven from 391 cases and 1286 close contacts identified by the Shenzhen CDC.

**Paediatric data from other countries**

In Singapore, there were 3 confirmed cases reported, who were very young (aged 6 months, 1 year, and 2 years) and had very mild symptoms (Wong JAMA 2020, see below).

Park (J Korean Med Sci 2020, see below) reported the first paediatric case of COVID-19 in Korea, a 10-year-old girl who presented mild clinical course of her pneumonia that did not require antiviral treatment.

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Pregnancy and newborns

In general, pregnancy is a physiological state in which women are more susceptible to respiratory pathogens and severe pneumonia, due to an immunosuppressive state and various physiological adaptive changes (e.g., diaphragm elevation, increased oxygen consumption, and oedema of respiratory tract mucosa). It is therefore reasonable to predict that pregnant women might be at greater risk for severe illness. Previous epidemics of other strains of CoV, such as SARS-CoV and MERS-CoV, have typically resulted in severe complications during pregnancy such as maternal morbidity and mortality, perinatal infections and death (Wong Am J Obstet Gynecol 2004, see below; and Alfaraj J Microbiol Immunol Infect 2019, see below). Analysis of the literature up till now reveals that, unlike coronavirus infections of pregnant women caused by SARS and MERS, pregnant women are not more susceptible for COVID-19, nor are they at risk of more severe disease than the non-pregnant population. Importantly, and similar to pregnancies with SARS and MERS, there were no confirmed cases of vertical transmission of the virus.

We acknowledge that available clinical data on COVID-19 infection in pregnancy are limited at present, and most cases on which data are available presented in the third trimester of pregnancy. There is, therefore, a need to continue collecting data on clinical cases of COVID-19 infection in pregnancy, and to improve our understanding of the course of the disease throughout pregnancy.

Clinical characteristics of the pregnant woman with COVID-19 infection

Multiple studies, all however limited to small case series or case-control studies, have observed the clinical characteristics of COVID-19 pneumonia in pregnant women. These characteristics seem to be similar to those reported for non-pregnant adult patients who developed COVID-19 pneumonia.

A retrospective study in 9 pregnant women who had a caesarean section in their third trimester did not show any severe COVID-19 pneumonia or maternal death (Chen, Guo et al. Lancet 2020, see below). The clinical characteristics of COVID-19 pneumonia in pregnant women included mainly fever and cough. Other symptoms were myalgia, malaise, sore throat, diarrhoea and dyspnoea. Biochemical examination revealed lymphopenia and increased CRP in the majority of patients. CT scan of the thorax showed the typical patchy ground-glass shadows in the lungs. None of the patients developed severe disease or died. Similarly, a retrospective comparison of the pregnancy outcomes was done between 16 women with COVID-19 and 45 women without COVID-19 (Zhang Zhonghua Fu Chan Ke Za Zhi 2020, see below). Of the pregnant woman with COVID-19, 15 cases showed mild disease, while 1 case developed severe respiratory illness. All 16 neonates were delivered via caesarean section. No significant differences were observed in gestational age, birth weight and blood loss during operation. A study by Fan (Clin Inf Dis 2020, see below) reported two cases with a mild disease course, however one patient exhibited a more severe condition. They hypothesize this course of action is due to a high viral load associated placental proinflammatory cytokine release.

A joint mission by the WHO travelled to the affected regions of China between 16 and 24 February 2020 (Available from: https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf). An investigation of 147 pregnant women (64 confirmed, 82 suspected and 1 asymptomatic) revealed 8% to have severe disease and 1% to be critical. They concluded pregnant woman not to be at higher risk of severe disease. Fan et al. hypothesized that maternal antibodies to COVID-19 could be transferred to the newborn postpartum via breastfeeding and have a protective effect (Fan Clin Inf Dis 2020, see below).

Two reports studied the CT features in COVID-19 infected women with pneumonia. Liu (AJR AM J Roentgenol 2020, see below) observed in a case series of 15 pregnant women that the most common early finding on chest CT was pure ground-glass opacity. When disease progressed, more crazy paving pattern and consolidations were seen. CT images obtained before and after delivery showed no signs of pneumonia aggravation after delivery. A different study

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3 See for instance Chen Guo Lancet 2020; Liu, Chen J Inf 2020; Zhang Zhonghua Fu Chan Ke Za Zhi 2020; Chen Huang Zhonghua Bing Li Xue Za Zhi 2020; Fan Clin Inf Dis 2020; Wang Clin Inf Dis 2020; Li on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.03.10.20033605v1

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analyzed and compared CT features of 59 patients with COVID-19: 14 non-pregnant adults, 41 pregnant women and 4 children (Liu J Infect. 2020, see below). Their findings showed a predominant presence of ground-glass opacity in non-pregnant adults, while mixed consolidation and complete consolidation were more common the pregnant group. Ground-glass opacity with reticulation was less common in pregnant groups than for the non-pregnant adults.

**Anaesthesia for caesarean delivery**

Data regarding management and safety of epidural or general anaesthesia for caesarean delivery in parturients with COVID-19 has been published in one case report (Song Transl Perioper & Pain Med 2020: http://www.transpomed.org/articles/tppm/tppm-2020-7-118.pdf) and one case series including 17 pregnant women (Chen Can J Anaesth 2020, see below). All of them underwent caesarean delivery with anaesthesia performed according to standardized procedures (tracheal intubation in 3 patients, continuous epidural anaesthesia in 14 patients). Both epidural and general anaesthesia were safely used for caesarean delivery. There were no deaths or serious neonatal asphyxia events and none of the neonates were infected with COVID-19. Nevertheless, the incidence of hypotension during epidural anaesthesia appeared excessive with 12 out 14 patients experiencing significant intraoperative hypotension. Intraoperative hemodynamic parameters were not improved by left-lateral position, intravenous liquid loading, and/or vasoconstrictor treatment.

**Perinatal complications**

Pregnancy may not increase susceptibility to COVID-19 infection or influence the severity of the disease, but COVID-19 infection does seem to influence the pregnancy. Severe pneumonia during pregnancy (regardless of the causative agent) increases the risk of preterm delivery, foetal growth restriction, low birth weight and low Apgar score at birth.

Zhu (Transl Ped 2020, see below) presented the clinical features and outcomes of 10 neonates, including 2 twins, born to 9 mothers with confirmed 2019-nCoV infection. Six out of ten neonates were born preterm. Of the neonates, 6 had a Paediatric Critical Illness Score (PCIS) score of less than 90. Clinical symptoms included shortness of breath, fever, thrombocytopenia with abnormal liver function, rapid heart rate, vomiting and pneumothorax. Up to now, 5 neonates have been cured and discharged, 1 has died, and 4 neonates remain in hospital in a stable condition. Similarly, Chen, Guo et al. (Lancet 2020, see below) reported 6 out of 9 neonates to be born preterm. Liu, Chen et al. (J Inf 2020, see below) described 13 cases, in which five patients were delivered by emergency caesarean section because of pregnancy complications including foetal distress, premature rupture of the membrane and stillbirth. All of the women affected by COVID-19 who delivered, did so within a short period of time after onset of illness; foetal growth is unlikely to be affected in this time period. There was no data on foetal growth in ongoing pregnancies. Chen, Huang et al. (Zhonghua Bing Li Xue Za Zhi 2020, see below) studied placental pathology of 2019-nCoV infection in three pregnant women. The pathological study suggested that there were no morphological changes related to infection in the three placentas.

Therefore, evidence is building that perinatal COVID-19 infection may have adverse effects on neonates, such as foetal distress, premature labour, respiratory distress, thrombocytopenia and even death. Tough less serious than SARS-CoV, in which adverse outcomes were reported in 10 out of 12 pregnancies, COVID-19 pregnancy seems not to be without risk.

**Vertical transmission**

Until now, there have been no reports on intrauterine foetal infections caused by vertical transmission in women who develop COVID-19 pneumonia in late pregnancy.

Zhu (Transl Ped 2020, see below) collected pharyngeal swab specimens from 9 neonates, all of which showed negative results. Li (Em Inf Dis 2020, see below) reported a pregnant woman with SARS-CoV-2 infection who delivered a healthy infant. Chen, Guo et al. (Lancet 2020, see below) tested amniotic fluid, cord blood, neonatal throat swab, and breastmilk samples from six patients for SARS-CoV-2, and all samples tested negative for the virus. Furthermore, it
seems that there is no risk of transmission of the virus via breast feeding. The same study confirmed that the virus was not detected in the colostrum of COVID-19-infected patients.

All of the current data stems from COVID-19 infection acquired during late pregnancy. Currently, there is no data on foetal and perinatal complications when the infection is acquired in the first or early second trimester. Zheng (Reprod Dev Med 2020: http://www.repdevmed.org/preprintarticle.asp?id=278679) studied the maternal-foetal interface at single-cell resolution. Their results showed that expression of ACE2, in all kinds of early maternal-foetal interface cells, was very low. As ACE2 has been confirmed as the receptor for the receptor-binding domain of SARS-CoV-2, it can be speculated that the mother-to-foetus transmission will be significantly low. Whether the foetal/neonatal lung parenchymal cell’s expression pattern of the ACE2 receptor could predispose them to higher morbidity or mortality remains to be better assessed.

Case definition

Case definitions based on the current information available have been issued by WHO (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/surveillance-and-case-definitions). These might be revised as new information accumulates, or adapted by countries depending on their own epidemiologic situation. The Case definition of the Chinese Center for Disease Control and Prevention for instance, as reported by Wu (Lancet 2020, see below) somewhat differs from the WHO definition.

Suspect case
A. Patient with severe acute respiratory infection (fever, cough, and requiring admission to hospital), AND with no other aetiology that fully explains the clinical presentation AND a history of travel to or residence in China during the 14 days prior to symptom onset,

OR

B. Patient with any acute respiratory illness AND at least one of the following during the 14 days prior to symptom onset:

a) contact with a confirmed or probable case of SARS-CoV-2 infection, or

b) worked in or attended a health care facility where patients with confirmed or probable COVID-19 acute respiratory disease patients were being treated.

Probable case
Probable case: A suspect case for whom testing for SARS-CoV-2 is inconclusive⁴ or is tested positive using a pанcoronavirus assay and without laboratory evidence of other respiratory pathogens.

Confirmed case
A person with laboratory confirmation of SARS-CoV-2 infection, irrespective of clinical signs and symptoms.

Definition of severe disease
While WHO has not provided a definition of severe cases of COVID-19, various publications have classified disease cases according to severity. For instance, Zhang (Allergy 2020, see ) designated severe COVID-19 when the patients had one of the following criteria: 1) Respiratory distress with respiratory frequency ≥ 30/min; 2) Pulse Oximeter Oxygen Saturation ≤ 93% at rest; 3) Oxygenation index (artery partial pressure of oxygen/inspired oxygen fraction, PaO2/FiO2) ≤ 300 mmHg.

⁴ Inconclusive being the result of the test reported by the laboratory
Pathophysiology of COVID-19

The pathogenesis of COVID-19 is under investigation. Of note, a review on the comparative pathogenicity of the different human coronaviruses was published by Liu (J Med Vir 2020, see below).

Viral tropism

The S protein is responsible for coronavirus entry into the cell after by binding to a cell receptor and membrane fusion, two key steps in viral infection and pathogenesis (Benvenuto J Med Vir 2020, see below). Virus infectivity studies using HeLa cells expressing or not expressing ACE2 proteins from humans, Chinese horseshoe bats, civet, pig, and mouse showed that SARS-CoV-2 is able to use all but mouse ACE2 as an entry receptor in ACE2-expressing cells, but not cells without ACE2. ACE2 therefore appears as the likely cell receptor of SARS-CoV-2 (Zhou Nature 2020, see below). It was also demonstrated that SARS-CoV-2 does not use other coronavirus receptors, aminopeptidase N and dipeptidyl peptidase 4.

However, cell entry of coronaviruses depends not only on binding of the viral S proteins to cellular receptors but also on S protein priming by host cell proteases. Koffmann (Cell 2020, see below) demonstrated that SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the serine protease TMPRSS2 for S protein priming.

ACE2 is expressed in a variety of cells of different organs (endothelium, liver, lungs, etc.) and is part of the renin-angiotensin blood pressure regulation system. In the respiratory tract, it is expressed on the apical face of respiratory epithelial cells via which infection may be mediated. Along the respiratory tract, ACE2 has been detected in the trachea, main bronchus and alveoli, and occasionally also in the small bronchi. A recent expression study found ACE2 was mostly (83%) expressed by type II alveolar cells (AT2), and that this cell population also highly expressed other genes that positively regulate viral reproduction and transmission (Zhao on Biornxiv: https://www.biorxiv.org/content/10.1101/2020.01.26.919985v1).

By single cell sequencing, Weng (on BioRxiv https://www.biorxiv.org/content/10.1101/2020.02.08.926006v3.full.pdf) found a strong co-expression between ACE2 and TMPRSSs, especially TMPRSS1 and TMPRSS2, in lung AT2 cells, which was also the main infected cell type in SARS-CoV pneumonia. Moreover, he found the endocytosis-associated genes were highly expressed in AT2 cells, implying that endocytosis may also facilitate the entry of SARS-CoV-2 into host cells. As the alveolar stem-like cells, AT2 cells promote surfactant biosynthesis, self-renewal and immunoregulation. Thus, SARS-CoV-2 appears to not only damage the AT2 cells leading to the direct injury to alveoli, but also raise alveolar surface tension to induce dyspnoea.

Based on the public single-cell RNA-Seq datasets, Wu (https://www.medrxiv.org/content/10.1101/2020.02.11.20022228v2) found ACE2 expression in nasal epithelial cells. The size of this population of ACE2-expressing nasal epithelial cells appeared comparable with the size of the population of ACE2-expressing AT2 cells.

Using bulk RNA-seq profiles from two public databases and single-cell transcriptomes from an independent dataset generated in-house, Xu (Nature 2020, see below) found evidence of ACE2 expression in the oral cavity and suggested enrichment in epithelial cells. Moreover, among different oral sites, ACE2 expression was found higher in tongue than buccal and gingival tissues.

ACE2 and TMPRSSs are also highly co-expressed in absorptive enterocytes and upper epithelial cells of oesophagus, implying that intestinal epithelium and oesophagus epithelium may also be the potential target tissues. Liang (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.03.20020289v2) also reported that ACE2 mRNA was highly expressed in the healthy human small intestine. Besides, single-cell RNA sequencing data showed ACE2 to be significantly elevated in proximal and distal enterocytes.

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In addition, a manuscript by Lin (https://www.biorxiv.org/content/10.1101/2020.02.08.939892v1) reported the use of published kidney and bladder cell atlas data and an independent unpublished kidney single cell RNA-Seq data to evaluate ACE2 gene expressions in all cell types in healthy kidneys and bladders. Results showed the enriched expression of all subtypes of proximal tubule cells of kidney and low but detectable levels of expression in bladder epithelial cells. The data suggest that the urinary system may be a potential target for infection. Fan (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.12.20022418v1) also noted that ACE2 is highly expressed in renal tubular cells, Leydig cells and cells in seminiferous ducts in testis. He recommended renal function evaluation and special care of patients, especially in case of therapy with drugs associated with renal toxicity, and suggested that clinicians should pay attention to the risk of testicular lesions in patients. However, this hypothesis is not supported by the observations by Wang (see Clinical disease in China above).

Liu (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.28.20029181v1), using public datasets (bulk RNA-seq and single-cell RNA-seq), showed expression of ACE2 in pancreas (in both exocrine glands and islets), and related this observation to clinical data suggesting mild pancreatitis in some patients. Among 67 severe cases, 11 patients (16.41%) showed elevated levels of both amylase and lipase, and 5 patients (7.46%) showed imaging alterations.

To construct a risk map of different human organs, Zou (Front Med 2020, see below) analysed the single-cell RNA sequencing (scRNA-seq) datasets derived from major human physiological systems, including the respiratory, cardiovascular, digestive, and urinary systems, for ACE2 expression. Through scRNA-seq data analyses, the authors identified the organs at risk, such as lung, heart, esophagus, kidney, bladder, and ileum, and located specific cell types (i.e., type II alveolar cells (AT2), myocardial cells, proximal tubule cells of the kidney, ileum and esophagus epithelial cells, and bladder urothelial cells) as vulnerable to SARS-CoV-2 infection.

Considering that a conserved RGD (403–405:Arg-Gly-Asp) motif is present in the receptor-binding domain of the spike proteins of all SARS-CoV-2, Sigrist (Antivir Res 2020, see below) presented the hypothesis that SARS-CoV-2 acquired integrin-binding to promote virus entry into host cells. However, experimental proof of this is required. Binding to integrin may play a supplemental role to ACE2 binding, like facilitating endocytosis by signalling through the integrin. Alternatively, the virus could infect different target cells by binding to ACE2 or to integrins.

**Determinants of pathogenicity**

Information pertaining to the replication of SARS-CoV-2, and the interactions between the virus and its host, is still lacking. Extensive data is available to document the mechanisms involved during infection by other human CoVs (see for instance, Fung Ann Rev Microbiol 2019 below or Chen J Med Virol 2020 below).

**S protein and interaction with ACE2**

A manuscript by Meng (on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.02.08.926006v3.full.pdf) suggests enhanced S protein cleavage with SARS-CoV-2 compared to SARS-CoV. A SPRR insertion in the S1/S2 protease cleavage sites of SARS-CoV-2 spike protein was found to increase cleavage efficiency as assessed by protein sequence alignment and furin score calculation. Additionally, the insertion sequence facilitates the formation of an extended loop which was more suitable for protease recognition by homology modelling and molecular docking. Coutard (Antivir Res 2020, see below) and Wang (Virol Sin 2020, see below) also identified a peculiar furin-like cleavage site in the S protein of SARS-CoV-2, which is lacking in the other SARS-like CoVs. The authors hypothesised that this cleavage site may affect the viral cycle and pathogenicity.

**Infection of immune cells?**

It has been reported that SARS-CoV directly infects macrophages and T cells, a key feature in SARS-CoV-mediated pathogenesis. Whether SARS-CoV-2 infects immune cells is still unknown (Prompetchara Asia Pac J All Imm 2020, see below).
**Signs of liver injury**

Guan (Zhonghua Gan Zang Bing Za Zhi 2020, see below) investigated the possible mechanism of liver injury in patients. ALT and AST are indeed abnormally elevated in some patients, especially in severe disease cases. The author assumed that in addition to the over-activated inflammatory response in patients with COVID-19 pneumonia, the up-regulation of ACE2 expression in liver tissue caused by compensatory proliferation of hepatocytes derived from bile duct epithelial cells may also be the possible mechanism of liver tissue injury.

A publication by Xu (Liver Int 2020, see below) provided a summary of available information on SARS-CoV-2 and liver injury. The authors noted that the liver injury observed in COVID-19 patients might be caused by lopinavir/ritonavir, and that there is still a lack of reports that liver failure occurs in COVID-19 patients with chronic liver diseases.

**Other observations**

Angeletti (J Med Vir 2020, see below) used sequence analysis and modelling to predict features of SARS-CoV-2 pathogenicity. He suggested that the stabilizing mutation falling in the endosome-associated-protein-like domain of the nsp2 protein could account for COVID-19 high transmission capability, while the destabilizing mutation in nsp3 proteins could suggest a potential mechanism differentiating COVID-19 from SARS.

Fahmi (Infect Genet Evol. 2020, see below) showed that two non-structural proteins, NS7b and NS8, were exclusively conserved among SARS-CoV-2, βCoV_RaTG, and BatSARS-like Cov. NS7b and NS8 have previously been shown to affect immune response signalling in the SARS-CoV experimental model. Thus, the authors speculated that the properties of these accessory proteins, NS7b and NS8, in SARS-CoV-2 may affect its ability to infect humans.

**Antibody-dependent enhancement of infection?**

Antibody-dependent enhancement (ADE) occurs when antibodies facilitate viral entry into host cells and enhance viral infection in these cells. ADE has been observed for a variety of viruses, most notably flaviviruses (e.g., dengue virus). ADE has been observed for coronaviruses. Several studies have shown that sera induced by SARS-CoV spike enhance viral entry into Fc receptor-expressing cells (Wan, Shang, Sun et al J Vir 2020, see below). One study demonstrated that unlike receptor-dependent viral entry, serum-dependent SARS-CoV entry does not go through the endosome pathway. Additionally, it has long been known that immunization of cats with feline coronavirus spike leads to worsened future infection due to the induction of infection-enhancing antibodies. Wan et al. further studied the molecular mechanism of ADE using MERS-CoV and a monoclonal antibody as a model.

A publication by Tetro (preprint available in Microb Inf 2020: https://reader.elsevier.com/reader/sd/pii/S1286457920300344?token=0E0B1A0532BEFAD83CBA48B5118C612C3C0AB30D8736B57A27E49972594314494E48D01DF5E0F17D3215A26D15466C2) further described the hypothesis that ADE due to prior exposure to other coronaviruses could underlie the severity of cases in the Hubei province. In the context of identifying the priming coronavirus, he noted that as the introduction of SARS-CoV into humans has been suggested to have occurred in the Hubei Province. However, SARS-CoV is not likely to be a predominant priming virus for ADE to SARS-CoV-2. Seroprevalence studies have shown a very low level of SARS-CoV seroconversion in the population apart from workers with direct contact with animals such as traders.

Alternatively, Fu (Virol Sin 2020, see below) speculated that a mechanism of ADE of viral infection occurs in some patients with early, sub-optimal antibody activity that cannot completely clear the virus, but instead leads to persistent viral replication and inflammation.

**Pathological observations from biopsies and autopsies**

A manuscript by Tian (J Thorac Oncol 2020, see below) describes examinations of biopsies of 2 asymptomatic cancer patients who underwent surgery and were later found to have been infected with SARS-CoV-2. The lungs of both patients exhibited oedema, proteinaceous exude with globules, focal hyperplasia of pneumocytes with only patchy...
inflammatory cellular infiltration, and multinucleated giant cells. Hyaline membranes were not prominent. These observations likely represent an early phase of the lung pathology of COVID-19 pneumonia.

Xu (Lancet Resp Med 2020, see below) described for the first time pathology findings from biopsies collected at autopsy. The pathological features of COVID-19 greatly resemble those seen in SARS and MERS. In addition, the liver biopsy specimens of the patient with COVID-19 showed moderate microvascular steatosis and mild lobular and portal activity, indicating the injury could have been caused by either SARS-CoV-2 infection or drug-induced liver injury. There were a few interstitial mononuclear inflammatory infiltrates, but no other substantial damage in the heart tissue.

Zhang (Ann Int Med 2020, see below) presented the histopathologic changes seen on post-mortem transthoracic needle biopsies from a patient with COVID-19 who had respiratory failure and radiographic bilateral ground-glass opacities. Nonspecific findings consistent with diffuse alveolar damage were observed. Immunostaining of lung sections with an antibody to the Rp3 NP protein of SARS–CoV-2 revealed prominent expression on alveolar epithelial cells, including damaged, desquamated cells within the alveolar space. In contrast, viral protein expression was minimally detectable on blood vessels or in the interstitial areas between alveoli.

**Acute respiratory distress syndrome**

Similar to patients with SARS-CoV and MERS-CoV, some patients with COVID-19 develop acute respiratory distress syndrome (ARDS) with characteristic pulmonary ground glass changes on imaging (Zumla Lancet 2020). COVID-19 is also associated with increases in IL-6, IL-10, IL-2 and IFN-γ levels in the peripheral blood in the severe cases compared to those in the mild cases (Liu on MedRxiv: [https://www.medrxiv.org/content/10.1101/2020.02.16.20023671v1](https://www.medrxiv.org/content/10.1101/2020.02.16.20023671v1)).

In line with these observations, Liao (see First observations in COVID-19 patients above) showed that monocyte-derived FCN1+ macrophages, but not FABP4+ alveolar macrophages that represent a predominant macrophage subset in BALF from patients with mild diseases, overwhelm in the severely damaged lungs from patients with ARDS. These cells are highly inflammatory and enormous chemokine producers implicated in cytokine storm.

Fu (Virol Sin 2020, see below) explored the possible mechanisms of the inflammatory response observed in COVID-19 pneumonia. Based on previous studies of SARS-CoV, he separated the inflammatory responses in SARS-CoV-2 infection into primary and secondary responses (Figure 5). Primary inflammatory responses occur early after viral infection, prior to the appearance of neutralizing antibodies (NAb). These responses are mainly driven by active viral replication, viral-mediated ACE2 downregulation and shedding, and host antiviral responses. Secondary inflammatory responses begin with the generation of adaptive immunity and NAb. The virus-NAb complex can also trigger FcR-mediated inflammatory responses and acute lung injury.
Ji (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.24.20025437v1) described the use of TWIRLS, an automated topic-wise inference method based on massive literature, to suggest a possible mechanism of SARS-CoV-2 pathogenicity. The method yielded the hypothesis that after triggering functional changes in ACE2/AT2R, an imbalance in the steady-state cytokine regulatory axis involving the Renin-Angiotensin System and IP-10 leads to a cytokine storm.

Li, Bai et Hashikawa (J Med Vir 2020, see below), considering the similarities of the disease with SARS-CoV and MERS-CoV, proposed a potential neuroinvasion of SARS-CoV2 to be partially responsible for the acute respiratory failure of COVID-19 patients.

**Co-infections**

A study by Wang (manuscript non-peer reviewed on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.12.20022327v1.full.pdf) analysed 613 patients with fever who underwent multiple tests for 13 respiratory pathogens. Interestingly, 5.8% of patients with SARS-CoV-2 infection were reported to be co-infected with coronavirus (3/104), influenza A virus (2/104), rhinovirus (2/104), and/or influenza A H3N2 (1/104).

