Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chapter 30

Dethroning the crown. From the kinetics and dynamics of COVID−19 diagnosis to promising treatments

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30.1 Introduction

In early January 2020, a novel coronavirus was identified as the cause of a cluster of pneumonia cases in Wuhan, Hubei China. This virus, later named SARS−CoV−2 (severe acute respiratory syndrome coronavirus 2), created a pandemic, which has become a great stressor for the modern socioeconomic structure testing its strength and reflexes. National health systems are struggling to find their way through this crisis by intensively working on COVID−19 (corona virus disease 19) research and drug development (Tsatsakis et al., 2020a, 2020b) while enforcing strict restrictions to their citizens in order to halt its progression. Therefore a mainstay in understanding this novel disease is to investigate key aspects of effective diagnosis and treatment. In fact, SARS−CoV−2 exhibits special properties regarding the kinetics and dynamics for its diagnosis and treatment.

30.2 Epidemiology

In order to thoroughly understand COVID−19 a brief overview of its epidemiological behavior is necessary. Globally, over 87.5 million confirmed cases of COVID−19 have been reported with 1.89 million deaths. Based on regional and national reports all regions and geographic areas have been infected apart from Antarctica (Kujawski et al., 2020). However, serosurveillance studies have proved that the reported case counts tend to underestimate the actual burden of COVID−19, as only a fraction of actual infections are diagnosed and reported. In fact, such studies report the rate of prior exposure to SARS−CoV−2 exceeding the incidence of reported cases by approximately 10-fold. Table 30.1 presents the various symptoms related to COVID−19.

30.3 Transmission

Even though at the beginning of the Wuhan epidemic it was believed that exposure to contaminated seafood and material was the main route of transmission, it soon became clear that person-to-person transmission is the principal one (Struyf et al., 2020). Direct person-to-person transmission largely implies inhalation of droplets containing the virus resulting in respiratory infection. Generally, heavy respiratory droplets (like those produced while talking) can only reach approximately a meter from the person who exhaled them while smaller ones can even reach two meters or more if exhaled with force (as seen in coughs, sneezes) (Stancioiu et al., 2020). Moreover, heavier droplets tend to fall sooner than smaller ones that tend to persist in ambient air longer (Farsalinos et al., 2020). This implies that in order for a person to get infected, prolonged exposure to a patient, especially at a short distance, and without proper personal protective measures (such as appropriate face mask) is needed or prolonged stay in poorly ventilated closed and crowded spaces. Infection might also occur through hand-to-mouth and hand-to-eye routes (Medline ® Abstract for Reference 54 of Coronavirus disease 2019 (COVID-19): Management in hospitalized adults—UpToDate, no date).

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TABLE 30.1 Stratification of the various symptoms related to COVID−19.

Symptoms that rise clinical suspicion for COVID−19	
• Fever (>38°C)	Most common symptoms
• Dry Cough	
• Fatigue	
• Anosmia or other smell abnormalities	Less common symptoms
• Ageusia or other taste abnormalities	
• Sore throat	
• Myalgias	
• Chills/rigors	
• Headache	
• Rhinorrhea and/or nasal congestion	
• Nausea/vomiting	
• Diarrhea	
• Dyspnea (new or worsening over baseline)	Most serious symptoms
• Chest pain or pressure	

Period of transmission—The precise interval during which an individual with COVID−19 can transmit SARS−CoV−2 to others is uncertain. However, as with other viral infections, it is expected to begin prior to the development of symptoms and reach its peak early in the course of the disease. Moreover, transmission after the 7th to 10th day after symptom initiation is unlikely (Singanayagam et al., 2020)

Period of highest transmission—Patients are more likely to be infectious during the first few days of the disease. At that time it is exhibited that SARS−CoV−2 RNA levels are at their highest especially from upper respiratory specimens.

Asymptomatic transmission—Transmission of SARS−CoV−2 from individuals with infection but no disease (no clinical infection) is controversial (Cao et al., 2020). It has been reported that viral RNA levels and duration of persistence in the upper respiratory tract specimens of asymptomatic patients may be similar to those of symptomatic ones. However, there is a growing body of evidence stating that asymptomatic patients have lower viral load and therefore are less likely to transmit the virus (Cao et al., 2020; Singanayagam et al., 2020). Nevertheless, the actual extent to which asymptomatic may transmit the virus and thus contribute to the pandemic remains unknown.

