

Persistent positivity of SARS-CoV-2 nucleic acid in asymptomatic healthcare worker: infective virion or inactive nucleic acid?

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SUMMARY

Asymptomatic individuals positive for SARS-CoV-2 RNA constitute a significant proportion of the infected population and play a role in the transmission of the virus. We describe a healthcare worker who presented with fever and malaise and was diagnosed with mild COVID-19. The symptoms resolved within 4 days but there was persistent positivity of viral RNA in the upper respiratory tract for more than 58 days, which is the longest reported duration of persistence of SARS-CoV-2 in a healthcare worker. In this case report, we discuss clinical and administrative issues such as the role of asymptomatic cases in the transmission of the virus to patients and coworkers as an occupational hazard, interpretation of persistent positivity of nucleic acid test, duration of isolation and return-to-work guidelines pertinent to researchers and global health policymakers.

reported to be approximately 9 days, with around a quarter of cases showing viral persistence beyond 2 weeks.⁸ Early identification and isolation of active cases, potential sources and carriers, and eliminating routes of transmission are important ways to control the COVID-19 pandemic. Global testing strategies focus on screening of symptomatic individuals and contact tracing in some countries. Asymptomatic cases often do not seek medical assistance and may continue to spread the infection staying unrecognised and can be thought of as the Achilles heel of the COVID-19 pandemic, making it more challenging to rein this Trojan horse.⁹ With the rising global burden and opening up of non-essential services, it is important to maintain a tight-rope balance between early recognition, isolation, treatment and timely reintegration of asymptomatic individuals back into the working force.

BACKGROUND

COVID-19 caused by SARS-CoV-2, was declared a pandemic by the WHO on 11 March 2020. As of 28 January 2021, the global burden of COVID-19 has surpassed 100 million with more than 2 million deaths.¹ The spectrum of the disease varies from asymptomatic to multiorgan failure in adults and children.² In Italy, a COVID-19 incidence of 20% was reported among front-line healthcare workers (HCWs).³ There is no single 'gold standard' test for diagnosis of COVID-19. Several assays targeting different genes of SARS-CoV-2 have been developed using real-time reverse transcription PCR (RT-PCR). Worldwide, it has become the screening test of choice for SARS-CoV-2. The accuracy of the test markedly varies with prevalence, disease stage, site of sampling and use of single or multiple gene targets. Sensitivity and specificity have been reported to be 70% and 95%, respectively.⁴ Although the maximum sensitivity of the RT-PCR test has been observed within 1 week of symptom onset, the test can also detect asymptomatic infected individuals.⁵ Such asymptomatic cases who test positive for viral nucleic acid have garnered attention as potential carriers.

The reported incidence of asymptomatic patients among SARS-CoV-2-positive cases varies widely from 1.6% to 78%.^{6,7} Four clinical scenarios have been described pertaining to asymptomatic cases: 'true asymptomatic', 'pre-symptomatic', 're-test positivity' and 'persistent positivity' of recovered patients. The median persistence of the virus among asymptomatic COVID-19 cases has been

CASE PRESENTATION

A doctor in their 20s who was involved in the care of paediatric patients with COVID-19 in a tertiary-care level COVID-19 hospital in eastern India developed symptoms of low-grade fever associated with myalgia. There were no other associated complaints such as cough, coryza, anosmia, ageusia or breathlessness. Mental state, diet and sleep were fair, and bladder and bowel habits were normal. The doctor had been wearing full personal protective equipment (PPE) at work and thus had low-risk direct contact with patients with COVID-19. There was no history of domestic or international travel in the past 2 weeks. There was no history of SARS-CoV-2 infection in the family, and no history suggestive of any comorbidity, known allergies or hospitalisation in the past.

