

## Review of COVID-19 diagnosis and future treatment strategies

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**Abstract:** In 2019, COVID-19 had taken place in Wuhan, China, and then cause a global pandemic. In March 2020, the World Health Organization (WHO) declared COVID-19 as pandemic. This disease is caused by a novel coronavirus SARS-CoV-2 belonging to Coronaviridae family. The rapid spreading disease calls for fast and reliable diagnosis tools and effective treatment. Here in this review, we evaluated the most widely used diagnosis tools including reverse transcribed PCR and radiographic imaging. Current treatment approaches have also been discussed. Two major novel therapeutics targeting SARS-CoV-2 with ongoing clinical trials - PF07321332 and Molnupiravir - have been discussed in detail. A rapid spread and alarming data about COVID-19 accumulating and causing death on a daily basis call for fast and reliable diagnostic tools and timely treatment. One of the best diagnostic methods is reverse transcription polymerase chain reaction (RT-PCR). An imaging technique such as CT and X-ray is a supplementary tool for confirming the infection based on the changes in images. As a result of the urgency of pandemic control, various studies have been carried out to develop specific therapeutics targeting SARS-CoV-2. It has been applied with limited success to repurpose several antiviral drugs. Molnupiravir and PF07321332, two recently developed therapeutics, are undergoing clinical trials showing promising antiviral effects.

### 1. Introduction

The outbreak of COVID-19 has swept the world for almost two years. The virus causing this disease is SARS-CoV-2, one of RNA-based virus classified as coronavirus. The World Health Organization (WHO) declared a pandemic caused by SARS-CoV-2 [1]. The clinical manifestation can be grouped into asymptomatic or symptomatic. The latter ones can vary from mild unspecified symptoms such as dry cough, headache, fever and fatigue to severe even critical symptoms including acute respiratory syndrome and multiorgan failure [2-4]. This health care emergency requires us to equip with sensitive, reliable, fast and cost-effective diagnostic tool to detect the disease as soon as possible. Meanwhile, effective antiviral therapeutics have always been the focus for scientific research with the expectation to minimize virus' destructive impact.

#### 1.1 Diagnosis and RT-PCR findings

RT-PCR is a method widely applied to detect virus. SARS-CoV-2 can be detected using RT-PCR technology, which firstly reverse transcribe RNA into deoxyribonucleic acid (DNA) and undergo replication until the genetic material becomes detectable [5].

RT-PCR provide sensitive solution to diagnose COVID-19. There are studies that have included 454 examples, and there are 60 (13.2%) positive examples, and 394 (86.8%) were negative for SARS-CoV-2 RNA by RT-PCR exam. Testing the research facility in hypothetical COVID-19 cases and contact with an individual between 0 and 14 days, with an average of 3 days. The quick test of SARS-CoV-2 involved 98.33% (95% CI, 91.06-99.96%) for assignability and 98.73% (95% CI, 97.06-99.59%) for specificity. A phony negative test comes from an example of a continuous RT-PCR cycle, and five positives come from the patient's example before the activity [6, 7].

At the same time, it is essential to collect sample from the tissue with relatively large amount of raw material while applying RT-PCR. Comparing to pick the sample from bronchoalveolar lavage liquid, sputum, or nasal by swabs, the accuracy and sensitivity of RT-PCR in detecting SARS-CoV-2 is a lot of lower when sample was collected from another region like stool [8].

## 1.2 Radiographic Findings and Other Imaging Studies\

Despite infection of SARS-CoV-2 being determined microbiologically, imaging techniques has played a crucial role in assessing the severity of the infection, directing treatment, distinguishing complications, and analyzing the response to the treatment. Chest X-rays are the primary imaging method as they are easily accessible and prudent, regardless of whether they are acquired with regular X-ray suites or with convenient units. Regarding efficiency in confirming COVID-19 infection, chest X-rays are often not conclusive as early-stage diseases do not present striking changes. Along with progress of the disease, infected cases would show bilateral multifocal alveolar opacities [9].

Computed tomography (CT) check is more sensitive than X-ray which enables CT as an ideal choice to diagnose SARS-CoV-2 caused pneumonia, even at early stages of the infection.

The most common revelation from chest CT for patients with COVID-19 pneumonia is multifocal bilateral areas with 'ground glass' feature. This feature is considered to associate with consolidation especially in the lower lobes. Besides, patchy opacities with consolidated peripheral ring have also been observed frequently [10].

