

REVIEW

How strong is the evidence that it is possible to get SARS-CoV-2 twice? A systematic review

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Summary

With a large number of coronavirus disease 2019 (Covid-19) patients being discharged from hospital with negative test results for SARS-CoV-2, it has been reported that several recovered cases tested positive after discharge (re-positive, RP). This finding has raised several important questions for this novel coronavirus and Covid-19 disease. In this review, we have discussed several important questions, including: (1) Can the virus re-infect recovered individuals? (2) What are the possible causes of the re-positive reverse transcriptase-polymerase chain reaction (RT-PCR) test in recovered patients? (3) What are the implications of these re-positive cases concerning the spread of the virus? Understanding how recovery from Covid-19 confers immunity to decrease the risk of re-infection is needed to inform current efforts to safely scale back population-based interventions, such as physical distancing. We have also described what is currently known about the immune response to Covid-19, highlighted key gaps in knowledge, and identified opportunities for future research. Overall, the quality of the evidence is poor and we describe the features that should be described for future cases.

KEYWORDS

Covid-19, recurrence, re-infection, risk, SARS-CoV-2

1 | INTRODUCTION

Starting December 2019, Chinese scientists witnessed the emergence of a new virus which was named SARS-CoV-2.^{1,2} By March 2020, the World Health Organization (WHO) declared the Covid-19 outbreak to be a pandemic. Extreme control measures were taken, resulting in grave consequences both in terms of health outcomes and financial losses. The SARS-CoV-2, in general, is a mild infection, however, some patients, especially those in the elderly group (>60 years old) with co-morbidities, are prone to develop more severe symptoms of the disease.^{2,3} Although the global daily case fatality rate (CFR) of the ongoing Covid-19 pandemic is declining, there are many countries where CFR is still increasing, and the rate of new infections is significantly high.⁴

Recently, a large number of recovered patients with Covid-19 have been discharged from the hospital, with regular follow-up and

observation.² Surprisingly, a re-positive (RP) result of the SARS-CoV-2 RNA test has been reported in some of these patients, most of whom had complete clinical remission and two consecutive negative nasopharyngeal swabs on discharge.² Although the number of these RP patients is small, their potential impact and significance are high. These RP patients generally belong to a younger age group and present initially with mild or moderate symptoms.¹ No patients who experienced severe symptoms of the disease initially have so far become RP. Retrospectively, it was documented that the RP patients displayed milder symptoms and earlier RNA negative-conversion as compared to NRP patients. When re-admitted to the hospital, these RP patients did not display any significant clinical symptoms, but showed normal or improving CT imaging and decreasing inflammatory cytokines.²

Although PCR-based methods cannot distinguish between the infectious form of the virus and the non-infectious nucleic acid, the

positive RT-PCR of these recovered patients represents an important finding that should be studied in detail. Few studies so far have investigated the contagiousness of these RP patients but, if they carry live viruses, they might be a potential source of infection to the general population. In this article, we review potential reasons why the results of the SARS-CoV-2 RT-PCR tests were positive again in recovered patients.

2 | MATERIALS AND METHODS

2.1 | Objective

The review was done to study the possibility of re-infection in patients diagnosed with Covid-19, after being discharged from the hospital. In this article, we discuss in detail all the possible causes that could result in a re-positive RT-PCR in these convalescent patients after meeting the recent discharge criteria.

2.2 | Databases

The literature review for the research was performed on PubMed and Cochrane. Mesh terms Covid-19, 'recurrence', 'infection', and 'risk' were searched with all corresponding keywords and relevant articles were imported into Endnote. Moreover, we used the bibliographies of literature retrieved via a search of authoritative texts and hand searches in WHO reports making sure we do not miss any articles. All keywords are shown in Table 1.

2.3 | Inclusion criteria

1. All studies with cases of re-positive PCR and suspected re-infection were included
2. Observational studies, case studies, case reports, and editorials
3. Good or fair-quality studies on the quality assessment questionnaire
4. Studies published in the English Language

2.4 | Exclusion criteria

1. Review articles
2. Studies published in languages other than English

2.5 | Study selection

A total of 206 studies was imported into Endnote from the two databases. Inclusion and exclusion criteria were applied and a total of 42 articles was selected after going through titles and abstracts. These 42 full articles were extracted and independently passed

through the quality assessment questionnaire to finally select a total of 27 articles included in the final review. Figure 1 shows the PRISMA flow chart of study selection.

2.6 | Data extraction

Data extraction was done from selected studies in tabulated form. Extraction of data was performed by the same review authors who conducted the study selection independently, using a structured form that contained study characteristics, including the age of the patients, presence of fever, presenting symptoms, vitals, CT scan findings, and management. Any disagreement was discussed after completion of the data collection process and reviewers were consulted for each topic.

