Prolonged incubation of SARS-CoV-2 in a Patient on Rituximab Therapy

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Abstract:

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Abstract

The incubation period of SARS-CoV-2 is rarely greater than 14 days. We report a patient with hypogammaglobulinemia who developed SARS-CoV-2 infection with a confirmed incubation period of at least 21 days. These findings raise concern for a prolonged presymptomatic transmission phase, necessitating a longer quarantine duration in this patient population.

Introduction

SARS-CoV-2 was discovered in Wuhan, China, and has since become a global pandemic through person-to-person spread. SARS-CoV-2 exhibits pre-symptomatic transmission during the incubation period, where an individual is contagious prior to symptom onset. Defining the incubation period therefore has infection control and public health implications, as a longer incubation necessitates a longer quarantine duration after an exposure.

Mean incubation periods range from 5.0 to 7.2 days, and one study reported a median incubation period of 5.1 days. In two studies, the 95th percentiles of the distribution were reported as 12.5 days and 13 days, and another three reported the 99th percentile as 11.9 days, 14 days and 14.9 days. In the vast majority of cases the incubation period is far less than 14 days, which has helped to inform the CDC recommendations for quarantining 14 days after a known COVID-19 exposure. However, these cases represent the general population, and do not provide detailed information on subpopulations in whom the incubation period may differ. Herein we present a case with an objectively confirmed SARS-CoV-2 infection with a prolonged incubation period proven through viral culture.
Case Presentation

A 71-year old female on rituximab for granulomatosis and polyangiitis presented with shortness of breath and nonproductive cough.

Six weeks prior to admission, several family members were diagnosed with COVID-19 infection, prompting her to undergo testing despite being asymptomatic. Her nasopharyngeal (NP) swab polymerase chain reaction (PCR) test for SARS-CoV-2 was positive. She was self-isolating, and her only contact was a family member who had recovered from mild COVID-19 illness and had since been asymptomatic. Repeat NP PCR testing 13 days later was also positive. Twenty-one days from the first test, the patient developed progressive dyspnea on exertion, a minimally productive cough, significant fatigue, and non-bloody diarrhea.

She was admitted to hospital on day 36 from her first test. She was febrile to 38.8°C and saturating 93% on room air. She was placed on 2 liters/minute of supplemental oxygen. Computed tomography (CT) of the chest demonstrated bilateral peribronchovascular groundglass opacities (Supplemental Figure 1). Relative to the day of her first test, she had repeat SARS-CoV-2 NP PCR tests on days 36, 37, and 40 which were negative. Serology for SARS-CoV-2 was negative. Flow cytometry of peripheral blood demonstrated no circulating B-cells, and an immunoglobulin panel demonstrated low levels of IgM, IgG, and IgA consistent with a history of receiving rituximab. Bronchoalveolar lavage (BAL) on hospital day 5 revealed a positive SARS-CoV-2 PCR with N1 and N2 cycle thresholds of 29 and 28, respectively. The patient was weaned off supplemental oxygen and discharged on hospital day 9.
The patient’s BAL fluid was stored at -80°C then thawed and inoculated into Vero E6 cell culture. Viral supernatant was harvested on day 4 post inoculation for plaque assay demonstrating infectious virus with a titer of $1.3 \times 10^3$ pfu/ml on passage 1 (Figure 1A and 1B). Nucleic acid extraction from the cell lysate confirmed the presence of SARS-CoV-2 by reverse transcription real time PCR, and by polyacrylamide gel (Figure 1C). Isolate from the first passage of the BAL specimen was used to infect Vero E6 cells for 48 hours. Cell lysates were probed for protein analysis using an antibody raised against SARS-CoV 3a antibody which demonstrated bands consistent with SARS-CoV-2 3a protein (Figure 1D). These studies indicate that infectious SARS-CoV-2 virus was isolated from the patient’s BAL.