## 2. current treatment

Patients with confirmed diagnosis of SARS-Cov-2 were classified into three categories according to their symptoms: mild, moderate and severe disease. As there is no specific effective antiviral therapeutic targeting this virus yet, for patients with mild disease, the standard of care is to relieve symptom, prevent further transmission and keep close monitoring [11]. In China, herbal medicine prescribed according to Traditional Chinese Medicine (TCM) was applied for patients with mild symptoms such as dry cough, headache, fever over 37.5 °C and fatigue but no dyspnea. Paracetamol is usually applied to treat fever. European guidelines have suggested to use hydroxychloroquine (HCQ) for patients at this stage. For patients with tendency of deterioration such as increased respiratory rate and pulmonary infiltrates, prompt escalation of care is necessary. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) can be individualized. For patients with severe symptom, acetaminophen is generally recommended in hospital as the usage of NSAIDs has induced increased risk of bleeding and renal injury [12, 13]. Application of oxygen supplementation such as nasal cannula and high flow oxygen is recommended to relieve their symptom. Noninvasive and invasive mechanical ventilation is frequently demanded for critical cases [14].

Numerous clinical trials examining therapeutics against SARS-CoV-2 are underway competitively. The clinical trial with bamlanivimab and etesevimab has shown that, for patients with gentle to moderate symptoms, treatment with these two drugs have associated with significant decrease in SARS-CoV-2 viral burden at day 11 after infection comparing to placebo group [15]. No significant distinction in viral burden was observed for patients group using bamlanivimab monotherapy.

As the genomic sequence and essential enzymes for SARS-CoV-2 are similar (80-90%) to coronaviruses causing SARS and MERS (Middle East respiratory syndrome), it is mainstay to use existing anti-viral therapeutics to treat the COVID-19. Remdesivir was found to be able to reduce viral activity at concentration of 0.77  $\mu$ M with combination of chloroquine with EC50 of 1.13  $\mu$ M [16]. This therapeutic is developed to target Ebola virus whilst it currently has been investigated to fight against as a broad-spectrum anti-viral agent. In the clinical trial in treating COVID-19 patients, Remdesivir has shown significant anti-viral activity to reduce mortality rate from 11% to 7.1%. With similar mechanism, Arbidol and favipiravir, which possess anti-viral activity against several RNA and DNA viruses, have been applied in several clinical trials. It is shown that favipiravir has superior performance with clinical recovery rate of 61.21% comparing to Arbidol (51.67%) [17].

### 3. Future treatment

The crisis of COVID-19 has prompted researchers worldwide to look for effective anti-viral treatments. Two major ongoing novel antiviral therapeutics have shown significant activities in clinical trials.

Patients at the early stage of COVID-19 infection do not need to take specific therapeutics and can recover by resting. Whilst patients with the tendency to deteriorate to moderate disease need to take drugs. Most of the current drugs are re-use of anti-viral therapeutics targeting SARS rather than SARS-CoV-2, which prompted researchers investigating specific drug for the latter virus. Molnupiravir, the prodrug of -D-N4-hydroxycytidine (NHC), is an example of promising targeted drug for SARS-CoV-2. After rapid conversion from the prodrug to the active 5-triphosphate form by host kinases, it induces antiviral activity when it combines with nascent viral RNA since it is a substrate for RNA-dependent RNA polymerase (RdRp). In preclinical studies [18], molnupiravir treatment and prophylaxis have managed to reduce both of SARS-CoV and SARS-CoV-2 levels in humanized mouse models. It also suppressed virus replication and pathogenesis of SARS-like bat coronaviruses as well as Middle East respiratory syndrome coronavirus (MERS-CoV). Molnupiravir has shown full anti-viral activity in a trial with a ferret model of SARS-CoV-2. In phase II clinical trial, Molnupiravir has also shown significant effectiveness. Patients who had been tested positive for SARS-CoV-2 within 96 hours and manifested symptoms of COVID-19 within seven days after treatment initiation were eligible for the study. Antiviral activity, safety, and tolerability were assessed for 28 days after treatment with Molnupiravir. Infectious virus isolation and reverse transcriptase polymerase chain reaction (RT-PCR) were performed by collection of nasopharyngeal swabs on Days 1 (baseline), 3, 5, 7, 14 and 28. Participants received either 200 mg of molnupiravir or a matching placebo on Day 1; 400 or 800 mg of molnupiravir on Day 3; or a placebo on Day 28. Adverse events were monitored throughout the experiment. The number of participants who were positive with virus detection decreased significantly over time as evidenced by infection rates of 43.5% in baseline to 1.9% for participants treated with molnupiravir, versus to 16.7% for whom received placebo. Virus load detected by infectious virus isolation has also decreased significantly on Day 5 for participants administered 400 or 800 mg molnupiravir, with 0 being detected comparing to 11.1% of placebo recipients. Regarding the side effects, only four participants in the molnupiravir 800 mg group experienced treatment-associated adverse reaction. A total of 2 (1.4%) molnupiravir participants discontinued the drug comparing to 1 (16.6%) of the placebo group. Grade 3 or higher adverse events occurred in 5.0% and 8.1% of the molnupiravir participants, respectively. The participants with severe adverse events and hospitalizations included 1 (1.6%) patient who received a placebo, 2 (3%) patients who received 400 mg molnupiravir (cerebral vascular accident and decreased oxygen saturation) and there was 1 participant received Molnupiravir 800 mg progressed to acute respiratory failure. Treatment was discontinued in all 4 participants. Following completion, 1 death due to COVID-19 occurred in a participant administered placebo outside of the 28-day time window. Side effects associated with Molnupiravir assumption include cold and fever.

