Probable causes and risk factors for positive SARS-CoV-2 test in recovered patients: Evidence from Brunei Darussalam

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Abstract
Case reports of patients with coronavirus disease-2019 (COVID-19) who have been discharged and subsequently report positive reverse transcription-polymerase chain reaction again (hereafter referred as “re-positive”) do not fully describe the magnitude and significance of this issue. To determine the re-positive rate (proportion) and review probable causes and outcomes, we conduct a retrospective study of all 119 discharged patients in Brunei Darussalam up till April 23. Patients who were discharged are required to self-isolate at home for 14 days and undergo nasopharyngeal specimen collection post-discharge. Discharged patients found to be re-positive were readmitted. We reviewed the clinical and epidemiological records of all discharged patients and apply log-binomial models to obtain risk ratios for re-positive status. One in five recovered patients subsequently test positive again for severe acute respiratory syndrome coronavirus 2—this risk is more than six times higher in persons aged 60 years and above. The average Ct value of re-positive patients was lower predischarge compared with their readmission Ct value. Out of 111 close contacts tested, none were found to be positive as a result of exposure to a re-positive patient. Our findings support prolonged but intermittent viral shedding as the probable cause for this phenomenon. We did not observe infectivity potential in these patients.

KEYWORDS
COVID-19, re-positive, SARS-CoV-2, viral shedding

1 INTRODUCTION
Current consensus on safe criteria for de-isolation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positive patients recommend the need for both clinical recovery of symptoms, and for those countries where testing and hospitalization capacity permits, two negative reverse transcription-polymerase chain reaction (RT-PCR) tests from respiratory specimens at 24 hours interval taken at least 8 days after symptom onset.¹ Notably though, there are reports from China, South Korea, and others of a number of patients who have met the discharge criteria and yet report positive RT-PCR again, from 2 to 13 days postdischarge.²⁻⁴ As yet, no study has attempted to describe the magnitude and significance of this phenomenon which we term “re-positive.” This could complicate the management of patients with coronavirus disease-2019 (COVID-19).

Up till 23 April 2020, Brunei Darussalam recorded a total of 138 COVID-19 cases of which 119 have been discharged. We conducted a retrospective cohort study of all discharged patients to identify those who are re-positive, describe their clinical and epidemiological outcomes, and analyse the predictors of re-positive status.
METHODS

2.1 | Surveillance

COVID-19 surveillance in Brunei is mandatory under the Infectious Disease Act. All persons who meet the following criteria must undergo a nasopharyngeal (NP) swab for SARS-CoV-2 RT-PCR testing: (a) contact history in the last 14 days, regardless of symptoms, (b) travel history in the last 14 days, regardless of symptoms, (c) pneumonia, and (d) presents to a health care facility with respiratory symptoms for the second time within a 14 day period. In addition, sentinel surveillance is conducted at selected community health clinics and for the foreign worker population.

2.2 | Patient diagnosis and management

We confirmed SARS-CoV-2 by RT-PCR assay on NP specimens if the cycle threshold (Ct) values for Orf1ab was less than 40. A commercial kit (BGI Genomics) was used (sensitivity 82.5% and specificity 81.5%). Specimens with Ct value more than 30, were further re-tested for additional confirmation with TibMolBiol. All NP swabs (regardless of prior discharge status) are obtained by trained clinical staff (doctor or nurse) at a designated health care facility.

Newly diagnosed patients are admitted to the National Isolation Centre and monitored for at least 14 days from admission until two consecutive negative specimens collected at least 24 hours apart.

Discharged patients are required to self-isolate at home, initially for a period of 7 days and later extended to 14 days. Verbal and written instructions for postdischarge self-isolation at home were given, including the requirement to stay in a separate bedroom from other members of the household. They undergo NP specimen collection at day 11 postdischarge. For patients already past day 11, NP specimens were collected at the earliest opportunity. Discharged patients found to be re-positive were readmitted. Bloods, chest X-ray, and antibody testing using the VivaDiag Rapid Test were conducted on readmission. This is a qualitative lateral flow immunoassay that has a reported specificity of 94.9% for IgG+/or IgM and a percentage positivity that range from 62.9% at 6 to 10 days postonset to 90.0% at more than 20 days postonset.

Contact tracing was conducted for all close contacts of re-positive cases. A close contact was defined as any individual living in the same household, or someone who was within 1 m of a confirmed case in an enclosed space for more than 15 minutes. All close contacts of confirmed cases were tested with RT-PCR.

2.3 | Empirical analysis

We review all discharged cases that had their postdischarge swab. We calculate the re-positive rate (percentage) by gender, age, clinical severity on first admission, and use of antiretroviral treatment (400 mg lopinavir/100 mg ritonavir) on first admission and apply log-binomial models to obtain risk ratios. We also compare pre- and postdischarge RT-PCR Ct values of re-positive patients using a paired t test. Ethical approval for this study was obtained from the Brunei Medical and Health Research Ethics Committee.

RESULTS

3.1 | Clinical and epidemiological characteristics of re-positive patients

About 106 patients had a follow-up NP swab taken between 11 to 18 days post discharge. About 21 (19.8%) were found to be re-positive (Figure 1). About 12 were male and 9 female. The median age was 47.0 years. About 20 (95%) of the re-positive patients were asymptomatic.

