Vaccine Discovery and Development: Lessons from COVID-19

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RESEARCH ARTICLE

Current meta-analysis does not support the possibility of COVID-19 reinfections

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Abstract

Coronavirus disease 2019 (COVID-19) reinfections could be a major aggravating factor in this current pandemic, as this would further complicate potential vaccine development and help to maintain worldwide virus pockets. To investigate this critical question, we conducted a clinical meta-analysis including all available currently reported cases of potential COVID-19 reinfections. We searched for all peer-reviewed articles in the search engine of the National Center for Biotechnology Information. While there are over 30,000 publications on COVID-19, only about 15 specifically target the subject of COVID-19 reinfections. Available patient data in these reports was analyzed for age, gender, time of reported relapse after initial infection and persistent COVID-19 positive polymerase chain reaction (PCR) results. Following the first episode of infection, cases of clinical relapse are reported at 34 (mean) ± 10.5 days after full recovery. Patients with clinical relapse have persisting positive COVID-19 PCR testing results until 39 ± 9 days following initial positive testing. For patients without clinical relapse, positive testing was reported up to 54 ± 24 days. There were no reports of any clinical reinfections after a 70-day period following initial infection.

KEYWORDS
coronavirus, COVID-19, effective, measures, pandemic, spread

1 | INTRODUCTION

Repeated news of successful coronavirus disease 2019 (COVID-19) vaccine developments regularly raise global attention. However, there is still no data or proof of any effective vaccine preventing COVID-19 infections. While the COVID-19 pandemic is contained in some areas, national and international travel remains a potential key vector for further virus spread. By now we know that extensive containment measures and policies have resulted in reduced cases over time. Some heavily affected countries have put their hopes into vaccine development, while others have relied on the concept of herd immunity (Figures 1 and 2).

As time passes by, there is an increase in reports on potential COVID-19 reinfections following initial recovery. While it is not possible to foresee whether an effective vaccine can be successfully developed, knowing whether COVID-19 reinfections are in fact possible could help determine the probability of developing a vaccine. In addition, understanding the possibility of COVID-19

FIGURE 1 Meta-analysis of patients with clinical relapse and persisting coronavirus diesease 2019 (COVID-19) positive test results. Age distribution is presented on the left side. Distribution of beginning of clinical relapse as well as last time of positive COVID-19 testing after initial diagnosis is shown on the right. Box-plot model including minimum value, first quartile, median value, third quartile, and maximum value
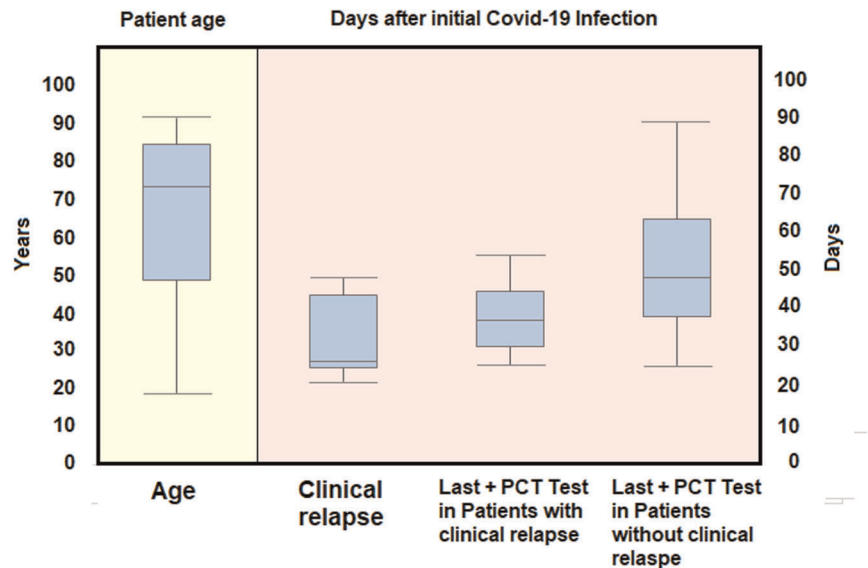
reinfections can help evaluate whether the amount of potentially infectable individuals is finite and if the concept of "herd immunity" discussed by some scientists is even theoretically feasible.[1] Until today, it has been impossible to develop a fully immunizing vaccine for any virus belonging to the Coronavirus family.[2]