**Discussion**

This case demonstrates an objectively confirmed asymptomatic SARS-CoV-2 infection, with symptom onset 21 days after her positive test. Furthermore, since a NP PCR can be falsely negative on the first day of infection, her incubation period may have been even greater.\(^9\) Lower respiratory tract sampling demonstrated viable SARS-CoV-2 virus, though the NP PCR was negative. A prior study demonstrated that NP PCR had a false negative rate of 66% by day 21, which may explain our observation.\(^9\)

Reports of incubation periods greater than 21 days are very rare. A patient with an incubation period of 24 days was reported, however incubation period was defined as the time between the earliest potential date of exposure to the first day of symptom onset, potentially leading to overestimation.\(^6\) A case report described a patient with an incubation period of at least 38 days based on a social history of limited contact with others after an exposure.\(^10\) Whether our patient’s
absence of circulating B-cells with subsequent hypogammaglobulinemia predisposed her to a prolonged incubation period is not known. Her negative serology suggests a poor humoral response to infection.

This report has significant implications for preventing the spread of COVID-19. For patients with known humoral immune deficits, until further data are available, one should exercise caution using a 14-day quarantine window based on the assumption of 14 days being the upper bound of the incubation period. It remains possible that this patient was shedding viable virus from the date of her initial positive test to beyond the date of her bronchoscopy 41 days later. This patient’s pre-symptomatic transmission window may have therefore been substantially greater than the estimated mean pre-symptomatic transmission window of 2.3 days in the general population.\(^1\) Whether prolonged incubation periods may occur in other immunosuppressing conditions remains to be evaluated, and further data in this area are needed to better define the appropriate quarantine period in this population.
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Conflicts of Interest

The authors report no conflicts of interest.
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Figure 1. Persistent SARS-CoV-2 viral replication in an immunocompromised patient. Vero E6 cells were mock-infected or inoculated with patient’s bronchoalveolar lavage fluid for 4 days. Viral supernatants from passage 1 were collected and used for plaque assay using Vero E6 cells. Representative plaque assay shown (1A). Plaques counted to deduce viral titer as plaque forming units (1B). Cell lysate used for qRT-PCR. SARS-CoV-2 virus (2019-nCoV/USA_WA1/2020) was used as a positive control (1C). Vero E6 cells were mock-infected or infected with virus isolated from passage 1 or a control SARS-CoV-2 virus (2019-nCoV/USA_WA1/2020) for 48 hours followed by immunoblot analysis of SARS-CoV-2 3a protein using an antibody against SARS-CoV 3a (1D). The primer sequences can be found in Supplementary Table 1.

A
Mock
Patient

B
10^1 10^2 10^3 10^4

C
Mock  Patient  SARS-CoV-2
E
N2 (CDC)
N (JH)
N (LM)
GAPDH

D
Mock  Patient  SARS-CoV-2
IB: SARS-CoV-3a
IB: GRP94

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Supplementary Figure 1. Computed tomography of the chest with intravenous contrast. Bilateral peribronchovascular groundglass opacities are noted consistent with COVID-19 infection.
**Supplementary Table 1** Primers for SARS-CoV-2 qRT-PCR assays.

| Target | Primer | Sequence | Ref |
|--------|--------|----------|-----|
| E      | E_Sarbeco_F | ACAGGTACGTAAATAGTTAATAGCGT | 1   |
|        | E_Sarbeco_R | ATATTGAGCAGTACGCACACA       |      |
| N2 (CDC)| N2_CDC_F | TTAACACATAGGCGGCAAAGGAA   | 2   |
|        | N2_CDC_R | GCCGACATCCGAAGAA           |      |
| N (JH) | N_JH_F  | CATTGGCATGGAAGTCACAC       |      |
|        | N_JH_R  | TCTGGGATAAGGCTGTGTT        |      |
| N (LM) | N_LM_F  | CTTCGACTCAACATGGGAAAGGAG  |      |
|        | N_LM_R  | GAGGAAGTTGTAGCAGATG        |      |
| GAPDH  | GAPDH_F | ACAACTTTGTATCGTGGGAAGG     |      |
|        | GAPDH_R | GCCATCAGGCCACAGTTGC        |      |

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