Similarly, respiratory virus, fungi and bacteria co-infections were reported by Ai (on MedRxiv https://www.medrxiv.org/content/10.1101/2020.02.13.20022673v1).

A case report on co-infection with SARS-CoV-2 and Influenza A Virus in a patient with pneumonia in China has also been presented (Wu Emerg Inf Dis 2020, see below).

Lin (Sci China Life Sci 2020, see below) reported on the use of a multiplex RT-PCR method (multiplex rapid detection kit 2.0, Uni-MEDICA Tech, Shenzhen), which can simultaneously detect 15 respiratory tract infection pathogens including the SARS-CoV-2, was employed to screen the pathogen agents in the patients. These 15 respiratory pathogens are SARS-CoV-2, influenza A/B, coronavirus NL63, coronavirus, parainfluenza virus type 1/2/3(PIV1/2/3), adenovirus, rhinovirus (hRV), human bocavirus, coronavirus HKU1 (HKU1), coronavirus OC43, human metapneumovirus (hMPV) and respiratory syncytial virus (RSV). 186 suspected COVID-19 cases were tested. In the 92 SARS-CoV-2 (49.46%) positive patients, the common respiratory viruses RSV, hRV, hMPV, PIV2 and HKU1 were also simultaneously detected in six patients (3.2%) respectively, of which four patients (2.2%) were positive for at least two
detected viruses. The co-infections in these six patients were further verified in parallel testing using a second-day sampling from the same patients.

**Animal models**

On the basis of sequence analyses, Wan, Shang, Graham et al. (J Vir 2020, see below) predicted that either SARS-CoV-2 or laboratory mice and rats would need to be genetically engineered before a robust mouse or rat model for COVID-19 would become available. By contrast, the authors noted that pigs, ferrets, cats, and non-human primates contain largely favourable SARS-CoV-2 -contacting residues in their ACE2 and hence may serve as animal models for SARS-CoV-2.

**Transgenic mice**

A manuscript by Bao (on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.02.07.939389v2) presented data supporting the suitability of the SARS-CoV transgenic mouse model for SARS-CoV2. The hACE2 transgenic mice were inoculated intranasally at a dosage of $10^5$ TCID50 per mouse. Weight loss of up to 5% was observed for 10 dpi only in the infected mice. Other clinical symptoms were not observed. The typical histopathology was interstitial pneumonia with significant inflammatory cells infiltration around the bronchioles and blood vessels, and viral antigens were observed in bronchial epithelial cells and alveolar epithelial cells. The phenomenon was not found in wild type mice infected with SARS-CoV-2.

**Ferrets**

A communication by CSIRO, the Commonwealth Scientific and Industrial Research Organisation in Australia, on March 9, suggested that ferrets are susceptible to SARS CoV-2, the team claiming that the virus replicates in the animal host (https://www.csiro.au/en/Research/Health/Infectious-dieases-coronavirus/Latest-updates).

**Cynomolgus macaques**

A manuscript by Rockx (on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.03.17.995639v1) indicates that SARS-CoV-2 infection in cynomolgus macaques results in COVID-19-like disease with prolonged virus excretion from nose and throat in absence of clinical signs.

**Rhesus macaques**

Callaway (Nature 2020, see below) provided a summary of the current status of research on COVID-19 animal models. He pointed to the preprint by Chao Shan at the Chinese Academy of Sciences Wuhan Institute of Virology, who found that rhesus macaques infected with SARS-CoV-2 had a fairly mild illness. None developed fevers, but X-rays of their lungs showed signs of pneumonia similar to those in humans with COVID-19. This was confirmed after some of the monkeys were euthanized and their lungs dissected. The researchers killed two monkeys three days after infection and another pair after six days. They monitored two further animals for three weeks; these monkeys lost some weight, but didn’t seem to have other serious symptoms.

Deng (non-peer-reviewed manuscript on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.03.13.990036v1.full.pdf) presented data suggesting that macaques can be infected with SARS-CoV-2 via the conjunctival route. Viral load and distribution in the macaques infected by this route were comparatively high in the nasolacrimal system, while relatively mild and local in the lung compared with those in macaques inoculated via intratracheal routes. This publication refers to the contrasting observation that no SARS-CoV-2 could be detected by RT-PCR in 114 conjunctival swabs samples 28 from patients with COVID-19 pneumonia.

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https://www.biorxiv.org/content/10.1101/2020.03.13.990036v1.full.pdf

Bao (non-peer-reviewed manuscript on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.03.13.990226v1.full.pdf) studied the possibility of reinfection in a rhesus macaque model of infection. Following the initial intratracheal infection, none of the animals developed fever, but three of the four monkeys showed weight loss ranging from 200 g to 400 g. Other clinical signs such as reduced appetite, increased respiration rate and hunched posture were transient after the initial challenge. Viral loads in nasal and pharyngeal swabs peaked at 3 days post-infection (dpi) and then declined naturally. Similarly, viral loads from anal swabs reached the peak at 3 dpi and then declined to undetectable level at 14 dpi. Chest X-ray at 7 dpi showed that the upper lobe of the right lung had varying degrees of the localized infiltration and interstitial markings, showing the mild to bilateral ground-glass opacification. Two infected monkeys (M3 and M4) were intratracheally re-challenged at 28 dpi. None of the monkeys showed the weight loss after re-exposure, but a transient elevation of body temperature was observed in both re-exposed monkeys. Viral loads in nasopharyngeal and anal swabs tested negative after the re-exposure of SARS-CoV-2. The neutralizing antibody titer of one of the 2 monkeys increased after re-challenge (Table 9). The authors concluded that SARS-CoV-2 infection could protect rhesus macaques from subsequent exposure.

Table 9 SARS-CoV-2 neutralizing antibody titers in infected monkeys (from Bao on BiorXiv)

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Primary challenge</th>
<th>Rechallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21 dpi</td>
<td>28 dpi</td>
</tr>
<tr>
<td>M1(^a)</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>M2(^b)</td>
<td>1:16</td>
<td>1:16</td>
</tr>
<tr>
<td>M3(^c)</td>
<td>1:8</td>
<td>1:8</td>
</tr>
<tr>
<td>M4</td>
<td>1:16</td>
<td>1:16</td>
</tr>
</tbody>
</table>

Notes: a M1 was euthanized and necropsied at 7 dpi. NE, not examined.

b M2 was continuously monitored without rechallenge. NE, not examined.

c M3 was euthanized and necropsied at 5 dpi. NE, not examined.

**Epidemiology**

**Disease emergence**

On 31 December 2019, the Wuhan Municipal Health Commission announced a cluster of cases of viral pneumonia of unexplained aetiology (Wu Eurosurv 2020, see below). The Southern China Seafood Wholesale Market in Wuhan was suspected to be related to 27 pneumonia cases without identified pathogenic agents that were reported in late December 2019. Most of the early cases were reportedly either shop owners, largely in the West District of the Southern China Seafood Wholesale Market, or people who visited the market before symptom onset. This market is a large open complex including sections selling seafood, fresh meat, produce, other perishable goods, and a very wide variety of live wild animals for consumption. Environmental disinfection of the Southern China Seafood Wholesale Market was initiated on 30 December 2019 and the market was closed on 1 January 2020.
Transmission and maintenance

Host range & search for intermediate animal hosts
In both the SARS-CoV and MERS-CoV epidemics, the viruses have likely originated from bats and then jumped into another amplification mammalian host [the Himalayan palm civet (Paguma larvata) for SARS-CoV and the dromedary camel (Camelus dromedarius) for MERS-CoV] before crossing species barriers to infect humans (Chan Em Micr Inf 2020, see below). While phylogenetic analysis indicates a bat origin of SARS-CoV-2, the virus also potentially recognizes the ACE2 receptor from a diversity of animal species (except mice and rats), implicating these animal species as possible intermediate hosts or animal models for SARS-CoV-2 infections (Wan J Virol 2020, see below). Ji (J Med Virol. 2020, see below) suggested that snake is the most probable wildlife animal reservoir for SARS-CoV-2 based on its relative synonymous codon usage bias resembling snake compared to other animals. However, this hypothesis was received with skepticism (https://www.nature.com/articles/d41586-020-00180-8). It was not supported by the bioinformatics protein structure and sequence analyses by Zhang (manuscript on Arxiv: https://arxiv.org/abs/2002.03173).

Chinese researchers of the South China Agricultural University in Guangzhou found 99% sequence similarity in the S RBD region between SARS-CoV-2 isolated from infected human subjects and coronaviruses taken from pangolins (Manis javanica) (https://www.nature.com/articles/d41586-020-00364-2). Researchers had noted previously that coronaviruses are a possible cause of death in pangolins, and that SARS-CoV-2 and coronaviruses from pangolins use receptors with similar molecular structures to infect cells. A manuscript by Lam (on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.02.13.945485v1.full.pdf) also reported the identification of SARS-CoV-2-related coronaviruses in pangolins seized in anti-smuggling operations in southern China. Metagenomic sequencing identified pangolin-associated CoVs that belong to two sub-lineages of SARS-CoV-2-related coronaviruses, including one very closely related to SARS-CoV-2 in the receptor-binding domain. Cyranoski (Nature 2020, see below) subsequently summarized the investigations pertaining to the animal source of SARS-CoV-2. He noted that the previously communicated 99% homology between SARS-CoV-2 and a pangolin virus only applied to the S RBD region. The homology with pangolin viruses when considering the whole genome did not exceed 92.4%. A subsequent report by Zhang (Current Biology 2020, see below) reached similar conclusions. By contrast, SARS-CoV shared 99.8% of its genome with a civet coronavirus.

A systematic comparison and analysis to predict the interaction between the RBD of the S protein and the ACE2 receptor suggested that not only pangolins, but also turtles (C. picta bellii, C. mydas, and P. sinensis) may act as potential intermediate hosts transmitting SARS-CoV-2 to human (Liu Xiao et al. J Med Vir 2020, see below).

Field studies and laboratory challenge data remain important to conduct for better understanding the zoonotic transmission of SARS-CoV-2. Of note, while controversies about the source of the virus and its intermediate host remain, Li (Infect Genet Evol 2020, see below) evaluated coronaviruses derived from five wild animals, including Paguma larvata, Paradoxurus hermaphroditus, Civet, Aselliscus stoliczkanus and Rhinolophus sinicus (Chinese rufous horseshoe bat). Genome and ORF1a homology showed that SARS-CoV-2 is not the same coronavirus as the coronavirus derived from these five animals, whereas the authors confirmed the highest homology with Bat coronavirus isolate RaTG13.

Human to human transmission
Han (Infl Other Resp Inf 2020, see below) published a very good summary of available information on the different transmission modes of SARS-CoV-2.

Of note, a new resource is a repository developed by Xu (Lancet Inf Dis 2020), which provides open-access information on COVID-19 cases detected in Wuhan and the rest of the world: https://docs.google.com/spreadsheets/d/1itaohdPiAeniCNlnNztZ_oRvjh0HsGuJXUJWET008/edit#gid=0

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Paper version: dd. 23 MAR 2020

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Early observations
In the 99 patients cohort reported by Chen (Lancet 2020, see below), 49 (49%) patients had a history of exposure to the Huanan seafood market, where wild animals were served at a restaurant. Among them, there were 47 patients with long-term exposure history, most of whom were salesmen or market managers, and two patients with short-term exposure history, who were shoppers. None of the patients were medical staff. These early data suggested that a point-source zoonotic (animal-to-human) route was likely the main mode of transmission of the disease (Nishiura J Clin Med 2020, see below). However, the reporting of a family cluster including a family member, who did not travel to Wuhan, but became infected with the virus after several days of contact with four of the family members, soon provided strong evidence of human-to-human transmission (Chan Lancet 2020, see below). In this cluster none of the family members had contacts with Wuhan markets or animals, although two had visited a Wuhan hospital. In line with this observation, the genetic epidemiology data suggest that from the beginning of December, 2019, when the first cases were retrospectively traced in Wuhan, the spread of infection has been almost entirely driven by human-to-human transmission, not the result of continued spillover (see https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30374-3/fulltext and above).

Transmission mode
The mechanisms of transmission of SARS-CoV-2 remain largely unknown. As for other respiratory viruses, contact, droplet and airborne routes of transmission are suspected (Shiu 2019, see below). However, indirect transmission of the virus may also occur. Cai (Em Inf Dis 2020, see below) investigated a cluster of COVID-19 cases associated with a shopping mall in Wenzhou, China. Data suggested transmission perhaps resulting from virus contamination of common objects, virus aerosolization in a confined space, or spread from asymptomatic infected persons.

Respiratory secretions
Virus shedding has been demonstrated in respiratory secretions by RT-PCR testing (see for instance Zou NEJM 2020 below). However, how SARS-CoV-2 viral load correlates with culturable virus still needs to be determined. Identification of patients with few or no symptoms and with modest levels of detectable viral RNA in the oropharynx for at least 5 days suggests that additional data are required to determine transmission dynamics and inform screening practices.

Conjunctiva
Two samples of tear and conjunctival secretions obtained from a COVID-19 patient with conjunctivitis yielded positive RT-PCR results (Xia J Med Vir 2020, see below). A manuscript by Sun (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.26.20027938v1) reported a similar observation, with SARS-COV-2 RNA detected in ocular discharges in one patient with conjunctivitis. Although conjunctivitis is a rare symptom of COVID-19 (observed in 2.8% of patients in this study), the authors suggested a potential route of nosocomial infection through the eyes after occupational exposure. However, a subsequent publication by Peng (J Med Vir 2020, see) rather indicated that the detection of SARS-CoV-2 RNA in tears and conjunctival secretions of very few COVID-19 patients complicated with conjunctivitis is a coincident event, rather than a causal event of SARS-CoV-2 infection of the conjunctiva.

Faecal excretion
Zhang (in Emerg Micr Inf 2020, see below) also reported presence of the virus in anal swabs and blood, with more anal swab positives than oral swab positives in a later stage of infection. Xiao (https://www.medrxiv.org/content/10.1101/2020.02.17.20023721v1) found 53.42% of patients testing positive in stool. 23.29% of the patients remained positive in faeces even after the viral RNA decreased to undetectable level in respiratory tract. The observation that 14 out of 138 patients (10 percent) in a Wuhan hospital (Wang JAMA 2020, see below) initially presented with diarrhoea and nausea one or two days prior to development of fever and dyspnoea also supported the hypothesis of faecal transmission of the virus. A similar observation had already been made with
the first U.S. patient diagnosed with COVID-19, who also experienced loose bowel movements for two days and subsequent viral RNA detection in stool.

**Vertical transmission**

A preliminary study suggested the absence of vertical transmission of SARS-CoV-2 (Chen Lancet 2020, [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30360-3/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30360-3/fulltext)). Subsequent reports seem to support this contention (see Pregnancy and newborns above).

**Blood products**

SARS-CoV-2 shedding in plasma or serum is common, which leads to a theoretical risk of transmission of coronaviruses through transfusion of blood products. Chang (Transfus Med Rev 2020, see below) noted that because more and more asymptomatic infections are being found among COVID-19 cases, considerations of blood safety and coronaviruses have arisen especially in endemic areas.

**Social drivers of transmission**

While the basic reproductive number only captures the average dynamics of transmission, a crucial question for control is whether specific situations and settings might be driving the epidemic (Liu Lancet 2020, see below). The secondary attack rate (SAR), defined as the probability that an infection occurs among susceptible people within a specific group (i.e., household or close contacts), can provide an indication of how social interactions relate to transmission risk.

Drawing on data from nine recent reports of secondary transmission associated with a specific event such as a meal or holiday visit, Liu estimated that 48 secondary infections occurred among 137 attendees. Assuming that all these secondary infections were generated by a single primary case, which is probable given the short-term nature of the exposure events, would imply a SAR among close contacts of 35% (95% CI 27–44). More data are needed to reliably estimate the true within-household and between-household transmission for SARS-CoV-2; recent reports might be biased towards larger transmission events. However, if it transpires that most at-risk contacts have a close relationship with cases, and superspreading events tend to occur at large gatherings of these close contacts measures to reduce infection risk during such gatherings and subsequent tracing of close contacts of cases might have a disproportionate effect on reducing overall transmission.

**Travel**

A retrospective analysis of early data found a significant association between domestic travel by train and the number of COVID-19 cases in China, whereas the associations of the other two means of transportation (car, flight) failed to reach statistical significance (Zhao Travel Med Inf Dis 2020, see below). However, a subsequent analysis by Chen (Chin Med J 2020, see below), based on cases up to Jan 30 and population migration data extracted from Baidu Qianxi, found a correlation coefficient between the provincial number of cases and emigration from Wuhan up to 0.943.

Zheng (Trav Med Inf Dis 2020, see below) studied the spatial transmission of COVID-19 via public and private transportation in China, and found a significant and positive association between the frequency of flights, trains, and buses from Wuhan and the daily as well as the cumulative numbers of COVID-19 cases in other cities with progressively increased correlations for trains and buses.

Qifang Bi on MedRxiv ([https://www.medrxiv.org/content/10.1101/2020.03.03.20028423v1](https://www.medrxiv.org/content/10.1101/2020.03.03.20028423v1)) studied 391 cases and 1286 close contacts identified by the Shenzhen CDC. In this dataset, cases were found older than the general population (mean age 45) and balanced between males (187) and females (204). Cases were isolated on average 4.6 days after developing symptoms; contact tracing reduced this by 1.9 days. Household contacts and those travelling with a case where at higher risk of infection (ORs 6 and 7) than other close contacts. The household secondary attack rate was 15%.
Mass gatherings
Ebrahim (Trav Med Inf Dis 2020, see below) highlighted the fact that mass gatherings, both those clearly defined and those spontaneously occurring, are key determinants of epidemiologic expansion of disease outbreaks. The authors noted that COVID-19 has already provided examples of both clearly planned event cancellations such as the Umrah suspension in Saudi Arabia, and situations where outbreaks and events were continued.

Superspreading events
Frieden (Em Inf Dis 2020, see below) noted that there have been multiple reports of superspreading events, which are associated with both explosive growth early in an outbreak and sustained transmission in later stages. The authors highlighted a major limitation of the concept of $R_0$, the basic reproductive number, which is presented as a mean or median value and does not capture the heterogeneity of transmission among infected persons.

Transmission by asymptomatic or pre-symptomatic subjects
Numerous reports provide data supporting the contention that asymptomatic (or pre-symptomatic) subjects can transmit COVID-19 with high efficiency (Chang Lancet Resp Med 2020, see below). The possibility of transmission by asymptomatic individuals is a critical question, as it directly impacts public health responses to the epidemic.

Evidence obtained both from epidemiological observations and laboratory testing of asymptomatic subjects is available:

- A boy aged 10 years who was infected with COVID-19 had no symptoms but had visible changes in lung imaging and blood markers of disease.
- Another patient undergoing surgery in a hospital in Wuhan infected 14 health-care workers (HCWs) even before fever onset.
- A patient who travelled from Shanghai to attend a meeting in Germany was subclinical until on the flight back to China. However, two of this patient’s close contacts and another two patients attending the meeting without close contact were found to be infected with COVID-19.
- Yu (J Inf Dis 2020, see below) reported on a familial cluster of four patients in Shanghai, of which one was an 88 year-old man with moving difficulties who was only exposed to his asymptomatic family members who developed symptoms later.
- Hoehl (NEJM 2020, see below) reported that in the effort to evacuate 126 people from Wuhan to Frankfurt, a symptom-based screening process was ineffective in detecting SARS-CoV-2 infection in 2 persons who later were found to have evidence of SARS-CoV-2 in a throat swab.
- Zhou (NEJM 2020, see below) analysed the viral load in one asymptomatic patient and found it similar to that in symptomatic patients, which suggests the transmission potential of asymptomatic or minimally symptomatic patients.
- Bai (JAMA 2020, see below) described a case of transmission from a presumed asymptomatic carrier with one positive PCR, but normal chest CT findings.
- Luo (Chin Med J 2020, see below) identified a confirmed case of asymptomatic SARS-CoV-2 infection in a 50-year old woman. Despite largely normal laboratory and chest CT findings, her persistent positivity of the virus nucleic acid in her throat swabs and anal swabs for at least 17 days suggested that she was very likely a healthy carrier.
- Tang (Emerg Infect Dis 2020, see below) reported on an asymptomatic child who was positive for SARS-CoV-2 by RT PCR in a stool specimen 17 days after the last virus exposure. The child was virus positive in stool specimens for at least an additional 9 days.
- Kam (Clin Inf Dis 2020, see below) described a 6-month-old infant with COVID-19, who had persistently positive nasopharyngeal swabs to day 16 of admission, but no clinical signs or symptoms apart from a single transient temperature of 38.5°C.
• Pan (Lancet Inf Dis, see below) described two individuals who were under active surveillance because of a history of exposure to infected patients and showed positive results on RT-PCR a day before disease onset.
• Tong (Emerg Infect Dis. 2020, see below) described 2 infections resulting from contact with a potentially pre-symptomatic traveller from the city of Wuhan.
• Du (non-peer reviewed manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.19.20025452v1) analyzed 468 infector-feeetee pairs with confirmed COVID-19 cases reported in China between January 21, 2020, and February 8, 2020. Interestingly, 12.1% of reports indicated pre-symptomatic transmission.

Overall, available data show that not only can subclinical patients transmit the virus effectively but patients can also shed high amounts of the virus and infect others even after recovery from the acute illness (see Transmission by recovered patients below).

Hu (Sci China Life Sci 2020, see below) presented the clinical characteristics of 24 cases with asymptomatic infection screened from close contacts and the transmission potential of asymptomatic COVID-19 virus carriers in Nanjing, China. None of the 24 asymptomatic cases presented any obvious symptoms when nucleic acid screening was performed. Five cases (20.8%) developed symptoms (fever, cough, fatigue, etc.) during hospitalization. Twelve (50.0%) cases showed typical CT images of ground-glass chest and 5 (20.8%) presented stripe shadowing in the lungs. The remaining 7 (29.2%) cases showed normal CT image and had no symptoms during hospitalization. These 7 cases were younger (median age: 14.0 years; P=0.012) than the rest. None of the 24 cases developed severe COVID-19 pneumonia or died. The median communicable period, defined as the interval from the first day of positive nucleic acid tests to the first day of continuous negative tests, was 9.5 days (up to 21 days). A typical asymptomatic transmission to the cohabiting family members, which caused severe COVID-19 pneumonia.

Of note, an early report on this topic had been more controversial. On January 30th, Rothe (N Engl J Med. 2020, see below) reported indeed a case of possible asymptomatic transmission, describing a Chinese woman who passed the virus to her German coworkers, triggering a 10-person cluster, reportedly without any symptoms when she flew back to China. The authors of the report, however, had not talked to the Chinese woman, according to a subsequent publication in Science (https://www.sciencemag.org/news/2020/02/paper-non-symptomatic-patient-transmitting-coronavirus-wrong). Citing sources familiar with the case, it was said that Robert Koch Institute scientists did talk to the woman, who said she actually did have symptoms in Germany, which included fatigue and muscle pain, for which she took fever medication.

As indicated by Nishiura (Int J Inf Dis 2020, see below), the asymptomatic ratio is conventionally estimated using sero-epidemiological data. However, collection of such data requires significant logistical effort, time, and cost. Instead, the authors estimated the asymptomatic ratio by using information on Japanese nationals that were evacuated from Wuhan, China on chartered flights. Based on this very small sample size, the asymptomatic ratio was estimated at 30.8% (95% confidence interval (CI): 7.7%, 53.8%) among evacuees. Mizumoto (Euro Surveill 2020, see below) derived the delay-adjusted asymptomatic proportion of infections cases on board the Diamond Princess cruise ship. The estimated asymptomatic proportion reached a somewhat lower value of 17.9% (95% credible interval (Crl): 15.5-20.2%), overlapping the confidence interval of the estimate of Nishiura.

Li (Science 2020, see below) used observations of reported infections within China, in conjunction with mobility data, a networked dynamic metapopulation model and Bayesian inference, to infer critical epidemiological characteristics associated with SARS-CoV2, including the fraction of undocumented infections and their contagiousness. The authors estimated 86% of all infections were undocumented (95% CI: 82%–90%) prior to 23 January 2020 travel restrictions. Per person, the transmission rate of undocumented infections was 55% of documented infections (46%–62%), yet, due to their greater numbers, undocumented infections were the infection source for 79% of documented cases.

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These findings help explain the rapid geographic spread of SARS-CoV2 and indicate containment of this virus will be particularly challenging.

**Transmission by recovered patients**

Lan (JAMA 2020, see below) reported data suggesting that at least a proportion of recovered patients still may be virus carriers. In this study four patients with COVID-19 who met criteria for hospital discharge or discontinuation of quarantine in China (absence of clinical symptoms and radiological abnormalities and 2 negative RT-PCR test results) had positive RT-PCR test results 5 to 13 days later, while they were continuing the quarantine protocol at home for 5 days. All patients had 3 repeat RT-PCR tests performed over the next 4 to 5 days and all were positive. An additional RT-PCR test was performed using a kit from a different manufacturer and the results were also positive for all patients. The patients continued to be asymptomatic by clinician examination and chest CT findings showed no change from previous images. They did not report contact with any person with respiratory symptoms. No family member was infected.

