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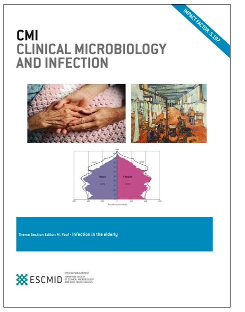

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COVID-19 Pandemic – a focused review for clinicians

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Abstract

Background

The COVID-19 pandemic caused by SARS-CoV-2 remains a significant issue for global health, economics and society. A wealth of data has been generated since its emergence in December 2019 and it is vital for clinicians to keep up with this data from across the world at a time of uncertainty and constantly evolving guidelines and clinical practice.

Objectives

Here we provide an update for clinicians on the recent developments about virology, diagnostics, clinical presentation, viral shedding, and treatment options for COVID-19 based on current literature.

Sources

We considered published peer-reviewed papers and non-peer-reviewed pre-print manuscripts on COVID19 and related aspects with an emphasis on clinical management aspects.

Content

We describe the virological characteristics of SARS-CoV-2 and clinical course of COVID-19 with an emphasis on diagnostic challenges, duration of viral shedding, severity markers and current treatment options.

Implications

The key challenge in managing COVID-19 remains the patient density. However, accurate diagnoses as well as early identification and management of high-risk severe cases are important for many clinicians. For improved management of cases, there is a need to understand test probability of serology, qRT-PCR and radiological testing, and the efficacy of available treatment options that could be used in severe cases with a high risk of mortality.

Introduction

The first cases of atypical pneumonia of unidentified aetiology were reported on December 30, 2019, from Wuhan, China. By January 7, 2020, a novel betacoronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV-2) was identified, while the disease has been named COVID-19. COVID-19 has now been declared a pandemic, affected nearly every country, with over 2.3 million confirmed cases and >160,000 deaths. The initial clinical case series from China largely comprised of hospitalised patients with severe pneumonia. Further data suggested that approximately 80% patients have mild disease, 20% require hospital admission, and approximately 5% require intensive care admission [1]. Mortality rates are higher among people over 60 years and with coexisting conditions; hypertension, diabetes and cardiovascular disease being the most common. Here we provide an update for clinicians on the recent developments about virology, diagnostics, clinical presentation, and treatment options for COVID-19 based on current literature.

Virology

Metagenomic sequencing and targeted real-time polymerase chain reaction (qRT-PCR) assays identified a novel human CoV (SARS-CoV-2) in bronchoalveolar lavage fluid taken from the initial patient cluster in Wuhan [2]. Infectious SARS-CoV-2 has been cultured on monkey Vero, human Huh7 and primary human airway epithelial cells [3], where it is cytopathic. Furthermore, serum antibodies (IgM and IgG) from cases neutralized SARS-CoV-2 in cell culture and detected virus-infected cells by indirect immunofluorescence [3].

Phylogenetic analysis reveals that SARS-CoV-2 is closely related to SARS-CoV (~80% similar) in the *Sarbecovirus* sub-family (genus *Betacoronavirus*) [2]. While an intermediate host has yet to be determined, it shares strong genetic similarity (>95%) to known bat coronaviruses from China, suggesting a likely bat origin. Relatively similar coronaviruses have been found in pangolins whose receptor-binding domain (RBD) of Spike (S) glycoprotein is more like to SARS-2-CoV-2 than known bat viruses [4].

SARS-CoV-2 shares most of its gene content with SARS-CoV, including the S glycoprotein, the RNA-dependent RNA polymerase (Nsp12) and two proteases papain-like protease (PLpro) and 3C-like protease (3CLpro) [3]. There is also substantial antigenic cross-reactivity between SARS-CoV-2 and SARS-CoV [3, 5]. A recent study confirmed that the angiotensin-converting enzyme 2 (ACE2), expressed in the human respiratory tract epithelium, is the entry receptor for SARS-CoV-2 similar to SARS-CoV and has been shown to cause pneumonia in lab mice only expressing human ACE2 [6, 7]. This is likely mediated by the RBD of the S glycoprotein [8]. Although there is obvious homology between SARS-CoV and SARS-CoV-2, and cross neutralization has been observed [9], significant biological differences, specifically in the S glycoprotein have been noted [5, 10, 11].