Relation between prolonged SARS−CoV−2 RNA detection and potential for transmission—The duration of viral RNA excretion is found to fluctuate among patients but is generally longer in older and in cases with severe illness. Detectable SARS−CoV−2 RNA, however, should not necessarily be correlated with the potential for transmission. However, an exact threshold of viral RNA level over which a patient may still be contagious has yet to be found. Interestingly though, there are cases where SARS−CoV−2 was isolated from respiratory or fecal specimens more than 10 days following symptom onset, an event mostly seen in patients with severe COVID−19.

30.4 Diagnosing SARS−CoV−2 infection

COVID−19 diagnosis has become great challenge given the similarity of the symptoms proclaimed with other viral respiratory infections. National and local authorities have endorsed COVID−19 testing both for symptomatic and asymptomatic individuals who fulfill certain criteria and for public health or infection control purposes.

Timing of COVID-19 testing—In patients presenting COVID−19 symptoms, especially those with high fever, differential diagnosis should include SARS−CoV−2 infection. Moreover, clinical suspicion should be risen in cases of severe lower respiratory tract infection (Long et al., 2020). The possibility of SARS−CoV−2 infection should also be taken into account in patients who reside in or have traveled within the prior 14 days to a location where there is

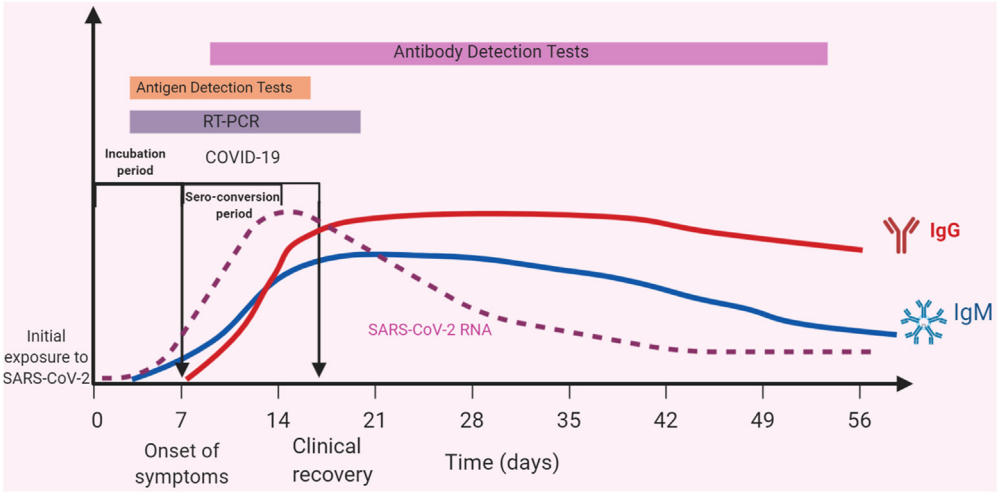

FIGURE 30.1 Graphic representation of the kinetics and dynamics of immune response and the suggested period of use for each form of SARS−CoV−2 testing.

community transmission of SARS−CoV−2 or report close contact with a confirmed or suspected case of COVID−19 during the last two weeks. In general, a close contact is considered any type of contact within approximately two meters from the patient for more than a few minutes while not wearing personal protective equipment or having direct contact with infectious secretions unprotected. While respiratory symptoms are the principal ones, a number of patients present extrapulmonary complications that have been associated with SARS−CoV−2 infection such as acute cardiac injury, ischemic stroke, and other thromboembolic events and inflammatory complications. The optimal time to test an individual for SARS−CoV−2 is largely unclear. Nonetheless, based on the data collected at the early stages of the pandemic, an individual should be tested 5−7 days after a confirmed exposure. Fig. 30.1 presents the suggested kinetics and dynamics of diagnosing SARS−CoV−2 in relation to the host's immune response.