3 | RESULTS

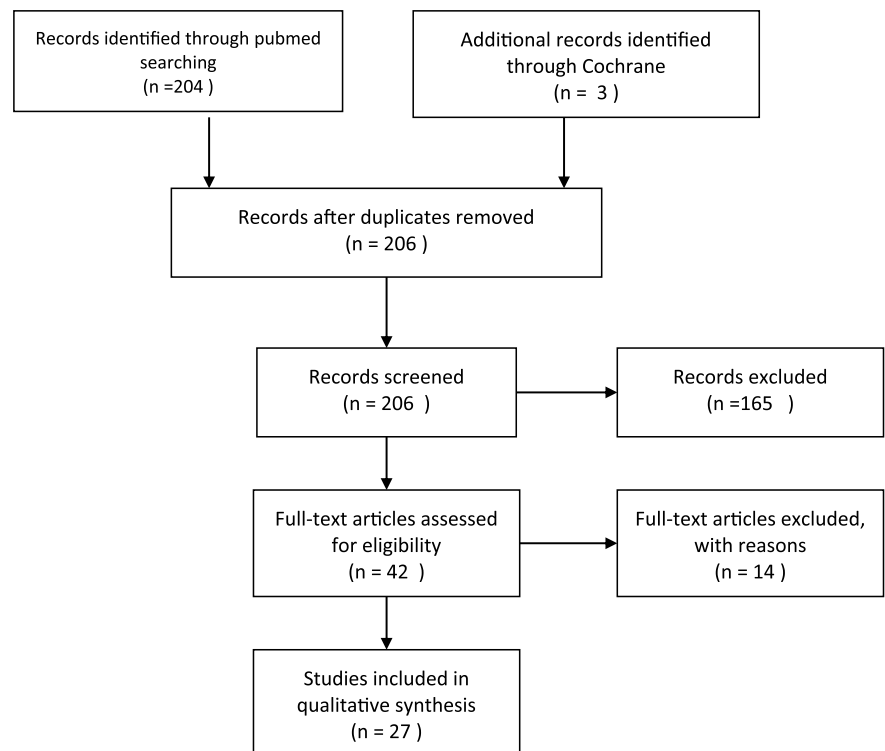
A total of 27 studies was included in our review, reporting a total of 253 cases who tested positive the second time after an average period of 11.5 days from the last negative RT-PCR test. The mean age of the RP positive cases was 47.85 years (Table 2). The sample most commonly collected was oropharyngeal swab ($n = 25/253$) followed by nasopharyngeal ($n = 21/253$) and in a few cases, saliva ($n = 1$), sputum ($n = 2$), or feces ($n = 4$); Table 3). In a few cases, samples from different sites were collected to reconfirm the initial positive results while in others the samples collected from secondary sites were positive suggesting viral shedding.

Data of initial presentation was available for 29, of whom 28 cases were symptomatic. Twenty-eight were febrile on the first presentation, six were afebrile while no data were available for the rest. There were significant findings on chest computed tomography (CT) scan in 18 patients with varying degrees of focal unilateral involvement, focal bilateral involvement, and diffuse bilateral lung involvement. They were initially managed with oxygen ($n = 7$), antibiotics ($n = 6$), antivirals ($n = 11$), steroids ($n = 10$), traditional Chinese medicine ($n = 1$), or anticoagulants ($n = 4$). Out of 253 patients who were RP positives in RT-PCR results, data from the second presentation was available for 28 out of which 18 were symptomatic on the second presentation while there were no significant clinical findings in 10 patients and were tested for mandatory reasons where a negative test was needed, for example, traveling or job. Six were febrile on the second presentation while 15 were afebrile. The chest CT scan showed positive findings in 22 patients, most of which were resolving ($n = 7$). Symptomatic cases were managed on the same line of management that is, oxygen ($n = 4$), antibiotics ($n = 6$), steroids ($n = 4$), antivirals ($n = 7$), Traditional Chinese Medicine ($n = 5$), and anticoagulants ($n = 1$). Serology results were available for ($n = 14$). IgM antibodies were positive in six patients and IgG in eight patients. Discharge RT-PCR data was available for 14. Twelve had negative RT-PCR results, while two were still positive and were sent home with instructions on precaution and isolation.

TABLE 1 MeSH terms

Mesh term	Covid-19	Recurrence	Infection	Risk
Entry terms	<ul style="list-style-type: none"> • 2019 novel coronavirus disease • COVID19 • COVID-19 pandemic • SARS-CoV-2 infection • COVID-19 virus disease • 2019 novel coronavirus infection • 2019-nCoV infection • Coronavirus disease 2019 • Coronavirus disease-19 • 2019-nCoV disease • COVID-19 virus infection • 2019-nCoV • Wuhan coronavirus • SARS-CoV-2 • 2019 novel coronavirus • COVID-19 virus • Coronavirus disease 2019 virus • COVID19 virus • Wuhan seafood market pneumonia virus 	<ul style="list-style-type: none"> • Recurrences • Recrudescence • Recrudescences • Relapse • Relapses 	<ul style="list-style-type: none"> • Infection and infestation • Infestation and infection • Infections and infestations • Infestations and infections • Infection 	<ul style="list-style-type: none"> • Risks • Relative risk • Relative risks • Risk, relative • Risks, relative

FIGURE 1 PRISMA



4 | DISCUSSION

Covid-19 has affected more than 55 million people worldwide with deaths exceeding one million.⁵ The chance of getting re-infected with the same virus poses a threat of another wave of the pandemic and has been a source of concern. In our study, we assessed whether reinfection is possible and other immunological, virological, and sampling errors which might have led to RP result.

4.1 | Low sensitivity of RNA detection kits

There is a possibility of RT-PCR rendering false-negative results, due to improper sampling procedures, sources of samples, or poor sensitivity/specificity of the nucleic acid test kit.

The low sensitivity of commercial RNA detection kits has been speculated to be one of the major causes of RP.⁶ Some commercial kits have only a 30%–50% positive rate of detection. According to the