Clinical presentation

A key difference between COVID-19 and seasonal influenza-associated pneumonia is the potential severity of disease even in young adults without comorbidities [12]. In a study that compared three well-conducted Chinese case series to a reference group of patients with influenza-associated pneumonia from 73 German sentinel hospitals, the severity of pneumonia even in adults aged <60 years without chronic preconditions was significantly greater in COVID-19. For instance, 28% of COVID-19 patients treated on the ICU had no reported comorbidity. The rate of ARDS and mechanical ventilation was markedly higher among COVID-19 patients. The median duration of ventilation was 9 days for non-invasive, and 17 days for invasive ventilation [12].

Across all studies, the most common symptoms at onset of illness were fever, cough, fatigue, and myalgia. However, available data suggest that only half of patients are febrile at the time of admission [13, 14]. Gastrointestinal symptoms, including anorexia, nausea, vomiting and diarrhoea are also common, reported in nearly 40% patients in some cohorts [15, 16]. Furthermore, up to 10% patients present with gastrointestinal symptoms without respiratory symptoms or fever [17]. COVID-19 has been associated with a hypercoagulable state with increased risk of venous thromboembolism[18]. Neurological manifestations, including headache, dizziness, altered consciousness, ischaemic and haemorrhagic strokes, as well as muscle injury, have also been reported [19]. A third of patients reported taste or olfactory disorders in a small Italian cohort, including anosmia [20]. Other extrapulmonary manifestations include skin and ocular manifestations. An Italian study reported cutaneous manifestations in 20% patients [21]. Lastly, ocular manifestations consistent with conjunctivitis was reported in 32% COVID patients in a Chinese case series [22].

The estimated mean incubation period is reported as 3-6 days (range 1.3-11.3) [12]. The duration from symptom onset to dyspnoea was 5-6 days [13, 17] On average, disease progresses further requiring hospitalisation at 7-8 days from symptom onset. Patients may initially appear relatively stable, but they often rapidly deteriorate with severe hypoxia [13, 17]. The key feature seen in these cases is acute respiratory distress syndrome (ARDS) [13, 17]. The interval from symptom onset to the development of ARDS is approximately 8-12 days [13]. In addition, the incidence of cardiovascular manifestations such as myocardial injury seems to be high, likely due to the systemic inflammatory response and immune system disorders during disease progression[23].

Illness severity and development of ARDS are associated with older age and underlying medical conditions [17]. Additionally, neutrophilia, raised lactate dehydrogenase and D-dimer, lymphocyte counts, CD3 and CD4 T-cell counts, AST, prealbumin, creatinine, glucose, low-density lipoprotein, serum ferritin, and prothrombin time were also associated with higher risk of severe disease and ARDS [17]. In a cohort of 191 patients with a definitive clinical outcome (137 discharged and 54 died), mortality was independently associated with older age, higher qSOFA score, d-dimer >1 μg/mL on admission, and the majority had severe disease and experienced complications, such as ARDS, acute kidney injury, and sepsis [13]. Factors most associated with critical illness were admission oxygen saturation <88%, first d-dimer>2500, first ferritin >2500, and first CRP >200 [24]. Furthermore, patients with cardiovascular disease were shown to be more likely to develop severe symptoms[23] in keeping with picture seen in MERS-CoV and SARS.

In comparison, most children appear to have mild disease. Among 1391 asymptomatic and symptomatic children (median age: 6.7 years) with known COVID19 contact in Wuhan Children's Hospital [25], 171 (12.3%) were SARS-CoV2-positive; 27 (15.8%) had no symptoms or radiologic features of pneumonia, 33 (19.3%) had upper respiratory symptoms, and 64.9% had pneumonia. Three patients (with coexisting conditions) required intensive care and 1 death.

In terms of co-infections, a pre-print examining >8000 samples of COVID-19 contacts tested for SARS-CoV2 in China reported viral co-infections in 5.8% of COVID-19 positive individuals (including seasonal coronaviruses, influenza A virus and rhinoviruses [26]. Another study of 1206 patients identified viral co-infection in 24 of 116 (21%) SARS-CoV2-positive patients; rhino/enterovirus, respiratory syncytial virus, and seasonal CoVs were most common [27]. Bacterial and fungal co-infections with SARS-CoV-2 have been documented especially in the ICU setting, including *Acinetobacter baumanii* and *Klebsiella pneumoniae* [28]. Among 191 patients, non-survivors were more likely to have sepsis based on qSOFA score and secondary infection, although detailed bacteriology results were not reported [13]. Secondary infection and positive association between steroid administration and secondary infection should be explored further.