Furthermore, reports suggest that not all patients with COVID-19 have a measurable antibody titer following their recovery.[3] These findings can be interpreted as potentially unfavorable when creating a possible vaccine with a high targeted immunization rate. While the development of vaccines for measles, polio, smallpox and others has been very successful, it has remained impossible to create vaccines preventing HIV and hepatitis C infections. Challenges in local containment,[4] spread by international travel,[5,6] sufficient testing,[7] consequences of failed containment have been previously analyzed and discussed.[8] However, if vaccine development fails or experiences delay, the virus threat may continue for an extended period of time and even be aggravated if COVID-19 reinfections prove possible. This would mean that even populations with widespread contact with COVID-19 would not experience any reduced susceptibility toward the virus. These considerations have been recently identified as critical.[9,10] By means of this study, we aim to investigate if in fact available data supports COVID-19 reinfections following initial recovery as well as understand how to interpret positive COVID-19 test results weeks to months after initial positive testing.
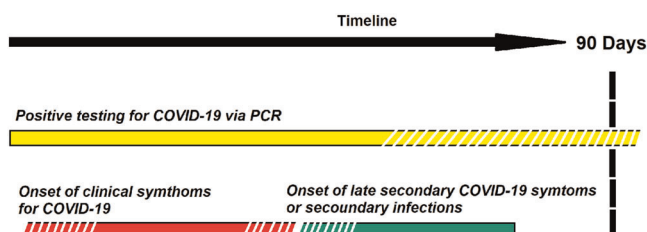

FIGURE 2 Model of overlapping recurrent COVID-19 symptoms or late secondary infections within the timeframe of positive COVID-19 positive PCR results. COVID-19, coronavirus disease 2019; PCR, polymerase chain reaction

2 | MATERIALS AND METHODS

2.1 | Data sources

Analyses of current data available on COVID-19 relapse and reinfection was challenging. While an excess of 30,000 articles is available on COVID-19, there are less than 15 articles which focus on potential clinical relapse and virus reinfection. We conducted a meta-analysis on the available clinical data presented in these articles. Seven of these peer-reviewed publications focused on COVID-19 reinfections. In five publications, there were at total of 15 patients with clinical manifest and confirmed COVID-19 reinfections. The other two studies presented persistent positive COVID-19 test results in asymptomatic patients.

2.2 | Confirmed coronavirus disease 2019 cases

All available reports were peer-reviewed publications which were accessible through the United States National Library of Medicine at the National Institutes of Health (see Table 1). Calculations included mean, median, standard deviation, as well as maximum and minimum for age and date of clinical relapse. Additionally, the last reported day of positive COVID-19 testing via reverse transcription-polymerase chain reaction (RT-PCR) was noted and its average with standard deviation was calculated for patients with and without clinical COVID-19 relapse.

2.3 | Statistical analysis

All data are expressed as mean ± SEM. $p < .05$ were considered statistically significant. All data was analyzed on GraphPad Prism (GraphPad Software 8.0.1; La Jolla). Data was additionally presented in quartiles.