Ling (Chin Med J 2020, see below) analysed, in 66 convalescent patients, the clearance time and factors influencing viral RNA detection in different samples from patients with COVID-19. A majority of patients had a longer duration until stool specimens were negative for viral RNA than for throat swabs, with a median delay of 2.0 (1.0-4.0) days. Only 6.9% urine samples were positive for viral nucleic acid; viral RNA was still present in three patients’ urine specimens after throat swabs were negative. Using a multiple linear regression model (F = 2.669, P = 0.044, and adjusted R2 = 0.122), the analysis showed that the CD4+ T lymphocyte count may help predict the duration of viral RNA detection in patients’ stools (t = −2.699, P = 0.010). The duration of viral RNA detection from oropharyngeal swabs and faecal samples in the glucocorticoid treatment group was longer than that in the non-glucocorticoid treatment group (15 days vs. 8.0 days, respectively; t = 2.550, P = 0.013) and the duration of viral RNA detection in faecal samples in the glucocorticoid treatment group was longer than that in the non-glucocorticoid treatment group (20 days vs. 11 days, respectively; t = 4.631, P < 0.001).

Chen (Int J Inf Dis 2020, see below) also reported a confirmed case of COVID-19 whose oropharyngeal swab test of SARS-CoV-2 RNA turned positive during convalescence.

Yuan (non-peer reviewed manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.03.06.20031377v1.full.pdf) reported recurrence of RT PCR positivity in 25 COVID-19 discharged patients. The positive results were observed in the absence of symptoms, after 2 to 13 days.

Of note, A case report by Qu (Trav Med Inf Dis 2020, see below) pointed to the importance of the specimen choice. Both a throat swab and sputum were collected before the patient was discharged. SARS-COV-2 nucleic acid was still detectable in sputum while the throat swab was negative.

**Estimation of the serial interval**

The serial interval of COVID-19 is defined as the time duration between a primary case (infector) developing symptoms and secondary case (infectee) developing symptoms (Du Em Inf Dis 2020, see below). Obtaining robust estimates for the distribution of COVID-19 serial intervals is a critical input for determining the reproduction number which can indicate the extent of interventions required to control an epidemic. The serial intervals reported by Du had a mean of 3.96 days (95% confidence interval: 3.53-4.39), a standard deviation of 4.75 days (95% confidence interval: 4.46-5.07), and 12.6% of reports indicating pre-symptomatic transmission.

Subsequently, from the analysis of a total of 28 infector-infectee pairs, Nishiura (Int J Inf Dis 2020, see below) estimated the median serial interval at 4.0 days (95% credible interval [Crl]: 3.1, 4.9). Limiting our data to only the most certain pairs, the median serial interval was estimated at 4.6 days (95% Crl: 3.5, 5.9). Considering that the serial interval of COVID-19 is close to or shorter than its median incubation period, the data suggest that a substantial proportion of secondary transmission may occur prior to illness onset.

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Zhao (Infect Control Hosp Epidemiol. 2020, see below) evaluated 48 transmission events including 21 in Hong Kong and 27 in Shenzhen. The authors found that the serial interval had been decreasing by 0.4 (95%CI: 0.1–0.7) per day, or 6.2% (95%CI: 0.4–11.6%) in percentage, from January 10 to February 2 in Hong Kong and Shenzhen. The Pearson correlation coefficient between the serial interval and calendar date was estimated at −0.37 with p-value < 0.01. The serial interval of male primary cases was 3.5 days (95%CI: 1.2–5.7) shorter than that of a female primary case, or 49.7% (95%CI: 15.3–70.1%) less in percentage.

Estimation of the reproductive number
Different estimates of the basic reproductive number ($R_0$) were reported from a number of studies since the start of the epidemic (see Table 10).


Liu (J Trav Med 2020, see below) had previously presented an overview of published $R_0$ estimates for the disease, and found the average $R_0$ to be 3.28 and median 2.79.

Zhao (J Trav Med 2020, see below) subsequently demonstrated that using an overestimated serial interval leads to overestimation of $R_0$, and found an $R_0$ at 2.0 when using a more recent estimate of the serial interval at 4.6 days.

Table 10 Published estimates of $R_0$ (adapted from Liu J Trav Med 2020)

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Study date</th>
<th>Methods</th>
<th>Approaches</th>
<th>$R_0$ estimates (average)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>China</td>
<td>18 January 2020</td>
<td>/</td>
<td>/</td>
<td>1.4–2.5 (1.95)</td>
<td>/</td>
</tr>
<tr>
<td>Shen</td>
<td>Hubei province</td>
<td>12–22 January 2020</td>
<td>Mathematical model, dynamic compartmental model with population divided into five compartments: susceptible individuals, asymptomatic individuals during the incubation period, infectious individuals with symptoms, isolated individuals with treatment and recovered individuals</td>
<td>$R_0 = \beta/\alpha\beta = \text{mean person-to-person transmission rate/day in the absence of control interventions, using nonlinear least squares method to get its point estimate } \alpha = \text{isolation rate } = 6$</td>
<td>6.49</td>
<td>6.31–6.66</td>
</tr>
<tr>
<td>Liu</td>
<td>China and overseas</td>
<td>23 January 2020</td>
<td>Statistical exponential Growth, using SARS generation time = 8.4 days, SD = 3.8 days</td>
<td>Applies Poisson regression to fit the exponential growth rate $R_0 = 1/M(−r)M$ = moment generating function of the generation time distribution $\hat{r}$ = fitted exponential growth rate</td>
<td>2.90</td>
<td>2.32–3.63</td>
</tr>
<tr>
<td>Liu</td>
<td>China and overseas</td>
<td>23 January 2020</td>
<td>Statistical maximum likelihood estimation, using SARS generation time = 8.4 days, SD = 3.8 days</td>
<td>Maximize log-likelihood to estimate $R_0$ by using surveillance data during a disease epidemic, and assuming the secondary case is Poisson distribution with expected value $R_0$</td>
<td>2.92</td>
<td>2.28–3.67</td>
</tr>
<tr>
<td>Read</td>
<td>China</td>
<td>1–22 January 2020</td>
<td>Mathematical transmission model assuming latent</td>
<td>Assumes daily time increments with Poisson-distribution and apply a deterministic SEIR metapopulation</td>
<td>3.11</td>
<td>2.39–4.13</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Location</th>
<th>Date Range</th>
<th>Methodology</th>
<th>Model Details</th>
<th>Transmission Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Majumder, Wuhan</td>
<td>December 8, 2019 to January 26, 2020</td>
<td>Mathematical Incidence Decay and Exponential Adjustment (IDEA)</td>
<td></td>
<td>Adopted mean serial interval lengths from SARS and MERS ranging from 6 to 10 days to fit the IDEA model,</td>
<td></td>
</tr>
<tr>
<td>Cao, China</td>
<td>January 23, 2020</td>
<td>Mathematical model including compartments Susceptible-Exposed-Infectious-Recovered-Death-Cumulative (SEIRDc)</td>
<td></td>
<td>$R = K \times (L + D) + K(L + D) + 1L = \text{average latent period} + \text{average latent infectious period} = 9, K = \text{logarithmic growth rate of the case counts}$</td>
<td></td>
</tr>
<tr>
<td>Zhao, China</td>
<td>January 10-24, 2020</td>
<td>Statistical exponential growth model method</td>
<td></td>
<td>Corresponding to 8-fold increase in the reporting rate $R_0 = 1/M(-r)$, $r$ = intrinsic growth rate</td>
<td></td>
</tr>
<tr>
<td>Zhao, China</td>
<td>January 10-24, 2020</td>
<td>Statistical exponential growth model method</td>
<td></td>
<td>Corresponding to 2-fold increase in the reporting rate $R_0 = 1/M(-r)$, $r$ = intrinsic growth rate</td>
<td></td>
</tr>
<tr>
<td>Imai (2020), Wuhan</td>
<td>January 18, 2020</td>
<td>Mathematical model, computational modelling of potential epidemic trajectories</td>
<td></td>
<td>Assume SARS-like levels of case-to-case variability in the numbers of secondary cases and a SARS-like generation time with 8.4 days, and set number of cases caused by zoonotic exposure and assumed total number of cases to estimate $R_0$ values for best-case, median and worst-case</td>
<td></td>
</tr>
<tr>
<td>Julien and Althaus, China and overseas</td>
<td>January 18, 2020</td>
<td>Stochastic simulations of early outbreak trajectories</td>
<td></td>
<td>Stochastic simulations of early outbreak trajectories were performed that are consistent with the epidemiological findings to date</td>
<td></td>
</tr>
<tr>
<td>Tang, China</td>
<td>January 22, 2020</td>
<td>Mathematical SEIR-type epidemiological model incorporates appropriate compartments corresponding to interventions</td>
<td></td>
<td>Method-based method and Likelihood-based method</td>
<td></td>
</tr>
<tr>
<td>Qun Li, China</td>
<td>January 22, 2020</td>
<td>Statistical exponential growth model</td>
<td></td>
<td>Mean incubation period = 5.2 days, mean serial interval = 7.5 days</td>
<td></td>
</tr>
</tbody>
</table>

5 J Med Vir 2020  
6 https://www.medrxiv.org/content/10.1101/2020.02.07.20021154v1  
7 https://www.medrxiv.org/content/10.1101/2020.02.10.20021675v1  
8 NEJM 2020  
9 Int J Infect Dis 2020  
10 Int J Infect Dis 2020  
11 Eurosurv 2020  

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Of note, some authors (e.g. Cao, manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.01.27.20018952v1.full.pdf; or Lai J Med Vir 2020, see below) have referred to the effective reproduction number (R, the expected number of secondary cases generated by an infectious case once an epidemic is underway), which has been presented as a more accurate definition than the basic reproduction number.

<table>
<thead>
<tr>
<th>Author</th>
<th>Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhou</td>
<td>1.4 – 3.8</td>
</tr>
<tr>
<td>Wu</td>
<td>2.68 – 2.86</td>
</tr>
<tr>
<td>Liu</td>
<td>2.8</td>
</tr>
<tr>
<td>Shao</td>
<td>3.25 ≤ R ≤ 3.4</td>
</tr>
</tbody>
</table>

Liu (on MedRxiv: https://www.biorxiv.org/content/10.1101/2020.01.25.919787v1.full) reported that the temporal distribution of R values showed a declining trend from 7.93 (95%CI: 5.00-12.00) to 2.60 (95%CI: 0.57-5.17). Such temporal effect was also observed by Kucharski (Lancet Inf Dis 2020, see below), who estimated that the median daily reproduction number (Rt) in Wuhan declined from 2.35 (95% CI 1.15–4.77) 1 week before travel restrictions were introduced on Jan 23, 2020, to 1.05 (0.41–2.39) 1 week after. Interestingly, Kucharski’s model also found that in locations with similar transmission potential to Wuhan in early January, once there are at least four independently introduced cases, there is a more than 50% chance the infection will establish within that population.

**At risk populations**

Early publications provided preliminary analyses of the main risk factors of COVID-19. For instance, a retrospective, single-centre study, including all confirmed cases of COVID-19 in Wuhan Jinyintan Hospital from Jan 1 to Jan 20, 2020 described 99 patients with PCR-confirmed COVID-19 pneumonia (Chen Lancet 2020, see below). Forty-nine (49%) had a history of exposure to the Huanan seafood market. The average age of the patients was 55.5 years (SD 13.1), including 67 men and 32 women. Fifty (51%) patients had chronic diseases. In this study, the disease was found more likely to affect older males with comorbidities. Subsequently, Yan (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.10.20021675v1) reported that out of a total of 8866 patients including 4021 (45.35%) laboratory confirmed patients, nearly half were aged 50 years or older (47.7%). There was a gender difference in incidence with 0.31 (male) vs. 0.27 (female) per 100,000 people (P<0.001).

Epidemiological data were also reported from other countries. For instance, a report of the Korean Society of Infectious Diseases; Korean Society of Pediatric Infectious Diseases; Korean Society of Epidemiology; Korean Society for Antimicrobial Therapy; Korean Society for Healthcare-associated Infection Control and Prevention; and Korea Centers for Disease Control and Prevention (J Korean Med Sci 2020, see below) provided the key epidemiological features of the disease in Korea.

**At risk of infection**

A retrospective study involving COVID-19 cases reported through February 11, 2020, and corresponding to 72,314 patient records - 44,672 (61.8%) confirmed cases, 16,186 (22.4%) suspected cases, 10,567 (14.6%) clinically diagnosed cases (Hubei Province only), and 889 asymptomatic cases (1.2%), - was reported by the Novel Coronavirus Pneumonia Emergency Response Epidemiology Team (Zhonghua Liu Xing Bing Xue Za Zhi 2020, see below; and Wu Jama 2020, 12, https://onlinelibrary.wiley.com/doi/epdf/10.1111/jebm.12376 13 Lancet 2020 14 https://academic.oup.com/jtm/advance-article/doi/10.1093/jtm/taaa021/5735319 15 https://www.medrxiv.org/content/10.1101/2020.02.17.20023747v2

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Among confirmed cases, most were aged 30-79 years (86.6%), diagnosed in Hubei (74.7%). The male-to-female ratio was 0.99:1 in Wuhan, 1.04:1 in Hubei, and 1.06:1 in China overall.

Yang (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.28.20028068v1), who analysed 55 cases in Beijing, also showed that compared with patients without pneumonia, those with pneumonia were 15 years older and had a higher rate of hypertension.

The characteristics of patients may at least in part reflect the movement and the social activities of individuals in different societies (Korean Society of Infectious Diseases J Korean Med Sci 2020, see below). In the Korean situation, the predominance of females and subjects in their 20s may be due to the fact that the outbreak related to a religious group in Daegu.

**Age**

Table 8 presented in Case fatality rate above, shows the risk of infection according to age as detected in the analysis of 72 314 patient records in China.

**Occupational risks**

As noted by Koh (Occup Med 2020, see below), a significant proportion of cases are related to occupational exposure. As this virus is believed to have originated from wildlife and then crossed the species barrier to infect humans, it is not unexpected that the first documented occupational groups at risk were persons working in seafood and wet animal wholesale markets in Wuhan. At the start of the outbreak, workers and visitors to the market comprised 55% of the 47 cases with onset before 1 January 2020, when the wholesale market was closed. Health care workers (HCWs) were next recognized as another high-risk group to acquire this infection. In a case series of 138 patients treated in a Wuhan hospital, 40 patients (29% of cases) were HCWs. Among the affected HCWs, 31 (77.5%) worked in the emergency department, and 2 (5%) in the intensive care unit (ICU). In Singapore, among the first 25 locally transmitted cases, 17 cases (68%) were probably related to occupational exposure. They included staff in the tourism, retail and hospitality industry, transport and security workers, and construction workers.

Sabino-Silva (Clin Oral Investig 2020, see below) highlighted the risks associated with the virus in saliva, especially for dentists and healthcare professionals that perform aerosol-generating procedures.

**At risk of severe disease**

The retrospective study involving 44 672 confirmed COVID-19 cases reported by the Novel Coronavirus Pneumonia Emergency Response Epidemiology Team in China (Zhonghua Liu Xing Bing Xue Za Zhi 2020, see below) described a proportion of 13.8% severe cases and 4.7% critical cases in their database. They reported more severe disease in Wuhan than outside the province of Hubei. Another study by Yang (Lancet 2020, see below), which studied 52 critically ill adult patients noted that 35 of these patients (67%) were men and 21 (40%) had chronic illness. In the cohort of 78 patients reported by Liu (Chin Med J 2020, see below), the patients in the progression group were also older than those in the disease improvement/stabilization group (66 [51, 70] vs. 37 [32, 41] years, U = 4.932, P = 0.001). The progression group had a significantly higher proportion of patients with a history of smoking than the improvement/stabilization group (27.3% vs. 3.0%, χ² = 9.291, P = 0.018).

Wu (JAMA Int Med 2020, see below) found that diabetes and hypertension to be more frequent in those who developed ARDS than those who did not. Fang (Lancet Resp Med 2020, see below) noted that the most frequent comorbidities reported in studies of patients with COVID-19 are often treated with angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type-I receptor blockers (ARBs) (even though treatment was not assessed in these published studies), which increase ACE2 expression. The authors therefore hypothesized that such treatment would increase the risk of developing severe and fatal COVID-19 in patients with cardiac diseases, hypertension, or diabetes.
At risk of death

Chen (Lancet 2020, see below) observed that the characteristics of patients who died were in line with the MuLBSTA score (calculated from 6 indexes consisting of multilobular infiltration, lymphopenia, bacterial co-infection, smoking history, hypertension, and age), an early warning model for predicting mortality in viral pneumonia.

Another report (Wang J Med Virol 2020, see below) reported the details of the first 17 deaths up to 24:00 pm 22 Jan 2020. The deaths included 13 males and 4 females. The median age of the deaths was 75 (range 48-89) years. The median days from first symptom to death were 14.0 (range 6-41) days, and tended to be shorter among people 70 years of age and above (11.5 [range 6-19] days) than those with ages below 70 years (20 [range 10-41] days, P=0.033).

Zhang (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.26.20028191v1) presented the clinical characteristics of 82 death cases of laboratory-confirmed as SARS-CoV-2 infection in Wuhan. Most of these death cases were male (65.9%). More than half of dead patients were older than 60 years (80.5%) and the median age was 72.5 years. The bulk of death cases had comorbidity (76.8%), including hypertension (56.1%), heart disease (20.7%), diabetes (18.3%), cerebrovascular disease (12.2%), and cancer (7.3%).

Similarly, Ruan (Intensive Care Med 2020, see below) conducted a retrospective multicenter study of 68 death cases and 82 discharged cases with laboratory-confirmed infection. The authors found a significant difference in age between the death group and the discharge group (p < 0.001) but no difference in the sex ratio (p = 0.43). A total of 63% (43/68) of patients in the death group and 41% (34/82) in the discharge group had underlying diseases (p = 0.0069). It should be noted that patients with cardiovascular diseases have a significantly increased risk of death when infected with SARS-CoV-2 (p < 0.001). A total of 16% (11/68) of the patients in the death group had secondary infections vs. 1% (1/82) of the patients in the discharge group (p = 0.0018).

The retrospective analysis of cases registered in China through February 11th (Novel Coronavirus Pneumonia Emergency Response Epidemiology Team Zhonghua Liu Xing Bing Xue Za Zhi 2020, see below; and Wu Jama 2020, see below) concluded that the ≥80 years age group had the highest case fatality rate of all age groups at 14.8%. Case fatality rate for males was 2.8% and for females was 1.7%. By occupation, patients who reported being retirees had the highest case fatality rate at 5.1%, and patients in Hubei Province had a >7-fold higher case fatality rate at 2.9% compared to patients in other provinces (0.4%). While patients who reported no comorbid conditions had a case fatality rate of 0.9%, patients with comorbid conditions had much higher rates - 10.5% for those with cardiovascular disease, 7.3% for diabetes, 6.3% for chronic respiratory disease, 6.0% for hypertension, and 5.6% for cancer.

Similarly, Shi (Crit Care 2020, see below) found age, occupation, hypertension (p<0.001 for the comparisons between mild and severe cases), gender and cardiovascular disease (p<0.003) as risk factors.

In line with the observations made in China, Porcheddu (J Infect Dev Ctries 2020, see below) observed that fatalities in Italy mostly occurred in the elderly with known comorbidities.

Smoking as a risk factor?

Cai (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.05.20020107v3) observed significantly higher ACE2 gene expression in former smoker's lung compared to non-smoker's lung. Also, the authors found higher ACE2 gene expression in Asian current smokers compared to non-smokers, but not in Caucasian current smokers, which may indicate an existence of gene-smoking interaction. In addition, they found that ACE2 gene is expressed in specific cell types related to smoking history and location. In bronchial epithelium, ACE2 is actively expressed in goblet cells of current smokers and club cells of non-smokers. In alveoli, ACE2 is actively expressed in remodelled AT2 cells of former smokers. Together, this study indicates that smokers especially former smokers may be more susceptible to COVID-19 and have infection paths different with non-smokers (at least in Asia).
Based on a review of published data as of 17 March 2020, Vardavas (Tobacco Induced Diseases 2020, see below) concluded that smoking is most likely associated with the negative progression and adverse outcomes of COVID-19. Indeed, the authors identified five studies reporting data on the smoking status of patients infected with COVID-19. Notably, in the largest study that assessed severity (Guan NEJM 2020, see below), there were higher percentages of current and former smokers among patients that needed ICU support, mechanical ventilation or who had died, and a higher percentage of smokers among the severe cases. From these published data Vardavas calculated that the smokers were 1.4 times more likely (RR=1.4, 95% CI: 0.98–2.00) to have severe symptoms of COVID-19 and approximately 2.4 times more likely to be admitted to an ICU, need mechanical ventilation or die compared to non-smokers (RR=2.4, 95% CI: 1.43–4.04).

However, another review published in parallel by Lippi (Eur J Int Med 2020, see below) reached different conclusions from the analysis of almost the same studies. The authors reported that in only one study (the study by Guan referred to by Vardavas), active smoking was found to be a significant predictor of COVID-19 severity, whilst in the other four studies the association was not statistically significant.

Cai (Lancet Resp Med 2020, see below) also considered that the relatively small proportion of current smokers in reported studies compared with the proportion of male smokers in China (50.5%) are unlikely to be associated with incidence or severity of COVID-19.

Moreover, a study by Shi (Crit Care 2020, see below) based on 487 patients, not included in any of these 2 reviews, did not identify smoking as a risk factor.

**Distribution**

A disease situation dashboard is available on the WHO website, which presents the number of confirmed cases globally over time, cases in China by provinces, regions and cities, as well as confirmed cases outside China by country (http://who.maps.arcgis.com/apps/opsdashboard/index.html#/c88e37cfc43b4ed3baf977d77e4a0667).

In addition, the Johns Hopkins University developed its own dashboard to visualize and track the reported cases of COVID-19 on a daily timescale (Dong Lancet Infect Dis 2020, see below). The complete set of data is downloadable as a google sheet (https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6). The case data visualized is collected from various sources, including WHO, U.S. CDC, ECDC China CDC (CCDC), NHC and DXY. DXY is a Chinese website that aggregates NHC and local CCDC situation reports in near real-time, providing more current regional case estimates than the national level reporting organizations are capable of, and is thus used for all the mainland China cases reported in the dashboard (confirmed, suspected, recovered, deaths). U.S. cases (confirmed, suspected, recovered, deaths) are taken from the U.S. CDC, and all other country (suspected and confirmed) case data is taken from the corresponding regional health departments. A snapshot of this dashboard data as of March 23 2020 10:33 CET is presented below (Figure 6).

The data is directly linked to the case definitions that are used for reporting (see Case definition above). A change in the case definition in China in February led to a spike in the reported figures (see for instance https://www.sciencemediacentre.org/expert-reaction-to-the-latest-change-in-case-definitions-in-china-for-covid-19/).
HealthMap has made an interactive map for SARS-CoV-2 available at [https://www.healthmap.org/covid-19/](https://www.healthmap.org/covid-19/). It offers near-real-time geolocated updates from various sources to better understand the progression of the pandemic. HealthMap is a team of researchers, epidemiologists and software developers at Boston Children’s Hospital founded in 2006, utilizing online informal sources for disease outbreak monitoring and real-time surveillance of emerging public health threats.

The repository developed by Xu (Lancet Inf Dis 2020, see below) provides information on COVID-19 cases detected in the Hubei province and the rest of the world ([https://docs.google.com/spreadsheets/d/1itaohdPlaeniCXNiNntNztZ_oRvjhoHsGuJXUJWET008/edit#gid=0](https://docs.google.com/spreadsheets/d/1itaohdPlaeniCXNiNntNztZ_oRvjhoHsGuJXUJWET008/edit#gid=0)).

Of note, WHO issued guidance to member states on the implementation of global surveillance of COVID-19 ([https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov)](https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov)). The objectives of this global surveillance are to monitor trends of the disease where human to human and/or zoonotic transmission occurs; rapidly detect new cases in countries where the virus is not circulating; provide epidemiological information to conduct risk assessment at the national, regional and global level; and provide epidemiological information to guide response measures.

In terms of research activities, various modelling studies analyse available information and make attempts at forecasting future spread of the disease. For instance, Thompson (J Clin Med 2020, see below) estimated the probability that an imported case is followed by sustained human-to-human transmission to 0.41 (credible interval [0.27, 0.55]).
Virus persistence in the environment

Kampf (J Hosp Infect 2020, see below) reviewed the literature on all available information about the persistence of human and veterinary coronaviruses on inanimate surfaces as well as inactivation strategies with biocidal agents used for chemical disinfection, e.g. in healthcare facilities. This analysis revealed that human coronaviruses such as SARS and MERS CoVs or endemic human CoVs can persist on inanimate surfaces like metal, glass or plastic for up to 9 days, but can be efficiently inactivated by surface disinfection procedures with 62-71% ethanol, 0.5% hydrogen peroxide or 0.1% sodium hypochlorite within 1 minute. Other biocidal agents such as 0.05-0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate are less effective.

Similarly, Yeo (Lancet Gastroenterol Hepatol 2020, see below) indicated that observations made with SARS and MERS CoVs support a relatively good viability of these viruses on surfaces depending on temperature and humidity. SARS-CoV RNA was found in the sewage water of two hospitals in Beijing treating patients with SARS. When SARS-CoV was seeded into sewage water obtained from the hospitals in a separate experiment, the virus was found to remain infectious for 14 days at 4°C, but for only 2 days at 20°C.

Related to this topic, a report by Ong (JAMA 2020, see below) described the detection of virus RNA in the environment of 3 COVID-19 cases in isolation in Singapore. Virus culture was not performed, but the link between PCR detection of the virus, but the authors were able to link virus detection to clinical characteristics of the patients.