Among the 21 re-positive cases, only one reported symptoms postdischarge, this was in patient D, the initial re-positive patient.
that was first identified. Patient D is a 39-year-old man with a background history of type 2 diabetes and sleep apnea, who was initially confirmed positive on March 12. He had mild symptoms of fever and diarrhea on admission, however, his clinical condition deteriorated and on March 19, he developed left lower lobe pneumonia with radiographic changes. He was started on 400 mg lopinavir/100 mg ritonavir twice daily on March 19 and intravenous amoxicillin-clavulanate, piperacillin-tazobactam, and ceftazidime. His condition resolved, and he was discharged on March 28 following two negative RT-PCR. He ended his SIN on April 4. On April 8, he developed a mild cough and on April 10, presented to his general practitioner. In view of his previous COVID-19 history, an NP specimen was obtained, and his result was confirmed positive.

Routine blood and chest X-ray for all re-positive patients were normal. About 14 (67%) of the re-positive patients had both IgM and IgG detected. We used Ct value as a proxy for viral load, with the value inversely related to RNA copy numbers. The average Ct value of re-positive patients was lower predischarge compared with their readmission Ct value. This was statistically significant (P-value < .001) (Supplementary Table).

Contact tracing identified 111 close contacts. All were tested for SARS-CoV-2. One household contact of patient D tested positive; however, epidemiological investigation concluded that the likely exposure had occurred more than a month earlier.

### 3.2 Factors associated with re-positive status

We compared re-positive rates of subgroups of four variables in Table 1. The highest re-positive rate was observed in patients aged 60 and above (46.2%), followed by those with moderate to critical conditions (33.3%) and lopinavir/ritonavir treatment (30.3%). Multivariable log-binomial model identified age as the only significant risk variable, with the highest risk in those aged 60 years and above (risk ratio 6.21, 95% confidence interval, 1.20-32.09).

### DISCUSSION

#### 4.1 Key findings

We report a 19.8% re-positive proportion. One patient reported mild symptoms on readmission, while the others were asymptomatic. Other studies have reported a lower re-positive proportion. A large national study in South Korea identified that 292 (3.3%) out of 8922 recovered patients subsequently have at least one positive test postdischarge, however, does not describe if all recovered patients were tested (as in our study), or if only those cases who were symptomatic were tested postdischarge. This potential difference in case ascertainment criteria could account for the higher observed re-positive rate in our study.

There were some observed differences between the two groups (re-positive and consistent negative). The associations with clinical severity and antiviral treatment were strongly attenuated in the multivariable model suggesting age as the key variable under consideration. No significant difference was observed for gender.

#### 4.2 Sampling and detection of SARS-CoV-2

We cannot exclude test performance or operator deficiencies in specimen collection as a contributing factor to our observed high re-positive rate. For other respiratory viruses, some studies have reported an overall sensitivity of between 65% and 78% by RT-PCR. NP swabs may be less sensitive for SARS-CoV-2 during the...
convalescent period and as such could have resulted in false negatives on initial discharge, however, the need for two consecutive negatives should mitigate against this.\textsuperscript{11} Also, sampling and detection deficiencies cannot explain the higher risk in older individuals.

4.3 | Reinfecion, reactivation, and residual infection

Some reports suggest reinfection as a possible cause; our findings do not support this.\textsuperscript{12} There was no evidence of infection among close contacts which would have been likely if they were reinfected (given the need for an infective source). Moreover, 67\% of re-positive patients in our study had antibodies on admission (however, the correlates of protection are still to be defined which is necessary to be able to comment on the possibility of reinfection).\textsuperscript{13}

About 20 (95\%) patients were asymptomatic on redetection, and negative RT-PCRs were observed in 16 patients soon (within 1-3 days) after they were readmitted, suggesting that reactivation (a phenomenon not observed in other human coronaviruses) is also unlikely.

Other virological studies have demonstrated prolonged viral shedding in SARS-CoV-2 positive individuals. Genetic studies on SARS suggest that host responses may result in undetectable levels of NP virus shedding at certain times.\textsuperscript{14} Our findings support prolonged but intermittent viral shedding as the most plausible explanation. First, we observed oscillation between positivity and negativity, particularly when Ct values were at the detection limit. Second, prolonged viral shedding is observed in SARS-CoV-2 positive individuals up to 37 days after the onset of symptoms among adult patients, consistent with our observations of repeat positive RT-PCR at 27 to 34 days following first diagnosis.\textsuperscript{15} Third, older patients are more likely to have severe disease and to encounter prolonged viral RNA shedding, consistent with the higher re-positive risk among older patients.\textsuperscript{16, 17}

4.4 | Infectivity

Viral RNA shedding of SARS-CoV-2 does not equate with infectivity, unless there is proof that the virus can be isolated and cultured from the particular samples. While we did not culture the samples in our study population, Wolfel et al\textsuperscript{18} reported on attempts at live virus isolation from clinical samples and found that no isolates were obtained from samples taken after day 8 in spite of ongoing high viral loads.\textsuperscript{18} Our epidemiological findings support the virological observations—of the 111 close contacts tested, none were found to be positive as a result of exposure to a re-positive patient.

4.5 | Implications for clinical practice

We observed that the re-positive rate is higher than commonly reported, with increased risk in older age groups. Our findings support prolonged but intermittent viral shedding as the probable cause for this phenomenon. We do not identify infectivity potential in these patients, however, given the high re-positive rate observed, it would be dangerous to exclude this entirely. Based on our findings, we suggest that patients should be isolated for an extended period of time even after discharge.

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CONFLICT OF INTERESTS
All the authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS
All authors provided critical feedback on the manuscript and contributed to the study design. JW wrote the manuscript with input from all authors. WCK and LN conducted the data analysis. RNM, MFA, and NF extracted the data.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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