TABLE 1 Available research data extracted from the United States National Library of Medicine at the National Institutes of Health

Cases (references in parenthesis)	Patients	Time of clinical relapse	Diagnostic confirmation	Outcome	Important observations
Case report, USA[11] (1 patient)	82 Years, male, discharged	Day 48 after initial presentation	Positive RT-PCR	Cured	
Case report, France[12] (3 patients)	84 Years, female	Day 41 after initial presentation	Positive RT-PCR Negative COVID-19 antibodies at relapse	Fatal	Relapse during in-clinic rehabilitation
	90 Years, female, discharged	6–7 weeks after initial presentation	Positive RT-PCR	Fatal	
	84 Years, female λ	Day 22	CAT scan positive nasopharyngeal sample twice negative	Fatal	Relapse during in-clinic rehabilitation
Cumulative case report, France[13] (11 patients)	P1 19 years, female; P2 32 years, female P3; 33 years, female; P4 43 years, female; P5 85 years, male; P6 54 years, male; P7 91 years, female; P8 55 years, male; P9 72 years, male; P10 73 years, male; P11 84 years, female	Day 26, Day 37, Day 27, Day 24, Day 44, Day 45, Day 25, Day 27, Day 27, Day 24, Day 49	positive RT-PCR tests both initially and during relapse; all patients showed typical signs of acute COVID-19 infection in CAT-scan during relapse	Cured, cured, cured, cured, cured, fatal, cured, cured, fatal, cured, fatal	
Case report, China[14] (1 patient)	34 years, male, discharged	No relapse	RT-PCR positive 59 days after initial symptoms	Cured	RT-PCT positive after 59 days
Clinical study, China[15] (8 patients)	All patients were asymptomatic CAT-scans showed no abnormalities		Readmission due to positive RT-PCR	Cured	Patients were cleared after three consecutive tests were negative. In two cases positive results persisted for >90 days
In vivo study[16]	Study specimen were macaques				Study suggests formation of neutralizing antibodies after COVID-19 infection
Clinical study on coronavirus family, USA[17] (191 patients)	Beta-coronaviruses HKU1 and OC43	34 Weeks after enrollment/ first infection[2]		No significant association between testing positive at least once and relapse of betacoronavirus	

Abbreviation: RT-PCR, reverse transcription-polymerase chain reaction.

3 | RESULTS

3.1 | Research subject

Current analyses of available literature reveal that only little research was conducted on potential COVID-19 reinfections. In fact, out of 30,000 COVID-19 related articles, less than 15 articles specifically targeted this subject. However, many of these reports were also only recently published. The subject of COVID-19 reinfection is either studied by means of clinical data or laboratory in-vitro or in-vivo experiments.

3.2 | Clinical data

Available clinical data were presented as single or cumulative case reports. Some of these case reports focused on clinically observed disease relapse or recurrence with typical, identified COVID-19 symptoms following initial COVID-19 infection recovery. Other reports concentrated on how long patients with COVID-19 are tested positive. In some cases, patients tested positive for the entire duration of the case whereas in others, a positive test result was obtained after two previous tests were negative. However, in these studies patients typically do not display any residual COVID-19 symptoms.

3.3 | Studies with predominantly clinical data

All 15 analyzed patient reports on COVID-19 reinfections are from the United States and France. These patients all presented with typical COVID-19 symptoms at the time of supposed reinfection. Beside their typical clinical symptoms, they were also tested positive for COVID-19 via PCR.[11-13] The medium age of these patients was 65.4 years (range: 19–91 years). Clinical disease relapse along with COVID-19 symptoms and positive test results occurred at 34 ± 10.5 days after initial COVID-19 diagnosis. In 40% of cases (n = 6), patients did not survive disease relapse. The average time of last positive PCR-testing was 39 ± 9 days.

Two studies investigated patients who had positive COVID-19 PCR results over an extended time period despite being clinically asymptomatic.[14,15] These two studies included a total of nine patients. In two of these patients, PCR testing remained positive for at least 90 days after initial COVID-19 diagnosis. The average time of the last positive PCR testing was 54 ± 24 days.