Environmental surveillance was also performed by Cheng (Inf Contr Hosp Epidem 2020, see below) in a patient with viral load of 3.3x10^6 copies/ml (pooled nasopharyngeal/ throat swab) and 5.9x10^6 copies/ml (saliva) respectively. SARS-CoV-2 revealed in 1 (7.7%) of 13 environmental samples, but not in 8 air samples collected at a distance of 10 cm from patient’s chin with or without wearing a surgical mask.

van Doremalen (NEJM 2020, see below) found that SARS-CoV-2 remained viable in aerosols for at least 180 minutes, with a reduction in infectious titer 3 hours post-aerosolization from 10^{3.5} to 10^{2.7} CID50/L (mean across three replicates). This reduction in viable virus titer was relatively similar to the reduction observed in aerosols containing SARS-CoV-1. The virus was most stable on plastic and stainless steel. Viable virus could be detected up to 72 hours post application, though by then the virus titer was greatly reduced (polypropylene from 10^{1.7} to 10^{0.6} TCID50/mL after 72 hours, stainless steel from 10^{1.7} to 10^{0.6} TCID50/mL after 48 hours,
Current management of patients

WHO issued a document intended for clinicians taking care of hospitalised adult and paediatric patients with severe acute respiratory infection (SARI) when a SARS-CoV-2 infection is suspected (https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected). It is not meant to replace clinical judgment or specialist consultation but rather to strengthen clinical management of these patients and to provide up-to-date guidance. The document addresses the following topics:

- Triage: recognize and sort patients with SARI
- Immediate implementation of appropriate infection prevention and control (IPC) measures
- Early supportive therapy and monitoring
- Collection of specimens for laboratory diagnosis
- Management of hypoxemic respiratory failure and acute respiratory distress syndrome (ARDS)
- Management of septic shock
- Prevention of complications
- Specific anti-COVID-19 treatments
- Special considerations for pregnant patients.

An increasing number of reports describe the disease course and clinical management of patients in China as well as in other countries where cases have occurred.

Triage and patient flow

A publication by Zhang (Lancet Resp Med 2020, see below) indicates that one effective strategy for disease control in Wuhan was the establishment of fever clinics for triaging patients. The clinical strategies that were used in these adult fever clinics for COVID-19 management is illustrated by the flowchart presented in Figure 7.

Many aspects of this algorithm would not be feasible in developing countries setting, as chest CT, differential blood counts, and C-reactive protein testing are not available. Ayebare (Lancet Resp Med 2020, see below) proposed a modified COVID-19 screening algorithm for use in resource-limited settings that do not have established local transmission.
Current treatment practices

Wang, Chen et al. (Biosci Tr 2020, see below) reported on the diagnosis and treatment of four patients with mild or severe COVID-19 pneumonia. All patients received antiviral treatment, including lopinavir/ritonavir (Kaletra®, lopinavir 400 mg/ritonavir 100 mg, q12h, po), arbidol (0.2 g, tid, po), and Shufeng Jiedu Capsule (2.08 g, tid, po). The duration of antiviral treatment was 6-15 days. In addition, all patients were all given antibiotic treatment and started on supplemental oxygen, delivered by nasal cannula after admission to hospital.

Liu (Crit Care 2020, see below) reported that patient management in Shenzhen was largely supportive, including intubation, early prone positioning, neuromuscular blockade, and extracorporeal membrane oxygenation (ECMO).
according to the recommendations updated by China’s National Health Committee. Low-dose systematic corticosteroids, lopinavir/ritonavir, and atomization inhalation of interferon were encouraged.

Murthy (JAMA 2020, see below) noted that evidence-based treatment guidelines for ARDS should be followed, including conservative fluid strategies for patients without shock following initial resuscitation, empirical early antibiotics for suspected bacterial co-infection until a specific diagnosis is made, lung-protective ventilation, prone positioning, and consideration of extracorporeal membrane oxygenation for refractory hypoxemia.

Wang (Lancet 2020, see below) reported on the classification of COVID-19 patients in 3 types for effective triage in a hospital in Wuhan. Patients with pneumonia were classified as type A. Basic treatments were provided, such as antivirals, antibiotics, oxygen therapy, and glucocorticoids. Type B patients had disease accompanied by serious comorbidities. Their pneumonia was managed and specific treatment plans were developed, including antihypertensives, hypoglycaemic therapy, and continuous renal replacement therapy. Critically ill patients were classified as type C. Attention was paid to organ function in these patients and necessary protective measures, including mechanical ventilation, glucocorticoids, antivirals, symptomatic treatments, and anti-shock therapy.

Based on the experience with 631 confirmed cases of COVID-19 (with a portion of critically ill patients whose ages ranged from 9 months to 96 years old) Sun (Ann Intensive Care 2020, see below) reported a cure rate of confirmed cases of 96.67% in Jiangsu Province, far exceeding that of national Chinese data. The authors noted that essential strategies to improve outcomes consist of early detection of high-risk and critically ill patients. In Jiangsu Province, critical care was shifted forward. All COVID-19 patients were screened twice every day and respiratory rate (RR), heart rate (HR), SpO2 (room air) were monitored regularly. Once SpO2 < 93%, RR > 30/min, HR > 120/min or any signs of organ failure were observed, patients would be transferred to ICU. Intervention to prevent the progression of disease were then three-fold: (1) For patients with ARDS or pulmonary extensive effusion in CT scan, high-flow nasal cannula oxygen therapy or non-invasive mechanical ventilation was used to maintain positive end expiratory pressure to prevent alveolar collapse even if some of these patients did not have refractory hypoxemia. (2) Restrictive fluid resuscitation under the premise of adequate tissue perfusion was performed to relieve pulmonary oedema. (3) Awake prone position was attempted in patients which showed significant effects in improving oxygenation and pulmonary heterogeneity.

Respiratory support
To reduce respiratory symptoms and improve prognosis, respiratory support is the most important means of life support, and non-invasive respiratory support systems, including various conventional oxygen therapies, non-invasive positive pressure ventilation (NPPV), and high-flow nasal cannula (HFNC), are most commonly used (Xia Chin Med J 2020, see below). However, their efficacy and safety remain unclear, and whether they increase the risk of aerosol dispersion and disease transmission is particularly controversial (see Safety of procedures below, and Namendys-Silva Lancet Respir Med 2020, see below). The retrospective epidemiological study of 99 COVID-19 pneumonia patients in China revealed that NPPV is the most commonly used mechanical ventilation method for acute respiratory failure, with reported rates of using non-invasive and invasive mechanical ventilation of 13% and 4%, respectively. For strictly selected early-stage patients with mild-to-moderate (partial pressure of arterial oxygen [PaO2]/fraction of inspired oxygen [FiO2] > 200 mmHg) hypoxic respiratory failure and especially for units with limited numbers of invasive ventilators, it has been recommended that NPPV be attempted for short periods of time (1-2 hours) and to intubate immediately if no improvement is observed.

Of note, MacLaren (JAMA 2020, see below) commented on the WHO interim guidelines making general recommendations for treatment of ARDS in the context of the COVID-19 epidemic, including that consideration be given to referring patients with refractory hypoxemia to expert centres capable of providing ECMO. ECMO being a resource-intensive, highly specialized, and expensive form of life support with the potential for significant complications, he recommended limiting support with ECMO to the most critically ill patients in regions with the...
extensive resources required to provide this therapy. In less well-resourced countries, his hypothesis is that many more lives will be saved by ensuring oxygen and pulse oximetry are widely available. Nevertheless, Li (Chin Med J 2020, see below) reported experience compared with that in patients receiving only conventional respiratory care, the fatality of those who had received ECMO was significantly lower (100% vs. 65%).

**Diagnostics**

Interim guidance to laboratories and stakeholders involved in laboratory testing of patients who meet the definition of suspected case of pneumonia associated with SARS-CoV-2 has been provided by WHO (https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117). Until validated diagnostic tests become available, the goals of diagnostic testing are to detect conventional causes of pneumonia early, to support disease control activities, and to work with reference laboratories that can perform pan-coronavirus detection and directed sequencing.

WHO has taken a three-pronged approach to enhance diagnostic capacity for COVID-19:

- Forming a network of specialized referral laboratories with demonstrated expertise in the molecular detection of coronaviruses. These international labs can support national labs to confirm COVID-19 cases and troubleshoot their molecular assays;
- Strengthening national capacity for detection of COVID-19 so that diagnostic testing can be performed rapidly without the need for overseas shipping. One way this has been achieved is through working with existing global networks for detection of respiratory pathogens such as, notably, the National Influenza Centers that support the Global Influenza Surveillance and Response System;
- Ensuring test availability. This has involved a) screening of SARS-CoV-2 PCR protocols from academic laboratories for validation data (e.g. limits of detection, specificity), b) looking for sequence alignment of established commercial coronavirus assays (e.g. SARS) to see if any were likely to be able to detect 2019-nCoV with high sensitivity, and c) working with commercial and non-commercial agencies with capacity to manufacture and distribute newly-developed SARS-CoV-2 PCR assays.

**Specimen**

Lower respiratory specimens were soon considered likely to have a higher diagnostic value than upper respiratory tract specimens for detecting SARS-CoV-2 infection. WHO recommended that lower respiratory specimens such as sputum, endotracheal aspirate, or bronchoalveolar lavage be collected for SARS-CoV-2 testing where possible (https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov)). However, Yang (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.11.20021493v1) noted that no data on the difference of viral shedding between the upper and lower respiratory tract specimens was available. He reported that while viral RNAs could be detected in all the lower respiratory tract of severe cases, the situation was different for mild cases. Sputum specimen were recommended as most accurate for laboratory diagnosis of COVID-19, followed by nasal swabs.

A study by Zou (NEJM 2020, see below) analysed the viral load in nasal and throat swabs obtained from 17 symptomatic patients in relation to day of onset of any symptoms. Higher viral loads were detected soon after symptom onset, with higher viral loads detected in the nose than in the throat. This analysis suggests that the viral nucleic acid shedding pattern of patients infected with SARS-CoV-2 resembles that of patients with influenza and appears different from that seen in patients infected with SARS-CoV.

A small study in 12 hospitalized patients suggested the feasibility of using self-collected saliva as specimen for diagnostic purposes (To J Vir 2020, see below).
While describing 2 cases, Han (Lancet Inf Dis 2020, see below) suggested that sputum induction might be more helpful than throat swabs for the detection of SARS-CoV-2 RNA in convalescent patients.

Zhang (J Med Virol 2020, see below) presented PCR testing results on stool and oropharyngeal swabs specimens from 14 patients.

A larger study analysed a total of 1070 specimens of different types that were collected from 205 patients with COVID-19 (Wang JAMA 2020, see below). 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%), fibrobronchoscope brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), faeces (44 of 153; 29%), and blood (3 of 307; 1%). None of the 72 urine specimens tested positive.

Testing methods
A list of assays commercially available for diagnosis of COVID-19 is updated by FIND (https://www.finddx.org/covid-19/). Assays that are still in development stage are also presented.

Molecular methods

RT-PCR
In acute respiratory infection, RT-PCR is routinely used to detect causative viruses from respiratory secretions. Early reports presented various assays for COVID-19 diagnosis. A real-time reverse-transcription PCR (rtRT-PCR) was used to identify SARS-CoV-2 through preliminary and validation detection of its E gene, RNA-dependent RNA polymerase (RdRp) gene, and N gene (Yu Micr Inf 2020, see below). Chu (Clin Chem 2020, see below) reported the development of two 1-step quantitative rtRT-PCR assays detecting the ORF1b and N regions of the viral genome. The primer and probe sets were designed to react with SARS-CoV-2 and its closely related viruses, such as SARS coronavirus. These assays were evaluated using a panel of positive and negative controls and shown to have a dynamic range of at least seven orders of magnitude (2x10^4-2000 TCID50/reaction).

Fluorescence-based quantitative PCR kit were rapidly distributed by the Chinese CDC for laboratory confirmation of disease in China. And a whole array of commercial tests became available. Wang (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.12.20022327v1.full.pdf) reported for instance the use of a detection kit (Bioperfectus, Taizhou, China) to detect the ORF1ab gene and the N gene using real-time RT-PCR. Positive results on both the ORF1ab gene and the N gene are required for laboratory confirmation of the disease.

In Europe, the envelope (E)-gene screening test as published by Corman (Euro Surv 2020, see below) has been widely implemented.

Sharfstein (JAMA 2020, see below) provided a detailed explanation of the issues faced in the U.S., which delayed COVID-19 PCR testing in the country.

Improving PCR assays
Chan (J Clin Micro 2020, see below) developed a novel real-time RT-PCR assay targeting the RNA-dependent RNA polymerase (RdRp)/helicase (Hel) (COVID-19-RdRp/Hel assay). The assay has a low limit of detection (1.8 TCID50/ml with genomic RNA and 11.2 RNA copies/reaction with in vitro RNA transcripts). It was compared to the RdRp-P2 assay currently used in European laboratories. Among 273 specimens from 15 patients with laboratory-confirmed COVID-19 in Hong Kong, 77 (28.2%) were positive by both the COVID-19-RdRp/Hel and RdRp-P2 assays. The COVID-19-RdRp/Hel assay was positive for an additional 42 RdRp-P2-negative specimens [119/273 (43.6%) vs 77/273 (28.2%), P<0.001], including 29/120 (24.2%) respiratory tract specimens and 13/153 (8.5%) non-respiratory tract specimens. The mean viral load of these specimens was 3.21x10^4 RNA copies/ml (range, 2.21x10^2 to 4.71x10^5 RNA copies/ml). The COVID-19-RdRp/Hel assay did not cross-react with other human-pathogenic coronaviruses and respiratory...
pathogens in cell culture and clinical specimens, whereas the RdRp-P2 assay cross-reacted with SARS-CoV in cell culture.

Won (ExpNeurobiol 2020, see below) presented a low cost, rapid alternative RT PCR protocol for COVID-19 diagnosis, composed of specimen self-collection by the patient via pharyngeal swab, Trizol-based RNA purification, and SYBR Green-based RT PCR.

Automated platforms
Pfefferle (Euro Surveill 2020, see below) evaluated the performance of a molecular assay for detection of SARS-CoV-2 on a high-throughput platform, the cobas 6800, using the 'open channel' for integration of a laboratory-developed assay. The authors observed good analytical performance in clinical specimens. The fully automated workflow enabled high-throughput testing with minimal hands-on time, while offering fast and reliable results.

Cepheid announced that it has received Emergency Use Authorization (EUA) from the U.S. FDA for Xpert® Xpress SARS-CoV-2, a rapid molecular diagnostic test for qualitative detection of SARS-CoV-2 (http://cepheid.mediaroom.com/2020-03-21-Cepheid-receives-emergency-use-authorization-from-FDA-for-Rapid-SARS-CoV-2-Test). The test has been designed to operate on any of Cepheid’s automated GeneXpert® Systems, with a detection time of approximately 45 minutes.

Validation data
Xie (Int J Inf Dis 2020, see below) compared nucleic acid amplification testing performed with 3 different fluorescent RT-PCR kits on different samples, including oropharyngeal swab, blood, urine and stool. Nine out of the 19 patients tested were found positive for SARS-CoV-2 using oropharyngeal swab samples, and the virus nucleic acid was also detected in eight of these nine patients using stool samples. None of positive results was identified in the blood and urine samples. Similar data were obtained with the 3 kits.

Of note, a lack of assay sensitivity was reported by Xie (Radiol 2020, see below), who described five patients with SARS-CoV-2 infection who had initial negative RT-PCR results in mouth swabs but typical imaging findings, including ground-glass opacity (5 patients) and/or mixed ground-glass opacity and mixed consolidation (2 patients). All patients were eventually confirmed with SARS-CoV-2 infection by repeated swab tests. Similar cases were reported by various authors:

- Huang (Radiol 2020, see below).
- Ai (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.13.20022673v1) illustrated the challenges of diagnosing COVID-19 and showed the limited sensitivity of RT-PCR.
- Winichakoon (J Clin Microb 2020, see below) reported a case of COVID-19 pneumonia diagnosed from bronchoalveolar lavage fluid in Thailand, who initially had negative tests from nasopharyngeal/oropharyngeal swabs.
- A publication by Wang, Kang et al. (J Med Vir 2020, see below) further illustrates the sensitivity limitation of current RT-PCR based diagnosis, previously reported by others. Although the paper does not provide details, the authors described a COVID-19 case not confirmed by SARS-CoV-2 RT-qPCR testing at the first three evaluations within three weeks, before bronchoalveolar lavage fluid was acquired and results from both RT-qPCR and next-generation sequencing (NGS) testing became positive for SARS-CoV-2.
- Ruan (Chin Med J 2020, see below) presented a case with negative RT PCR result until day 11 of disease onset.

Other molecular techniques
Other possible molecular-based detection techniques include for example, reverse transcription loop-mediated isothermal amplification (RT-LAMP), an RNA amplification technique that detects the N gene of MERS-CoV and the ORF1a gene. One-pot reverse transcription loop-mediated isothermal amplification (one-pot RT-LAMP) is the
optimized RT-LAMP, while RT-LAMP-VF is the deformation of RT-LAMP, which is the combination of reverse transcription loop-mediated isothermal amplification and vertical flow visualization strips. Both are used to detect the N gene of MERS-CoV, making detection easier, faster, more efficient and highly specific. Besides these three methods, reverse transcription recombinase polymerase amplification assay (RT-PRA) is also used to identify MERS-CoV.

Lamb (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.19.20025155v1) demonstrated the feasibility of rapid screening diagnosis completed in under 30 minutes, using Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP). No validation data have been presented yet. Only simulated patient samples were used, which were created by spiking serum, urine, saliva, oropharyngeal swabs, and nasopharyngeal swabs with a portion of the COVID-19 nucleic sequence.

Yu (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.20.20025874v1) developed an isothermal LAMP based method for COVID-19, amplifying a fragment of the ORF1ab gene. The assay detected synthesized RNA equivalent to 10 copies of virus. Reaction time varied from 15-40 minutes, depending on the loading of virus in the collected samples. 42/43 patient samples initially diagnosed with RTqPCR showed consistent signal after 40 min incubation with the new assay (97.6% sensitivity).

Hou (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.22.20025460v1) reported the development of an isothermal, CRISPR-based diagnostic. The assay demonstrated a near single-copy sensitivity. It was evaluated on 61 specimen with suspected infection (52 positives) and showed great clinical sensitivity with a shorter turn-around time (40 min) than RT-PCR. Proof-of-principle of another CRISPR-based detection method was also described by Curti (on BioRxiv: https://www.biorxiv.org/content/10.1101/2020.02.29.971127v1).

Guan (Chin Med J 2020, see below) reported a case with inconsistent fluorescence quantitative-PCR results, for which high-throughput sequencing was used to make a further diagnosis of SARS-CoV-2 infection. Although high-throughput sequencing appears too costly and labour-intensive for routine diagnosis, the authors believe that it can be used for further diagnosis of COVID-19 patients with unclear PCR results under the condition of strict operation and quality control.

Serological methods

Maohua Li (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.20.20025999v2) presented the development of polyclonal and monoclonal antibodies generated by immunizing animals with synthetic peptides corresponding to different areas of the Nucleoprotein (N) of SARS-CoV-2.

A SARS-CoV-2 IgM and IgG ELISA has been reported by Zhang (Em Micr Inf 2020, see below). The assay is based on recombinant NP. A preliminary evaluation was conducted in 16 patients (incl. 3 patients with severe disease). As shown on Figure 8, an increase of specific antibodies was seen in part of the patients on day 5.
Xiang (manuscript on MedRxiv [https://www.medrxiv.org/content/10.1101/2020.02.27.20028787v1]) reported the evaluation of 2 serological assays: an IgG and IgM ELISA and a colloidal gold-immunochromatographic assay kit for detection of COVID-19. Using 63 samples for the ELISA and 91 plasma samples for the colloidal gold-immunochromatographic assay, they found a sensitivity of the combined ELISA IgM and ELISA IgG of 55/63 (87.3%), and that of the colloidal gold-immunochromatographic IgM and IgG assay of 75/91 (82.4%). Both methods displayed a specificity of 100%.

**Rapid test for antibody detection**

Li, Yi et al. (J Med Vir 2020, see below) reported the development of a rapid and simple point-of-care lateral flow SARS-CoV-2 immunoassay which can detect IgM and IgG antibodies simultaneously in human blood within 15 minutes. The clinical detection sensitivity and specificity of this test were measured using blood samples collected from 397 PCR-confirmed COVID-19 patients and 128 negative patients at 8 different clinical sites. The overall testing sensitivity reached 88.66% and specificity 90.63%. The assay was evaluated on fingerstick blood samples, as well as serum and plasma from venous blood.

**Rapid test for antigen detection**

According to the company website, Bioeasy (Shenzen, China) has developed 2 different rapid tests for SARS-CoV-2 antigen detection: a Fluorescence test and a GICA colloidal gold enhanced Rapid Test for the qualitative detection of SARS-CoV-2 antigen (http://en.bioeasy.com/product?kind=milk).

**Chest CT for COVID-19 detection**

Fang (Radiol 2020, see below) reported that in a series of 81 patients, the sensitivity of chest CT was found greater than that of RT-PCR (98% vs 71%, respectively, p<.001). Subjects with initial negative RT-PCR became positive upon retest 1 to 7 days later. Possible reasons for the low efficiency of viral nucleic acid detection may include: 1) immature development of nucleic acid detection technology; 2) variation in detection rate from different manufacturers; 3) low patient viral load; or 4) improper clinical sampling. These results provided first evidence of a possible role for chest CT for screening patients with clinical and epidemiologic features compatible with COVID-19 particularly when RT-PCR testing is negative.

Ai (Radiol 2020, see below) reported a large study further supporting the diagnostic value of chest CT. Of 1014 patients included in the study, 59% had positive RT-PCR results, and 88% had positive chest CT scans. The sensitivity of chest CT in suggesting COVID-19 was 97% based on positive RT-PCR results. In patients with negative RT-PCR results, 75% (308/413) had positive chest CT findings; among them, 48% were considered as highly likely cases, 33% as probable...
cases. The mean interval time between the initial negative to positive RT-PCR results was 5.1 ± 1.5 days. Moreover, 60% to 93% of cases had initial positive CT consistent with COVID-19 prior (or parallel) to the initial positive RT-PCR results. Interestingly, 42% (24/57) cases showed improvement in follow-up chest CT scans before the RT-PCR results turning negative.

**Deep-learning analysis methods**

Wang (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.14.20023028v2) suggested the application of Artificial Intelligence's deep learning methods to extract COVID-19's specific graphical features from radiographical changes in CT images. The internal validation of the new method achieved a total accuracy of 82.9% with specificity of 80.5% and sensitivity of 84%. The external testing dataset showed a total accuracy of 73.1% with specificity of 67% and sensitivity of 74%. Subsequently, a similar deep learning approach developed by Xu (on ArXiv: https://arxiv.org/abs/2002.09334) was reported to yield an overall diagnostic accuracy of 86.7%.

Chen (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.25.20021568v1) even suggested a better performance of this approach. His model achieved a per-patient sensitivity of 100%, specificity of 93.55%, accuracy of 95.24%, PPV of 84.62%, and NPV of 100%; a per-image sensitivity of 94.34%, specificity of 99.16%, accuracy of 98.85%, PPV of 88.37%, and NPV of 99.61% in a retrospective dataset. For 27 prospective patients, the model achieved a comparable performance to that of an expert radiologist with much shorter reading time (41.34s [IQR 39.76-44.48] vs. 115.50s [IQR 85.69-118.17] per patient).

A manuscript by Xu (on ArXiv: https://arxiv.org/abs/2002.09334) confirmed previous publications suggesting that artificial intelligence deep learning applied to the analysis of CT scans might be the basis of a novel diagnostic approach for COVID-19. The models developed in this study were reported to yield an overall diagnostic accuracy of 86.7%.

**Combination of chest CT and RT-PCR**

A publication in Lancet (Shi Lancet Inf Dis 2020, see below), which presents clinical imaging data from a large cohort of 81 patients, also states that combining imaging assessments with clinical and laboratory findings could help identify SARS-CoV-2 infections early. A similar conclusion was reached by Ren (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.25.20027755v2) based on 87 confirmed COVID-19 cases and 481 exclusion cases. Combination of RT-PCR and CT had higher sensitivity (91.9%) than RT-PCR alone (78.2%) or CT alone (66.7%) or combination of two RT-PCR tests (86.2%).

**Virus isolation**

The first SARS-CoV-2 was successfully isolated by inoculating human airway epithelial cells with bronchoalveolar-lavage fluid samples from a patient with pneumonia (Zhu NEJM 2020, see below). Since human airway epithelial cells (because of their resemblance to pseudostratified mucociliary epithelium) require 4-6 weeks to differentiate in vivo, isolation of SARS-CoV-2 using Vero cells or Caco-II cells is more convenient. Kim (Osong Public Health Res Perspect 2020, see below) showed virus replication in Vero cells, with cytopathic effects observed. The author indicated that further studies are needed to select more sensitive cell lines suitable for virus isolation from low viral load samples. Harcourt (Em Inf Dis 2020, see below) presented data showing that the virus replicates to high titer in Vero-CCL81 cells and Vero E6 cells in the absence of trypsin.

Matsuyama (PNAS 2020, see below) showed that a TMPRSS2-expressing Vero E6 cell line is highly susceptible to SARS-CoV-2 infection, making it useful for isolating and propagating the virus.

**Alternative diagnostic methods**

Prevention and control strategies

Tanne (BMJ 2020, see below) provided a rapid overview of how selected countries (US, Canada, Australia, India, Japan, South Korea, Italy, Spain, France, Germany, Iran) are tackling the epidemic in March 2020.