TABLE 2 Characteristics of the re-positive individuals included in this review

Study	Median age		F 1°	F 2°	PC 1°	PC 2°	SaO ₂ 1°	CT 1°	CT 2°	Mx 1°	Mx 2°
	N	age (years)									
Bentivegna et al. ¹	1	69	F	AF	UTI, fever, cough	UTI	NA	B/L parenchymal consolidations	Accentuation of left parenchymal consolidation; resolution on right	HCO, LPV/r	None
Bongiovanni et al. ⁴²	1	81	F	AF	Severe acute RF	Malaise, muscle pain	83%	Severe interstitial pneumonia	NA	CPAP, HCO, heparin	None
Bongiovanni et al. ⁴²	1	85	F	F	Moderate RF	Malaise, muscle pain, dry cough	90%	Severe interstitial pneumonia	NA	O ₂ , HCO, heparin	None
Chena et al. ³	1	46	F	AF	Sore throat, cough, chest distress	NA	98%	NA	NA	NA	NA
Dou et al. ⁴³	1	34	F	AF	Cough, sore throat, dizziness, fatigue	Asymptomatic	91%	B/L GGO	Absorption of lung lesions	AHG, ribavirin, cefuroxime, LPV, IFN- α , human Ig	AHG, HCOe phosphate, IFN- α .
Lafaie et al. ⁴⁴	1	84	F	F	Cough, respiratory signs	Respiratory signs	79%	B/L GGO	B/L moderate lung lesions	Levofloxacin, MPS	O ₂ , NIV, MPS, levofloxacin, aztreonam, tocilizumab
Lafaie et al. ⁴⁴	1	90	F	NA	Cardiorespiratory decompensation	Dehydration with hypernatremia	93%	B/L Pleural Effusions	B/L diffuse lesion and lobar PE	Ofloxacin, MPS, anti-coagulation, diuretics	Palliative care
Lafaie et al. ⁴⁴	1	84	F	F	Asthenia, ageusia, respiratory signs with dry cough, polypnea	Dry cough, SaO ₂ 83%	93%	B/L GGO	B/L severe pneumonia	CTX, MPS, Rovamycine, methotrexate, MPS, O ₂	CTX, MPS, cotrimoxazole, plasma transfusion
Liu et al. ⁴⁵	1	35	F	F	Cough, sore throat, fatigue	Cough	NA	patchy GGO & hyperdense areas	No abnormalities	LPV, IFN- α , AHG, MPS	IAI, AHG
Loconsole et al. ⁴⁶	1	48	F	AF	Cough, shortness of breath, hyporexia	Dyspnea, chest pain.	90%	NA	Segmental & sub-segmental signs of arterial microembolism with ground glass areas	O ₂ , LPV/r, HCO, Enoxaparin, MPS	Anticoagulant
Qu et al. ⁴⁷	1	49	F	NA	Respiratory symptoms	Asymptomatic	NA	Multiple patchy GGO	Decreased inflammation	NA	NA

TABLE 2 (Continued)

Study	N	Median age (years)	F 1°	F 2°	PC 1°	PC 2°	SaO2 1°	CT 1°	CT 2°	Mx 1°	Mx 2°
Wang et al. ⁴⁸	1	33	F	NA	Cough	NA	NA	NA	NA	NA	Osetamivir, umifenovir, IFN
Zhou et al. ⁴⁹	1	40	F	F	Dyspnea	Respiratory symptoms	<80%	B/L multiple irregular areas of consolidation	Dense consolidation	AHG, MPS, Ig, O2, BIPAP	O2, MPS
Peng et al. ⁵⁰	1	67	F	AF	Cough	Asymptomatic	NA	NA	Abnormal	NA	NA
Peng et al. ⁵⁰	1	NA	AF	AF	Chills	Insomnia, anxiety	NA	NA	Abnormal	NA	NA
Peng et al. ⁵⁰	1	NA	AF	AF	Cough, expectoration	Insomnia, anxiety	NA	NA	Abnormal	NA	NA
Peng et al. ⁵⁰	1	NA	AF	AF	Cough	Asymptomatic	NA	NA	Normal	NA	NA
Peng et al. ⁵⁰	1	38	F	AF	NA	Asymptomatic	NA	NA	NA	NA	NA
Peng et al. ⁵⁰	1	29	F	AF	NA	Asymptomatic	NA	NA	Normal	NA	NA
Peng et al. ⁵⁰	1	21	F	AF	Itchy throat	Asymptomatic	NA	NA	Abnormal	NA	NA
Li et al. ⁵¹	1	41	F	NA	Critically ill	Chest pain, cough	90%	Flaky GGO close to the visceral pleura	Cellulosic exudation	NA	NA
Aming et al. ⁵²	1	58	F	NA	Cough with thick white sputum, chest pain, dizziness, poor appetite, fatigue	Mild cough, throat itching	NA	B/L patchy lung shadows	Absorption of opacities	NA	NA
Wang et al. ⁵³	8	46	F (n = 7)	F (n = 1)	Cough, fatigue, dyspnea	Cough, fatigue, expectoration, chest tightness, chest pain, palpitation, pharyngeal pain, nausea, myalgia, inappetence, vomiting, diarrhea, rhinorrhea	NA	Resolving infiltrates (n = 6), aggravating infiltrates (n = 1)	Resolution except B/L GGO (n = 1)	NA	TCM, cough medicine, O2, cortico steroids, expectorants
Lan et al. ⁵⁴	4	30–36	F (n = 3)	NA	Cough (n = 3)	Asymptomatic	NA	GGO	No changes	Osetamivir	NA
Liu et al. ⁵⁵	9	46 (14–70)	NA	NA	NA	Dry cough (n = 3), cough with sputum (n = 1)	NA	Focal GGO (n = 9), multiple GGO (n = 41), consolidation (n = 25)	Focal GGO (n = 5), multiple GGO (n = 12), consolidation (n = 1)	NA	NA

(Continues)

TABLE 2 (Continued)

Study	N	Median age (years)	F 1°	F 2°	PC 1°	PC 2°	SaO ₂ 1°	CT 1°	CT 2°	Mx 1°	Mx 2°
Liu et al. ⁵⁶	11	49 (37–62)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Zou et al. ⁵⁷	53	60 (22–98)	F	NA	Dry cough, fatigue, dyspnea, diarrhea, chest pain, myalgia, headache	One with cough, one with diarrhea	NA	NA	NA	NA	Osetamivir, Abx
Zheng et al. ⁵⁸	3	23–57	F	NA	NA	Improved symptoms	NA	NA	NA	NA	NA
An et al. ¹	38	14–60	F (n = 25)	AF	Respiratory symptoms (n = 28), GI symptoms (n = 3)	Mild cough, chest tightness	NA	NA	U/L lesions (n = 6), B/L lesions (n = 15), all lobes (n = 6)	Steroids (n = 4)	IAI (n = 1), low-flow O ₂ & TCM (n = 4)
Wang et al. ⁵⁹	35	32	F	AF	Fatigue (n = 5), dry cough (n = 16), expectoration (n = 9), runny nose (n = 5)	Asymptomatic (n = 32)	NA	B/L lung lesions (n = 30), lower lobe (n = 33), peripheral distribution (n = 25)	positive scans (n = 16), fully absorbed lung lesions (n = 5)	NA	NA
Yuan et al. ⁶⁰	20	46.4	NA	NA	Normal	NA	NA	NA	NA	NA	NA
Yuan et al. ⁶¹	25	16–42	F (n = 17)	AF	Cough (n = 14)	Asymptomatic	NA	NA	Improved (n = 12)	TCM	NA
Li et al. ⁶²	4	52.8	F	NA	Fever + fatigue (n = 4), cough (n = 2)	NA	NA	Inflammation	Absorption	O ₂ (n = 3), Abx (n = 2), antiviral (n = 4)	NA
Deng et al. ⁶³	4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Xiao et al. ⁶⁴	15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Xing et al. ⁶⁵	1	40 s	F	NA	NA	NA	NA	Infection in lower segment	NA	NA	NA
Xing et al. ⁶⁵	1	20 s	AF	NA	Headache, pharyngalgia	NA	NA	NA	NA	NA	NA