To investigate the possibility of COVID-19 reinfections, in-vitro and in-vivo studies have been conducted. However, results from these studies remain inconclusive. For example, in an in vivo COVID-19 study on macaques, the authors concluded that following initial COVID-19 infection, a stage was reached at which macaques were suspected to develop neutralizing antibodies.[16] However, a clinical study investigating patients suffering from beta-coronaviruses (HKU1 and OC43) before the COVID-19 outbreak came to the opposite conclusion, indicating that no immunization of described corona virus strains was attained.[17]

4 | DISCUSSION

The incorporated data does not include any cases beyond 90 days. Public awareness on the COVID-19 outbreak started in January 2020, and since most attention was directed toward containment strategies, reporting on reinfections became negligible. Reported cases of COVID-19 reinfections are either based on the reoccurrence of clinical symptoms or intermittent positive test results. In fact, in many cases, patients had already clinically improved despite continuous positive PCR testing. These observations call the likelihood of true COVID-19 reinfections into question.

Prolonged presence of genetic material in a host is common in many viral infections, even after virus clearance and resolution of symptoms.[18-20] Thus, detection of genetic material by sole RT-PCR does not necessarily prove active infection or infectivity.[21,22] Recent studies have reported that some patients have tested COVID-19 positive using RT-PCR days or even weeks after disease recovery, and previous negative results.[23,24] RNA detection, in any sample, does not necessarily mean the presence of the complete virus in the host nor an active infection. A positive RT-PCR result does not prove severe acute respiratory syndrome coronavirus 2 viability with certainty, even if genome sequencing is performed.[25] In fact, RT-PCR is not able to differentiate infective virus from noninfectious RNA.[26] Aside from this problem, the sensitivity of COVID-19 testing is also problematic. Several studies were able to demonstrate that repeated tests on patients with COVID-19 were negative. In follow-up series, the same cases were again subjected to testing and COVID-19 infection was finally detected as patients presented with severe clinical symptoms.[27]

The relapse of typical clinical symptoms occurred within a less-than-three-month time frame following initial diagnosis. While relapses predominantly seemed to affect older individuals, recurrence might have occurred due to inadequate care of COVID-19 infections, possibly enabling second episodes of viral replication. It is also important to consider the difficulty in distinguishing COVID-19 reinfections from secondary complications, such as pulmonary embolism or superinfections.[28]

However, such distinctions are especially difficult to make when PCR testing shows positive COVID-19 test results and secondary infections occur in the vulnerable time frame when traces of viral RNA are still detectable in respiratory samples. While patients may not be infectious to others, this does not mean that they are cured of COVID-19.[21] An inflammatory rebound triggered by an inappropriate immune response could provide an alternative explanation for the recurrence of clinical symptoms.

5 | CONCLUSION

Studies have repeatedly reported positive COVID-19 PCR test results for up to 90 days after initial infection. However, we currently do not see cases of COVID-19 reinfections occurring outside a time frame of

90 days from initial infection. Thus, in accordance with our data, we conclude that any observed COVID-19 relapse within a 90-day frame might be a protracted initial infection and not a reinfection. To diagnose a true COVID-19 reinfection, positive COVID-19 testing combined with recurrent clinical symptoms occurring outside of this time frame is required.

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

AUTHOR CONTRIBUTIONS
Mohamed Arafkas: data acquisition, drafting of the manuscript, final approval for publication. Tanja Khosrawipour: analysis and interpretation, drafting of the manuscript, and final approval for publication. Piotr Kocbach: data interpretation, drafting of the manuscript, and final approval for publication. Kacper Zielinski: data interpretation, drafting of the manuscript, and final approval for publication. Justyna Schubert: data interpretation, drafting of the manuscript, and final approval for publication. Agata Miko-lajczyk: data interpretation, critical revision for important intellectual content, and final approval for publication. Maria Celinska: analysis and interpretation, drafting of the manuscript, and final approval for publication. Veria Khosrawipour: data interpretation, conception and design of the work, critical revision for important intellectual content, and final approval for publication.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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