Disease surveillance guidelines

WHO issued guidance on implementation of global surveillance of COVID-19 by Member States (https://www.who.int/publications-detail/global-surveillance-for-human-infection-with-novel-coronavirus-(2019-ncov)). The objectives of this global surveillance are to monitor trends of the disease where human to human and/or zoonotic transmission occurs; rapidly detect new cases in countries where the virus is not circulating; provide epidemiological information to conduct risk assessment at the national, regional and global level; and provide epidemiological information to guide response measures.

Recommendations for follow-up of contacts
A contact is a person involved in any of the following:
- Providing direct care for COVID-19 patients, working with HCWs infected with novel coronavirus, visiting patients or staying in the same close environment of a COVID-19 patient.
- Working together in close proximity or sharing the same classroom environment a with COVID-19 patient
- Traveling together with COVID-19 patient in any kind of conveyance
- Living in the same household as a COVID-19 patient within a 14-day period after the onset of symptoms in the case under consideration.

Monitoring of contacts of probable and confirmed cases is recommended as follows:
- Contacts should be monitored for 14 days from the last unprotected contact.
- Contacts should self-limit travel and movements. Monitoring by public health authorities can be done through household or virtual visits or by telephone to check for symptoms.
- Any contact who becomes ill and meets the case definition becomes a suspect case and should be tested.
- Any newly identified probable or confirmed cases should have their own contacts identified and monitored.

Recommendations for laboratory testing
Any suspected case should be tested. However, depending on the intensity of the transmission, the number of cases and the laboratory capacity, only a randomly selected sample of the suspect cases may be tested. If resources allow, testing may be done more broadly (for instance through sentinel surveillance) to better assess the full extent of the circulation of the virus. Based on clinical judgment, clinicians may opt to order a test in a patient not strictly meeting the case definition, such as for a cluster of acute respiratory illness among HCWs.

Recommendations for reporting surveillance data to WHO
WHO requests that national authorities report probable and confirmed cases of novel coronavirus infection within 24 hours of identification, by providing the minimum data set outlined in the “Interim case reporting form for 2019 Novel Coronavirus of confirmed and probable cases”. For countries with extensive importation or human-to-human transmission, daily aggregated data are requested, with reporting of the number of new confirmed and probable cases and deaths by first administrative level (e.g. region, province, state, municipalities) if possible.

Of note, in a press conference on Feb 4, WHO mentioned they only received complete information about 38% of the cases reported outside of China (https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-technical-briefing-on-2019-novel-coronavirus).

Public health response in China
As of 26 January 2020, in China, 30 provinces initiated a level-1 public health response to control COVID-19. As described by Deng (J ClinMed 2020, see below), level-1 response means that during the occurrence of a particularly
serious public health emergency, the provincial headquarters shall organize and coordinate the emergency response work within its administrative area according to the decision deployment and unified command of the State Council. Fever observation rooms were to be set up at stations, airports, ports, and so on to detect the body temperature of passengers entering and leaving the area and implement observation/registration for the suspicious patients. The government under its jurisdiction, in accordance with the law, is to take compulsory measures to restrict all kinds of the congregation, and ensure the supply of living resources. They also ensure the sufficient supply of masks, disinfectants, and other protective articles on the market, and standardize the market order. The strengthening of public health surveillance, hygiene knowledge publicity, and monitoring of public places and key groups is required. Comprehensive medical institutions and some specialized hospitals are to be prepared to accept COVID-19 patients to ensure that severe and critical cases can be differentiated, diagnosed, and effectively treated in time. The health administration departments, public health departments, and medical institutions at all (province, city, county, district, township, and street) levels, and social organizations function in epidemic prevention and control and provide guidance for patients and close contact families for disease prevention.

Chen, Yang et al. (Lancet 2020, see below) also underlined the importance of the social distancing measures that were applied during the Chinese Lunar New Year holiday in China. People in China are indeed estimated to make close to 3 billion trips over the 40-day travel period, or Chunyun, of the Lunar New Year holiday. As part of these social distancing policies, the Chinese Government encouraged people to stay at home; discouraged mass gatherings; cancelled or postponed large public events; and closed schools, universities, government offices, libraries, museums, and factories. Only limited segments of urban public transport systems remained operational and all cross-province bus routes were taken out of service. As a result of these policies and public information and education campaigns, Chinese citizens started to take measures to protect themselves against COVID-19, such as staying at home as far as possible, limiting social contacts, and wearing protective masks when they needed to move in public. The Chinese Government even extended the Lunar New Year holiday, so that the duration of the holiday would be sufficiently long to fully cover the suspected incubation period of COVID-19.

**Public health response in other countries**

When COVID-19 cases started to appear outside China, affected countries implemented a variety of measures. The first of them consisted of contact tracing and recommending a set of precautions. In the United States, on January 20, state and local health departments, in collaboration with teams deployed from CDC, began identifying and monitoring all persons considered to have had close contact with patients with confirmed COVID-19 (Burke MMWR Morb Mortal Wkly Rep 2020, see below). The aims of these efforts were to ensure rapid evaluation and care of patients, limit further transmission, and better understand risk factors for transmission.

Legido-Quigley (Lancet 2020, see below) analysed the response in Hong Kong, Singapore and Japan. The three locations introduced appropriate containment measures and governance structures; took steps to support health-care delivery and financing; and developed and implemented plans and management structures. However, their response is vulnerable to shortcomings in the coordination of services; access to adequate medical supplies and equipment; adequacy of risk communication; and public trust in government. Moreover, it is uncertain whether these systems will continue to function if the requirement for services surges. Three important lessons have emerged. The first is that integration of services in the health system and across other sectors amplifies the ability to absorb and adapt to shock. The second is that the spread of fake news and misinformation constitutes a major unresolved challenge. Finally, the trust of patients, health-care professionals, and society as a whole in government is of paramount importance for meeting health crises.

The response of Singapore to contain the epidemic was also described in a publication by Lee (J Trav Med 2020, see below).

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Since January 30, the Italian Government has implemented extraordinary measures to restrict viral spread, including interruptions of air traffic from China, organised repatriation flights and quarantines for Italian travellers in China, and strict controls at international airports’ arrival terminals (Spina Lancet 2020, see below). Local medical authorities adopted specific WHO recommendations to identify and isolate suspected cases of COVID-19. Such recommendations were addressed to patients presenting with respiratory symptoms and who had travelled to an endemic area in the previous 14 days or who had worked in the health-care sector, having been in close contact with patients with severe respiratory disease with unknown aetiology. Suspected cases were transferred to preselected hospital facilities where the SARS-CoV-2 test was available and infectious disease units were ready for isolation of confirmed cases. Since the first case of SARS-CoV-2 local transmission was confirmed, the EMS in the Lombardy region (reached by dialling 112, the European emergency number) represented the first response to handling suspected symptomatic patients, to adopting containment measures, and to addressing population concerns. The EMS of the metropolitan area of Milan instituted a COVID-19 Response Team of dedicated and highly qualified personnel, with the ultimate goal of tackling the viral outbreak without burdening ordinary EMS activity. More details on the consequences of the COVID-19 outbreak on critical care capacity were provided by Grasselli (JAMA 2020, see below).

Johnson (Euro Surv 2020, https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.9.2000202) characterised three sequential scenarios for the spread of SARS-CoV-2 in the EU/EEA, with the third scenario divided in two sub-scenarios based on impact on the healthcare system (Figure 9). The scenarios are: (1) short, sporadic chains of transmission, (2) localised sustained transmission, (3a) widespread sustained transmission with increasing pressure on the healthcare system and (3b) widespread sustained transmission with overburdened healthcare system. These scenarios were presented together with suggested control measures to limit the impact of the epidemic. At different points in time, it was expected that different countries may find themselves in different scenarios.

Figure 9 Scenarios for the potential spread and impact of COVID-19 in the EU/EEA, with suggested actions for containment and mitigation, March 2020 (from Johnson Euro Surv 2020)

1. Short, sporadic chains of transmission

   **Description:**
   - Limited number of distinct introductions
   - Transmission limited to clusters with known epidemic links
   - Up to two generations of transmission within the EU/EEA in all but a couple of clusters

   **Suggested control measures:**
   - Conduct active case finding, including contact tracing
   - Isolate cases
   - Manage contacts following recommendations
   - Evaluate suitability of influenza surveillance systems for COVID-19 surveillance
   - Review pandemic preparedness plan
   - Identify alternative supply chains for personal protective equipment and other healthcare consumables
   - Consider stockpiling
   - Ensure regular, transparent risk communication

2. Localised sustained transmission

   **Description:**
   - Increasing number of importations
   - Majority of European cases attributable to local transmission rather than importation
   - Three or more generations of transmission in at least three distinct, well defined, clusters within a country

   **Additional control measures:**
   - Activate pandemic preparedness plan
   - Ensure stakeholders are prepared to activate healthcare system surge capacity
   - Assess the risk related to mass gatherings
   - Review and update contingency plan
   - Explain and justify to the public any changes in the public health measures applied
   - Monitor public perceptions

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Screening approaches

Thailand has 58 international flights connecting with Wuhan (Sriwijitalai Int J Prev Med 2020, see below). Active screening at the airport (by body temperature scanning and clinical history taking) has been done to identify possible infected cases. In the first month of the epidemic, active screening identified 12 cases with positive result. However, the final diagnosis by molecular diagnostic tests could identify only one case with SARS-CoV-2 infection, which was the first case report of infection outside China. The other 11 cases were infected with influenza virus.

As reported by Ge (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.20.20025973v1), symptom-based mass screening and testing intervention (MSTI) can identify a large fraction of infected individuals during an infectious disease outbreak. China is currently using this strategy for the COVID-19 outbreak. The authors noted that this might lead to increased transmission if not properly implemented. The outcome of a modelling study suggested that the approach can be useful if the probability of transmission at testing sites is less than the probability that a symptomatic person is infected with SARS-CoV-2. This type of data is important to generate, as it may support recommendations such as for instance the use of dedicated testing sites separate from the usual healthcare facilities.

Gostic (Elife 2020, see below) estimated the impact of different screening programs given current knowledge of key COVID-19 life history and epidemiological parameters. Even under best-case assumptions, the authors predicted that screening will miss more than half of infected people. Most cases missed by screening are fundamentally undetectable, because they have not yet developed symptoms and are unaware that they were exposed.

Rao (Infect Control Hosp Epidemiol 2020, see below) proposed to use machine learning algorithms to help improve possible COVID-19 case identifications using a mobile phone-based web survey capturing with the most common manifestations of disease, along with basic travel history.

For safe and efficient screening for COVID-19, drive-through screening centres have been designed and implemented in South Korea. Kwon (J Korean Med Sci 2020, see below) presented the overall concept, advantages, and limitations of these screening centres. The steps of the drive-through centres include registration, examination, specimen collection, and instructions (Figure 10). The entire service takes about 10 minutes for one testee without leaving his
or her cars. Increased testing capacity over 100 tests per day and prevention of cross-infection between testees in the waiting space are the major advantages, while protection of staff from the outdoor atmosphere is challenging.

Figure 10 Illustration of drive-through COVID-19 screening centre provided for the public in South Korea (from Kwon J Korean Med Sci 2020)

Contact tracing
When several unknown epidemiological and clinical characteristics of the disease remain and an effective medical intervention is lacking (as in the case of COVID-19), contact management becomes one of the core strategies to minimize additional transmission. Among the first 10 patients with travel-related confirmed COVID-19 reported in the United States, a total of 445 persons (range = 1-201 persons per case) who had close contact with one of the 10 patients on or after the date of the patient’s symptom onset were identified (Burke MMWR Morb Mortal Wkly Rep 2020, see below). 222 (50%) were health care personnel. Active symptom monitoring of the 445 close contacts, consisting of daily telephone, text, or in-person inquiries about fever or other symptoms for 14 days following the last known exposure to a person with confirmed COVID-19, was conducted by local health jurisdictions.

Traditional investigative methods, depending on the patient or proxy interview, has the limitation of omissions and errors associated with recalling previous activities (COVID-19 National Emergency Response Center Osong Public Health Res Perspect 2020, see below). In South Korea for instance, the methods used to overcome recall and confirmation biases that can occur while determining the location of the contact include checking medical facilities records, phone-based global positioning system (GPS), card transaction records, and closed-circuit television (CCTV).

Containment measures
China has taken draconian measures to contain the outbreak, including the quarantine of at least 30 million residents of Wuhan and neighbouring cities (Kickbusch British Med J 2020, see below). Countrywide interventions include delaying resumption of school after the spring festival holiday, encouraging citizens to work from home and stay at home, using personal protective equipment such as face masks, and cancelling all mass gatherings. Vehicular traffic in Wuhan was banned. Authorities closed public transit and cancelled outbound transportation (air, train, and long-haul buses). China also imposed a ban on overseas travel with tour groups and suspended sale of flight and hotel packages. Authorities cancelled Lunar New Year gatherings in Beijing as well as intraprovince bus service into the nation’s capital. China’s Finance Ministry announced ¥1 billion (U.S. $145 million) to fund the response as well as the rapid construction of 2 hospitals in Wuhan to treat those affected (Phelan JAMA 2020, see below).
Most districts of Hangzhou announced in a statement that every community would be kept under closed management, and only one family member was allowed to leave his house and buy daily living supplies outdoors every two days (Diao Infect Control Hosp Epidemiol 2020, see below). Furthermore, "non-contact delivery", a new delivery method, was adopted by many express delivery companies, which could reduce contagion risk. Fourth, in order to reduce the concentration of personnel to avoid the risk of cross-infection, online working and network teaching were encouraged for workers and students, respectively, which were supported by mobile technology companies. Fifth, to meet the need of resumption of production and curb the transmission of the virus as far as possible, Hangzhou arranged chartered transportation to help numbers of migrants return to workplaces. Lastly, in cooperation with Alipay, Hangzhou adopted the health QR code system on February 11, 2020, which were designated by green, yellow or red. People who wanted to get into Hangzhou needed to submit their travel history and health information online in advance. Residents with a green code indicated they had a low current risk of being infected, while residents with yellow or red codes were quarantined for seven or fourteen days and required to report their health condition every day to exclude infection before the codes turned green.

The Hong Kong Special Administrative Region declared its highest-tier emergency, curtailed public events, and barred travellers from Hubei Province. Travelers from mainland China must complete health declarations. Hong Kong has also closed schools and universities at least until mid-February.

Governments have not yet felt the need to ban travel from China, with 2 exceptions: North Korea has prohibited entry of all Chinese travellers and Kyrgyzstan has closed its border with China. During previous outbreaks like SARS and Ebola, governments curtailed travel and trade, so future directives seem reasonably foreseeable. Multiple countries (e.g., Australia, Thailand, South Korea, Japan, India, Italy, Singapore, Malaysia, and Nigeria) have commenced temperature screening, symptom screening, and/or questionnaires for arriving passengers from China. The U.S. Centers for Disease Control and Prevention launched enhanced, non-invasive screening of travellers from Wuhan at 20 major airports, while the U.S. State Department issued its highest-level travel advisory for Hubei Province: level 4, “do not travel.” The State Department now advises that people should “reconsider travel” for all of mainland China.

A manuscript by Tian (on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.01.30.20019844v3) provides a preliminary evaluation of the efficacy of control measures implemented in China. The Wuhan city travel ban is considered to have slowed the dispersal of infection to other cities by an estimated 2.91 days (95% CI, 2.54-3.29) on average. Among the other urban centres across mainland China, cities that implemented control measures preemptively, before the first case was reported, had 37% fewer cases in the week following the first reported case (13.0, 95%CI, 7.1-18.8) compared with cities starting control after the first case (20.6, 95%CI, 14.5-26.8). Among individual control measures investigated, the most effective were suspending intra-city public transport, and closing entertainment venues and banning public gatherings.

**A role for telemedicine**

Zhai (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.02.20.20025957v1) described the Emergency Telemedicine Consultation System (ETCS), a telemedicine-enabled outbreak alert and response network, established by the National Telemedicine Center of China in Zhengzhou. ETCS was built upon a doctor-to-doctor (D2D) approach, in which health services can be accessed remotely through terminals across hospitals. The system architecture of ETCS comprises three major architectural layers: (1) telemedicine service platform layer, (2) telemedicine cloud layer, and (3) telemedicine service application layer. The ETCS has demonstrated substantial benefits in terms of the effectiveness of consultations and remote patient monitoring, multidisciplinary care, and prevention education and training.

Hollander (NEJM 2020, see below) presented the benefits that can be expected from telemedicine. Direct-to-consumer (or on-demand) telemedicine allows patients to be efficiently screened. It is both patient-centered and conducive to self-quarantine, and it protects patients, clinicians, and the community from exposure. It can allow...
physicians and patients to communicate 24/7, using smartphones or webcam-enabled computers. Health care providers can easily obtain detailed travel and exposure histories. Automated screening algorithms can be built into the intake process, and local epidemiologic information can be used to standardize screening and practice patterns across providers. Interestingly, more than 50 U.S. health systems already have such programs, and systems lacking such programs can outsource similar services. However, the authors also identified the numerous challenges to be faced (incl. reimbursement) before such approach can be used in the management of COVID-19 in the U.S.

Greenhalgh (BMJ 2020, see below) reported that video could be useful for people with heightened anxiety (for whom a video consultation may be more reassuring than a phone call), those with mild symptoms suggestive of coronavirus (for which visual cues may be useful), and those with more severe symptoms (when a video consultation may reduce the need to visit a potentially contagious patient). Well patients seeking general advice could be directed to a website or recorded phone message. Moreover, there may be a trade-off between staying at home and coming to clinic for a full examination—for example, in frail older patients or immunosuppressed patients.

Specific disease management guidelines

Pregnancy and delivery

Guidance regarding manner of delivery:

- The U.S. CDC states that COVID-19 infection is not an indication for delivery and states that vaginal delivery can be pursued in the event of spontaneous labour and good maternal condition;
- Effective implementation of protection measures during delivery, such as negative-pressure delivery room and shortening of the second stage, may help prevent the infant from acquiring COVID-19;
- In the event of caesarean section, measures should be undertaken to protect the medical staff (see below)

Guidance regarding delayed cord clamping:

- Consensus guidance from China: ‘delayed cord clamping is not recommended’ in order to reduce the risk of vertical transmission (Wang Ann Transl Med 2020, see below)
- RCOG guidance does not concur, advising that delayed cord clamping should be practiced as normal. If vaginal delivery is permitted, with exposure to maternal secretions and blood, it could be argued that 1 min of further perfusion via the placenta is unlikely to alter the risk of vertical transmission.

Guidance regarding routine separation of infants from mothers affected by COVID-19:

- Consensus guidance from China recommends routine separation of neonates from mothers infected by COVID-19 (Wang Ann Transl Med 2020, see below)
- Experts from Switzerland, The United States and France also recommended this approach (Favre Lancet Infect Dis 2020, see below)
- Guidance from the U.S. CDC is less clear but is still precautionary
- In the UK, the Royal College of Obstetricians and Gynaecologists advises against routine separation of mother and baby and gives guidance on individualized care (Mullins Ultrasound Obstet Gynecol. 2020, see below)

Care of children

Li (Ann Pall Med 2020, see below) noted that health care providers are in urgent need of a guideline to assist the diagnosis and management of SARS-COV-2 infected children. The authors aim to develop a rapid advice guideline using a multidisciplinary and collaborative approach. The guideline will follow the methods for developing WHO rapid advice guidelines.

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**Novel technologies**

Kamel Boulou (Int J Health Geogr 2020, see below) described a range of online/mobile geographic information systems (GIS) and applications for tracking the coronavirus epidemic and associated events as they unfold around the world. Some of these dashboards and applications are receiving data updates in near-real-time (at the time of writing). One of them is meant for individual users (in China) to check if the app user has had any close contact with a person confirmed or suspected to have been infected with SARS-CoV-2 in the recent past.

**Infection control in health care settings**

As reported by Wang (J Hosp Inf 2020, see below), by 24th February, the National Health Commission of the People’s Republic of China reported in a press conference of WHO-China Joint Mission on COVID-19 that 3 387 healthcare workers had been confirmed with COVID-19, with 22 (0.6%) deaths. More than 90% of infected HCWs were from Hubei province. The director of National Hospital Infection Management and Quality Control Centre summarized some reasons for such high number of infected HCWs during emergency outbreak. These included inadequate personal protection of HCWs at the beginning of the epidemic; long-time exposure to large-scale of infected patients, which directly increased the risk of infection for HCWs; pressure of treatment, work intensity, and lacking of rest, which indirectly increased the probability of infection for HCWs; shortage of personal protective equipment (PPE); and inadequate training to infection prevention and control for front-line HCWs (except infectious disease physicians).

Guidance on infection prevention and control strategies for use when COVID-19 is suspected has been issued by WHO (https://www.who.int/publications-detail/infection-prevention-and-control-during-health-care-when-novel-coronavirus-(ncov)-infection-is-suspected-20200125). This guidance is intended for HCWs, healthcare managers and IPC teams at the facility level but it is also relevant for the national and district/provincial level. Additional recommendations are issued at country level, as done for instance in Canada (Wax Can J Anaesth 2020, see below).

**Personal protection of health-care workers**

Available epidemiological data show that not only can subclinical patients transmit the virus effectively but patients can also shed high amounts of the virus and infect others even after recovery from the acute illness. Chang (Lancet Resp Med 2020, see below) concluded that these findings warrant aggressive measures (such as N95 masks, goggles, and protective gowns) to ensure the safety of HCWs during this COVID-19 outbreak, as well as future outbreaks, especially in the initial stages where limited information about the transmission and infective potency of the virus is available.

A manuscript by Wang on MedRxiv supports the efficacy of N95 respirators, disinfection and hand washing to reduce risk of COVID-19 transmission among medical staff at Zhongnan Hospital of Wuhan University (https://www.medrxiv.org/content/10.1101/2020.02.18.20021881v1).

Of note, a medical expert, who visited Wuhan to investigate the COVID-19 outbreak, after returning to Beijing, initially exhibited conjunctivitis of the lower left eyelid before the appearance of catarrhal symptoms and fever. The individual tested positive for COVID-19, suggesting the virus tropism to non-respiratory mucosal surfaces, limiting the effectiveness of face masks (Chang Lancet Resp Med 2020, see below).

Yan (Dermatol Ther 2020, see below) presented a consensus of Chinese experts on protective measures and advice on hand-cleaning- and medical-glove-related hand protection, mask- and goggles-related face protection, UV-related protection, eye protection, nasal and oral mucosa protection, outer ear and hair protection. The authors noted that insufficient and excessive protection will have adverse effects on the skin and mucous membrane barrier and that using moisturizing products is highly recommended to achieve better protection.

Zhao (J Cardiothorac Vasc Anesth 2020, see below) described more specifically the level 3 personal protective measures for healthcare workers to be used for emergency procedures in patients with confirmed or suspected SARS-
CoV-2 infection in China. They included hand disinfection, wearing a cap, a medical protective mask, goggles/face screens/eye protective surgical masks, isolation gowns/protective suits, shoe-covers and gloves.

Facilities
As of February 19th, the Chinese government converted 13 large-scale public places in Wuhan into makeshift hospitals for patients with COVID-19 with mild symptoms. Chen (J Hosp Inf 2020, see below) noted that insufficient ventilation in these makeshift hospitals may increase infection risk of opportunistic airborne transmission.

Patient flow and triage
An innovative approach was developed in the United Kingdom to stop unnecessary ambulance use and hospital visits, whereby people with suspected COVID-19 are being tested in their homes (Mahase BMJ 2020, see below). The community testing scheme started at the end of January at North West London NHS Trust and has now been implemented in other trusts. More than 130 patients have been reported to be tested in two weeks. Mahase (BMJ 2020, see below) subsequently indicated that in Wales, 90% of suspected cases are managed at home. Members of the public who call NHS or 111 and are assessed as a possible case, are evaluated for their suitability for home testing on the basis of their self-reported health status and their ability to self-isolate at home. Public Health Wales’s microbiology team then coordinates with the relevant health board community testing teams to arrange home testing within 12-36 hours.

Safety of procedures
Wong (Can J Anaesth 2020, see below) described the outbreak response measures of the anaesthetic department of 2 hospitals in Singapore. These included engineering controls such as identification and preparation of an isolation operating room, administrative measures such as modification of workflow and processes, introduction of personal protective equipment for staff, and formulation of clinical guidelines for anaesthetic management.

Aerosol-generating procedures, such as non-invasive ventilation (NIV), high-flow nasal cannula (HFNC), bag-mask ventilation, and intubation are of particularly high risk when dealing with COVID-19 patients. Cheung described the approach developed by a local intensive care unit in a Hong Kong hospital to managing the risks to health-care staff, while maintaining optimal and high-quality care. They do not recommend using NIV or HFNC until the patient is cleared of COVID-19. Airway devices providing 6 L/min or more of oxygen are considered high-flow and they discourage their use if an airborne infection isolation room is unavailable. They recommend that endotracheal intubation is done by an expert specialised in the procedure, and early intubation considered in a patient with deteriorating respiratory condition. They recommend avoiding bag mask ventilation for as long as possible; and optimising preoxygenation with non-aerosol-generating means. Methods include the bed-up-head-elevated position, airway manoeuvres, use of a positive end expiratory pressure valve, and airway adjuncts.

Zuo (Chin Med Sci J. 2020, see below) noted that endotracheal intubation may put the anaesthesiologists at high risk of nosocomial infection. In fact, SARS-CoV-2 infection of anaesthesiologists after endotracheal intubation for confirmed COVID-19 patients have been reported in hospitals in Wuhan. The expert panel of airway management in Chinese Society of Anaesthesiaology drafted a recommendation to guide the performance of endotracheal intubation by frontline anaesthesiologists and critical care physicians.