Abbreviations: 1°, initial infection; 2°, re-detectable positive infection; Abx, antibiotics; AF, afebrile; AF, atrial fibrillation; AHG, arbidol hydrochloride granules; B/L, bilateral; BIPAP, Bi-level positive airway pressure; BP, blood pressure; CPAP, continuous positive airway pressure; CTX, ceftriaxone; F, fever; GGO, ground-glass opacities; GI, gastrointestinal; HCQ, hydroxychloroquine; HR, heart rate; IAI, interferon α 2b atomization inhalation; LPV/r, lopinavir/ritonavir; MPS, methylprednisolone; Mx, management; N, number of cases; NIV, non-invasive ventilation; NP, nasopharyngeal; O₂, oxygen; OP, oropharyngeal; PC 1°, presenting complaints; PE, pulmonary embolism; RF, respiratory failure; RT-PCR, reverse transcriptase-polymerase chase reaction; SaO₂, oxygen saturation; TCM, traditional Chinese medicine; U/L, unilateral; UTI, urinary tract infection.

TABLE 3 Characteristics of SARS-CoV-2 related investigations among re-positive individuals

Study	N	Median age (years)	Days from last negative RT-PCR	Sample site	Discharge RT-PCR result	Serology
Bentivegna et al. ¹	1	69	23	NP	(-)	IgG (2.7 signal/cut-off)
Bongiovanni et al. ⁴²	1	81	2	NP	(-)	NA
Bongiovanni et al. ⁴²	1	85	5	NP	(-)	NA
Chena et al. ³	1	46	2	NP	NA	NA
Dou et al. ⁴³	1	34	14	NP	(-)	NA
Lafaie et al. ⁴⁴	1	84	>1 month	NP	NA	NA
Lafaie et al. ⁴⁴	1	90	Not specified	NP	NA	Not performed
Lafaie et al. ⁴⁴	1	84	No diagnostic test in asymptomatic phase	NP	NA	NA
Liu et al. ⁴⁵	1	35	11	NP, rectal	NP (+), rectal (-)	IgM (-), IgG (+) on reinfection
Loconsole et al. ⁴⁶	1	48	15	NP	NA	IgM and IgG (+)
Qu et al. ⁴⁷	1	49	3	OP	Sputum PCR (+)	NA
Wang et al. ⁴⁸	1	33	NA	OP	(-)	IgM and total antibody test (+)
Zhou et al. ⁴⁹	1	40	5	OP	(-)	IgM(19.27 to 36.44 AU/ml)IgG (24.68 to 28.81 AU/ml)
Peng et al. ⁵⁰	1	67	NA	OP	NA	NA
Peng et al. ⁵⁰	1	NA	NA	OP, NP	NA	NA
Peng et al. ⁵⁰	1	NA	NA	OP, NP	NA	NA
Peng et al. ⁵⁰	1	NA	NA	OP, NP	NA	NA
Peng et al. ⁵⁰	1	38	NA	Anal, OP	NA	NA
Peng et al. ⁵⁰	1	29	NA	Anal, OP	NA	NA
Peng et al. ⁵⁰	1	21	NA	NP	NA	NA
Li et al. ⁵¹	1	41	14 days	NP, sputum, fecal	NA	NA
Aming et al. ⁵²	1	58	22 days	OP	(-)	IgM(91.29),IgG(203.85)
Wang et al. ⁵³	8/131	46.5	1-2 weeks; 3-4 weeks	NA	(-)	NA
Lan et al. ⁵⁴	4	30-36	5-13	OP	(-)	NA
Liu et al. ⁵⁵	9/51	46.6	14 days	OP	(-)	NA
Liu et al. ⁵⁶	11/150	49	NA	OP	NA	IgG 243.0 (164.9-353.1) IgM 9.6 (4.1-24.9)
Zou et al. ⁵⁷	53/257	60.37	1-12 days	OP	(-)	IgG and IgM antibodies (+)150/257 patients
Zheng et al. ⁵⁸	3/20	23-57	7 days	Fecal, saliva	(-)	NA
An et al. ¹	38/262	14-60	14 days	Pharyngeal, anal	NA	NA
Wang et al. ⁵⁹	35/420	32	10 (7-16 days)	NP, OP, anal	NA	NA
Yuan et al. ⁶⁰	20/182	46.4	7-14 days	OP, anal	NA	NA
Yuan et al. ⁶¹	25	16-42	6 days (4-10)	NP, OP, cloacal	NA	NA

(Continues)

TABLE 3 (Continued)

Study	N	Median age (years)	Days from last negative RT-PCR	Sample site	Discharge RT-PCR result	Serology
Li et al. ⁶²	04/13	52.8	14 days (n = 1), 7 days (n = 1) 6 days (n = 1), 5 days (n = 1)	NP, OP, sputum, fecal	NA	NA
Deng et al. ⁶³	4/17	NA	3 days	NP, OP	NA	NA
Xiao et al. ⁶⁴	15/70	NA	NA	OP, NP	NA	NA
Xing et al. ⁶⁵	1	40s	NA	OP	NA	NA
Xing et al. ⁶⁵	1	20s	NA	OP	NA	NA