The doctor presented to the influenza clinic after 2 days of illness onset, with a heart rate of 88 per minute, respiratory rate of 20 per minute, peripheral oxygen saturation (SpO₂) of 98% on room air and axillary temperature of 37°C measured with a digital thermometer. General and systemic examinations were unremarkable. A nasopharyngeal swab was collected in a standard vial containing viral transport media (HiMedia Lab) and sent under cold chain to inhouse COVID-19 testing laboratory recognised by the Indian Council of Medical Research (ICMR) for RT-PCR testing. RNA was extracted by silica column-based technology (Qiagen Viral RNA Mini Kit) and was subjected to one-step multiplex real-time PCR in Biorad CFX96 using PCR kits (TRUPCR SARS-CoV-2 RT qPCR



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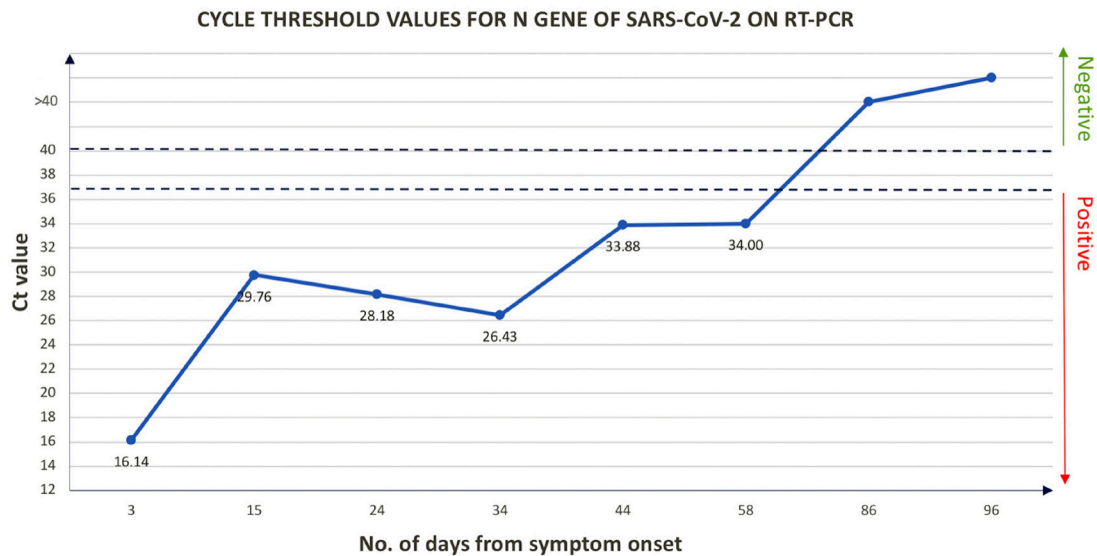


Figure 1 Cycle threshold (Ct) values for N gene of SARS-CoV-2 on reverse transcription (RT)-PCR test.

Kit V.2.0 (I); 3B BlackBio Biotech India) approved by the ICMR for detection of SARS-CoV-2. The results were interpreted as per the manufacturer's instruction. The test was positive for SARS-CoV-2 so home isolation was advised for mild COVID-19. No other laboratory test was considered at this time and oral paracetamol and nutritional supplements were prescribed.

The doctor stayed under regular follow-up through telemedicine, with self-monitoring of temperature, respiratory rate, heart rate and SpO₂ using a portable pulse oximeter. During home isolation, personal protective measures such as frequent hand-washing, using alcohol-based hand sanitiser, wearing mask and maintaining social distancing of 1.8 metre were followed. Clinical recovery was documented within 4 days of illness onset. A repeat RT-PCR for SARS-CoV-2 done 10 days after symptom onset was positive so home isolation was extended. The HCW was allowed to resume work 3 weeks after illness onset. To minimise infection spread to coworkers, the nature of work was changed from front-line clinical work to academic and research over the next few days. The HCW was kept under regular follow-up after resuming work. The viral nucleic acid showed persistent positivity in the upper respiratory tract for more than 58 days despite clinical recovery (figure 1). On day 40 of illness, the IgG antibody titres to SARS-CoV-2 spike glycoprotein (S1) antigen were >20 units (ADVIA Centaur SARS-CoV-2 IgG). Considering the persistence of infection, inflammatory markers for COVID-19 illness were done on day 44 and all were within normal limits (table 1). The last two nasopharyngeal swabs tested for SARS-CoV-2 on days 86 and 96 of illness onset were

negative. None of the coworkers or family members developed symptoms of COVID-19 during this period to suggest any potential source of infection.