Another drug that arose quite attention is PF-07321332, a covalent inhibitor of 3CL protease acting directly on its catalytic cysteine residue (Cys145). It is developed by Pfizer and currently is undergoing phase III clinical trial [19, 20].

As part of phase III trials for COVID-19, PF-07321332 is being combined with ritonavir to maintain higher levels of circulating drug. Ritonavir serves to slow down PF-07321332 metabolism by cytochrome enzymes. PF-07321332 has dosed the first patient in a phase II/III clinical trial of the experimental antiviral drug. Pfizer announced positive phase 2/3 results, including an 89% reduction in hospitalizations within three days of symptom onset. PF-07321332 is a protease inhibitor intended for use in non-hospitalized patients with SARS-CoV-2 virus symptoms but are not at risk of developing severe disease. It works by inhibiting the main enzyme required by the virus for replication. The metabolism or breakdown of the antiviral drug is expected to slow down when combined with

ritonavir, making it a great facilitator to keep PF-07321332 more stable in the body. The latest double-blind Phase II/III trial will compare the effects of PF07321332 plus ritonavir treatment with those of a placebo every 12 hours for five days [21]. Another global two-arm study is being conducted to assess whether the treatment is safe and effective for symptomatic outpatients over 18 years old who do not have severe illness or are at low risk of developing severe illness [22].

#### 4. Discussion

Fast shift to intensive care unit (ICU) or medical emergency has to be executed for patients progress to septic shock and acute respiratory distress syndrome (ARDS) with COVID-19 pneumonia. Vital signs must be monitored closely. In terms of ventilation facilities, besides artificial ventilation by non-invasive and invasive approaches, extracorporeal membrane oxygenation (ECMO) is necessary for patients with refractory hypoxemia. Glucocorticoids and broad-spectrum antibiotics should be applied for these patients to increase their pulmonary function. For critical case with septic shock, intravenous crystalloids and vasoactive agents should be administered.

#### 5. Conclusion

Rapid spread and the alarming data about accumulative and daily new cases and death caused by COVID-19 call for fast and reliable diagnostic tools and timely treatment. RT-PCR is the standard diagnostic approach with high sensitivity. Imaging approaches such as CT and X-ray are supplementary diagnostic tools to confirm the infection based on image changes. The urgent requirements of the pandemic control attracted various investigations on development of specific therapeutics targeting SARS-CoV-2. Repurposing of several anti-viral drugs have been applied with limited efficiency. Newly developed therapeutics including Molnupiravir and PF07321332 are undergoing clinical trials showing promising anti-viral activities.