Abbreviations: N, number of cases; NP, nasopharyngeal; OP, oropharyngeal.

study by An et al.¹ the use of higher sensitive methods increased the detection of viral RNA in the samples of RP patients with initially negative results. Another possible, albeit less plausible reason, include contamination of the samples, even though most centres ensure that the testers change PPE (personal protective equipment) in between patients.⁷ Despite positive RT-PCR test results, most patients were asymptomatic and had unchanged clinical imaging, indicating that a positive RT-PCR does not necessarily signify reinfection and fails to correlate clinically.⁷ Hoang et al.⁸ also showed that recurrences could be persistent infections with false-negative PCR results at discharge. A high false-negative rate (48/384, 12.5%) of RT-PCR results for SARS-CoV-2 detection has been recorded.⁸ Therefore, future studies should improve both the sensitivity and specificity of the detection kit.

4.2 | Defective sampling techniques

A pharyngeal specimen is the most commonly collected specimen to detect the virus as the virus initially appears in the upper respiratory tract. It is collected either via nasopharyngeal or oropharyngeal swabs. The specimen should be collected correctly via a flocked tapered swab from the nasopharynx and then stored in a sterile environment. In the case of an oropharyngeal specimen, a swab is inserted into the posterior pharynx and tonsillar areas. The swab should rub over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums. If the swab is not inserted deep enough, it may not collect sufficient viral particles. To minimize the chances of false negatives, it is recommended that two samples from both nostrils should be collected.⁹ Initially, the virus appears in the upper respiratory tract, but as the infection progresses, it appears in the lower respiratory tract or other locations such as the gut or blood.¹⁰

4.3 | PCR techniques

Different RT-PCR assays are commonly used for targeting different SARS-CoV-2 genomic regions, including ORF8 regions, ORF1b, spike (S), nucleocapsid (N), envelope (E) genes, or RNA-dependent RNA

polymerase.¹¹ These gene-specific primers may also affect the results of the tests through the variation in the viral RNA sequences that are targeted. These limitations of Covid-19 tests can be recognized by applying the intact virus to yield better detection of actual samples compared with the use of nucleotide sequences. Hence, improved PCR techniques with greater amplification efficiency should be routinely used, such as the addition of a second primer pair or a multiple-target gene amplification, and the use of probing primer sets that are designed to minimize misdetection.¹²

4.4 | Residual viral particles

The PCR test relies on the amplification of nucleic acid in the sample, not fully active viral particles. Studies have shown that the presence of these inactive viral RNA particles outlasts the infectious viral particles in the body.¹³ While the immune system generates antibody responses to the surface protein of viral particles, the genetic material (RNA, DNA) left behind degrades over time.¹⁴ Thus, RP positive PCR results may not necessarily signify reinfection, but rather the presence of leftover genetic material from a previous active infection. Wolfel et al.¹⁵ isolated live virus from individuals infected with SARS-CoV-2 but noticed that after day 8 of infection, the live virus was not able to be isolated despite high overall viral loads. An et al.¹ showed that young patients with a mild episode of Covid-19 seem to constitute most of the RP patients after discharge. According to the study Bentivegna et al.,⁶ most of the patients with post-recovery positive RT-PCR were either asymptomatic or mildly symptomatic. All contacts of RP patients were tested negative for SARS-CoV-2 RNA, and no suspicious clinical symptoms were reported.

Virus culture can be used to identify whether the prolonged PCR positivity is just a result of non-viable viral RNA shedding or the result of persistent, infectious viral RNA shedding. If the culture is negative for the viable virus, then the detected viral RNA from the PCR is a likely result of non-viable viral RNA shedding and not an ongoing infection or re-infection. If a viable virus is identified through culture, further investigation is needed to assess whether the viable virus from the second episode is the result of a secondary infection by a different viral strain.¹⁶

4.5 | Low viral load

Viral load is a strong indicator of the severity of infection.¹⁷ Normally, PCR testing amplifies the genetic material from the collected specimen in cycles; the fewer the cycles required to detect it, the greater will be the initial viral load.¹⁸ Accurate detection and measurement of viral load are crucial for clinical practice and decision-making. RT-PCR could be used to directly quantify viral load by observing the fluorescence signal that proportionally increases with the amount of nucleic acid. This test serves to confirm the positivity of a case under investigation based on a specified threshold of detected fluorescence and a certain number of PCR cycles. A high cycle threshold (Ct) value indicates low viral load. A Ct (number of cycles required to detect viral particles) value of 40 is a cut-off point and should be determined in routine laboratories.¹⁸

4.6 | Intermittent viral shedding

One potential reason for RP is intermittent viral shedding. The GI system is one of the main potential shedding sites due to the presence of ACE-2 receptors.¹⁹ Xing et al.²⁰ reported persistent viral shedding in two children during the convalescent phase. This led to the surveillance of faecal matter of paediatric patients and their families with negative nasal PCR tests. In another study, the faecal shedding of the viral matter was postulated to be not infectious in nature.²¹

Gupta et al.²² conducted a systematic review to identify the incidence and timing of positive faecal testing in clinically recovered patients. In his review, he deduced that faecal testing remains positive for 1 to 33 days after a negative nasopharyngeal swab. So, even if there is a negative nasopharyngeal swab, the faecal test can be positive for days as the upper respiratory tract clears the virus faster than the gut.²³ Such a positive faecal test may be mistaken for recurrence and future reports should explicitly document this.

Dou et al.²⁴ confirmed the presence of characteristic lesions detected on serial CT imaging that were not resolved in re-positive patients. Prolonged viral shedding was noted with PCR on respiratory swab samples in a 71-year-old woman 60 days after the onset of symptoms, and 36 days after symptoms had subsided.²⁵ Researchers have reported delayed admission and mechanical ventilation as the factors correlating with delayed viral shedding.²⁶ Therefore, prolonged viral shedding may explain persistent re-positive results.