GLOBAL HEALTH PROBLEM LIST

- ▶ HCWs are at high risk of exposure to SARS-CoV-2. Undiagnosed HCWs can transmit COVID-19 to negative patients and pose an occupational hazard to coworkers. Their protection, timely identification and isolation become even more important in developing countries with a low doctor to population ratio, where trained human resource is a critical limiting resource.
- ▶ There are many reported cases of persistence of SARS-CoV-2 nucleic acid among asymptomatic individuals beyond 2 weeks of illness onset. It is unclear whether persistent positivity is due to live infective virion or inactive viral nucleic acid, and how this translates into infectivity and further transmissibility.¹⁰
- ▶ Prolonged viral persistence brings forth challenging questions to healthcare institutions, such as when to end isolation for an infected front-line HCW with persistent positivity and allow them to return to work. Finding its answer is important to ensure a safe environment for patients and other HCWs while mitigating staffing shortages during the pandemic.¹¹

GLOBAL HEALTH PROBLEM ANALYSIS

Asymptomatic carriers play an important role in the propagation of disease pandemics. The role of asymptomatic cases in the transmission of SARS-CoV-2 remains unclear. The incidence rate of asymptomatic individuals among RT-PCR-positive cases has been reported to be ranging from 1.6% to 78%.^{6,7} As testing strategies for SARS-CoV-2 in most countries focus on symptomatic individuals, contact tracing and testing of high-risk clusters, the proportion of asymptomatic cases is likely to be under-reported. Due to the nature of their work, HCWs are at high risk of exposure to SARS-CoV-2 and are more likely to have a positive COVID-19 test compared with the general population (adjusted HR 11.61, 95% CI 10.93 to 12.33).¹² In Italy, a COVID-19 incidence of 20% was reported among front-line HCWs responding to the pandemic.³ As with other respiratory viruses, appropriate

Table 1 Blood investigation results performed on days 40 and 44 from symptom onset

Test	Results	Reference range and unit
IgG antibody to SARS-CoV-2 S1 antigen	>20.0	<1 unit: negative
Haemoglobin	148	130–170 g/L
Haematocrit	42.6	40.0%–50.0%
Total leucocyte count	9.1	4–10 ×10 ⁹ /L
Platelets	303	150–450 ×10 ⁹ /L
C reactive protein	<2.8	0–5 mg/L
Serum ferritin	53.8	22–322 ng/mL

PPE can prevent SARS-CoV-2 infection among approximately 88% of HCWs.¹³ The HCW in our case did not report any shortages or reuse of PPE and wore full PPE at work. Occupational exposure may be attributed to the aerosol-generating procedures performed in the paediatric intensive care unit where the HCW was deployed. Undetected HCWs carrying SARS-CoV-2 can cause nosocomial transmission of the virus. Mass testing of asymptomatic individuals and HCWs is not a cost-effective and pragmatic approach to curb this pandemic. In a pooled analysis, the probability of a false-negative RT-PCR test is found to be minimum on day 8 of exposure (third day of symptom onset), suggesting the role of vigilant contact tracing to test individuals during this window period.⁵ Our hospital developed a contact tracing algorithm for resource-limited settings based on the recommendation by the European Centre for Disease Prevention and Control.¹⁴ It includes classifying individuals as having high-risk or low-risk exposure. Contacts with high-risk exposure are quarantined and tested between days 5 and 14 of exposure. Low-risk contacts continue to work and self-monitor for symptoms for 14 days. All HCWs showing symptoms of COVID-19 within 14 days of exposure are tested.¹⁵ Our case had the typical symptoms of COVID-19 while being involved in the care of paediatric patients with COVID-19 and was therefore tested for SARS-CoV-2.