Molecular and Serological Diagnosis

The first genome sequence for SARS-CoV-2 was released on virological.org on 10 January (GenBank accession number MN908947). This allowed the rapid development of several sensitive and specific qRT-PCR assays [29]. Many laboratories worldwide are now able to test for SARS-CoV-2. Assays have been described that detect <10 copies of SARS-CoV-2 per reaction and will not cross-react with SARS-CoV or other human coronaviruses [29]. However, sensitivity and specificity of these tests remain unknown and there is no clear consensus on which is preferred.

Viral RNA loads by qRT-PCR were substantially higher in sputum compared to throat swabs [3, 30, 31], suggesting that the type of sample may also influence the outcome of the test. Therefore, currently submission of both lower and upper respiratory tracts samples is advised.

Precise molecular detection is hampered by the variability in viral loads in the upper respiratory tract, especially at later stages of infection. In a study from China, among 241 COVID-19 patients with at least one positive SARS-CoV-2 qRT-PCR test result, in the first test, 384 (63.0%) were negative [32]. In addition, several tests at different points were variable from the same patients during the course of diagnosis and treatment.[32]. Therefore, a single positive test should be confirmed by a second qRT-PCR assay targeting a different SARS-CoV-2 gene. Although, similar studies in Taiwan and Hong Kong reported less false-negatives[33]. Secondly, a single negative SARS-CoV-2 test (especially if from upper respiratory tract specimen) or a positive test result for another respiratory pathogen result should not be used to exclude COVID-19 infection. These findings indicate that qRT-PCR has low probability of ruling out an infection and in clinically high suspicious cases repeat sampling and also CT images may need to be used to guide the diagnosis.

Antibody-based methods to detect seroconversion in serum or plasma based upon enzyme-linked immunosorbent assays (ELISA), indirect-immunofluorescence or virus neutralisation have been reported [34-36]. Around 40-50% patients develop an antibody response to SARS-CoV-2 infection after 7 days, and the majority by 14 days [35, 37]. S1 has been shown to be more specific than S as an antigen for SARS-CoV-2 in serological diagnosis [36]. The commercial S1 IgG and IgA assays have lower specificity but IgA showing higher sensitivity [36]. Recently, an ELISA assay based on detection of recombinant S protein by serum antibodies demonstrated robust and scalable determination of seroconversion that will facilitate screening of potential exposed individuals for evidence of past infection [38]. Since seroconversion occurs relatively late in infection, rapid antibody tests have a limited role in the diagnosis of acute infection; qRT-PCR remains the ‘gold standard’.

There is an ongoing work to understand protective antibody level and immunological marker. Among 175 recovered laboratory-confirmed COVID-19 patients, neutralizing antibodies (NAb) peaked at 10 to 15 days after disease onset. However, approximately 30% failed to develop good level of NAb titres (ID50: < 500)[39]. In addition, patients who did not generate NAbs at the time of discharge did not develop NAbs thereafter. These results highlight that some patients with SARS-CoV-2 would recover without developing high titers of virus-specific NAbs. These findings have some implications for vaccine development and also for convalescent plasma treatment as the donor plasma should be titrated before use in passive therapy. There is less information available on the T cell response during SARS-CoV-2 infection and how it correlates with the NAb titres.

Duration of viral shedding and isolation period

SARS-CoV-2 RNA has been identified by qRT-PCR in respiratory tract samples 1-2 days prior to symptom onset and can persist for 7-12 days in moderate cases, and up to 2 weeks in severe cases [35, 40]. SARS-CoV-2 has also been detected in whole blood [41], saliva [42], faeces [43], and urine [44] by qRT-PCR (Table 1).. In several case series with serial sampling, viral loads were highest soon after symptom onset [35, 45]. Patients with severe COVID-19 had significantly higher viral load and longer period of viral shedding than mild cases [46]. Prolonged viral RNA shedding has been reported from throat swabs up to 37 days among adult patients [13], and in faeces, for over one month after illness onset in children[40, 47]. However, detection of viral RNA by qRT-PCR does not necessarily equate with infectious virus. No live virus was cultured from 9 mild COVID-19 cases beyond day 8 after symptom onset in throat swabs or sputum despite ongoing high viral load [35]. Persistently high levels of RNA were also identified in the stool of the mild cases, but no live virus was cultured [35]. These findings suggest that patients may continue to shed RNA in various samples for a long period, but this does not equate to infectiousness potential (Table 1). This supports the current guidance of 7-14 days self-isolation from symptom onset. Certain hospitals following a protocol to confirm viral clearance prior to transfer out of dedicated COVID-19 wards, however, this may not be required given the prolonged RNA shedding without the evidence of viable virus. However, whether faecal-oral or faecal-respiratory transmission occurs, and the role of shedding in severe cases in transmission requires further exploration.

