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Article

SARS-CoV-2 PCR Persistence and Duration of COVID-19 Symptoms in Health Professionals

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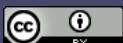
Abstract: Background and Objectives: Most individuals infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are asymptomatic or have mild symptoms of COVID-19, which usually resolve after few days. Regardless of symptoms, infected people can transmit the virus to others especially on the first days of infection. Quantitative reverse transcription-polymerase chain reaction (RT-qPCR) is used to confirm SARS-CoV-2 infection; some individuals show persistent PCR-positivity after recovering from COVID-19. In this study, 12 individuals who showed persistence of COVID-19 symptoms and of SARS-CoV-2 PCR-positivity were followed-up. **Methods:** nasopharyngeal samples were collected for SARS-CoV-2 detection by RT-qPCR; clinical and epidemiological data were analyzed. **Results:** that persistence of SARS-CoV-2 PCR positivity was associated with duration of symptoms ($r_s = 0.81338$), which varied between one and 49 days, with 75% of the individuals reporting symptoms for more than two weeks; 83.33% of cases remained positive after two weeks of onset of symptoms, despite decreases in viral load. **Conclusion:** neither RT-qPCR test nor a symptom-based approach alone are sufficient to evaluate discontinuation of patient isolation; other factors such as viral loads and symptom severity should also be considered. Additional studies are needed to understand how RT-PCR-positivity is related to symptoms and the risk of viral transmission, and to better support isolation guidelines.

Keywords: SARS-CoV-2; PCR persistent positive; respiratory infection; COVID-19

1. Introduction

The rapid spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the coronavirus disease COVID-19, became the main concern of health authorities. Controlling the pandemic is still a challenge as new viral variants emerge; in addition, despite vaccines have been developed, 68.2% of the world population has received at least one dose of a COVID-19 vaccine.¹ Protocols and guidelines to control viral transmission are constantly changing as new studies unravels the biology of COVID-19.

Clinical manifestation of SARS-CoV-2 infection ranges from asymptomatic to mild, moderate, or severe symptoms characteristic of the coronavirus disease COVID-19. Currently, nucleic acid amplification tests, such as RT-qPCR specific for SARS-CoV-2 RNA, are considered the gold-standard assays to confirm infection, based on which anyone who



tests positive for SARS-CoV-2 is considered infected regardless of symptoms.² The highest viral loads are observed within the first week of infection, decreasing afterwards; this is also the period during which an infected person is most likely to transmit the virus to other people.³ Notwithstanding, some studies have detected SARS-CoV-2 RNA by RT-qPCR in samples collected weeks after the infection; however, recovery of replication-competent virus after 10 days of infection has only been reported in some individuals with severe COVID-19, most of who are immunocompromised patients.^{3,4}

Based on these studies, ongoing recommendations for duration of patient isolation in case of SARS-CoV-2 infection are mostly based on symptoms rather than on molecular diagnosis; according to the World Health Organization (WHO) guidelines, asymptomatic individuals should maintain isolation for 10 days after testing positive for SARS-CoV-2 in RT-qPCR, whereas patients with mild to moderate COVID-19 symptoms and a positive SARS-CoV-2 RT-qPCR should stay isolated for at least 10 days after symptom onset, without fever.⁵ The Centers for Disease Control and Prevention (CDC) recommends stay home for at least five days and the end isolation is based on how serious your COVID-19 symptoms were. If the patient had no symptoms may end isolation after day five. If symptoms are not improving continue to isolate until fever-free for 24 hours (without the use of fever-reducing medication).⁶

Though persistence of SARS-CoV-2 PCR positivity does not necessarily indicate that an individual still carries replication-competent virus, a recent study was successful in recovering viable viral particles from two patients with mild COVID-19, 24 and 37 days after symptom onset.⁷ These findings raise again concerns about long-term virus persistence and the possibility of an individual transmitting the virus even after full recovery. More studies showing persistence of viral RNA in asymptomatic, mild and moderate COVID-19 cases will help understanding the course of SARS-CoV-2 infection.

This study reports 12 cases of SARS-CoV-2 infection that tested positive in RT-qPCR for longer than 10 days (SARS-CoV-2 PCR persistence) after symptom onset and discusses symptoms along the course of infection.

2. Methods

This was an observational study reporting 12 cases of SARS-CoV-2 infection with persistent positivity in RT-qPCR between June and December 2020. Guidelines in force at the time recommended that symptomatic individuals with a RT-qPCR positive test should maintain isolation at home for at least 10 days after symptom onset, in addition to 24 hours without symptoms.^{8,9} All individuals reported at least two symptoms indicative of COVID-19, and because for most of them symptoms did not resolve after 10 days, they were monitored for SARS-CoV-2 detection by RT-qPCR.