Studies on familial clusters of cases with COVID-19 from China have suggested the potential role of asymptomatic carriers in the transmission of the virus.^{16,17} Transmission efficiency of asymptomatic individuals is reported to be about one-third that of symptomatic cases.¹⁸ Four clinical scenarios have been described in this regard. In the first scenario, the patient remains asymptomatic throughout the course of the disease. The second scenario is of 'pre-symptomatic' cases, who are asymptomatic at the time of RT-PCR testing but develop symptoms a few days after the test. In the third scenario, patients with COVID-19 who become asymptomatic and test negative for SARS-CoV-2 at the time of discharge may show 're-test positivity' during follow-up. The fourth scenario is when COVID-19 symptoms resolve and the patient enters into convalescent phase, yet SARS-CoV-2 shows persistent positivity. Meyerowitz *et al*¹⁹ have described that true asymptomatic patients with COVID-19 do not have any typical or atypical or minor symptoms of COVID-19 during a short follow-up period of 14 days but test positive for SARS-CoV-2 by RT-PCR or serological tests. Such cases remain undetected and are identified only through active surveillance or contact tracing. They act as 'asymptomatic carriers' in community transmission of the disease. Inconsistent definitions and lack of follow-up often lead to the mischaracterisation of presymptomatic or mild cases as asymptomatic. Meyerowitz *et al*¹⁹ highlight the need for use of consistent diagnostic criteria for such individuals in future studies to better understand their pathogenesis and transmissibility. In a nursing facility outbreak of COVID-19, Arons *et al*²⁰ found that 24 out of 48 (50%) residents who tested positive were presymptomatic and 3 (6.25%) were asymptomatic. In another such outbreak, Kimball *et al*²¹ reported that 10 out of 23 (43.5%) residents who tested positive were presymptomatic and 3 (13%) were asymptomatic. Both studies found comparable high viral loads among symptomatic, presymptomatic and asymptomatic cases with no statistical difference in the mean cycle threshold (Ct) values among these groups, indicating an important role of asymptomatic and presymptomatic cases in the transmission of SARS-CoV-2. The management and the public health implications of 're-test positivity' of recovered patients with COVID-19 are another dilemma faced by clinicians. In a Chinese study, 38 of 262 (14.5%) recovered patients

with COVID-19 showed retest positivity.²² None of the 21 close contacts of these patients reported any symptoms of COVID-19, nor tested positive for viral RNA. Thus, the role of these individuals in disease transmission is questionable.

Several studies have documented persistence of SARS-CoV-2 nucleic acid in asymptomatic cases beyond 2 weeks of illness. Saurabh *et al*⁸ reported a median viral persistence of 9 days (maximum 25 days) in 44 asymptomatic COVID-19 cases. In another study on asymptomatic COVID-19-positive HCWs, the median duration from symptom onset to negative RT-PCR test was 24 days (maximum 37 days).²³ An isolated case report has documented the persistence of SARS-CoV-2 RNA until 92 days after illness onset in a patient with COVID-19 pneumonia.²⁴ Multiple samples from the patient's body and surrounding environment were negative for SARS-CoV-2 and no verified transmission occurred during home isolation in that patient. In our report, environmental testing could not be done, but none of the close contacts or coworkers developed symptoms of COVID-19 during the period of positivity. In another case report, a nurse showed prolonged COVID-19 illness for 48 days with negative RT-PCR test on day 50 from symptom onset.²⁵ In our case, the HCW was diagnosed with mild COVID-19, recovered clinically within 4 days of illness onset and showed persistent positivity of SARS-CoV-2 nucleic acid in the upper respiratory tract for more than 58 days despite clinical resolution of symptoms. This is the longest duration of persistence of SARS-CoV-2 in an HCW reported in the medical literature to the best of our knowledge.