Transmission patterns

A review of modelling studies based on Chinese case numbers report a median basic reproduction number (R_0) of 2.79 [48], though R_0 as high as 5.7 have been reported [49].These estimates are substantially higher than the reproduction number for seasonal influenza (~1.3) [50], and indicate that control measures would need to prevent >60% transmission to stop the epidemic. Of note, R_0 will vary by setting, and can be substantially reduced by countermeasures, as have been observed in China [51].

It is now clear that a significant proportion of individuals with COVID-19 have very mild or no symptoms. Asymptomatic infection at the time of laboratory testing have been reported [52, 53], though a large proportion go on to develop symptoms. For instance, among 55 asymptomatic carriers with positive qRT-PCR for SARS-CoV-2 in pharyngeal swab samples, 14 went on to develop mild, 39 ordinary, and 2 severe COVID-19 [54]. There have been several reports of SARS-CoV-2 transmission from asymptomatic or presymptomatic persons [55, 56], which poses significant challenges to contact tracing. Nevertheless, the relative contribution of asymptomatic or pre-symptomatic transmission on the overall transmission dynamics of the pandemic remains uncertain. Thus, household studies to study secondary human transmission of SARS-CoV-2 and serosurveys to determine the incidence of asymptomatic and subclinical infections are needed.

A further consideration is superspreading events, whereby a small number of cases are responsible for a disproportionate number of secondary cases. This was a feature of both SARS- and MERS-CoV, responsible for multiple nosocomial outbreaks [57, 58]. Several superspreading events has been reported for COVID-19 [17]. Rapid identification and mitigation of these events will be crucial to controlling this pandemic.

Treatment options in clinical trials

At present, there are no approved antivirals for SARS-CoV-2. Several antivirals that have shown promise against SARS- or MERS-CoV in vitro and in vivo are currently being evaluated in clinical trials for COVID-19. Lopinavir/ritonavir (LPV/r), a protease inhibitor used as an antiretroviral, showed inconclusive findings for the treatment of SARS, but demonstrated strong *in vitro* and *in vivo* antiviral activity against MERS-CoV when combined with interferon-beta (IFNb) [59]. The first of a number of clinical trials involving LPV/r was recently published [60]. Among 199 seriously ill laboratory-confirmed COVID-19 patients, no significant difference in clinical improvement, mortality or viral clearance was observed between LPV/r (n=99) and standard care (n=100) arms. However, treatment was instituted late in infection; median time from symptom onset to treatment was 13 days, and >40% of patients had undetectable viral load before or during treatment. The results were complicated by the variable use of other treatments, including interferon, glucocorticoids and antibiotics. Of note, day 28 mortality was lower (not significantly) in those with early treatment (19% vs. 27%) and those who received LPV/r also had lower vasopressor and non-invasive ventilation use. Another promising drug is remdesivir, a novel nucleotide analogue that interferes with nsp12 polymerase [61]. It has shown *in vitro* activity against a wide range of RNA viruses including SARS and MERS-CoV [62, 63], and has also demonstrated superior antiviral activity compared to LPV/r-IFNb against MERS-CoV in a mouse model [59]. Against SARS-CoV-2, it has shown promising antiviral activity in Vero E6 cells and Huh7 cells [64]. Remdesivir has been given to a small number of patients with severe COVID-19 through compassionate use, however, given the lack of randomisation and control group interpretation of the findings is difficult [65]. There are ongoing RCTs assessing its efficacy and safety in patients with COVID-19 worldwide, and a study in France evaluating its impact on viral shedding in high and moderate risk contacts in confirmed COVID-19 cases (NCT04259892).