Whom to test for COVID−19—Ideally all patients presenting with respiratory tract infection should be tested for COVID−19. However, given the limited capacity of diagnostic kits (as evidenced at the very beginning of the pandemic in numerous countries), testing has to be narrowed to those patients who are either more likely to be infected (high clinical suspicion) or those who work as healthcare workers, those who reside in congregate living settings, and those considered as frail. However, there are instances when selective testing among asymptomatic individuals may also be needed for public health or infection control purposes. A promising application of asymptomatic testing can be found in mass transportation and participation in condenced settings such as working environments and leisure. In such cases, a recent negative antigen test will be a prerequisite. Moreover, owing to the widespread of antigen testing platforms in a reasonable price, more asymptomatic people are expected to be tested. Fig. 30.2 is a schematic representation of the suggested groups to be tested.

Testing modalities for COVID-19—Even though intensive effort has been made to identify and validate affordable and easy to use diagnostic tests for SARS−CoV−2, those currently in use are nucleic acid amplification tests (NAAT), mostly represented by reverse-transcription polymerase chain reaction (RT−PCR) assay to detect SARS−CoV−2 RNA, SARS−CoV−2 antigen testing, and serologic tests to detect antibodies against SARS−CoV−2 (Lee et al., 2020) (Fig. 30.3). Occasionally, given the higher expenses of RT−PCR, antigen testing may be the first test to be used followed by RT−PCR if indicated. In the United States, the current recommendation for specimen testing dictates testing from the upper respiratory tract by nasopharyngeal swab specimen; nasal swab specimen from both anterior nares (using a flocked or spun polyester swab); nasal midturbinate swab (using a flocked taper swab); nasal or nasopharyngeal wash/aspirate; oropharyngeal swab; and saliva specimen (1−5 mL) (Tu et al., 2020; Wang et al., 2020). Collectively, the aforementioned specimens are tested with RT−PCR for SARS−CoV−2 RNA. A detailed presentation of the various tests and their special characteristics is presented in Table 30.2. However, it is unclear which type of upper respiratory tract specimen presents optimal results (both sensitivity and specificity) with oropharyngeal specimens exhibiting the less favorable results (Wong et al., 2020). In cases of patients suffering from lower respiratory tract infections, expectorated sputum should be collected while tracheal aspirate or bronchoalveolar lavage would be an alternative solution for intubated ones.

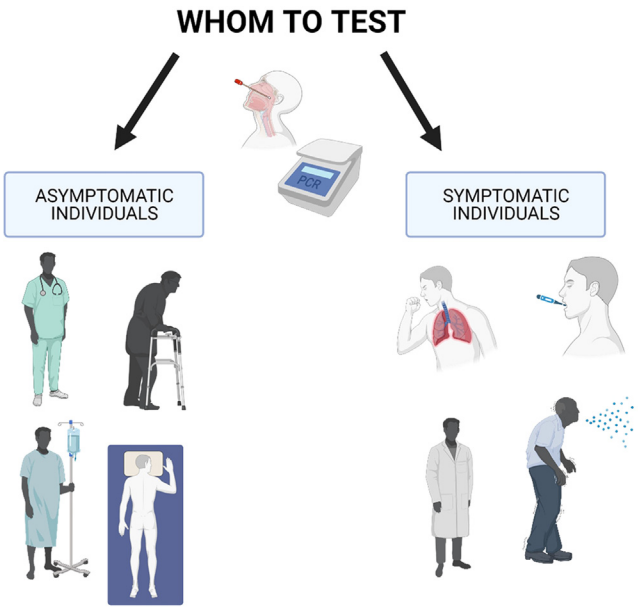

FIGURE 30.2 According to the latest guidelines it is suggested that given the limited capacity of diagnostic kits, testing has to be focused on symptomatic patients (especially those with high clinical suspicion) and asymptomatic individuals on high risk such as healthcare workers, those who reside in congregate living settings, those considered as frail, and those who are going to undergo an operation.