Zhang (Anesthesiology 2020, see below) also reported that the Wuhan Union Hospital’s Department of Anaesthesiology drafted the “Perioperative Care Provider’s Considerations in Managing Patients with COVID-19” and carried out 45 surgical procedures on such patients. An upgraded surgical safety checklist for patients with suspected or confirmed COVID-19 was drawn up and implemented, along with infection-control guidelines for the care of such patients. Task forces dedicated to procedure standardization, infection control, and staff scheduling within anaesthesia were quickly assembled in most hospitals across the country. Monitoring was implemented to ensure that anaesthesia providers wore and removed personal protective equipment before working in the perioperative

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Paper version: dd. 23 MAR 2020 Transdisciplinary Insights - Living Paper
environment. Drills were held to ensure the optimal management of emergencies, with mandatory multidisciplinary participation across anaesthesia, surgery, critical care, paediatrics, and obstetrics and gynaecology.

Of note, another report noted that during pandemics the number of intensive care unit beds for mechanical ventilation through tracheal intubation could rapidly become insufficient. Therefore, non-invasive ventilation could be required outside the intensive care unit. To increase safety during NIV, use of a helmet has been suggested by Cabrini (Lancet 2020, https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30359-7/fulltext).

In order to limit the risk of nosocomial transmission, Chen, Tian et al. (Lancet Inf Dis 2020, see below) reported the use of an innovative infection-control system in a Guangdong hospital, called the "observing system", whereby cameras cover the negative pressure isolation ward and infection control observers monitor medical staff and provide assistance in real time via computer monitors. The main responsibilities of these infection control observers are to maintain the normal operation of the negative pressure isolation wards, supervise the implementation of disinfection, ensure a sufficient supply of protective materials, arrange specimens for inspection, and relieve anxiety of the medical personnel while treating patients.

Guidelines
A rapid advice guideline suitable for the first frontline doctors and nurses, managers of hospitals and healthcare sections, as well as community residents or public health persons has been made available by Jin (Mil Med Res 2020, see below). This guideline covers disease screening and population prevention, diagnosis, treatment and control (including traditional Chinese Medicine), nosocomial infection prevention and control, and disease nursing of the 2019-nCoV.

Availability of medical supplies
A comment by Wang (Biosci Trends 2020, see below) addressed the importance of medical supplies availability. As the pandemic developed in China, a serious dearth of emergency medical supplies emerged, and especially an extreme shortage of personal protective equipment such as masks and medical protective clothing. This is considered as one of the major factors affecting the progress of epidemic prevention and control.

Modelling studies
A huge number of modelling studies has been reported. These studies aimed at characterizing the epidemiology of the disease in various countries, but also predicting the impact of various public health measures. The publications can be found at https://github.com/midas-network/COVID-19/wiki/Documents#estimate.
For instance:

- Gostic estimated the effectiveness of symptom and risk screening to prevent the spread of COVID-19 (Elife. 2020, see below)
- Anzai assessed the Impact of reduced travel on exportation dynamics of COVID-19 (J Clin Med 2020, see below)
- Pan (on MedRxiv https://www.medrxiv.org/content/10.1101/2020.02.19.20025387v3) described 2 mathematical models simulating the epidemic in Wuhan and other parts of China, taking into account the mobility of people. The data suggest that the peak of new asymptomatic cases per day in Wuhan occurred on February 6, and the peak of new symptomatic infections on February 3. The model predicts that COVID-19 cases will gradually wane by the end of April 2020, both in Wuhan and the other parts of China.
- Hellewell (Lancet Glob Health 2020, see below) used a mathematical model to assess if isolation and contact tracing are able to control onwards transmission from imported cases of COVID-19. The authors found that the probability of controlling an outbreak decreased with the number of initial cases, when R0 was 2.5 or 3.5 and with more transmission before symptom onset. Across different initial numbers of cases, the majority of scenarios with an R0 of 1.5 were controllable with less than 50% of contacts successfully traced. To control
the majority of outbreaks, for R0 of 2.5 more than 70% of contacts had to be traced, and for an R0 of 3.5 more than 90% of contacts had to be traced. The delay between symptom onset and isolation had the largest role in determining whether an outbreak was controllable when R0 was 1.5. For R0 values of 2.5 or 3.5, if there were 40 initial cases, contact tracing and isolation were only potentially feasible when less than 1% of transmission occurred before symptom onset.

- Chinazzi (Science 2020, see below) used a global metapopulation disease transmission model to project the impact of travel limitations on the national and international spread of the epidemic. The model was calibrated based on internationally reported cases, and shows that at the start of the travel ban from Wuhan on 23 January 2020, most Chinese cities had already received many infected travellers. The travel quarantine of Wuhan delayed the overall epidemic progression by only 3 to 5 days in Mainland China, but had a more marked effect at the international scale, where case importations were reduced by nearly 80% until mid-February. Modelling results also suggested that sustained 90% travel restrictions to and from Mainland China only modestly affected the epidemic trajectory unless combined with a 50% or higher reduction of transmission in the community.

- Wells (PNAS 2020, see below) estimated the impact of these control measures and investigated the role of the airport travel network on the global spread of the COVID-19 outbreak. Our results show that the daily risk of exporting at least a single SARS CoV-2 case from mainland China via international travel exceeded 95% on January 13, 2020. The authors found that 779 cases (95% CI: 632 to 967) would have been exported by February 15, 2020 without any border or travel restrictions and that the travel lockdowns enforced by the Chinese government averted 70.5% (95% CI: 68.8 to 72.0%) of these cases. In addition, during the first three and a half weeks of implementation, the travel restrictions decreased the daily rate of exportation by 81.3% (95% CI: 80.5 to 82.1%), on average.

- Based on the 199 first confirmed cases on the Diamond Princess cruise ship, Nishiura (J Clin Med 2020, see below) employed a back-calculation method to estimate the incidence of infection. Without the movement restriction policy imposed from 5 February, it was predicted that the cumulative incidence with and without close contact would have been as large as 1373 (95% CI: 570, 2176) and 766 (95% CI: 587, 946) cases, respectively, out of a total of 3711 persons (2666 passengers and 1045 crew members).

- Lau (J Trav Med 2020, see below) evaluated whether rigorous lockdown measures as implemented by China have the potential to slow down the virus’ spread. The authors reported a significant decrease in the growth rate of the epidemic. Moreover, a corresponding increase in the doubling time of COVID-19 cases within China was observed, from 2 days (95% Confidence Interval, CI): 1.9-2.6), to 4 days (95% CI: 3.5-4.3) after lockdown. However, the authors also noted that the number of cases outside lockdown areas have increased, and new epicenters are developing across the globe.

- Wang (manuscript on MedRxiv: https://www.medrxiv.org/content/10.1101/2020.03.03.20030593v1) divided the epidemic in China into four periods based on key events and interventions, compared epidemiological characteristics across periods and demographic groups, and developed a susceptible-exposed-infectious-recovered model to evaluate the impact of interventions. The authors found that the effective reproductive number dropped from 3.86 (95% credible interval 3.74 to 3.97) before interventions to 0.32 (0.28 to 0.37) post interventions. The interventions were estimated to prevent 94.5% (93.7 to 95.2%) infections till February 18. They noted that at least 59% of infected cases were unascertained in Wuhan, potentially including asymptomatic and mild-symptomatic cases.

- Liu (Biology 2020, see below) developed another mathematical model for the disease, which predictions emphasize the importance of major public health interventions such as isolation, quarantine, and public closings, to greatly reduce the final size of this epidemic, and make the turning point much earlier than without these measures.

- Karako (Biosci Trends 2020, see below) presented a stochastic transmission model by extending the Susceptible-Infected-Removed (SIR) epidemiological model with an additional modelling of the individual
action on whether to stay away from the crowded areas. The authors concluded that the infection spread in Japan would be gradually contained by reducing the time spent in the crowded zone to less than 4 hours.

In addition, a publication by Roy Anderson (Lancet 2020, see below) discussed the various unknowns that remain with regard to the epidemiology of the disease and the expected impact of various public health strategies. It provides a useful overview of the topic.

**Therapeutic interventions and research**

Identifying treatment options as soon as possible is critical for the response to the COVID-19 outbreak (Lu Biosci Trends 2020, see below). Various approaches, including evaluation of existing broad-spectrum antiviral drugs using standard assays, screening of a chemical library containing many existing compounds or databases, and the redevelopment of new specific drugs based on the genome and biophysical understanding of individual coronaviruses, can be used to identify potential therapies. Numerous candidates were proposed from the beginning of the epidemic, some of which were very soon administered to patients.

A list of candidate therapeutics has been published by WHO (https://www.who.int/blueprint/priority-diseases/key-action/overview-ncov-therapeutics.pdf?ua=1). The Chinese Academy of Sciences also suggested a list of 30 different compounds, with 12 HIV medicines, including Saquinavir, Indinavir, Lopinavir, ritonavir and Carfilzomib, two respiratory syncytial virus drugs, a schizophrenia medication and an immunosuppressant. Candidates also include certain Traditional Chinese Medicines. The efficacy and safety of these candidates for COVID-19 still need to be confirmed by robust clinical evaluations.

Subsequent publications on this topic presented additional lists of potential compounds. For instance, Li provided a longer list of anti-coronavirus agents, including preclinical compounds that could be considered for screening or starting points for optimizing antiviral agents (https://www.nature.com/articles/d41573-020-00016-0).

A manuscript by Yan (https://www.preprints.org/manuscript/202002.0254/v1) also indicated that in addition to synthetic compounds (including FDA-approved drugs), Chinese Patent Drugs (CPD) can also be a source of therapies against COVID-19. He compiled major components from 38 CPDs that are commonly used in the respiratory diseases and docked them against two drug targets, ACE2 receptor and viral main protease. Ten antiviral components, including hesperidin, saikosaponin, rutin, baicalin, glycyrrhizin, mulberroside A, puerarin, orientin, amygdalin, and ilexgenin A, were predicted as able to directly bind to both host cell target ACE2 receptor and viral target main protease, indicating their potential for treatment.

In *silico* work is still ongoing to identify potential drug candidates. Using network proximity analyses of drug targets and known human CoV-host interactions in the human protein-protein interactome, Zhou (Cell Discov 2020, see below) computationally identified 135 putative repurposable drugs for the potential prevention and treatment of human CoV infections. In addition, he prioritized 16 potential repurposable drugs (including melatonin, mercaptopurine, and sirolimus) that were further validated by enrichment analyses of drug-gene signatures and CoV-induced transcriptomics data in human cell lines. Finally, he presented three potential drug combinations (including sirolimus plus dactinomycin, mercaptopurine plus melatonin, and toremifene plus emodin) captured by the Complementary Exposure pattern: the targets of the drugs both hit the human CoV-host subnetwork, but target separate neighbourhoods in the human protein-protein interactome network.

As live SARS-CoV-2 handling requires high-level biosafety facilities, Fan (Chin Med J 2020, see below) suggested the use of a pangolin coronavirus model to facilitate *in vitro* studies of potential drug candidates against COVID-19. The drug candidates were screened for their ability to inhibit cytopathic effect upon GX_P2V/pangolin/2017/ Guangxi
strain infection of Vero E6 cells. The approach identified cepharanthine, selamectin and mefloquine hydrochloride as potential drugs.

Importantly, a publication in Nature (Maxmen 2020, see below) reported the launch of more than 80 clinical trials to test candidate coronavirus treatments.

Management of early symptoms
The French minister, Oliver Veran, tweeted on March 14th that people with suspected COVID-19 should avoid anti-inflammatory drugs. “Taking anti-inflammatory drugs (ibuprofen, cortisone . . .) could be an aggravating factor for the infection. If you have a fever, take paracetamol,” he said. His comments seem to have stemmed in part from remarks attributed to an infectious diseases doctor in southwest France (Day BMJ 2020, see below). She was reported to have cited four cases of young patients with covid-19 and no underlying health problems who went on to develop serious symptoms after using non-steroidal anti-inflammatory drugs (NSAIDs) in the early stage of their symptoms. Some experts in the UK backed this sentiment that for treating symptoms such as fever and sore throat, it seems sensible to stick to paracetamol as first choice. In parallel, in a press release yesterday, the EMA stated that there is currently no scientific evidence establishing a link between ibuprofen and worsening of COVID-19 (https://www.ema.europa.eu/en/news/ema-gives-advice-use-non-steroidal-anti-inflammatories-covid-19).

Antiviral drugs

Inhibitors of virus entry

Chloroquine, hydroxychloroquine and analogues
Chloroquine and its structural analogues such as hydroxychloroquine, amodiaquine, pamaquine, plasmoquine, primaquine, mefloquine or ferroquine, have been used for decades as the primary and most successful drugs against malaria (Al-Bari 2017). These drugs are also found to be effective against a wide variety of viral infections. Chloroquine is known to block virus infection by increasing endosomal pH required for virus/cell fusion, as well as interfering with the glycosylation of cellular receptors of SARS-CoV (Wang Cell Res 2020, see below). Besides its antiviral activity, chloroquine has an immune-modulating activity, which may synergistically enhance its antiviral effect in vivo. Chloroquine is widely distributed in the whole body, including lung, after oral administration. Devaux (Int J Antimicrob Ag 2020, see below) recently provided an overview of the possible mechanisms of chloroquine interference with SARS-CoV-2 replication.

Of note, several clinical studies of chloroquine and its analogues have been conducted for treatment of dengue, hepatitis C virus, chikungunya and HIV-1 infections. Disappointingly, the outcome of one of these clinical trials showed no benefit of chloroquine treatment of dengue virus infection (Tricou 2010). More recently, Garbern (2019) found a non-statistically significantly decreased risk of mortality in Ebola patients exposed to artesunate-amoquin during mass drug administrations as compared with Ebola patients not exposed to artesunate-amoquin.

Chloroquine was very recently found to potently inhibit infection of Vero E6 cells by a SARS-CoV-2 clinical isolate (EC50 = 1.13 μM; CC50 > 100 μM, SI > 88.50). The drug was shown to function at both entry, and at post-entry stages of the SARS-CoV-2 infection in Vero E6 cells. The EC90 value of chloroquine against SARS-CoV-2 was 6.90 μM, which can be clinically achievable as demonstrated in the plasma of rheumatoid arthritis patients who received 500 mg administration. The data therefore suggest that chloroquine, a cheap and safe drug, is potentially clinically applicable against COVID-19.

Subsequently, Yao (Clin Inf Dis 2020, see below) found hydroxychloroquine (EC50=0.72 μM) to be more potent than chloroquine (EC50=5.47 μM) in vitro. Based on physiologically-based pharmacokinetic models results, a loading dose of 400 mg twice daily of hydroxychloroquine sulfate given orally, followed by a maintenance dose of 200 mg given twice daily for 4 days would be recommended for SARS-CoV-2 infection, as it reached three times the potency of

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chloroquine phosphate when given 500 mg twice daily 5 days in advance. Similar results were reported by Liu (Cell Discov 2020, see below). At all conditions tested (MOI of 0.01, 0.02, 0.2, and 0.8), the EC50 for chloroquine (2.71, 3.81, 7.14, and 7.36 μM) was lower than that of hydroxychloroquine (4.51, 4.06, 17.31, and 12.96 μM). The differences in EC50 values were statistically significant at an MOI of 0.01 (P < 0.05) and MOI of 0.2 (P < 0.001).

A number of clinical trials have been quickly initiated in China to test the efficacy and safety of chloroquine or hydroxychloroquine in the treatment of COVID-19 associated pneumonia in more than 10 hospitals in Wuhan, Jingzhou, Guangzhou, Beijing, Shanghai, Chongqing, and Ningbo (https://www.nature.com/magazine-assets/d41573-020-00016-0/17663286). Thus far, results from more than 100 patients have been released by the China National Centre for Biotechnology Development and said to indicate that chloroquine phosphate is superior to control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus-negative conversion, and shortening the disease course, according to a news briefing (Gao BioSci Trends 2020, see below). Severe adverse reactions to chloroquine phosphate were not noted in the aforementioned patients.

Cortegiani (J Crt Care 2020, see below) identified 23 ongoing trials, all in China. The trials varied in study design, severity of the disease in the target population and in dosing and duration of the treatment. That so many such studies are being conducted in parallel suggests that that the scientific community is making a huge effort to clarify this question, but this effort is probably insufficiently coordinated. In support of this observation, the Chinese authorities have recently issued a directive to regulate and coordinate clinical trials studying potential pharmacological treatments for COVID-19. The results of these trials will be the first available on humans.

In addition, Gautret (International Journal of Antimicrobial Agents 2020: https://www.mediterranee-infection.com/wp-content/uploads/2020/03/Hydroxychloroquine_final.DOI_IJAA.pdf) reported the outcome of an open-label non-randomized clinical trial that evaluated hydroxychloroquine alone or combined with azithromycin compared to untreated patients from another centre and cases refusing the protocol. Twenty cases received treatment in this study and showed a significant reduction of the viral carriage at day 6 post inclusion compared to controls, and much lower average carrying duration than reported of untreated patients in the literature. Azithromycin added to hydroxychloroquine was significantly more efficient for virus elimination.

In order to guide and regulate the use of chloroquine in patients with novel coronavirus pneumonia, the multicenter collaboration group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for chloroquine in the treatment of novel coronavirus pneumonia recommended chloroquine phosphate tablet, 500mg twice per day for 10 days for patients diagnosed as mild, moderate and severe cases of novel coronavirus pneumonia and without contraindications to chloroquine (Zhonghua Jie He He Hu Xi Za Zhi 2020, see below).

The Dutch CDC suggested to treat severe infections requiring admission to the hospital and oxygen therapy or admitted to the ICU, with chloroquine (Cortegiani J Crt Care 2020, see below). However, the document also stated that treating patients only with optimal supportive care is still a reasonable option, due to lack of supportive evidence. The suggested regimen in adults consists of 600mg of chloroquine base followed by 300mg after 12 h on day 1, then 300mg × 2/die per os on days 2–5.

Another guideline document by the Italian Society of Infectious and Tropical disease (Lombardy section) recommends the use of chloroquine 500mg × 2/die or hydroxychloroquine 200mg die for 10 days, although the treatment may vary from 5 to 20 days according to clinical severity. The suggested target population ranged from patients with mild respiratory symptoms and comorbidities to patients with severe respiratory failure.

**Arbidol**

Arbidol (umifenovir), which is currently used as an antiviral in Russia and China, has been reported to have inhibitory effects on a diverse array of viruses. Studies aimed at determining the mechanism of action of arbidol implicate a
number of possible antiviral effects, including several steps of entry as well as later phases of the infectious cycle. According to a communication of the China National Center for Biotechnology Development at a press conference, the drug has been added to the list of possible treatments of COVID-19 in the sixth edition of the treatment and diagnosis plan published by the Chinese National Health Commission (https://www.thestar.com.my/news/regional/2020/02/18/chinese-experts-confirm-antimalarial-drug-is-effective-on-covid-19-infection).

Deng (J Inf 2020, see below) presented the results of a retrospective cohort study in 33 adults with laboratory-confirmed COVID-19 without invasive ventilation. The authors concluded that combined oral arbidol (at a dose of 200mg every 8 h) and lopinavir/ritonavir therapy was associated with a significant elevated negative conversion rate of COVID-19 RT-PCR at 7-day and 14-day, compared with lopinavir/ritonavir only. The combination therapy was also associated with a significantly improved the chest CT scans at the 7-day timepoint. However, data have to be interpreted with caution considering the non-randomized design of the study and its small size.

**Teicoplanin**

Teicoplanin, a glycopeptide antibiotic routinely used in the clinic to treat bacterial infection with low toxicity, had been previously reported to significantly inhibit the invasion of cells by Ebola virus, SARS-CoV and MERS-CoV, via specific inhibition of the activity of cathepsin L. The efficacy of teicoplanin against SARS-CoV-2 infection was recently tested: teicoplanin was found to potently prevent the entrance of S-HIV luc pseudoviruses into the cytoplasm, with an IC50 of 1.66 μM. Although the inhibitory effect upon replication of wildtype viruses ex vivo and in vivo remains to be determined, these preliminary result support a potential antiviral activity of teicoplanin (Zhang on BioRixv: https://www.biorxiv.org/content/10.1101/2020.02.05.935387v1).

**Nafamostat**

Nafamostat, an anticoagulant, is a potent inhibitor of MERS-CoV, preventing membrane fusion. The drug has been found inhibitive against SARS-CoV-2 in vitro infection (EC50 = 22.50 μM, CC50 > 100 μM, SI > 4.44) (Wang Cell Res 2020, see below).

**EK1**

Peptide OC43-HR2P, derived from the HR2 domain of human CoV OC43, has been shown to exhibit broad fusion inhibitory activity against multiple human CoVs. EK1, the optimized form of OC43-HR2P, showed substantially improved pan-CoV fusion inhibitory activity and pharmaceutical properties (Xia Sci Adv 2019, see below). Crystal structures indicated that EK1 can form a stable six-helix bundle structure with both short α-HCoV and long β-HCoV HR1s, further supporting the role of HR1 region as a viable pan-CoV target site. Intranasal application of EK1 peptide before or after viral challenge can protect human DPP4-transgenic mice from MERS-CoV infection (Jiang Em Micr Inf 2020, see below). The potential prophylactic and therapeutic efficacy of EK1 against SARS-CoV-2 infection remains to be evaluated.

**Niclosamide**

Niclosamide, an FDA-approved anthelminthic drug, was found to be effective against various viral infections with nanomolar to micromolar potency such as SARS-CoV, MERS-CoV, Zika virus, hepatitis C virus and human adenovirus, indicating its potential activity against SARS-CoV-2 (Xu ACS Infect Dis 2020, see below). However, experimental data with SARS-CoV-2 have not been reported yet.

**Baricitinib**

One of the known regulators of endocytosis is the AP2-associated protein kinase 1 (AAK1). Disruption of AAK1 might thus interrupt the passage of the virus into cells and also the intracellular assembly of virus particles. A high-affinity AAK1-binding drug is the janus kinase inhibitor baricitinib, which also binds the cyclin G-associated kinase, another regulator of endocytosis. Because the plasma concentration of baricitinib on therapeutic dosing (either as 2 mg or 4
mg once daily) is sufficient to inhibit AAK1, Richardson (Lancet 2020, see below) suggested it could be trialled, using an appropriate patient population with COVID-19 acute respiratory disease.

Using an Artificial Intelligence-derived knowledge graph, queried by a suite of algorithms, Stebbing (Lancet Inf Dis 2020, see below) also identified a group of approved drugs that could inhibit clathrin-mediated endocytosis and thereby inhibit viral infection of cells. The drug targets are members of the numb-associated kinase (NAK) family—including AAK1 and GAK—the inhibition of which has been shown to reduce viral infection in vitro. Baricitinib was identified as a NAK inhibitor. Further, baricitinib is a potent and selective JAK inhibitor and powerful anti-inflammatory that, as JAK–STAT signalling inhibitor, is proposed as likely to be effective against the consequences of the elevated levels of cytokines observed in patients with COVID-19.

Camostat mesylate
The in vitro data reported by Koffmann (Cell 2020, see below) suggested that the Japanese drug camostat mesylate (trade name: Foipan), a TMPRSS2 inhibitor, might constitute a treatment option for COVID-19.

RNA-dependent RNA polymerase inhibitors
Nucleoside analogues commonly target viral replication, particularly the viral DNA or RNA polymerase, and have succeeded clinically in treating multiple viral infections (Agostini mBio 2018, see below). However, identification and development of antiviral nucleosides against coronaviruses have been hampered by the presence of the unique CoV proofreading 3’-S’exoribonuclease (ExoN). While nucleoside analogues such as BCX4430 inhibit CoVs, several previously tested nucleoside analogues have been incapable of potently inhibiting CoV replication, and others have demonstrated poor selectivity indexes. CoV resistance to the mutagens 5-fluorouracil and ribavirin in vitro is attributed to their removal by the proofreading ExoN, supporting the hypothesis that an effective nucleoside analogue must evade proofreading to successfully interfere with CoV RNA synthesis.

Remdesivir
Remdesivir (GS-5734) is the monophosphoramidate prodrug of the C-adenosine nucleoside analogue GS-441524 (Agostini mBio 2018, see below). This drug candidate had been shown to inhibit in vitro infections with SARS-CoV, MERS-CoV, and bat CoV strains that are capable of replicating in primary human airway epithelial cells and mediate entry using human CoV receptors. Remdesivir (EC50 = 0.77 μM; CC50 > 100 μM; SI > 129.87) potently blocked infection of Vero E6 cells by a clinical isolate of SARS-CoV-2 at low-micromolar concentration and showed high selectivity index16 (Wang Cell Res 2020, see below).

In vivo, remdesivir demonstrated both prophylactic and therapeutic efficacy against SARS-CoV disease in a mouse model. In a Ces1c−/- hDPP4 mouse model of MERS-CoV infection, both prophylactic and therapeutic use of remdesivir improved pulmonary function and reduced lung viral loads and severe lung pathology (Sheahan Nat Commun 2020, see below).