Nasopharyngeal swabs (NP) were collected by a member of the study team either at the individual's workplace (health unit) or home and sent to the State Central Laboratory (LACEN-RS) for analysis. The first NP collection was within the first two days of onset of symptoms, and afterwards as described in the Results. Viral RNA extraction was performed with MagMAX™ Viral/Pathogen Nucleic Acid Isolation kit in a KingFisher™ Flex Purification System (ThermoFisher Scientific, USA). Viral detection was based on RT-qPCR using BIOMOL-OneStep/COVID-19 kit (IBMP, Brazil) in a 7500 Real Time PCR System (Applied Biosystems, USA). All protocols followed manufacturer's instructions. Spearman rank correlation coefficient was used to assess significant correlation. The study was approved by the Ethics Committee of Universidade Federal de Ciências da Saúde de Porto Alegre (Protocol n. 3.978.647, CAAE 30714520.0.0000.5345); the patients read and signed the free and informed consent form.

3. Results

All individuals included in this study tested positive for SARS-CoV-2 RT-qPCR and most presented symptoms for more than 10 days, therefore a follow-up analysis was performed. Duration of symptoms varied between one day and 49 days, with 75% of the

individuals reporting symptoms for more than two weeks, and 83.33% remaining PCR-positive after two weeks of symptoms onset (**Table 1**). After 20 days, Ct values increased (>30), except in one case for which a Ct of 29 was still obtained on the 47th day after symptom onset (**Figure 1**).

Table 1. Demographic and clinical characteristics from individuals included in this study.

Case	Sex	Age	Duration ^a		Reported Symptoms	Comorbidity	Occupation
			Symptoms	PCR positivity			
1	M	49	49	41	Fever, cough, headache	No	Health professional
2	F	48	43	35	Fever, cough, headache	No	Health professional
3	M	64	42	40	Fever, cough, dyspnea, anosmia, myalgia, fatigue, loss of appetite, headache	Neurological disease	Health professional
4	F	31	47	47	Sore throat, headache, myalgia, coryza, fatigue, cough, diarrhea, nasal obstruction	No	Health professional
5	F	24	14	12	Sore throat, cough, headache, loss of appetite, fatigue, diarrhea, nausea, nasal obstruction	No	Health professional
6	M	44	29	31	Headache, fatigue, nasal obstruction, myalgia, cough	Hypertension	Health professional relative
7	F	52	18	14	Sore throat, fatigue, myalgia, coryza	No	Health professional
8	M	25	15	14	Fever, myalgia, cough, nasal obstruction, anosmia, respiratory distress	No	Health professional
9	F	20	1	12	Fever, headache, nasal obstruction	No	Health professional
10	M	19	1	16	Headache, myalgia, diarrhea	No	Health professional relative
11	F	54	23	16	Cough, headache, myalgia, diarrhea	Diabetes	Health professional
12	M	36	10	21	Sore throat, myalgia, loss of appetite, headache, fever, fatigue, anosmia, nasal obstruction	No	Health professional relative