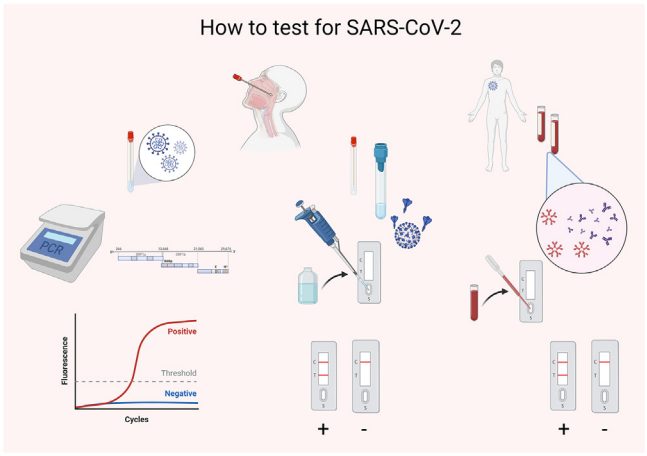

FIGURE 30.3 Based on the types of diagnostic kits that have been approved for use for COVID−19, an individual can be tested for SARS−CoV−2 infection either with a specimen from his upper respiratory tract (e.g., nasopharyngeal swab) with a blood sample in which there are anti-SARS−CoV−2 antibodies. Therefore viral antigen, viral RNA, and IgM/IgG presence can be detected.

RT−PCR result interpretation—Given the high specificity of RT−PCR assay, a result for SARS−CoV−2 generally confirms the presence of the virus. Thus there is no need for additional testing. Nonetheless, there may be indications when additional testing may be necessary in order to guide epidemiologic management (Fang et al., 2020). Such occasions may be healthcare professionals for whom a negative result is necessary to consider them safe to work (Yu et al., 2020). On the contrary, patients with COVID−19, especially those who suffered from a severe form of the disease, may have detectable SARS−CoV−2 RNA in upper respiratory tract specimens even weeks after symptoms initiation. However, this finding should be considered under the prism of the overall clinical status of patients in order to determine whether prolonged viral RNA detection represents an ongoing infection. On the contrary, for most asymptomatic individuals or those patients with low clinical suspicion of COVID−19, a single negative RT−PCR result is sufficient to exclude COVID−19 diagnosis. However, in cases with high clinical suspicion of SARS−CoV−2 infection, if initial testing returns negative, many local guidelines suggest empiric treatment as for COVID−19 and subsequent repeat of the test. Even though there is no common agreement regarding the optimal time window for repeating the testing, it is generally accepted that 24−48 hours after the initial test are enough but not earlier than that. For epidemiological purposes, in cases of negative test but with a rather high clinical suspicion, serology testing is an alternative. In detail, 3−4 weeks after the onset of symptoms and given that the patient has fully recovered, a positive IgG test would be suggestive of prior COVID−19 whereas a negative test could suggest a decreased likelihood but cannot exclude the diagnosis

TABLE 30.2 Presentation of the various categories of clinical tests for COVID−19 diagnosis and their special characteristics.

Type of testing	Clinical utility	Type of specimen	Special characteristics	Need for trained personnel
RT−PCR	Diagnosis of ongoing infection	Nasopharyngeal swab	• High sensitivity and specificity in ideal settings • Clinical value range based uppon the type, quality of the specimen, and the duration of illness at the time of testing	Yes
		Nasal swab specimen from both anterior nares		No
		Nasal midturbinate swab		No
		Nasal or nasopharyngeal wash/aspirate		Yes
		Oropharyngeal swab		Yes
		Saliva		No
Specific antibodies detection	Diagnosis of prior infection (epidemiology)	Blood	• Sensitivity and specificity vary considerably • Detectable antibody titers may take several days to develop • Chance for cross−reactivity with other coronaviruses	Yes
Antigen tests	Diagnosis of ongoing infection	Nasopharyngeal or nasal swabs	Less sensitive than RT−PCR	Depending on the specimen

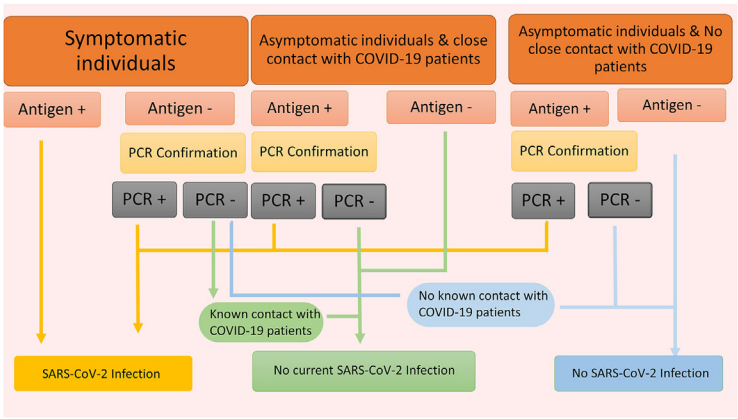

FIGURE 30.4 Graphic presentation of the current algorithm suggested for testing against SARS−CoV−2.