4.7 | Mutated strains

A mutation that can affect the biological conformation of the virus leading to altered infectivity and immunogenicity is called a strain, otherwise, it is called a variant. A total of 106 variants and 68 strains has been identified so far with most having little to no impact on the virulence of the virus.²⁷ As the cases of Covid-19 are now rising exponentially, recurrences due to different strains have also been reported.²⁷

In August, a 33-year-old man was diagnosed again with Covid-19 in Hong Kong after 142 days since the initial infection.²⁸ After an initially mild symptomatic episode in March 2020, he recovered and the serological testing did not reveal any detectable antibodies. In August 2020, he was tested positive on entry screening at the airport, after his return trip to Europe. The genomic testing revealed mutated viral particles making this the first confirmed case of reinfection due to a different strain. In comparison, there were four amino acid residues that differed in the spike protein between the first and second infection, including L18F, A222V, D614G, and Q780E. The A222V and D614G mutations affecting the spike protein of the virus were said to be significant in evading the immune response and causing reinfection. While there is no significant data on A222V mutation apart from the fact that A222V mutation occurs in one of the viral spike proteins that T-cells target, the D614G mutation has been studied widely.²⁹ The original strain of the virus from Wuhan had the D614 variant. The D614G mutation changed the amino acid at position 614 from D (aspartic acid) to G (glycine) giving birth to a new G614 strain. The mutation initially occurred in Europe and gradually noticed to be on the rise in North America, Oceania, and then Asia.³⁰ Currently, 70% of the general population has this mutation. The G614 mutation has been infamous for its increased infectivity, viral load, and rapid spread among the general population while further studies are being conducted to observe the difference in virulence.³¹

After the aforementioned case, reports of recurrent cases with a different strain emerged from Belgium, Nevada, Ecuador, and India.^{32–35} These recurrences have raised several questions regarding the longevity of immunity, the immune response to a new strain, and the future of vaccines in lieu of these mutations. The second episode in most of the reported cases was mild predicting residual immunity after the initial infection. Larson et al.³⁶ reported a severe episode after a mildly symptomatic initial infection. The limited testing and genomic studies make it difficult to calculate the extent of reinfection due to a new strain and further studies are warranted to predict the immune response to a different strain.

4.8 | Humoral immunity

Antibodies against other coronaviruses can wane over time (range: 12–52 weeks from the onset of symptoms).³⁷ Memory cells develop along with the killer T cells and next time the body is infected, the body takes a shorter time to combat the infection.

The antibody response developed against the novel SARS-CoV-2 depends upon the severity of the infection. A typical case develops immunity after 7–15 days.³⁸ Mild infections take longer to confer immunity and it is postulated that asymptomatic infections do not develop detectable antibodies. The antibody response once developed did not wane within the four months as concluded from a recent study done in Iceland.³⁹ Further longitudinal studies should be done to study the immune response to Covid-19 and its impact on the development of antibodies. Mantovani et al. has also hinted towards

the protective effect of prior vaccines, particularly BCG vaccine on the immune system. BCG increases the immune response to pathogens other than TB and conferring nonspecific protection against a wide range of infections by activating the innate immune system. It also helps the innate immune system to develop a 'memory' against other microorganisms, a phenomenon known as trained innate immunity.⁴⁰ However, Luciana et al. reported a unique case of acute tuberculosis infection superimposed on COVID-19 pneumonia, hinting towards the fact that BCG vaccine may have failed to train the immune system to fight off both infections.⁴¹ This hypothesis requires multiple scientific trials for confirmation. Furthermore, in lieu of the multiple mutated strains, highly sensitive antibody testing methods should be devised to detect antibodies against a range of epitopes found on multiple viral proteins. This will not only help us in studying protective immune response but also help in the development of suitable vaccines and the protection they confer. We can then assess the longevity of protective antibodies produced after vaccination.

5 | CONCLUSION

While the initial cases of re-positives can be due to sampling errors, immunological and viral factors that may have caused a false positive, recent studies have shown that reinfection by mutated viral particles is possible. The re-infection in most cases was either asymptomatic or mildly symptomatic hinting towards potential long-term immunity to the subsequent exposure. Genetic studies should be done to assess the effect of various mutations on the virulence and spread of the virus. Further studies are warranted to evaluate the immune response to the mutated particles, the longevity of antibodies, and the effect of these mutations on the development of the vaccine.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

L.F. and A.M. contributed equally in designing the study, database screening, data extraction, and writing manuscript. M.K.S. critically reviewed the paper for the final submission.