^aDuration in days

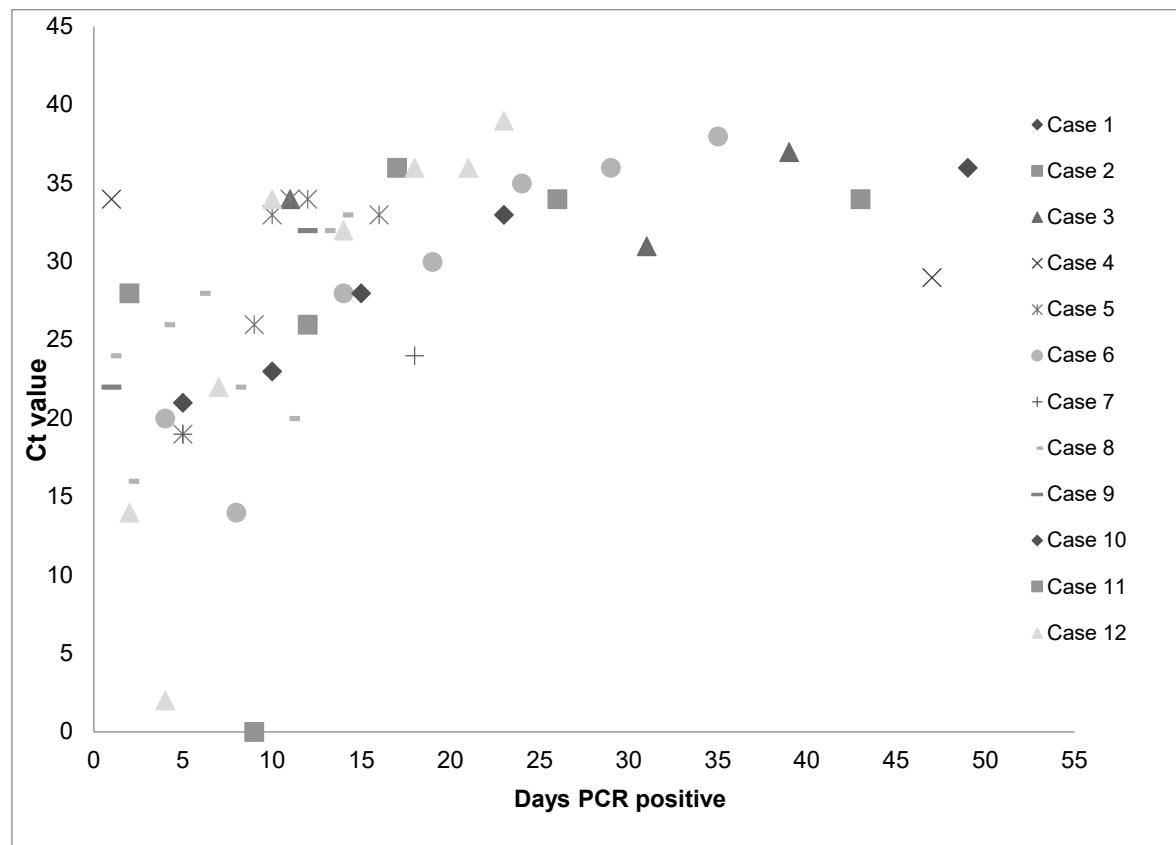


Figure 1. Ct values of SARS-CoV-2 RT-qPCR tests during the follow-up of each patient (Case 1 – Case 12).

Duration of detectable SARS-CoV-2 was positively associated with duration of symptoms; by normal standards, the association between these two variables would be considered statistically significant ($r=0.81338$, $p(2\text{-tailed})=0.00129$ **Figure 2A**). None of the cases were hospitalized, nevertheless one of the patients reported dyspnea. The most common symptoms were cough and headache (75%); myalgia and fatigue (58.33%); fever, sore throat, and nasal obstruction (50%); diarrhea (33.33%); anosmia and loss of appetite (30%); other symptoms like coryza, nausea and respiratory distress were also reported (**Figure 2B**). Most symptoms resolved after 15 days, nonetheless for some individuals cough, sore throat, headache, fatigue, and nasal obstruction lasted longer than 20 days (**Figure 2C**).