(He et al., 2020). However, the reliability of the serologic test result depends on the specific assay and the duration of illness. Finally, as with any test, RT−PCR may has a gray zone (indeterminate result). In such cases, good communication and sharing of clinical information between the clinician and laboratory staff in order to better interpret the result. An illustrated algorithm for SARS−CoV−2 testing and interpretation is presented in Fig. 30.4. Interestingly, Singanayagam et al. proved that there is a strong relationship between RT−PCR's cycle threshold (Ct) value (which represents a semiquantitative measure of SARS−CoV−2 viral load) and ability to harvest alive and infectious virus by infecting cell cultures. The estimated OR of harvesting infectious virus decreased by 0.67 for each unit increase in Ct value (95% CI: 0.58−0.77). The estimated probability of viral yield from samples with Ct > 35 was 8.3% (95% CI: 2.8%−18.4%). Thus a Ct value around 35 could potentially serve as a threshold for determining positivity of RT−PCR testing. In addition, detection of cultivable (infectious) viral shedding peaked around symptom onset. The culture

positivity rate was significantly higher during week 1 than week 2 (74% vs 20%; $p = 0.002$). Ten days after symptom onset, the probability of culturing virus declined to 6.0% (95% CI: 0.9%–31.2%) (Singanayagam et al., 2020).

RT–PCR Accuracy—Despite the fact that accuracy and predictive values of SARS–CoV–2 RT–PCR assays have not been systematically evaluated they are generally considered highly specific tests. However, it has been proven that there may be a considerable lack of specificity as their clinical performance is more variable. In fact, it is estimated that in clinical settings false-negative rates may range from less than 5% to 40% depending on the type and quality of the specimen obtained, the duration of illness at the time of testing, and the specific assay used (McCulloch et al., 2020).

RT–PCR performance by specimen type—Preliminary data indicate that in general lower respiratory tract specimens may have higher viral loads and thus be more suitable for diagnosis than upper respiratory tract specimens. On the other hand, studies focusing on the upper respiratory specimens indicate that viral RNA concentration may be higher in nasal specimens compared to oropharyngeal ones. In order to facilitate reduction of viral spread among healthcare professionals, self-collected saliva specimens by patients have also been evaluated exhibiting comparable sensitivity with nasopharyngeal ones harvested by professionals (He et al., 2020).

RT–PCR performance by illness duration—An increasing body of evidence indicates that the likelihood of detecting SARS–CoV–2 RNA depends widely on the duration of disease (time since symptom onset). During the first day of symptom onset detecting the virus is rather impossible, but viral RNA detection rate increases during the first 5–7 days and then decreases but not necessarily vanishes during the coming 14–20 days (Kucirka et al., 2020). Unfortunately, it is unknown how soon viral RNA can be detectable following exposure to SARS–CoV–2 (Wölfel et al., 2020).