#### References

- [1] Li Q, Guan X, Wu P, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. *N Engl J Med*. 2020;382(13):1199-1207.
- [2] Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol* 2020 175. 2020;17(5):533-535.
- [3] Zhang J, Litvinova M, Wang W, et al. Evolving epidemiology and transmission dynamics of coronavirus disease 2019 outside Hubei province, China: a descriptive and modelling study. *Lancet Infect Dis*. 2020;20(7):793-802.
- [4] He Y, Wang J, Li F, Shi Y. Main Clinical Features of COVID-19 and Potential Prognostic and Therapeutic Value of the Microbiota in SARS-CoV-2 Infections. *Front Microbiol*. 2020;11:1302.
- [5] Markoulatos P, Siafakas N, Moncany M. Multiplex polymerase chain reaction: A practical approach. *J Clin Lab Anal*. 2002;16(1):47.
- [6] C C, B K, N T, et al. Rapid SARS-CoV-2 antigen detection assay in comparison with real-time RT-PCR assay for laboratory diagnosis of COVID-19 in Thailand. *Virology*. 2020;17(1).
- [7] Liu R, Han H, Liu F, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Clin Chim Acta*. 2020;505:172-175.
- [8] Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA*. 2020;323(18):1843-1844.
- [9] Hao W, Li M. Clinical diagnostic value of CT imaging in COVID-19 with multiple negative RT-PCR testing. *Travel Med Infect Dis*. 2020;34:101627.

- [10] Waller J V., Kaur P, Tucker A, et al. Diagnostic Tools for Coronavirus Disease (COVID-19): Comparing CT and RT-PCR Viral Nucleic Acid Testing. 2020;215(4):834-838. doi:10.2214/AJR.20.23418
- [11] Salian VS, Wright JA, Vedell PT, et al. COVID-19 Transmission, Current Treatment, and Future Therapeutic Strategies. *Mol Pharm.* 2021;18(3):754-771.
- [12] Cumhur Cure M, Kucuk A, Cure E, NSAIDs may increase the risk of thrombosis and acute renal failure in patients with COVID-19 infection. *Therapie* 2020, 75(4), 387-388
- [13] Berg R, Chmel H, Mayo J, Armstrong D. *Corynebacterium equi* infection complicating neoplastic disease. *Am. J. Clin. Pathol.* 1977, 68 (1), 73-77
- [14] Interim Guidelines for Collecting, handling and testing clinical specimens for COVID-19. Centers for disease control and prevention. <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>
- [15] RL G, A N, P C, et al. Effect of Bamlanivimab as Monotherapy or in Combination With Etesevimab on Viral Load in Patients With Mild to Moderate COVID-19: A Randomized Clinical Trial. *JAMA.* 2021;325(7):632-644.
- [16] Schooley RT, Carlin AF, Beadle JR, et al. Rethinking remdesivir: Synthesis, antiviral activity, and pharmacokinetics of oral lipid prodrugs. *Antimicrob Agents Chemother.* 2021;65(10).
- [17] Blaising J, Polyak S. J., Pecheur E. I., Arbidol as a broadspectrum antiviral: an update. *Antiviral Res.* 2014, 107, 84-94
- [18] Singh A. K., Singh A, Singh A, Misra A, Molnupiravir in COVID-19: A systematic review of literature. *Diabetes Metab Syndr.* 2021 Oct 30; 15(6): 102329
- [19] Ahmad B, Batool M, Ain QU, Kim MS, Choi S. Exploring the Binding Mechanism of PF-07321332 SARS-CoV-2 Protease Inhibitor through Molecular Dynamics and Binding Free Energy Simulations. *Int J Mol Sci.* 2021;22(17).
- [20] Pfizer begins dosing in Phase II/III trial of antiviral drug for Covid-19. Accessed November 13, 2021.
- [21] First-In-Human Study To Evaluate Safety, Tolerability, And Pharmacokinetics Following Single Ascending And Multiple Ascending Doses of PF-07304814 In Hospitalized Participants With COVID-19. - Full Text View - ClinicalTrials.gov. Accessed November 13, 2021. <https://clinicaltrials.gov/ct2/s>
- [22] NCT04535167Pfizer's Novel COVID-19 Oral Antiviral Treatment Candidate Reduced Risk of Hospitalization or Death by 89% in Interim Analysis of Phase 2/3 EPIC-HR Study | Pfizer. Accessed November 13, 2021. <https://www.pfizer.com/news/press-release/press-release-detail/pfizers-novel-covid-19-oral-antiviral-treatment-candidate>