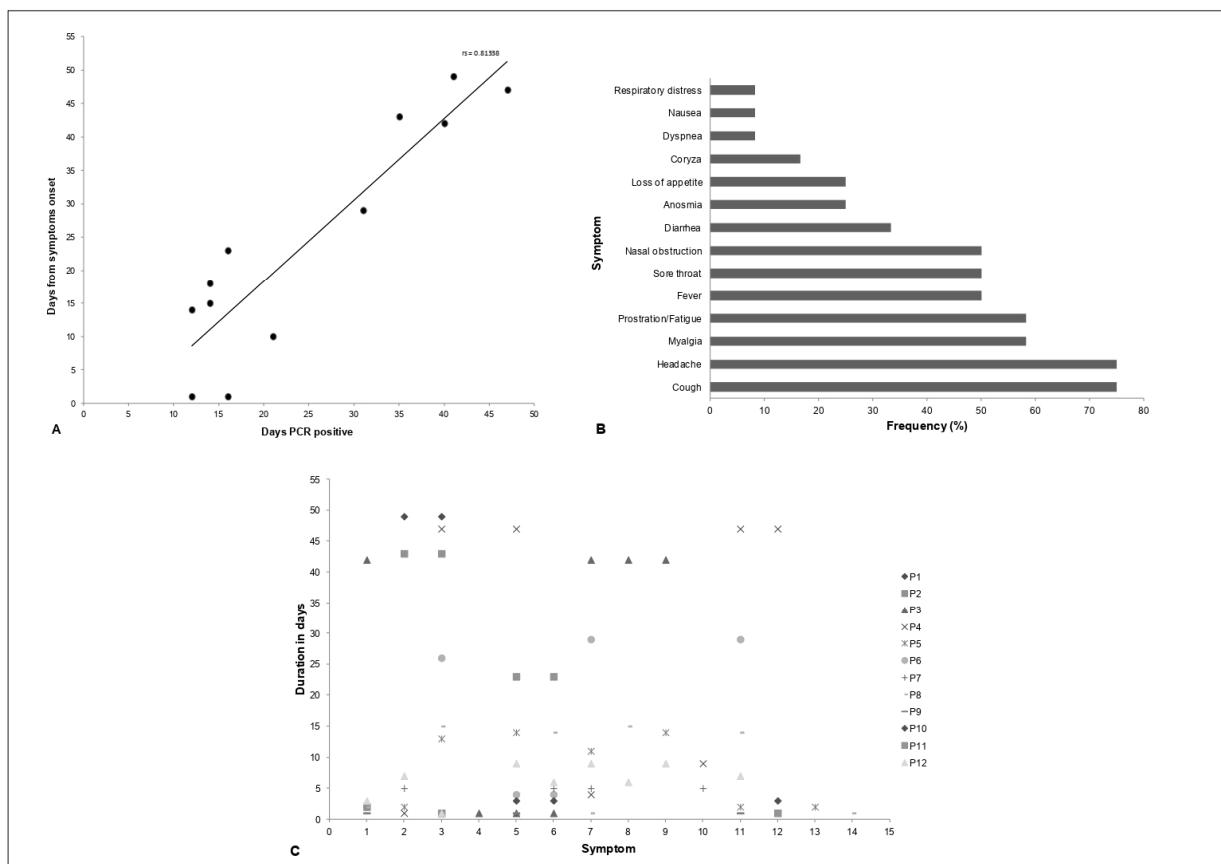


Figure 2. Duration of symptoms and SARS-CoV-2 RT-qPCR positivity. **A)** Association between number of days of PCR positivity and of days of symptoms. **B)** Frequency (%) of symptoms reported by individuals. **C)** Duration of symptoms (in days) observed for each individual (Case 1 – Case 12). Statistical analyses were by Spearman's correlation coefficient, and the analyses included all data points.

The median age was 40 years old, the youngest individual being 19 years old, and the oldest 64, and no differences were found regarding sex (50% females) (Table 1). With respect to comorbidities, nine individuals did not report any condition, whereas one had diabetes, one hypertension, and one neurological disease (Table 1). Of note, none of the individuals had immunodeficiency.

Nine individuals were health professionals who had contact with infected persons, except one, who despite not having contact with patients used public transportation daily. The other three had relatives who were health professionals, and one declared having contact with an infected person, one had traveled to another town, and the third was not sure about the source of contamination.

4. Discussion

In this study we performed an observational analysis of 12 patients that showed SARS-CoV-2 PCR persistence, most of whom were health professionals with symptoms that lasted for up to 49 days. Previous studies showing that SARS-CoV-2 PCR positivity usually lasts longer in hospitalized patients with severe illness than in outpatients with mild symptoms suggest that viral shedding time of SARS-CoV-2 is associated to disease severity.¹⁰ The present study found that duration of SARS-CoV-2 PCR positivity in outpatients may also be associated with duration of symptoms. Notably, our findings do not suggest any case of reinfection, as reinfection is considered two detectable SARS-CoV-2

RT-qPCR results with an interval \geq 90 days between tests,¹¹ and in our study the longest duration of PCR positivity was 47 days.

Most studies with patients who were followed-up after SARS-CoV-2 infection and showed persistence of viral detection by RT-qPCR did not succeed in isolating replication-competent virus;¹¹ notwithstanding, a recent study with two cases of persistent viral infection successfully obtained replicative virus from patients' samples 24 and 37 days after symptom onset.⁷ The capacity of infected individuals to transmit SARS-CoV-2 is higher on the first days or even before symptoms onset, however asymptomatic people can also be contagious.¹¹ On the other hand, carrying replication-competent virus is not sufficient for an individual to be infective, as most studies did not find an association between SARS-CoV-2 PCR persistence and transmissibility. In this sense, a recent study assessed cases of COVID-19 in people participating in the American basketball league games, including players, staff and vendors who were in close proximity, and found individuals who had fully recovered from COVID-19 symptoms and were still SARS-CoV-2 PCR positive after approximately 30 days of symptom onset; notably, no cases of SARS-CoV-2 transmission were found.¹² Another study with individuals who had recovered from COVID-19 found cases of persistent SARS-CoV-2 PCR positivity for up to 109 days from symptoms onset, and performed contact tracing for each of these individual to assess if they could be a source of SARS-CoV-2 transmission; no cases of SARS-CoV-2 infection was found among their contacts.¹¹ These studies concluded that PCR persistence does not indicate that an individual is a source of viral infection, however both studies were performed with individuals fully recovered from COVID-19, i.e. who did not have symptoms at the time of the study. Differently, in our study all individuals were symptomatic, and duration of symptoms showed association with duration of SARS-CoV-2 positivity; these are novel and relevant findings. The study was carried out during the lockdown period, so contacts were investigated as a standard of investigation and no case of infection among them was confirmed.