Alternative testing—Even though RT–PCR, as shown earlier, is considered as the gold standard for COVID–19 diagnosis, local authorities and health organizations have chosen to use SARS–CoV–2 antigen detection as an alternative solution (in outbreak settings and for screening purposes among high-risk populations), and in many cases as the first test to be used again within the first 5–7 days from the onset of symptoms. This is because antigen detection has the advantages of time and cost efficiency while being able to analyze it at the point of care. However, clinicians should bear in mind that antigen tests typically lack sensitivity resulting to a considerable rate of false-negative results and thus a negative result cannot rule out SARS–CoV–2 infection. Therefore in cases where clinical suspicion is high and the antigen test returns negative RT–PCR should also be performed. A comparative study among various antigen tests available in the United States showed that the sensitivity was highly variable ranging from 0% to 94% with an average of 56.2%. Nonetheless, sensitivity was increased (approximately to 93%) as the viral load increased while the average specificity was kept at a 99.5% (Paltiel, Zheng and Walensky, 2020). Another alternative to RT–PCR testing but with different intent is serology testing (Tostmann et al., 2020). In this case, detection of IgG, IgM, and IgA antibodies in blood samples against SARS–CoV–2 is the main goal in order to verify current or prior infection. However, antibodies may need several days, up to 4 weeks, to reach a detectable titer and for this reason they are not used for diagnosis (Makaronidis et al., 2020). What is more, it is also shown that antibody identification should better be performed 3–4 weeks after the onset of symptoms but the time frame cannot exceed the 5th week. In detail, serologic studies have shown IgM detection in 23% at the first week, 58% at the second week, and 75% at the third week. For the same time points, detection rates for IgG were 30%, 66%, and 88%, respectively. A rather interesting point is that long-term immunologic memory lasts for about 3–5 months after infection after which antibodies are no longer detectable.

30.5 Treating SARS–CoV–2 infection

Treatment of COVID–19 emphasizes three pillars: (1) empiric therapy against other microbes that may coinfect the patient (influenza, bacterial pneumonia); (2) prevention of venous thromboembolism; and (3) administration of COVID–19 specific therapy (Aristides Tsatsakis et al., 2020). Given that empiric therapy for other infections varies among each country and season this chapter will focus only on anticoagulation therapy and anti-SARS–CoV–2 treatment (Siemieniuk et al., 2020; Torequl Islam et al., 2020).

COVID–19 associated coagulopathy—Even though the exact pathophysiology of hypercoagulability in COVID–19 is not very well understood, current guidelines suggest COVID–19 detection in cases of unexplained coagulopathy or acute cardiac events. Generally coagulation may be approached by Virchow's triad (endothelial injury, stasis, and hypercoagulable state). Under this approach, COVID–19 patients seem to suffer mostly from hypercoagulability and thus present venous thromboembolic events (VTE), including extensive deep vein thrombosis (DVT) and pulmonary embolism (PE) (an event documented in up to one-third of patients in ICU). Even though venous thrombosis is the most common side effect, arterial thrombosis may also occur resulting in cardiac ischemic events, stroke, and ischemia of the extremities. In the case of hospitalized patients, typical laboratory examination should include complete blood

count (CBC) with special focus on platelets count, prothrombin time (PT) and activated partial thromboplastin time (aPTT), fibrinogen count, and D-dimer levels. The current practice of prevention and therapy of thrombotic events include low-molecular-weight heparin, unfractionated heparin (classical heparin), and tissue plasminogen activator (tPA) according to the therapeutic protocols each institution follows (Thachil et al., 2020).

COVID−19 specific therapy—Treating SARS−CoV−2 is still a matter of debate given that there is no specific treatment yet. The current practice of SARS−CoV−2 treatment is based greatly on symptomatic care with critical care support applied when heamodynamic, respiratory, or renal insufficiency is documented. Nonetheless, intensive research has led to several antiviral agents targeting the inhibition of viral entry (via the ACE2 receptor and TMPRSS2), viral membrane fusion and endocytosis or the activity of the SARS−CoV−2 3CL protease and the RNA-dependent RNA polymerase (Nitulescu et al., 2020). Moreover, agents that modulate the immune response such as human blood-derived products and immunomodulatory therapies are also being explored as adjunctive treatments for the management of moderate to critical infection. In addition to the antiviral medications and the immune-based therapies administration of antithrombotic therapy, vitamins C and D, and zinc is suggested (Calina et al., 2020; Skalny et al., 2020). A summary of the suggested therapeutic approaches is presented in Table 30.3. Main therapies used in Covid−19 to date are the following:

1. Remdesivir is the only antiviral agent that has been granted for use in hospitalized patients with severe COVID−19. Remdesivir is a nucleotide analog that interferes with viral RNA-dependent RNA polymerase. In the United States,

TABLE 30.3 Summary of the current guidelines for COVID−19 therapy as presented by the National Institutes of Health (United States).