Prolonged positivity raises questions regarding sampling issues, reinfection or persistence of infection.²⁶ Consecutive five nasopharyngeal samples collected at different points of time were positive for SARS-CoV-2 in the HCW, almost ruling out sampling or testing error. The HCW did not have any history of verified contact with COVID-19 case during this period, decreasing the likelihood of reinfection. Persistence of infection can be due to decreased clearance, possible carrier state of viral illness,²² viral mutations or detection of dead viral gene fragments (viral shedding) without the capability of active replication. It has been suggested that the presence of comorbidities such as diabetes mellitus and hypertension or receiving glucocorticoids may delay viral clearance.²⁶ Our case did not have any pre-existing conditions. In a study by Yao *et al*,²⁷ ultra-deep sequencing of viral genomic RNA isolated from 11 patients with COVID-19 identified 33 mutations, out of which 19 were novel. A case report has provided evidence of mutational diversity as a cause of persistence or reinfection. Next-generation sequencing of virus isolated from nasopharyngeal swabs showed that a 25-year-old man had reinfection with a genetically different variant of SARS-CoV-2.²⁸ Due to lack of facility, genomic analysis of the viral isolates could not be done in our case.

RT-PCR cannot differentiate between non-infective viral nucleic acid and infective virion. In an effort to predict infectiousness of SARS-CoV-2 RT-PCR samples, Bullard *et al*²⁹ incubated 90 positive samples on Vero cells. They found that positive cultures were observed from samples collected up to 8 days from symptom onset, and increasing symptom to test time was significantly associated with no viral growth on culture (OR 0.63, 95% CI 0.42 to 0.94, $p=0.025$). They also observed that samples with positive culture had significantly lower median Ct value of 17 (IQR 16–18) compared with culture-negative samples with a median Ct value of 27 (IQR 22–33) ($p<0.001$). Lower Ct values were significantly associated with positive viral culture (OR 0.64, 95% CI 0.49 to 0.84, $p<0.001$). Wölfel *et al*³⁰ also reported that no viral cultures were positive from upper respiratory tract samples taken after day 8 of

symptom onset despite high viral loads. In our case, RT-PCR was performed against multiple gene targets of SARS-CoV-2 (N, ORF, RNP, E genes) for samples obtained from the nasopharynx. Only the first nasopharyngeal sample taken on day 3 of illness onset had a Ct value of <18 (16.14) for N gene. Ct values of subsequent SARS-CoV-2-positive samples taken over 2 months ranged between 26 and 34 (figure 1). Infectiousness of these samples could not be established by viral culture, but none of the close contacts of the HCW reported any symptoms during the follow-up period. Also, despite persistent detection of SARS-CoV-2 RNA, anti-S1 receptor binding domain (RBD) antibody titre was >20 units on day 40 and inflammatory markers tested on day 44 were within normal limits (table 1). Antiviral antibodies (IgM and IgG) against SARS-CoV-2 can be produced against spike glycoprotein (S) or nucleocapsid (N). Spike is a transmembrane glycoprotein comprising S1 and S2 regions. S1 (comprising RBD) facilitates recognition and binding of the ACE2 viral receptor on the host cells, and S2 mediates viral fusion and entry into the cell.³¹ Antibodies against S1 RBD interfere with the RBD–ACE2 interaction, thereby blocking viral entry and may be neutralising in nature.³² It is still debatable whether the presence of these neutralising antibodies hastens viral clearance. Mild immune response has been reported in asymptomatic patients with COVID-19.³³ It is speculated to be due to lower ACE2 levels and weaker binding capacity to its receptor, thereby leading to less invasion of cells by SARS-CoV-2.⁶

As prolonged positivity of viral nucleic acid in asymptomatic cases does not necessarily mean infectivity, it is unclear whether antiviral therapy in such patients will reap any benefits. In our case, no antiviral therapy was prescribed. A pressing administrative question for us was when to end the isolation and allow them to safely join back work. Availability of trained HCWs is globally a limiting factor to run hospitals. This is especially true during the COVID-19 pandemic in countries where doctor to population ratio and nurse to population ratio are already poor. The Centers for Disease Control and Prevention advocates a symptom-based rather than testing-based strategy in return-to-work guidelines. It recommends that HCWs with mild-moderate illness who are not severely immunocompromised can join work after 10 days of symptom onset if they are afebrile for at least 24 hours with improvement of symptoms.¹¹ In this pandemic, ensuring a safe environment for patients and HCWs while mitigating staffing shortages remains a priority for healthcare institutions. At our hospital, it is allowed to perform a repeat RT-PCR test for COVID-19-positive HCWs at the end of their quarantine period before they return to work. Our case had globally the longest duration of persistent COVID-19 positivity in any HCW to date. The HCW stayed under home isolation for the initial 3 weeks. Subsequently, despite the positive status, the HCW was given academic and research work that involved minimal direct interaction with colleagues. Initial concerns of coworkers regarding the risk of disease transmission were resolved with available evidence in the literature, contemporary guidelines and efficacy of PPE used while working in the COVID-19 hospital.