Other candidate antivirals are studied in RCTs, including favipiravir and hydroxychloroquine, which has been shown to inhibit virus cell entry in vitro [66]. Hydroxychloroquine (HCQ), an analogue of chloroquine, has demonstrated anti-SARS-CoV-2 activity *in vitro* [67]. Among a small open-label non-randomised study, patients treated with HCQ and HCQ + Azithromycin showed viral load reduction compared to controls. However, there has been significant concerns and ethical issues about the content, the ethical approval of the trial and the peer review process prior to publication raised by several physicians and also the International Society of Antimicrobial Chemotherapy. In a small RCT of HCQ (n=30), there was no change in viral load or clinical outcome after 7 days [68]. Currently, there are 45 trials evaluating chloroquine or HCQ for the treatment and prophylaxis of COVID-19, including multi-centre RCTs in the UK (RECOVERY, ISRCTN50189673), Europe (DisCoVeRy, NCT04315948) and also globally involving >70 countries (SOLIDARITY, ISRCTN83971151).

Host-targeted therapeutic options are also being explored, such as inhibition of human cytokine interleukin-6 (IL-6), the abundance of which has been associated with worse prognosis [69]. A preprint including 21 patients that received Tocilizumab (an IL-6 receptor inhibitor) reported improvement in symptoms, hypoxaemia and CT changes in the majority of patients[70]. There are ongoing RCTs evaluating tocilizumab and sarilumab, also an IL-6 receptor inhibitor. With insufficient evidence of efficacy for any existing treatments, the IDSA recommends that experimental therapies should only be offered to patients in the context of a clinical trial [71].

There is no licenced vaccine to protect against COVID-19. However, a number of experimental candidates are in development with some already in early clinical trials. Most vaccine candidates focus on immunisation with only the S glycoprotein, which is the major target for neutralization antibodies. Candidate vaccines differ in the mode of S delivery and platforms dependent on recombinant protein, mRNA or viral vectored approaches are being tested. Passive immunisation through transfusion of convalescent sera or plasma containing neutralizing antibodies from recovered donors have been reported in several case series, with clinical improvement reported in recipients [72, 73]. Clinical trials evaluating convalescent plasma as treatment for severe COVID-19 are ongoing.

Conclusion

A wealth of data has been generated already on COVID-19 since early January 2020. Nevertheless, key questions remain regarding understanding the population at risk and age groups, proportion of individuals that have had asymptomatic infections and their transmission potential, endemicity and seasonality of COVID-19, and whether stringent physical distancing measures will be effective in countries outside China. The main challenge in managing COVID19 remains the patient density, however, accurate diagnoses as well as early identification and management of high-risk severe cases remains a daily battle for many clinicians. For improved management of cases, there is a need to understand test probability of serology, qRT-PCR and radiological testing, and the efficacy of available treatment options that could be used in severe cases with high risk of mortality.

Authors contributions

MC, CM, AH drafted the first and subsequent versions of the manuscript, and all authors provided critical feedback and contributed to the manuscript.

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Conflicts of interest

MC, CB and AT has nothing to disclose.

Table 1: Transmission routes

Source	Mode of transmission	RNA by PCR (Days since onset of symptoms)	Viable virus (Days since onset of symptoms)
Nasopharynx	Droplet	Up to 37 days	Up to 7 days (in mild cases)
Sputum	Droplet / airborne during aerosolize-producing procedures	Up to 37 days	Up to 7 days (in mild cases)
Stool	No evidence of faecal-oral transmission	> 30 days	Only 1 report; uncertain
Blood	No viable virus to date	Up to 14 days	No
Urine	No viable virus to date	No	No
Conjunctiva	No viable virus to date Macaques with corneal inoculation develop infection	Yes	No
Vertical	No strong evidence of vertical transmission to date	No	N/A

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Table 1: Transmission routes

Source	Mode of transmission	RNA by PCR (Days since onset of symptoms)	Viable virus (Days since onset of symptoms)
Nasopharynx	Droplet	Up to 37 days	Up to 7 days (in mild cases)
Sputum	Droplet / airborne during aerosolize-producing procedures	Up to 37 days	Up to 7 days (in mild cases)
Stool	No evidence of faecal-oral transmission	> 30 days	Only 1 report; uncertain
Blood	No viable virus to date	Up to 14 days	No
Urine	No viable virus to date	No	No
Conjunctiva	No viable virus to date Macaques with corneal inoculation develop infection	Yes	No
Vertical	No strong evidence of vertical transmission to date	No	N/A