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REFERENCES

- An J, Liao X, Xiao T, et al. Clinical characteristics of the recovered COVID-19 patients with re-detectable positive RNA test. *MedRxiv*. 2020.
- Chahrour M, Assi S, Bejjani M, et al. A bibliometric analysis of Covid-19 research activity: a call for increased output. *Cureus*. 2020; 12(3):7357. <https://doi.org/10.7759/cureus.7357>.
- Chen D, Xu W, Lei Z, et al. Recurrence of positive SARS-CoV-2 RNA in COVID-19: a case report. *Int J Infect Dis*. 2020;93:297-299.
- Elrashdy F, Aljaddawi AA, Redwan EM, Uversky VN. On the potential role of exosomes in the COVID-19 reinfection/reactivation opportunity. *Journal of Biomolecular Structure and Dynamics*. 2020; 1-12. <https://doi.org/10.1080/07391102.2020.1790426>.
- Coronavirus Update (live): 58,949,581 cases and 1,393,073 deaths from COVID-19 virus pandemic - Worldometer. <https://www.worldometers.info/coronavirus/>. 2020.
- Bentivegna E, Sentimentale A, Luciani M, Speranza ML, Guerritore L, Martelletti P. New IgM seroconversion and positive RT-PCR test after exposure to the virus in recovered COVID-19 patient. *Journal of Medical Virology*. 2020. <https://doi.org/10.1002/jmv.26160>.
- Chaturvedi R, Naidu R, Sheth S, Chakravarthy K. Efficacy of Serology Testing in Predicting Reinfection in Patients With SARS-CoV-2. *Disaster Medicine and Public Health Preparedness*. 2020;1-3. <https://doi.org/10.1017/dmp.2020.216>.
- Hoang VT, Dao TL, Gautret P. Recurrence of positive SARS-CoV-2 in patients recovered from COVID-19. *Journal of Medical Virology*. 2020;92(11):2366-2367. <https://doi.org/10.1002/jmv.26056>.
- CDC. *Interim guidelines for collecting, handling, and testing clinical specimens for COVID-19*. <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>.
- Chen Y, Bai W, Liu B, et al. Re-evaluation of retested nucleic acid-positive cases in recovered COVID-19 patients: report from a designated transfer hospital in Chongqing, China. *J Infect Public Health*. 2020;13(7):932-934.
- Carter LJ, Garner LV, Smoot JW, et al. Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Central Science*. 2020;6(5):591-605. <https://doi.org/10.1021/acscentsci.0c00501>.
- Osman AA, Al Daajani MM, Alsahafi AJ. Re-positive coronavirus disease 2019 PCR test: could it be a reinfection? *New Microbes and New Infections*. 2020;37:100748. <https://doi.org/10.1016/j.nmni.2020.100748>.
- Karia R, Nagraj S. A review of viral shedding in resolved and convalescent COVID-19 patients. *SN Compr Clin Med*. 2020;1-10.
- Chang D, Mo G, Yuan X, et al. Time kinetics of viral clearance and resolution of symptoms in novel coronavirus infection. *Am J Respir Crit Care Med*. 2020;201(9):1150-1152.
- Wolfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature*. 2020;581(7809):465-469.
- European Centre for Disease Prevention and Control. *Reinfection with SARS-CoV: Considerations for public health response*. ECDC; 2020.
- Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci*. 2020;63(3):364-374.

18. Mandavilli A. *Your coronavirus test is positive. Maybe it shouldn't be.* The New York Times. <https://www.nytimes.com/2020/08/29/health/coronavirus-testing.html>.
19. Zhang H, Kang Z, Gong H, et al. The digestive system is a potential route of 2019-nCoV infection: a bioinformatics analysis based on single-cell transcriptomes. *bioRxiv*. 2020.30 v1
20. Xing Y, Ni W, Wu Q, et al. Prolonged presence of SARS-CoV-2 in feces of pediatric patients during the convalescent phase. *medRxiv*. 2020.
21. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis*. 2020;20(4):411-412.
22. Gupta S, Parker J, Smits S, Underwood J, Dolwani S. Persistent viral shedding of SARS-CoV-2 in faeces - a rapid review. *Colorectal Dis* 2020;22(6):611-620.
23. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect*. 2020;9(1):386-389.
24. Dou P, Zhang S, Wang C, et al. Serial CT features in discharged COVID-19 patients with positive RT-PCR re-test. *European Journal of Radiology*. 2020;127:109010. <https://doi.org/10.1016/j.ejrad.2020.109010>.
25. Li J, Zhang L, Liu Baihui, Song D. Case Report: Viral Shedding for 60 Days in a Woman with COVID-19. *The American Journal of Tropical Medicine and Hygiene*. 2020;102(6):1210-1213. <https://doi.org/10.4269/ajtmh.20-0275>.
26. Xu K, Chen Y, Yuan J, et al. Factors Associated With Prolonged Viral RNA Shedding in Patients with Coronavirus Disease 2019 (COVID-19). *Clinical Infectious Diseases*. 2020;71(15):799-806. <https://doi.org/10.1093/cid/ciaa351>.
27. Li Q, Wu J, Nie J, et al. The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell*. 2020;182(5):1284-1294. <https://doi.org/10.1016/j.cell.2020.07.012>.
28. To KK-W, Hung I-F-N, Ip J-D, et al. Coronavirus Disease 2019 (COVID-19) Re-infection by a Phylogenetically Distinct Severe Acute Respiratory Syndrome Coronavirus 2 Strain Confirmed by Whole Genome Sequencing. *Clinical Infectious Diseases*. 2020. <https://doi.org/10.1093/cid/ciaa1275>.
29. Braun J, Loyal L, Frentsch M, et al. Presence of SARS-CoV-2 reactive T cells in COVID-19 patients and healthy donors. *Medrxiv*. Preprint. 2020;10(2020.04):17.20061440.
30. Korber B, Fischer WM, Gnanakaran S, et al. Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell*. 2020;182(4):812-827. e819.
31. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;33(7):1870-1874.
32. Tillett RL, Sevinsky JR, Hartley PD, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. *The Lancet Infectious Diseases*. 2021;21(1):52-58. [https://doi.org/10.1016/s1473-3099\(20\)30764-7](https://doi.org/10.1016/s1473-3099(20)30764-7).
33. Gupta V, Bhojar RC, Jain A, et al. Asymptomatic Reinfection in 2 Healthcare Workers From India With Genetically Distinct Severe Acute Respiratory Syndrome Coronavirus 2. *Clinical Infectious Diseases*. 2020. <https://doi.org/10.1093/cid/ciaa1451>.
34. Prado-Vivar B, Becerra-Wong M, Guadalupe JJ, et al. COVID-19 re-infection by a phylogenetically distinct SARS-CoV-2 variant. *First Confirmed Event in South America (September 3, 2020)*. 2020.
35. Van EJ, Vermeersch P, Vandervoort K, et al. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain. *Clinical Infectious Diseases*. 2020. <https://doi.org/10.1093/cid/ciaa1330>.
36. Larson D, Brodniak SL, Voegtly LJ, et al. A Case of Early Reinfection With Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clinical Infectious Diseases*. 2020. <https://doi.org/10.1093/cid/ciaa1436>.
37. Kellam P, Barclay W. The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection. *J Gen Virol*. 2020;101(8):791-797.
38. Zhao J, Yuan Q, Wang H, et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. *Clinical Infectious Diseases*. 2020;71(16):2027-2034. <https://doi.org/10.1093/cid/ciaa344>.
39. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. *New England Journal of Medicine*. 2020;383(18):1724-1734. <https://doi.org/10.1056/nejmoa.2026116>.
40. Mantovani A, Netea MG. Trained innate immunity, epigenetics, and Covid-19. *N. Engl J Med*. 2020;383(11):1078-1080.
41. Luciani M, Bentivegna E, Spuntarelli V, et al. Coinfection of tuberculosis pneumonia and COVID-19 in a patient vaccinated with Bacille Calmette-Guérin (BCG): case report. *SN Compr Clin Med*. 2020:1-4.
42. Bongiovanni M, Basile F. Re-infection by COVID-19: a real threat for the future management of pandemia? *Infect Dis (Lond)*. 2020;52(8):581-582.
43. Dou C, Xie X, Peng Z, et al. A case presentation for positive SARS-CoV-2 RNA recurrence in a patient with a history of type 2 diabetes that had recovered from severe COVID-19. *Diabetes Res Clin Pract*. 2020;166:108300.
44. Lafaie L, Célarier T, Goethals L, et al. Recurrence or Relapse of COVID -19 in Older Patients: A Description of Three Cases. *Journal of the American Geriatrics Society*. 2020;68(10):2179-2183. <https://doi.org/10.1111/jgs.16728>.
45. Liu F, Cai ZB, Huang JS, et al. Positive SARS-CoV-2 RNA recurs repeatedly in a case recovered from COVID-19: dynamic results from 108 days of follow-up. *Pathog Dis*. 2020;78(4). <https://doi.org/10.1093/femspd/ftaa031>.
46. Loconsole D, Passerini F, Palmieri VO, et al. Recurrence of COVID-19 after recovery: a case report from Italy. *Infection*. 2020;48(6):965-967. <https://doi.org/10.1007/s15010-020-01444-1>.
47. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733.
48. Wang P. Recurrent presence of SARS-CoV-2 RNA in a 33-year-old man. *J Med Virol*. 2020. <https://doi.org/10.1002/jmv.26334>.
49. Zhou X, Zhou J, Zhao J. Recurrent pneumonia in a patient with new coronavirus infection after discharge from hospital for insufficient antibody production: a case report. *BMC Infect Dis*. 2020;20(1):500.
50. Peng J, Wang M, Zhang G, Lu E. Seven discharged patients turning positive again for SARS-CoV-2 on quantitative RT-PCR. *Am J Infect Control*. 2020;48(6):725-726.
51. Li X-j, Zhang Z-w, Zong Z-y. A case of a readmitted patient who recovered from COVID-19 in Chengdu, China. *Critical Care*. 2020;24:1-3.
52. Luo A. Positive SARS-Cov-2 test in a woman with COVID-19 at 22 days after hospital discharge: A case report. *Journal of Traditional Chinese Medical Sciences*. 2020. <https://doi.org/10.1016/j.jtcm.2020.04.001>.
53. Wang X, Xu H, Jiang H, et al. The clinical features and outcomes of discharged coronavirus disease 2019 patients: a prospective cohort study. *QJM*. 2020;113(9):657-665.
54. Lan L, Xu D, Ye G, et al. Positive RT-PCR test results in patients recovered from COVID-19. *JAMA*. 2020;323(15):1502-1503.
55. Liu C, Ye L, Xia R, et al. Chest CT and clinical follow-up of discharged patients with COVID-19 in Wenzhou City, Zhejiang, China. *Ann Am Thorac Soc*. 2020;17(10):1231-1237.
56. Liu T, Wu S, Zeng G, et al. Recurrent positive SARS-CoV-2: immune certificate may not be valid. *J Med Virol*. 2020;92(11):2384-2386. <https://doi.org/10.1002/jmv.26074>.