In conclusion, larger studies are needed to ascertain the duration of viral RNA persistence among asymptomatic individuals and understand the underlying mechanism. Some studies suggest that persistent positivity of viral nucleic acid in asymptomatic cases may not correlate with infectivity in vitro. Translating this bench-side observation to bedside practice requires larger epidemiological studies to rule out transmission from such cases at the community level, and laboratory studies to detect infective virus via cell culture in addition to RT-PCR tests. It should be encouraged to formulate local guidelines on the duration of isolation

and return to work depending on the staffing strength and proportion of infected HCWs to ensure optimal patient care.

Patient's perspective

I am a doctor who came positive for COVID-19. Though there were no symptoms but repeatedly five samples showing my positive status for COVID-19 made me anxious. It was reassuring to have normal blood investigations and antibodies titre >20 units against the virus. It has been very hard to understand why the test is coming repeatedly positive despite everything else being fine.

Learning points

- ▶ Despite the availability of adequate and appropriate personal protective equipment, occupational exposure to SARS-CoV-2 among healthcare workers is possible.
- ▶ Clear contact tracing guidelines that allow timely identification and isolation of exposed healthcare workers are key to prevent nosocomial transmission of COVID-19.
- ▶ Asymptomatic individuals may show persistent positivity of SARS-CoV-2 RNA despite clinical recovery, attributable to sampling issue, reinfection or persistence of infection.
- ▶ Larger studies based on viral culture are needed to understand whether persistent positivity is due to live infective virion or inactive viral nucleic acid, particularly in asymptomatic cases.
- ▶ Epidemiological studies with larger sample size are needed to understand the infective potential of these cases in community transmission and to guide policies on return-to-work decisions.

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REFERENCES

- 1 WHO. Who coronavirus disease (COVID-19) Dashboard. Available: <https://covid19.who.int/> [Accessed 28 No 2020].
- 2 Tiwari L, Shekhar S, Bansal A, et al. COVID-19 associated arterial ischaemic stroke and multisystem inflammatory syndrome in children: a case report. *Lancet Child Adolesc Health* 2021;5:88–90.