The results presented herein are important to better understand SARS-CoV-2 PCR persistence in health professionals and in the general population and contribute for protocols and guidelines to control COVID-19 pandemic. The small number of cases analyzed was a limitation of this study, and this was due to reasons such as: i) limited human and laboratory resources; ii) necessity of well-organized logistics for sample collection at individuals' homes; iii) delay in notification, and consequently in detection of PCR-persistent cases that could have been included.

In summary, our findings reinforce that neither RT-qPCR test nor a symptom-based approach alone are sufficient to indicate SARS-CoV-2 infection. Other factors, including viral loads and symptom severity should also be evaluated before a patient discontinues isolation, especially considering that in some cases transmission potential cannot be completely ruled out.

Author Contributions: TSG: conceptualization, funding acquisition, resources, data acquisition and analysis, molecular analyses in the laboratory, writing-original draft preparation, writing and editing final manuscript. LFB: molecular analyses; writing-original draft. RSS: molecular analyses, data analysis and visualization. IAM: data acquisition. ABCV: funding acquisition, design of the study, data analysis and visualization, writing-reviewing draft, writing and editing final manuscript. All authors critically revised the manuscript, approved the final version to be published, and agree to be accountable for all aspects of the work.

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Data Availability Statement: Data except individuals' identifiers is available upon request (anabgv@ufcspa.edu.br).

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References

1. Our World in Data (2022). Coronavirus (COVID-19) Vaccinations. [<https://ourworldindata.org/covid-vaccinations>]. Accessed 11 October 2022.
2. Bullard J, Dust K, Funk D, et al (2020) Predicting infectious severe acute respiratory syndrome coronavirus 2 from diagnostic samples. *Clin Infect Dis* 71:2663–2666.
3. Kampen JJA van, Vijver DAMC van de, Fraaij PLA, et al (2021) Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). *Nat Commun* 12:8–13.
4. Lu J, Peng J, Xiong Q, et al (2020) Clinical, immunological and virological characterization of COVID-19 patients that test re-positive for SARS-CoV-2 by RT-PCR. *EBioMedicine* 59:102960.
5. World Health Organization (2020). Criteria for releasing COVID-19 patients from isolation: Scientific briefs. [<https://www.cdc.gov/coronavirus/2019-ncov/your-health/isolation.html>]. Accessed 11 October 2022.
6. Center for Disease Control and Prevention (2022). I Isolation and Precautions for People with COVID-19. [<https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html> (URL)]. Accessed 11 October 2022.
7. Mendes Correa MC, Leal FE, Villas Boas LS, et al (2021) Prolonged presence of replication-competent SARS-CoV-2 in mildly symptomatic individuals: A Report of 2 Cases. *J Med Virol* 93:5603–5607.
8. SES/RS (2021). NOTA INFORMATIVA 30 COE/SES-RS. [chrome-extension://efaidnbmnnibpcajpcglclefindmkaj/viewer.html?pdfurl=https%3A%2F%2Fcoronavirus.rs.gov.br%2Fupload%2Farquivos%2F202102%2F18162639-nota-informativa-30-18-02-2021.pdf&clen=1533198&chunk=true (URL)]. Accessed 26 August 2021.
9. SES/RS (2020). NOTA INFORMATIVA 12 COE/SES-RS. [<https://www.cachoeiradosul.rs.gov.br/portal/download/arquivos/CKPPw/> (URL)]. Accessed 26 August 2021.
10. Vibholm LK, Nielsen SS, Pahus MH, et al (2021) SARS-CoV-2 persistence is associated with antigen-specific CD8 T-cell responses. *EBioMedicine* 64:103230.
11. Rhee C, Kanjilal S, Baker M, Klompas M (2021) Duration of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infectivity: When Is It Safe to Discontinue Isolation? *Clin Infect Dis* 72:1467–1474.
12. Mack CD, DiFiori J, Tai CG, et al (2021) SARS-CoV-2 Transmission Risk Among National Basketball Association Players, Staff, and Vendors Exposed to Individuals With Positive Test Results After COVID-19 Recovery During the 2020 Regular and Postseason. *JAMA Intern Med* 181:960–966.