Remdesivir has been evaluated in a phase 2/3 controlled safety and efficacy clinical trial for the treatment of people with Ebola virus disease, which also tested 3 Ebola-specific monoclonal antibody (mAb) candidates (NCT03719586, https://clinicaltrials.gov/ct2/show/NCT03719586?term=remdesivir&draw=2&rank=1). The results of an interim analysis showed superiority of two of the mAb-based treatments over remdesivir with respect to mortality, and the trial was continued without a remdesivir arm (Mulangu New Engl J Med 2019, see below). In this study, 175 patients received remdesivir. One serious adverse event was determined to be possibly related to remdesivir: a patient in this study group had hypotension that resulted in cessation of a loading dose of remdesivir and that was followed rapidly by cardiac arrest. Another clinical trial of remdesivir assessed its antiviral activity, longer-term clearance of Ebola virus, and safety in male Ebola survivors with evidence of Ebola virus persistence in semen (NCT02818582, 16 Importantly, in the same study, high concentrations of three nucleoside analogs including ribavirin, penciclovir and favipiravir were required to reduce viral infection

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Remdesivir inhibits murine hepatitis virus (MHV) with similar 50% effective concentration values (EC50) as SARS-CoV and MERS-CoV (Agostini mBio 2018, see below), and this model was used to assess virus resistance to remdesivir. Passage of wild type MHV in the presence of the remdesivir parent nucleoside selected two mutations in the nsp12 polymerase at residues conserved across all CoVs that conferred up to 5.6-fold resistance to remdesivir, as determined by EC50. The resistant viruses were unable to compete with wild type virus in direct coinfection passage in the absence of remdesivir. Introduction of the MHV resistance mutations into SARS-CoV resulted in the same in vitro resistance phenotype and attenuated SARS-CoV pathogenesis in a mouse model. Finally, an MHV mutant lacking ExoN proofreading was found significantly more sensitive to remdesivir. Combined, the results indicate that remdesivir interferes with the nsp12 polymerase even in the setting of intact ExoN proofreading activity and that resistance can be overcome with increased, nontoxic concentrations of the drug candidate.

An overview of the various arguments supporting the use of remdesivir to treat COVID-19 has also been made available by Ko (Int J Antimicrob Ag, see below).

Holshue (New Engl J Med 2020, see below) reported on the case report of the first U.S. patient. Given the radiographic findings, the decision to administer oxygen supplementation, the patient’s ongoing fevers, the persistent positive SARS-CoV-2 RNA at multiple sites, and published reports of the development of severe pneumonia at a period consistent with the development of radiographic pneumonia in this patient, clinicians pursued compassionate use of remdesivir therapy. Treatment with intravenous remdesivir was initiated on the evening of hospital day 7 (illness day 11), and no adverse events were observed in association with the infusion. On hospital day 8 (illness day 12), the patient’s clinical condition improved. As of January 30, 2020, the patient remained hospitalized. He was afebrile, and all symptoms had resolved with the exception of his cough, of decreasing severity.


Ribavirin
While in vitro data have not identified ribavirin as a lead candidate, a randomized clinical trial of the drug used in combination with pegylated interferon has been reported in China for COVID-19 (ChiCTR2000029387) (https://www.nature.com/magazine-assets/d41573-020-00016-0/17663286).

Favipiravir
Randomized trials of favipiravir have been reported in China for COVID-19 therapy (ChiCTR2000029544, ChiCTR2000029600) (https://www.nature.com/magazine-assets/d41573-020-00016-0/17663286). On March 17, Zhang Xinmin released in the media data from a Chinese trial that evaluated favipiravir (http://www.chinadaily.com.cn/a/202003/17/W020200317758957517277f5bd.html). The Third People's Hospital of Shenzhen in Guangdong province conducted a clinical trial on 80 patients, with 35 receiving the drug. The results showed that patients treated with favipiravir took four days before being tested negative, whereas the control group took 11 days. The lung conditions of 91.43 percent of the treated group improved as shown in chest imaging, compared with 62.22 percent of the control group. In another comparative trial on 120 patients conducted by Zhongnan Hospital of Wuhan University, the results were said to have shown that the treated group had a higher recovery rate at the end of treatment and took less time to reduce fever and relieve cough. Scientific publication of these data is now awaited.
**Other RNA-dependent RNA polymerase inhibitors**

Lung (J Med Vir 2020, see **below**) screened chemical structures from traditional Chinese medicinal compounds proven to show anti-viral activity in SARS-CoV and similar chemical structures through a molecular docking study to target the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2, SARS-CoV and MERS-CoV. Theaflavin was identified as a potential SARS-CoV-2 RdRp inhibitor.

**Protease inhibitors**

Involved in the formation of the coronavirus replication complex, the viral main protease (3-chymotrypsin-like cysteine protease, 3CLpro, also called Mpro) represents an attractive target for therapy. The structure of Mpro has been resolved and made publicly available to facilitate global efforts to develop novel drug candidates.

**Lopinavir/ritonavir**

Lopinavir and ritonavir are used as a combination therapy for the treatment and prevention of HIV/AIDS. However, they soon appeared as candidate of choice for COVID-19 therapy. Yao (J Med Vir 2020, see **below**) published a review of the literature on the efficacy of lopinavir *in vitro* and *in vivo*, especially in patients with SARS and MERS.

Lin (manuscript on MedRxiv: [https://www.biorxiv.org/content/10.1101/2020.01.31.929695v2.full.pdf](https://www.biorxiv.org/content/10.1101/2020.01.31.929695v2.full.pdf)) presented evidence supporting the mode of action of lopinavir, ritonavir and dapenavir through their predicted interactions with SARS-CoV-2 proteases. He suggested that the therapeutic effect of ritonavir and lopinavir on COVID-19 may be mainly due to their inhibitory effect on coronavirus endopeptidase C30, with ritonavir appearing to have stronger efficacy; the inhibitory effect of darunavir on SARS-CoV-2 and its potential therapeutic effect may be mainly due to its inhibitory effect on papain-like viral protease.

Several clinical trials are currently ongoing to evaluate lopinavir and/or ritonavir (± other drug candidates) in COVID-19 (see for instance study NCT04252885: [https://clinicaltrials.gov/ct2/show/NCT04252885?term=lopinavir&recrs=ab&draw=2&rank=3](https://clinicaltrials.gov/ct2/show/NCT04252885?term=lopinavir&recrs=ab&draw=2&rank=3), or NCT04255017: [https://clinicaltrials.gov/ct2/show/NCT04255017?term=lopinavir&recrs=abd&draw=2&rank=7](https://clinicaltrials.gov/ct2/show/NCT04255017?term=lopinavir&recrs=abd&draw=2&rank=7)).

Cao (NEJM 2020, see **below**) reported the outcome of an open-label trial involving hospitalized adult patients with confirmed SARS-CoV-2 infection in Wuhan. 199 patients were randomly assigned in a 1:1 ratio to receive either lopinavir-ritonavir (400 mg and 100 mg, respectively) twice a day for 14 days, in addition to standard care, or standard care alone. Treatment with lopinavir-ritonavir was not associated with a difference from standard care in the time to clinical improvement (hazard ratio for clinical improvement, 1.24; 95% confidence interval [CI], 0.90 to 1.72). Mortality at 28 days was similar in the lopinavir-ritonavir group and the standard-care group (19.2% vs. 25.0%; difference, 5.8 percentage points; 95% CI, -17.3 to 5.7). The percentages of patients with detectable viral RNA at various time points were similar. Moreover, lopinavir-ritonavir treatment was stopped early in 13 patients (13.8%) because of adverse events. The interpretation of these data in the editorial by Baden (NEJM 2020, see **below**) is also of interest. For instance, it highlighted the fact that patients recruited for this study were late in infection and already had considerable tissue damage.

**Other candidates targeting Mpro**

Zhang ([https://www.biorxiv.org/content/10.1101/2020.02.17.952879v1](https://www.biorxiv.org/content/10.1101/2020.02.17.952879v1)) determined the crystal structure of the unliganded Mpro at 1.75 A resolution and used this structure to guide optimization of a series of alpha-ketoamide inhibitors. The main goal of the optimization efforts was improvement of the pharmacokinetic properties of the compounds.

Using a computational strategy, based on the synergy of virtual screening, docking and molecular dynamics techniques, Macchiagodena (on ArXiv: [https://arxiv.org/abs/2002.09937](https://arxiv.org/abs/2002.09937)) identified lead compounds for the non-covalent inhibition of Mpro of SARS-CoV-2. Ligands were found to share a common binding pattern with aromatic moieties connected by rotatable bonds in a pseudo-linear arrangement. Molecular dynamics calculations confirmed the

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Paper version: dd. 23 MAR 2020  |  Transdisciplinary Insights - Living Paper
stability in the Mpro binding pocket of most potent binder identified by docking, namely a chlorophenyl-pyridyl-carboxamide derivative.

Tahir ul Qamar (manuscript on Preprints: https://www.preprints.org/manuscript/202002.0193/v1) analysed the Mpro sequence, constructed a 3D homology model, and screened it against a medicinal plant library containing 32 297 potential anti-viral phytochemicals/traditional Chinese medicinal compounds. These analyses revealed nine hits that may serve as potential anti-SARS-CoV-2 lead molecules for further optimisation and drug development to control COVID-19.

Ton (Mol Inf 2020, see below) developed a novel deep learning platform - Deep Docking (DD) which provides fast prediction of docking scores of Glide (or any other docking program) and, hence, enables structure-based virtual screening of billions of purchasable molecules in a short time. The authors applied DD to all 1.3 billion compounds from ZINC15 library to identify top 1000 potential ligands for SARS-CoV-2 Mpro protein.

Other drug candidates

**Nitazoxanide**

Nitazoxanide, a commercial antiprotozoal agent with antiviral potential against a broad range of viruses including human and animal coronaviruses, inhibited infection of Vero E6 cells by a clinical isolate of SARS-CoV-2 at a low-micromolar concentration (EC50 = 2.12 μM; CC50 > 35.53 μM; SI > 16.76). Further in vivo evaluation of this drug against SARS-CoV-2 infection was recommended by Wang (Cell Res 2020, see below).

**Biological response modifiers**

Biological response modifiers (BRM) are substances that interact with and modify the host immune system by acting on a therapeutic target considered important in the pathogenic process of the disease (Lacoma Front Imm 2019). They are now established as therapies for malignancies, transplant rejection, as well as several immune disorders, and can also provide protection against infectious diseases. They include immunostimulatory agents capable of enhancing host defence mechanisms, as well as compounds offering protection against the negative consequences of immune responses. They include antimicrobial peptides, therapeutic small molecules, therapeutic antibodies, cytokines and other immunomodulators. Controlling cytokine production and inflammatory response appears as a desirable objective, given that they are responsible for the accumulation of cells and fluids. However, as pointed out by Li (J Med Virol 2020, see below), this strategy remains challenging as long as immune response parameters that can be inhibited specifically without compromising the beneficial host defence have not been identified. For instance, completely blocking a proximal event in the immune response (e.g., activation of interferon response-related pattern recognition receptors) seems unwise considering its general role in regulating host defence.

**Interferon-α**

During the SARS outbreak in 2003, an animal study revealed that recombinant human IFN-α2b spray can prevent SARS CoV infection in Rhesus monkey model by inhibiting virus infection and replication (Shen World J Pediatr 2020, see below). Further clinical evaluation revealed that recombinant human IFN-α2b spray can effectively reduce the infection rate of respiratory syncytial virus, influenza virus, adenovirus and SARS-CoV. The “Novel Coronavirus Infection Pneumonia Diagnosis and Treatment Standards (fourth edition) and Diagnosis, treatment and prevention of 2019 novel coronavirus infection in children: experts’ consensus statement” of the National Health Commission of People’s Republic of China also listed IFN-α atomization as a treatment option for COVID-19 pneumonia.

**Anti-inflammatory therapies**

**Corticosteroids**

Corticosteroids were widely used during the outbreaks of SARS and MERS CoVs and are being used in patients with COVID-19 in addition to other therapeutics. However, current interim guidance from WHO on clinical management of
severe acute respiratory infection when novel coronavirus (COVID-19) infection is suspected (https://www.who.int/docs/default-source/coronaviruse/clinical-management-of-novel-cov.pdf) advises against the use of corticosteroids unless indicated for another reason. The same conclusion was reported by Russell (Lancet 2020, see below) who concluded from a literature review that no unique reason exists to expect that patients with COVID-19 will benefit from corticosteroids, and that they might be more likely to be harmed with such treatment. However, a subsequent publication by Shang (Lancet 2020, see https://www.thelancet.com/action/showPdf?pii=S0140-6736%2820%2930361-5) noted that the existing evidence on this topic is inconclusive, and even systematic reviews and meta analyses on this topic reach differing conclusions. The authors recommended short courses of corticosteroids at low-to-moderate dose, used prudently, for critically ill patients with COVID-19 pneumonia. A similar recommendation was made by Zhou (Signal Transduct Target Ther 2020, see below).

**Immune checkpoint inhibitors**

Immune checkpoint inhibitors are being considered for their potential to augment the host response in sepsis. PD-1 and PD-L1 are indeed key mediators in T cell depletion in sepsis patients. Animal models have shown that blocking PD-1 or PD-L1 can prevent T cell death, regulate cytokine production, reduce organ dysfunction and reduce death in sepsis. Previous experience showed the clinical safety of anti-PD-1 antibody in sepsis patients through randomized, placebo-controlled trials. A phase 2 clinical trial is currently planned in 120 COVID-19 patients to evaluate anti-PD-1 antibody treatment vs. thymosin vs. control (NCT04268537, https://clinicaltrials.gov/ct2/show/NCT04268537?term=anti-PD-1&cond=COVID-19&draw=2&rank=1).

**Antimicrobial peptides**

Antimicrobial peptides (AMPs), also termed host defence peptides, can be produced as part of the host’s innate immune system during an infection process (Cardoso Int J Mol Sci 2019, Brice Curr Med Chem 2019). These peptides belong to a broad group of molecules produced by many tissues and cell types in a variety of organisms, including plants, invertebrates, vertebrates, fungi and bacteria. The majority of AMPs are composed of relatively small (<10 kDa), cationic and amphipathic molecules, mostly consisting of 6 to 50 amino acid residues. The different amino acid compositions lead to structural properties in terms of amphipathicity, net positive charge, shape and size, which favour interaction with microbial surfaces, insertion into lipid bilayers and induction of membrane damage. It is therefore not surprising that human AMPs display activity against enveloped viruses as well as bacteria and fungi (Brice Curr Med Chem 2019). However, these peptides also exhibit activity against a wide range of non-enveloped viruses, acting at a number of different steps in viral infection. Recent studies have begun to elucidate the antiviral properties of AMPs as well as their role in regulation of inflammation and chemotraction. AMPs have been suggested as promising therapies against viral pathogens (Ahmed Viruses 2019), even though experimental data are still needed to support this proposal.

The antiviral activity of defensins, a class of AMPs, was first reported in 1986. Since then, defensins have demonstrated *in vitro* effects against HIV, influenza A virus, human adenovirus, human papillomavirus, RSV, herpes simplex virus and SARS-CoV. However, few studies in animal models of virus infection have been reported. A murine β-defensin 1-deficient mouse model showed that MBD1, the murine counterpart of HBD1, participated in the protection of mice from influenza infection via a mechanism other than the inhibition of viral replication (Park 2018). Innovation Pharmaceuticals has announced the consideration of its defensin mimetic drug candidate Brilacidin for the potential treatment of Covid-19, the disease caused by the coronavirus (https://www.pharmaceutical-technology.com/news/innovation-pharmaceuticals-covid-19-drug/). Brilacidin is a small molecule in late-phase development. The drug is said to have shown antibacterial, anti-inflammatory and immunomodulatory activity in different clinical studies.

**Therapeutic antibodies**

Several antibodies specific for host targets that are developed in the context of lung disease might appear as promising for COVID-19 therapy. Some antibodies may indeed have the potential to reduce prolonged damaging cellular

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Infiltration during severe lung infections (Elbahesh Front Imm 2019). For instance, angiopoietin-like 4 (ANGPTL4) is a soluble angiogenic regulating protein. Following proteolytic cleavage, the C-terminal portion (cANGPTL4) is involved in integrin-dependent wound repair and can regulate vascular permeability. ANGPTL4 is significantly elevated in lung biopsies from influenza virus-induced pneumonia patients. In mouse studies, neutralizing anti-ANGPTL4 antibodies reduced pulmonary tissue leakiness, significantly accelerating lung recovery. Vascular leakage is a hallmark of many infectious diseases, including those caused by SARS and MERS CoVs (Li Oncotarget 2015). The roles of ANGPTL4 in nCoV infection is still unclear, but warrant future investigations.

**Antibodies**

**Polyclonal antibodies**

**Convalescent plasma**

While at present the use of convalescent plasma for treatment of COVID-19 is not supported by any data, the effectiveness of convalescent plasma for the treatment of SARS, as reviewed by Mair-Jenkins (J Infect Dis 2015, see below), was assessed by 8 studies reporting outcomes for 214 patients with SARS in total. The absolute reduction in the risk of mortality varied from 7% (95% CI, -2.39 to 18.68) to 23% (95% CI, 5.59–42.02) in 2 studies at medium to high risk of bias. Subgroup analyses suggested that early treatment was beneficial. Four non-comparative studies found that the case-fatality rate varied from 0% (0/1) to 12.5% (10/80) in treated subjects. Increased antibody levels were detected up to day 5 after treatment in 1 study of HCWs (which was at high risk of bias).

Experience with convalescent plasma infusion has also been obtained in the context of MERS-CoV infections. Ko (Antivir Ther 2018, see below), based on experience with 3 patients, suggested that for effective convalescent plasma infusion against MERS, donor plasma with a neutralization activity (PRNT titre) ≥1:80 should be used. However, the observation that convalescent plasma infusion led to possible transfusion-related acute lung injury (TRALI) in a MERS patient in Korea suggests that convalescent plasma therapy should be cautiously approached (Chun Ann Lab Med 2016, see below).

In a recent publication, Zhang (J Med Vir 2020, see below) identified this approach as a potential treatment for COVID-19. Up to now there has been no data reported on this topic, but information released in the media indicates that the procedure is being evaluated clinically (https://www.scoop.it/topic/virusworld/p/4115315422/2020/02/14/china-seeks-plasma-from-recovered-patients-to-treat-virus). A plasma donation program has been launched in Zhejiang Province (http://www.xinhuanet.com/english/2020-02/19/c_138799179.htm). The plasma donated by recovered coronavirus patients is said to be used for treatment of COVID-19 patients in critical condition.

**Purified immune globulin**

SAB-301 is a fully-human polyclonal IgG immunoglobulin (SAB-301) produced from hyperimmune plasma of transchromosomic cattle immunized with purified MERS-CoV spike protein nanoparticles vaccine (Beigel Lancet Inf Dis 2018, see below). In a phase 1 trial, single infusions of SAB-301 up to 50 mg/kg appear to be safe and well-tolerated in healthy participants. Single dose pharmacokinetics (PK) demonstrated relatively linear and dose-proportional increases in maximal concentration and area-under-the-concentration-time curve (AUC₀₋₂₄), and the PK strongly correlated with the microneutralization assay. Whether SAB-301 purified immune globulin is able to neutralize SARS-CoV-2 remains to be evaluated.

**Monoclonal antibodies**

The SARS-CoV and MERS-CoV neutralizing monoclonal antibodies (mAbs) and nanobodies with protective efficacy are specific to the S1 subunit of S protein, particularly the receptor-binding domain (RBD) (Jiang Em Micr Inf Dis 2020, see below). Therefore, the SARS-CoV-2 S-RBD can be anticipated to be a key target for developing SARS-CoV-2-neutralizing mAbs. Neutralizing mAbs targeting non-RBD regions, including the NTD and S2 of SARS-CoV and/or MERS-CoV S could also be identified, although their neutralizing potency is generally lower than that of RBD-specific mAbs. One of the
rapid approaches to develop a mAb against SARS-CoV-2 is to evaluate the currently available SARS-CoV neutralizing antibodies with cross-neutralizing and protection activity against SARS-CoV-2 infection. SARS-CoV S-RBD-specific neutralizing mAbs and sera have been shown able to cross-neutralize bat-SL-CoVs, such as bat-SL-CoV-W1V1 and bat-SL-CoV-SHC014, suggesting that they might also cross-neutralize SARS-CoV-2.

A whole range of mAbs have been listed by WHO as potential candidates against COVID-19 (https://apps.who.int/iris/bitstream/handle/10665/330680/WHO-HEO-RDBlueprint%28nCoV%29-2020.1-eng.pdf?ua=1) (Table 11). Their ability to neutralize SARS-CoV-2 in vitro and in vivo remains to be confirmed.

<table>
<thead>
<tr>
<th>Product type and candidate</th>
<th>Target disease</th>
<th>Description</th>
<th>Status of development</th>
<th>Preliminary results</th>
</tr>
</thead>
<tbody>
<tr>
<td>80R mAB S3.1 m396</td>
<td>SARS</td>
<td>Human monoclonal antibodies</td>
<td>In vitro</td>
<td>inhibited different SARS-CoV subtypes Didn’t neutralize GD03 strain</td>
</tr>
<tr>
<td>GD27, Gd33, MCA1, JCS7-14, MERS-4, CDC2-C2, VHH-83, HCAb-83, CVHs, NbMx10, NbM10-Fc LCA60</td>
<td>MERS</td>
<td>HmAbs/ Fab-RBD, HmAbs/ Fab-RBD, HmAbs/ Fab-RBD Macaque mAbs/ FabRBD HmAbs/ Fab-RBD HmAbs/ Fab-RBD Dromedary VHHs Dromedary VHHs Llama VHHs Llama VHHs Human survivors</td>
<td>In vitro and in vivo (transgenic mice)</td>
<td>Most of mAbs can neutralize pseudotype or live MERS-CoV and some shown protection in animal models in vivo</td>
</tr>
<tr>
<td>REGN3048 and REGN3051 antibody cocktail</td>
<td>MERS</td>
<td>Double-blind, placebo-controlled Phase I study. Single ascending dose cohorts safety, 48 subjects. NCT03301090</td>
<td>No results posted</td>
<td></td>
</tr>
</tbody>
</table>

A review on FcR and antibody engineering by Chenoweth (Immunol Cell Biol 2020, see below) could be of particular interest to the development of therapeutic mAbs against COVID-19.

**Traditional Chinese Medicine**

The utilization of Traditional Chinese Medicine (TCM) in managing COVID-19 is substantial in China. All confirmed COVID-19 cases in Shanghai started integrative Chinese-Western medicine treatment (Yuan and Qiu. Forty-one patients with new coronavirus pneumonia were treated with traditional Chinese medicine. Xinhua Net, Shanghai, 2020). National guideline recommended herbal formulations according to clinical stages and severity of COVID-19. Although national/provincial/local guidelines could differ in terms of treatment strategy, most guidelines defined COVID-19 as endemic, toxic, dampness or warm infectious disease (Chan Am J Chin Med 2020, see below). The six most commonly used herbs were Astragali Radix (Huangqi), Glycyrrhiza Radix Et Rhizoma (Gancao), Saposhnikoviae Radix (Fangfeng), Atractylidis Macrocephalae Rhizoma (Baizhu), Lonicerae Japonicae Flos (Jinyinhua), and Forsythiae Fructus (Lianqiao). Some of them are the core components of classical herbal formula: Yupingfeng (powder), for tonifying qi to protect from external pathogens, and Yinqiao san (powder), used to prevent and treat respiratory infectious diseases (Luo Chin J Integr Med 2020, see below).

Between 23 January and 8 March 2020, 382 new trials related to management of patients with COVID-19 were registered on the WHO’s International Clinical Trials Registry Platform (ICTRP). 98 out of these 382 trials evaluate TCM,
which includes 48 named TCMs, 27 unspecified methods; 18 combinations with unspecified Western therapies, and 5 others (e.g. acupuncture) (Aronson at https://www.cebm.net/oxford-covid-19/covid-19-registered-trials-and-analysis/). Among the ongoing largest COVID-19 clinical trials in terms of participant size, one trial (GDCT0379500), involving 600 participants in Hubei in China, is aiming to determine if the addition of TCM to standard health education is more effective than health education alone in preventing COVID-19 (https://www.clinicaltrialsarena.com/comment/covid-19-clinical-trials/).

Therapies targeting the acute respiratory distress syndrome

Acute respiratory distress syndrome is a common cause of respiratory failure in critically ill patients and is defined by the acute onset of non-cardiogenic pulmonary oedema, hypoxaemia and the need for mechanical ventilation (Matthay Nat Rev Dis Primers 2019). Despite some improvements, it remains associated with a high level of mortality (30-40%) in most studies. One approach to improve disease outcome is to identify patients earlier in their clinical course, so that supportive care with lung-protective ventilation, prone positioning and a conservative fluid approach can be implemented. Up to now, pharmacological agents did not prove very helpful in the management of acute respiratory distress syndrome. A recent review (Lewis Cochrane Database Syst Rev 2019) found insufficient evidence to determine with certainty whether corticosteroids, surfactants, N-acetylcysteine, statins, or beta-agonists were effective at reducing mortality, or duration of mechanical ventilation, or at increasing ventilator-free days. The list of unsuccessful therapies also includes agents such as prostaglandin E1, activated protein C, anti-oxidants, omega-3 supplementation, ketoconazole, lisofylline, factor VIIa, IFN-β1α, or granulocyte macrophage-stimulating factor. However, it remains possible that the clinical trials that evaluated these products were not designed in the most suitable way.
**Vaccine development**

A draft landscape of the candidate SARS-CoV-2 vaccines currently under development is regularly updated by WHO: [https://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus-landscape-ncov.pdf?ua=1](https://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus-landscape-ncov.pdf?ua=1). According to other sources, within two months of the SAR-CoV-2 outbreak, at least 37 biopharmaceutical companies or academic sectors were reported to be in the race to develop a prophylactic vaccine by using several platforms including mRNA, DNA, adenoviral vector and recombinant protein (Promptpatchar Asia Pac J All Imm 2020, see below). Full-length S or S1 which contains receptor binding domain (RDB) might be considered as a good vaccine antigen as it could induce neutralizing antibodies preventing host cell attachment and infection. The S antigen has been included in different types of vaccines against infections by CoVs (Yu Micr Inf 2020, see below).