- 3 The Lancet COVID-19: protecting health-care workers. *The Lancet* 2020;395:922.
- 4 Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. *BMJ* 2020;369:m1808.
- 5 Kucirka LM, Lauer SA, Laeyendecker O, et al. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. *Ann Intern Med* 2020;173:262–7.
- 6 Gao Z, Xu Y, Sun C, et al. A systematic review of asymptomatic infections with COVID-19. *J Microbiol Immunol Infect* 2021;54:12–16.
- 7 Day M. Covid-19: four fifths of cases are asymptomatic, China figures indicate. *BMJ* 2020;369:m1375.
- 8 Saurabh S, Kumar R, Gupta MK, et al. Prolonged persistence of SARS-CoV-2 in the upper respiratory tract of asymptomatic infected individuals. *QJM* 2020;113:556–60.
- 9 Gandhi M, Yokoe DS, Havlir DV. Asymptomatic transmission, the Achilles' heel of current strategies to control Covid-19. *N Engl J Med* 2020;382:2158–60.
- 10 Widders A, Broom A, Broom J. SARS-CoV-2: the viral shedding vs infectivity dilemma. *Infect Dis Health* 2020;25:210–5.
- 11 CDC. Criteria for return to work for healthcare personnel with SARS-CoV-2 infection (interim guidance), 2020. Available: <https://www.cdc.gov/coronavirus/2019-ncov/hcp/return-to-work.html> [Accessed 9 Oct 2020].
- 12 Nguyen LH, Drew DA, Joshi AD, et al. Risk of COVID-19 among frontline healthcare workers and the general community: a prospective cohort study. *medRxiv* 2020. doi:10.1101/2020.04.29.20084111. [Epub ahead of print: 25 May 2020].
- 13 Tian Z, Stedman M, Whyte M, et al. Personal protective equipment (PPE) and infection among healthcare workers - What is the evidence? *Int J Clin Pract* 2020;74:e13617.
- 14 European Centre for Disease Prevention and Control. Contact tracing: public health management of persons, including healthcare workers, who have had contact with COVID-19 cases in the European Union – third update. Stockholm ECDC; 2020.
- 15 Tiwari L. All India Institute Of Medical Sciences Patna Standard Operating Procedure & Hand Book For COVID-19 MANAGEMENT, Version 3. Available: https://www.aiimspatna.org/advertisement/COVID_SOP_AIIMS_P_version3.0.pdf [Accessed 10 Jan 2021].
- 16 Bai Y, Yao L, Wei T, et al. Presumed asymptomatic carrier transmission of COVID-19. *JAMA* 2020;323:1406–7.
- 17 Yu P, Zhu J, Zhang Z, et al. A familial cluster of infection associated with the 2019 novel coronavirus indicating possible person-to-person transmission during the incubation period. *J Infect Dis* 2020;221:1757–61.
- 18 Zhao H, Lu X, Deng Y, et al. COVID-19: asymptomatic carrier transmission is an underestimated problem. *Epidemiol Infect* 2020;148:e116.
- 19 Meyerowitz EA, Richterman A, Bogoch II, et al. Towards an accurate and systematic characterisation of persistently asymptomatic infection with SARS-CoV-2. *Lancet Infect Dis* 2020;30837–9.
- 20 Arons MM, Hatfield KM, Reddy SC, et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. *N Engl J Med* 2020;382:2081–90.
- 21 Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and presymptomatic SARS-CoV-2 infections in residents of a long-term care skilled nursing facility — King County, Washington, March 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:377–81.
- 22 An J, Liao X, Xiao T. Clinical characteristics of the recovered COVID-19 patients with re-detectable positive RNA test. *medRxiv* 2020.
- 23 Gomar S, Chang M, Hogan CA, et al. Persistent detection of SARS-CoV-2 RNA in patients and healthcare workers with COVID-19. *J Clin Virol* 2020;129:104477.
- 24 Wang J, Hang X, Wei B, et al. Persistent SARS-CoV-2 RNA positivity in a patient for 92 days after disease onset: a case report. *Medicine* 2020;99:e21865.
- 25 Buselli R, Corsi M, Necciarri G, et al. Sudden and persistent dysphonia within the framework of COVID-19: the case report of a nurse. *Brain Behav Immun Health* 2020;9:100160.
- 26 Kang H, Wang Y, Tong Z, et al. Retest positive for SARS-CoV-2 RNA of "recovered" patients with COVID-19: Persistence, sampling issues, or re-infection? *J Med Virol* 2020;92:2263–5.
- 27 Yao H-P, Lu X, Chen Q. Patient-Derived mutations impact pathogenicity of SARS-CoV-2. *SSRN Journal* 2020.
- 28 Tillett RL, Sevinsky JR, Hartley PD, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. *Lancet Infect Dis* 2021;21:52–8.
- 29 Bullard J, Dust K, Funk D. Predicting infectious SARS-CoV-2 from diagnostic samples. *Clin Infect Dis* 2020:ciaa638.
- 30 Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;581:465–9.
- 31 Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* 2020;11:1620.
- 32 Ju B, Zhang Q, Ge J, et al. Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* 2020;584:115–9.
- 33 Hu ZB, Song C. [Screening and management of asymptomatic infection of 2019-novel coronavirus]. *Zhonghua Yu Fang Yi Xue Za Zhi* 2020;54:484–5.

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