57. Zou Y, Wang BR, Sun L, et al. The issue of recurrently positive patients who recovered from COVID-19 according to the current discharge criteria: investigation of patients from multiple medical institutions in Wuhan, China. *J Infect Dis.* 2020;222(11):1784–1788. <https://doi.org/10.1093/infdis/jiaa301>.
58. Zheng KI, Wang XB, Jin XH, et al. A case series of recurrent viral RNA positivity in recovered COVID-19 Chinese patients. *J Gen Intern Med.* 2020;35(7):2205–2206.
59. Wang LF, Yan H, Zhang D, et al. Clinical Characteristics and Imaging Findings of 35 Patients Recovered from COVID-19 with Recurrent Positive RT-PCR Test Results. *SSRN Electronic Journal.* <https://doi.org/10.2139/ssrn.3564400>.
60. Yuan B, Liu H-Q, Yang Z-R, et al. Recurrence of positive SARS-CoV-2 viral RNA in recovered COVID-19 patients during medical isolation observation. *Scientific Reports.* 2020;10(1). <https://doi.org/10.1038/s41598-020-68782-w>.
61. Yuan J, Kou S, Liang Y, Zeng J, Pan Y, Liu L. Clinical characteristics on 25 discharged patients with COVID-19 virus returning. *medRxiv.* 2020.
62. Li Y, Hu Y, Yu Y, et al. Positive result of Sars-Cov-2 in faeces and sputum from discharged patient with COVID-19 in Yiwu, China. *Journal of Medical Virology.* 2020;92(10):1938–1947.
63. Chen Y, Bai W, Liu B, et al. Re-evaluation of retested nucleic acid-positive cases in recovered COVID-19 patients: Report from a designated transfer hospital in Chongqing, China. *Journal of Infection and Public Health.* 2020;13(7):932–934. <https://doi.org/10.1016/j.jiph.2020.06.008>.
64. Xiao AT, Tong YX, Zhang S. False negative of RT-PCR and prolonged nucleic acid conversion in COVID-19: Rather than recurrence. *J Med Virol.* 2020.
65. Xing Y, Mo P, Xiao Y, Zhao O, Zhang Y, Wang F. Post-discharge surveillance and positive virus detection in two medical staff recovered from coronavirus disease 2019 (COVID-19), China, January to February 2020. *Eurosurveillance.* 2020;25(10):2000191.

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