Several candidate vaccines had completed Phase 1 clinical trials against SARS-CoV and MERS-CoV ([https://www.who.int/blueprint/priority-diseases/key-action/prioritization-candidate-vaccines-ncov2019.pdf?ua=1](https://www.who.int/blueprint/priority-diseases/key-action/prioritization-candidate-vaccines-ncov2019.pdf?ua=1)). However, despite some level of sequence homology between SARS-CoV-2 and SARS-CoV, and to a lesser extent MERS-CoV, vaccine candidates developed against SARS-CoV and MERS-CoV are not expected to generate adequate levels of cross-reactive antibodies. Current efforts are thus focused on engineering and advancing vaccines that include antigens from the SARS-CoV-2 strain.

Lu (Emerg Microbes Inf 2020, see below) described both the reasons why a COVID-19 vaccine is needed, and the challenges to be faced.

**RNA vaccines**

Stermirna Therapeutics Co., Ltd. and Shanghai East Hospital of Tongji University announced a project for the co-development of an mRNA vaccine targeting COVID-19 ([http://www.xinhuanet.com/english/2020-01/28/c_138739378.htm](http://www.xinhuanet.com/english/2020-01/28/c_138739378.htm)). According to sources with the Chinese CDC, preclinical evaluation of this vaccine candidate in a mouse model is ongoing ([http://www.xinhuanet.com/english/2020-02/10/c_138771569.htm](http://www.xinhuanet.com/english/2020-02/10/c_138771569.htm)).

Moderna, Inc. and the Coalition for Epidemic Preparedness Innovations (CEPI) announced a new collaboration to develop an mRNA vaccine against COVID-19 ([https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-funding-award-cepi-accelerate-development](https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-funding-award-cepi-accelerate-development)). Under the terms of the agreement, Moderna will manufacture an mRNA vaccine, which will be funded by CEPI. The Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases (NIAID) collaborated with Moderna to design the vaccine. Moderna announced the initiation of the phase 1 trial of the mRNA-1273 vaccine on March 16 ([https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-first-participant-dosed-nih-led-phase-1-study](https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-first-participant-dosed-nih-led-phase-1-study)). Information on this phase 1 clinical trial has been posted on the clinicaltrials.gov website (Safety and Immunogenicity Study of 2019-nCov Vaccine (mRNA-1273) to Treat Novel Coronavirus - Full Text View - ClinicalTrials.gov). Three dose levels of mRNA-1273 (25, 100, 250 μg) are administered on a two-dose vaccination schedule, given 28 days apart. A total of 45 healthy adults will be enrolled in the study. Participants will be followed through 12 months after the second vaccination. The primary objective is to evaluate the safety and reactogenicity of a two-dose vaccination schedule of mRNA-1273. The secondary objective is to evaluate the immunogenicity to the SARS-CoV-2 S protein.


**DNA vaccines**

Advaccine-To-Advance-INO-4800-Vaccine-Against-New-Coronavirus-In-China/default.aspx). The company subsequently announced that it is collaborating with Beijing Advaccine Biotechnology Co. to advance the vaccine candidate development in China. The goal of this collaboration is to leverage Advaccine’s expertise to run a Phase 1 trial in China in parallel with Inovio's clinical development efforts in the U.S. Inovio and Advaccine will also work together to attract additional grant funding and further collaborations with larger vaccine companies in China to increase the speed of future testing of INO-4800.

Interestingly, INO-4700 (GLS-5300), Inovio’s MERS-CoV vaccine, has already undergone Phase 1 clinical testing: the vaccine candidate appeared well-tolerated; it induced antibody responses in 94% of subjects after 3 injections; neutralizing antibodies were detected in 50% of participants, and T cell responses in 78% of study participants (Modjarrad Lancet Inf Dis 2019, see below). Immune responses were dose-independent, and durable through 1 year of follow-up.

**Subunit vaccines**

**Virus-like particles**

On March 12 2020, Medicago announced the successful production of Virus-Like Particle (VLPs) of SARS-CoV-2 (https://www.medicago.com/en/covid-19-programs/). Preclinical studies are to be initiated in a very short timeframe.

**Nanoparticles**

According to a press release, Novavax has initiated development of a vaccine against COVID-19. The company previously used its technology to develop vaccine candidates against SARS-CoV and MERS-CoV (see for instance, Coleman Vaccine 2014, see below). Purified full-length MERS and SARS S proteins formed ~25 nm diameter particles consisting of multiple S protein molecules. The antigens were combined with Matrix M1 adjuvant and evaluated in mice. The company is currently assessing multiple nanoparticle vaccine candidates in animal models prior to identifying an optimal candidate for human testing, which is expected to begin by the end of spring 2020 (http://ir.novavax.com/news-releases/news-release-details/novavax-advances-development-novel-covid-19-vaccine).

**Fusion protein-based approach**

Viral fusion proteins undergo structural rearrangements from a metastable pre-fusion conformation to a highly stable post-fusion conformation (https://www.pharmaling.com/detail.php?uid=66499). Traditional approaches to recombinant expression of these proteins typically result in premature triggering and a conformational shift to the structurally more stable post-fusion form. The “molecular clamp” approach developed by the University of Queensland, Australia, uses a polypeptide moiety and has been shown to display increased stability over alternate stabilizing trimerization domains (https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018176103). This technique has already been used to produce chimeric polypeptides that mimic the pre-fusion conformations of HIV, respiratory syncytial virus, influenza, measles and Ebola viruses. The University of Queensland has been requested to use this technology to develop a vaccine candidate against COVID-19 (https://www.uq.edu.au/news/article/2020/01/race-develop-coronavirus-vaccine).

**Adjuvanted vaccines**

CEPI and Glaxo Smith Kline (GSK) announced a collaboration to develop a vaccine against COVID-19, which will leverage GSK’s pandemic vaccine adjuvant technology (https://www.gsk.com/en-gb/media/press-releases/cepi-and-gsk-announce-collaboration-to-strengthen-the-global-effort-to-develop-a-vaccine-for-the-COVID-19-virus/). The adjuvant is designed to reduce the amount of antigen needed per patient and thereby to help stretch vaccine supplies. As part of the arrangement, an agreement between GSK and the University of Queensland will support early stage research on the molecular clamp vaccine. In a subsequent announcement, the University of Queensland reported they had
created the first vaccine candidate in the laboratory and were moving into further development before formal pre-

In addition, the U.S. Department of Health and Human Services and Sanofi Pasteur announced a collaboration
targeting the development of a COVID-19 vaccine candidate (https://www.hhs.gov/about/news/2020/02/18/hhs-
/media/Project/One-Sanofi-Web/Websites/Global/Sanofi-COM/Home/media-room/press-releases/2020/2020-02-
18-16-00-00-1986380-en.pdf). The candidate will be based on proteins found on the surface of the virus and be
produced by Sanofi Pasteur’s (formerly Protein Sciences) baculovirus expression platform, which was initially
developed to manufacture large quantities of pandemic influenza vaccines. According to Sanofi’s press release, the
vaccine candidate will be formulated. Even though not clearly stated in the announcement, it can be expected that an
adjuvant will be used.

Another adjuvanted vaccine candidate is based on the S-trimer subunit vaccine candidate of Clover
Biopharmaceuticals Inc. A research collaboration with GSK targeting evaluation of this candidate with GSK pandemic
adjuvant system has indeed been announced (https://www.gsk.com/en-gb/media/press-releases/clover-and-gsk-
around-research-collaboration-to-evaluate-coronavirus-covid-19-vaccine-candidate-with-pandemic-adjuvant-
system/).

**Vectored vaccines**

**Adenovirus vector**

The Ad5-nCoV vaccine of CanSino Biologics is a replication-defective adenovirus type 5 vector expressing SARS-CoV-2
S protein. According to company communication, results from preclinical animal studies of Ad5-nCoV have shown that
the vaccine candidate can induce strong immune response in animal models, and preclinical animal safety studies
demonstrated a good safety profile (http://www.canstech.com/articles/article/show/56/153.html). The GMP
clinical batches have passed quality testing and are ready for Phase 1 Clinical Trial, which has been approved. This trial
is a single-center, open and dose-escalation phase I trial, testing safety and tolerance of Ad5-nCoV in healthy adults,
ages 18 to 60 years. The low-, middle- and high-dosage groups will each see 36 patients, who will receive 5e10vp, 1e11vp and 1e11vp of Ad5-nCoV, respectively (http://www.chictr.org.cn/showprojen.aspx?proj=51154).

According to company communication, Altimmune develops a single-dose intranasal COVID-19 vaccine candidate,
based on the technology used for their influenza vaccine candidate, NasoVAX, which is known to induce mucosal
immunity as well as cell and IgG responses (https://ir.altimmune.com/static-files/f9e406df-9cc0-4fb7-9d9c-
52d780679780). The candidate is a replication-deficient adenovirus 5 vector expressing SARS-CoV-2 S protein.

Johnson & Johnson also announced that it has initiated efforts to develop a vaccine candidate against COVID-19
threat). The vaccine program will leverage Janssen’s AdVac® and PER.C6® technologies that provide the ability to
rapidly upscale production of the optimal vaccine candidate. These are the same technologies that were used in the
development and manufacturing of Janssen’s investigational Ebola adenovirus type 26 vector vaccine, which is
currently deployed in the Democratic Republic of the Congo and Rwanda. They were also used to construct the
Company’s Zika, respiratory syncytial virus and HIV vaccine candidates.

**Modified Vaccinia Ankara vector**

GeoVax Labs, Inc., together with BravoVax, a vaccine developer in Wuhan, China, today announced the signing of a
Letter of Intent to jointly develop a vaccine against COVID-19. Under the collaboration, GeoVax will use its MVA-VLP
vaccine platform and expertise to design and construct the vaccine candidate using genetic sequences from the
ongoing coronavirus outbreak. BravoVax will provide further development, including testing and manufacturing

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**Other vectors**

**Live-attenuated vaccines**
Non-vaccine approaches to better host resistance

Host resistance to viral infections can be increased in multiple ways. While there has been no report to date of such studies in the context of COVID-19, there is evidence available to support further investigations in this area.

Traditional Chinese Medicine

In China, the use of Traditional Chinese Medicine (TCM) to prevent epidemics of infectious diseases was traced back to ancient Chinese practice cited in Huangdi’s Internal Classic (Huang Di Nei Jing), which was written about 2000 years ago (Luo Chin J Integr Med 2020, see below). It suggested two aspects which should be employed to prevent the spread of epidemics. One was to maintain and improve the healthy qi in the body by taking preventive medicine (Xiaojin dan, the first recommended formula of TCM to prevent pestilence), healthy diet care, exercise and so on, so as to resist the invasion of external pathogen, and the other was to avoid the source of infection. These two principles of epidemic disease prevention have been followed by TCM practitioners until now. In 2003, TCM approaches were used to prevent and treat SARS, and in 2009, during the influenza A(H1N1) pandemic, the National Administration of TCM of China issued a TCM prevention program, which included four Chinese herbal formulae for adults of different body constitutions and one for children. A literature review identified three studies using TCM for prevention of SARS and four studies for H1N1 influenza. None of the participants who took Chinese medicine contracted SARS in the three studies. The infection rate of H1N1 influenza in the TCM group was significantly lower than non-TCM group (RR 0.36, 95% CI 0.24-0.52; n=4). For prevention of COVID-19, 23 provinces in China issued TCM programs. The main principles of TCM use were to tonify qi to protect from external pathogens, disperse wind and discharge heat, and resolve dampness. The most frequently used herbs included Astragali (Huangqi), Glycyrrhizae (Gancao), Saposhnikoviae (Fangfeng), Atractylodis Macrocephalae (Baizhu), Lonicerae Japonicae (Jinyinhua), and Forsythiae (Lianqiao).

Manipulating the commensal microbiota?

A plethora of evidence suggests that the commensal microbiota regulates and is in turn regulated by invading viruses through diverse mechanisms, thereby having stimulatory or suppressive roles in viral infections (reviewed by Li Front Immun 2019, see below). Such knowledge could help design alternative approaches to the control of a number of viral infections, including COVID-19. A trial investigating the underlying mechanism of development of lower respiratory tract infection (LRTI) after viral infection showed for instance that patients with a higher abundance of butyrate-producing bacteria in their faecal samples had a 5-fold lower possibility of developing viral LRTI. Considering that butyrate-producing bacteria are favoured by a diet rich in fibers, similar studies on COVID-19 appear useful to undertake.

Curcumin?

Curcumin is considered as the major active compound in the rhizome of turmeric (Curcuma longa). Curcumin has been used extensively in Ayurveda, Siddha medicine and traditional Chinese medicine for centuries, as it has been associated with a variety of therapeutical properties including antioxidant, analgesic, anti-inflammatory, antiseptic activity, and anti-carcinogenic activity (reviewed by Mathew J Funct Foods 2018). Curcumin’s antiviral effects were observed against numerous viruses including parainfluenza virus type 3, vesicular stomatitis virus (VSV), herpes simplex virus, and RSV. Curcumin also appeared as a potent inhibitor when tested for its in vitro activity against SARS-CoV on Vero E6 cells (Wen J Med Chem 2007).

Various clinical trials provided promising results suggesting a low toxicity of curcumin. However, many questions and challenges still exist. Curcumin has been reported as an unstable, reactive, non-bioavailable compound (Nelson J Med Chem. 2017), and the lack of placebo-controlled trials to support its efficacy in humans has been pointed out (Nelson ACS Med Chem Lett. 2017). The distinction between turmeric (the plant), curcuminoids (contained in turmeric and in extracts of turmeric) and curcumin also needs to be highlighted. Curcuminoids, as typically available commercially, contain not only curcumin but three primary components and approximately 15% of oleoresins and essential oil (Nelson 2017b).

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Paper version: dd. 23 MAR 2020
Vector control and disease control in animals

Available evidence on SARS-CoV-2 and previous experience with other coronavirus (MERS-CoV and SARS-CoV) and other respiratory viruses (e.g., avian influenza) suggest that there may be zoonotic transmission associated with SARS-CoV-2. The following recommendations were therefore issued by WHO (https://www.who.int/health-topics/coronavirus/who-recommendations-to-reduce-risk-of-transmission-of-emerging-pathogens-from-animals-to-humans-in-live-animal-markets).

As of to date, the recommendations remain very general, as the animal species that may be involved in such transmission remain unknown.

As a general precaution, general hygiene measures are recommended to anyone visiting live animal markets, wet markets or animal product markets. These include regular hand washing with soap and potable water after touching animals and animal products, avoiding touching eyes, nose or mouth with hands, and avoiding contact with sick animals or spoiled animal products. It is also recommended to avoid contact with other animals possibly living in the market (e.g., stray cats and dogs) and with potentially contaminated animal waste or fluids on the soil or structures of shops and market facilities. A last recommendation is to avoid consumption of raw or undercooked animal products.

People with underlying medical conditions are considered at higher risk of severe disease. Therefore, individuals with these underlying medical conditions are recommended to avoid contact with live animal markets, stray animals and wild animals, and should not eat animal raw meat.

Good personal hygiene is specifically recommended to slaughterhouse workers, veterinarians in charge of animal and food inspection in markets, market workers, and those handling live animals and animal products. Use of protective gowns, gloves, masks as well as frequent disinfection of equipment and working stations, is also recommended.
Social interventions

Psychological intervention for affected people
It has been claimed that the mental health needs of patients with confirmed COVID-19, patients with suspected infection, quarantined family members, and medical personnel have been poorly handled in China, and that the organisation and management models for psychological interventions must be improved (Duan Lancet Psych 2020, see). With disease progression, clinical symptoms become severe and psychological problems in infected patients change; therefore, psychological intervention measures should be targeted and adapted as appropriate. Studies have confirmed that individuals who have experienced public health emergencies still have varying degrees of stress disorders, even after the event is over, or they have been cured and discharged from hospital, indicating these individuals should not be ignored. It is recommended that interventions are based on a comprehensive assessment of risk factors leading to psychological issues, including poor mental health before a crisis, bereavement, injury to self or family members, life-threatening circumstances, panic, separation from family and low household income.

Brooks (Lancet 2020, see below) reviewed the psychological impact of quarantine. Most reviewed studies reported negative psychological effects including post-traumatic stress symptoms, confusion, and anger. Stressors included longer quarantine duration, infection fears, frustration, boredom, inadequate supplies, inadequate information, financial loss, and stigma. In situations where quarantine is deemed necessary, the author recommended officials to quarantine individuals for no longer than required, provide clear rationale for quarantine and information about protocols, and ensure sufficient supplies are provided. Appeals to altruism by reminding the public about the benefits of quarantine to wider society are presented as favourable.

Social media and information to the general public
Using data collected during the 2015 Middle East Respiratory Syndrome coronavirus (MERS-CoV) outbreak in South Korea, a study reported by Oh (Health Comm 2020, see below) explored the relationships among social media use, risk perception, and preventive behaviours by examining the mediating role of two self-relevant emotions: fear and anger. The findings demonstrate that social media use is positively related to both of these emotions, which are also positively related to the public's risk perception. The findings also indicate that social media use can significantly increase preventive behaviours via the two self-relevant emotions and the public's risk perception.

In China, the government strives to improve the public's awareness of prevention and intervention strategies by providing daily updates about surveillance and active cases on websites and social media (Bao Lancet 2020, see below). Increasingly, psychologists and psychiatrists use the internet and social media (e.g., WeChat, Weibo, etc) to share strategies for dealing with psychological stress. For example, experts from Peking University Sixth Hospital made six suggestions for the public to cope with mental stress. These included assessing the accuracy of information disclosed, enhancing social support systems (e.g., families and friends), eliminating stigma associated with the epidemic, maintaining a normal life under safe conditions, and using the psychosocial service system, particularly telephone-based and internet-based counselling for health-care staff, patients, family members, and the public. Liu (Lancet Psych 2020, see below) even reported that several artificial intelligence (AI) programmes have been put in use as interventions for psychological crises during the epidemic. For example, individuals at risk of suicide can be recognised by the AI programme Tree Holes Rescue,5 by monitoring and analysing messages posted on Weibo, and alerting designated volunteers to act accordingly.

Outside China, at the start of the epidemic, the emergence of misinformation and racism against patients and Chinese visitors has been reported (Shimizu Lancet 2020, see below). Excess demand for surgical masks among the general public also became a serious concern in countries such as Japan, as it lowered provision for medical facilities including emergency and critical care centres. It has been recommended that mass media take responsibility for providing correct information and creating comprehension among citizens. Effective communication may contribute to lessening...
the risk for inappropriate behaviour, such as unnecessary visits to health-care facilities, as well as help eliminate fake news and discrimination against patients and Chinese visitors.

However, just as the coronavirus itself, misinformation has spread far and wide, drowning out credible sources of information (Mian BMC Med 2020, see below). Over the last couple of months, posts from the WHO and the US CDC have cumulatively only achieved several hundred thousand engagements, considerably eclipsed by hoax and conspiracy theory sites, which have amassed over 52 million. This serves to emphasise the popularity of unverified sources of information.

Gonçalves-Sá (Nat Med. 2020, see below) also highlighted the staggering amount of misinformation propagating online on the topic of COVID-19, including the most concerning conspiracy theory circulating online related to the factitious claim that the virus was engineered by the Chinese, with political or economic goals. The author noted that the decision to delete this misinformation publicly might reinforce conspiracy theories. As an alternative, it was suggested that social-media platforms could attempt to implement simple nudges: asking people whether they are sure they want to share something could activate their best judgment and reduce over-confidence; and introducing time delays on the publication of dubious information, while it is being checked, could slow the spreading process and eventually prevent its publication.

Mian (BMC Med 2020, see below) noted that the disconnect between scientific consensus and members of the public has progressively worsened as society has become further divided in the political climate of today. Calisher (Lancet 2020, see ) published a statement of solidarity to fight against COVID-19 and to promote scientific evidence and unity over misinformation and conjecture.

**Mental support for health care workers in hospitals**

Several reports from China describe the importance of maintaining staff mental health when dealing with the epidemic. Various measures of psychological intervention were reported (see for instance Chen Lancet Psych 2020, see below). First, the hospital provided a place for rest where staff could temporarily isolate themselves from their family. The hospital also guaranteed food and daily living supplies, and helped staff to video record their routines in the hospital to share with their families and alleviate family members' concerns. Second, in addition to disease knowledge and protective measures, pre-job training was arranged to address identification of and responses to psychological problems in patients with COVID-19, and hospital security staff were available to be sent to help deal with uncooperative patients. Third, the hospital developed detailed rules on the use and management of protective equipment to reduce worry. Fourth, leisure activities and training on how to relax were properly arranged to help staff reduce stress. Finally, psychological counsellors regularly visited the rest area to listen to difficulties or stories encountered by staff at work, and provide support accordingly.

**Vulnerable groups**

**Elderly people in China**

The outbreak of COVID-19 has raised great challenges for mental health services for older adults in the community in China. Yang (Lancet Psych 2020, see below) noted that older adults have limited access to internet services and smart phones, and as such only a small fraction of older adults can benefit from such service provision. In addition, in most areas of China, clinically stable older adults with psychiatric disorders or their guardians usually need to visit psychiatric outpatient clinics monthly to obtain the maintenance medications. The mass quarantines and restrictions to public transport have inevitably become a major barrier to access maintenance treatments for this group.

**International migrant workers**

Regardless of their communities’ self-reliance and resilience, Liem (Lancet Psych 2020, see below) noted that addressing the health needs of international migrant workers should be made an urgent public health priority.
Compared with other international migrants, migrant workers encounter more barriers in accessing health services in host countries. Under normal conditions, they have a high burden of common mental disorders (e.g., depression) and a lower quality of life than local populations. This situation could worsen during the COVID-19 epidemic due to the potential and fear of governmental-imposed quarantine and lost income. In the absence of reliable information in their own language, international migrant workers may not recognise the seriousness of the epidemic or receive accurate information on how to protect themselves from infection. However, most international migrant workers have smartphones, which can be a useful aid in providing informational and social support during the epidemic. For instance, WeChat (a Chinese social network platform) is used by international migrant workers in Hong Kong and Macau for sharing key health messages and official information to the community and providing one another with emotional support. It can, however, also spread inaccurate information and panic that could lead to IMWs delaying visits to health centres due to stigmatisation of those who are infected.

**Homeless people**
Tsai (Lancet Resp Med 2020, see below) described various issues, which are unique to people experiencing homelessness, with regards to the COVID-19 epidemic. For instance, when cities impose a lockdown to prevent COVID-19 transmission, it is unclear whether shelter is provided for the large number of people experiencing homelessness, especially when considering that closures of shelters and other high-density communal settings (eg, drop-in centres and soup kitchens) are possible.
REFERENCES


Authors: Martine Denis, Valerie Vandeweerdt, Diane Van der Vliet

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Authors: Martine Denis, Valerie Vandeweerdt, Diane Van der Vliet

Day M. Covid-19: ibuprofen should not be used for managing symptoms, say doctors and scientists. BMJ. 2020 Mar 17;368:m1086. https://www.bmj.com/content/368/bmj.m1086


Authors: Martine Denis, Valerie Vandeweerdt, Diane Van der Vliet
Paper version: dd. 23 MAR 2020


Greenhalgh T, Wherton J, Shaw S, Morrison C. Video consultations for covid-19. BMJ. 2020 Mar 12;368:m998. https://www.bmj.com/content/368/bmj.m998.long


Authors: Martine Denis, Valerie Vandeweerdt, Diane Van der Vliet
Paper version: dd. 23 MAR 2020

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Authors: Martine Denis, Valerie Vandeweerdt, Diane Van der Vliet
Paper version: dd. 23 MAR 2020
Transdisciplinary Insights - Living Paper | 107


Mahase E. Coronavirus: home testing pilot launched in London to cut hospital visits and ambulance use. BMJ. 2020 Feb 14;368:m621. https://www.bmj.com/content/368/bmj.m621.long

Mahase E. Coronavirus: Wales tests 90% of suspected patients in their own home. BMJ. 2020 Feb 20;368:m698. https://www.bmj.com/content/368/bmj.m698.long


Mahase E. Coronavirus: home testing pilot launched in London to cut hospital visits and ambulance use. BMJ. 2020 Feb 14;368:m621. https://www.bmj.com/content/368/bmj.m621.long

Mahase E. Coronavirus: Wales tests 90% of suspected patients in their own home. BMJ. 2020 Feb 20;368:m698. https://www.bmj.com/content/368/bmj.m698.long


Authors: Martine Denis, Valerie Vandeweerd, Diane Van der Vliet
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Authors: Martine Denis, Valerie Vandeweerd, Diane Van der Vliet

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Authors: Martine Denis, Valerie Vandeweerd, Diane Van der Vliet


Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, Li SB, Wang HY, Zhang S, Gao HN, Sheng JF, Cai HL, Qiu YQ, Li LJ. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-CoV-2) outside of Wuhan, China: retrospective case series. BMJ. 2020 Feb 19;368:m606. https://www.bmj.com/content/368/bmj.m606.long


Authors: Martine Denis, Valerie Vandeweerd, Diane Van der Vliet
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Authors: Martine Denis, Valerie Vandeweerdt, Diane Van der Vliet
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