Specific Therapies for COVID−19 (COVID−19 Treatment Guidelines, no date)

Drug name	Adverse effects	Recommendation for use
Chloroquine	• Cardiac arrhythmias Gastrointestinal effects • Hepatitis • Hypoglycemia • Hemolysis (especially in patients with G6PD deficiency) • Myopathy • Rash	Against (strong recommendation)
Hydroxychloroquine	Same with Chloroquine	Against (strong recommendation)
Lopinavir/Ritonavir	• Gastrointestinal effects • Transaminase elevation • Cardiac arrhythmias	Against (strong recommendation)
Ivermectin		Against (strong recommendation)
Remdesivir	• Nausea • ALT and AST elevations • Hypersensitivity • Increases in prothrombin time • Renal toxicity	Approved for hospitalized COVID−19 patients with low needs for O2 delivery
Dexamethasone		Approved for hospitalized COVID−19 patients with high needs for O2 delivery (plus remdesivir)
Non-SARS−CoV−2-specific intravenous immunoglobulin		Against (strong recommendation)
Mesenchymal stem cells		Against (strong recommendation)
SARS−CoV−2) immunoglobulins		Recommendation for hospitalized patients
COVID−19 convalescent plasma		Recommendation for hospitalized patients
Vitamin C		Recommendation for out patients
Vitamin D		Recommendation for out patients
Zinc		Recommendation for out patients

the Food and Drug Administration (FDA) has approved remdesivir for hospitalized children ≥12 years and adults with COVID−19, regardless of disease severity. However, better clinical results are exhibited among patients with severe disease who do not require high-flow oxygen supplementation or ventilation. Among hospitalized patients with nonsevere disease, remdesivir may have only a modest benefit, which is yet to be fully identified (Beigel et al., 2020).

2. Convalescent plasma administration is another promising therapy for hospitalized patients with COVID−19. It is harvested from individuals who have recovered from COVID−19. Convalescent plasma provides passive antibody-based immunity. The main idea behind convalescent plasma administration is the opportunity to aid patients' immune system with preformed neutralizing antibodies against SARS−CoV−2 while other immune mediators may also play a role. It is reported that when given early in the course of disease (i.e., in patients who do not require mechanical intubation) will have better results (Li et al., 2020).
3. Monoclonal antibodies that have been developed to neutralize SARS−CoV−2 by targeting the SARS−CoV−2 spike protein and preventing viral cell entry are also a promising treatment. However, given the preliminary phase of these trials, such a therapy cannot be suggested yet (Chen et al., 2020).
4. Lastly, immunomodifying therapy may also be a choice especially for severely ill patients with COVID−19. In that case, dexamethasone or other glucocorticoids (e.g., hydrocortisone, methylprednisolone, or prednisone) may be used (Angus et al., 2020; Group, 2020). However, it should be made clear that immunomodifying therapy is not recommended for either prevention or treatment of mild to moderate COVID−19 (patients not needing oxygen supplementation) (Sterne et al., 2020).

30.6 Conclusion

The COVID−19 pandemic has become an undisputable stressor of the global community. Unfortunately, despite the enormous death toll, intensive research has made only little progress on unveiling the pathophysiology related with the disease and the virus per se. Therefore finding the missing parts of this pathophysiology puzzle may allow sooner and safer recovery from this pandemic. Such missing parts are understanding of the transmission patterns, identification of potential vectors, proper and reliable diagnosis and finally effective treatment. Regarding the transmission patterns, it is relative safe to say that the majority of COVID−19 cases are caused from close interpersonal contact. This is because, while at the first steps of the pandemic domestic animals and after them young or asymptomatic individuals were considered to be to main vectors of SARS−CoV−2, it seems that this may not be the case. It is rather shown that prosymptomatic and symptomatic patients who poorly comply with personal protection measures, are those who transmit the virus. In addition, in order to restrict transmission, in-depth understanding of the various means of diagnosis is also needed in order to take proper measures against false-positive and more importantly false-negative test results. Understanding the inherited strengths and weaknesses of each test, policy and opinion makers will be able to indorse effective protective measures and allow for access in proper treatment those individuals who are most likely to benefit from it. Finally, it is important to underline the great potential provided by the COVID−19 pandemic regarding the great advances on drug and vaccine innovation which will not only benefit COVID−19 patients but also future non-COVID−19 patients.

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