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RESEARCH ARTICLE

Predictive Factors for a New Positive Nasopharyngeal Swab Among Patients Recovered From COVID-19

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Introduction: As an emerging infectious disease, the clinical and virologic course of COVID-19 requires better investigation. The aim of this study is to identify the potential risk factors associated with persistent positive nasopharyngeal swab real-time reverse transcription–polymerase chain reaction tests in a large sample of patients who recovered from COVID-19.

Methods: After the acute phase of SARS-CoV-2 epidemic infection, the Fondazione Policlinico A. Gemelli IRCSS of Rome established a post-acute care service for patients discharged from the hospital and recovered from COVID-19. Between April 21 and May 21, 2020, a total of 137 individuals who officially recovered from COVID-19 were enrolled in this study. All patients were tested for the SARS-CoV-2 virus with nucleic acid RT-PCR tests. Analysis was conducted in June 2020.

Results: Of the 131 patients who repeated the nasopharyngeal swab, 22 patients (16.7%) tested positive again. Some symptoms such as fatigue (51%), dyspnea (44%), and coughing (17%) were still present in a significant percentage of the patients, with no difference between patients with a negative test and those who tested positive. The likelihood of testing positive for SARS-CoV-2 infection was significantly higher among participants with persistent sore throat (prevalence ratio=6.50, 95% CI=1.38, 30.6) and symptoms of rhinitis (prevalence ratio=3.72, 95% CI=1.10, 12.5).

Conclusions: This study is the first to provide a given rate of patients (16.7%) who test positive on RT-PCR test for SARS-CoV-2 nucleic acid after recovering from COVID-19. These findings suggest that a significant proportion of patients who have recovered from COVID-19 still could be potential carriers of the virus. In particular, if patients continue to have symptoms related to COVID-19, such as sore throat and rhinitis, it is reasonable to be cautious by avoiding close contact, wearing a face mask, and possibly repeating a nasopharyngeal swab.

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INTRODUCTION

The new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is spreading daily throughout the world, reaching more than 5 million patients in May 2020, with more than 2 million recovered patients. Almost all studies are primarily focused on the description of the epidemiologic, clinical, biological, and radiological characteristics of patients

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with confirmed coronavirus disease 2019 (COVID-19).[1,2] However, only a few studies, mainly case reports, have addressed the importance of the follow-up of recovered patients.[3,4] To date, the clinical and virologic course of SARS-CoV-2 infection remains to be investigated. In particular, there are no conclusive data showing how long patients with COVID-19 continue to have symptoms and test positive for the SARS-CoV-2 virus, even after 2 consecutive negative real-time reverse transcription–polymerase chain reaction (RT-PCR) tests.[5,6]

The WHO criteria for hospital discharge or discontinuation of COVID-19 quarantine have been described.[7] Nevertheless, data show that some patients are positive on nasopharyngeal swab after being declared recovered, and thus the appropriate timing of ending COVID-19 quarantine remains undetermined.[8,9] Overall, there is no information about signs and symptoms that can predict a new positive test in patients declared recovered from COVID-19. The fact that some patients could develop a potentially long-lasting viral presence highlights an important point of vigilance for controlling the pandemic both at the individual and collective level.[10]

This study aimed to identify the potential risk factors associated with a new positive nasopharyngeal swab RT-PCR test (after 2 negative tests) in a large sample of patients who recovered from COVID-19.

METHODS

The Gemelli Against COVID-19 Post-Acute Care (GAC19-PAC) project was an initiative developed by the Department of Geriatrics, Neuroscience and Orthopedics of the Catholic University of the Sacred Heart (Rome, Italy) to answer an important open question: *Once recovered from COVID-19, what happens to patients, and how has the virus impacted their body?* In this respect, the Fondazione Policlinico Universitario A. Gemelli IRCSS has set up a multidisciplinary healthcare service called Day Hospital Post-COVID-19 for all patients recovered from SARS-CoV-2 infection. The complete GAC19-PAC study protocol has been described in detail elsewhere.[11,12]

This study was approved by the Catholic University/Fondazione Policlinico Gemelli IRCCS Institutional Ethics Committee (protocol Identification Document Number: 0013008/20). Written informed consent was obtained from the participants. The manuscript was prepared in compliance with the STROBE reporting guidelines for observational studies.

Study Sample

Only the patients with COVID-19 who met the following WHO criteria for discontinuation of quarantine were admitted to the follow-up study project: (1) being fever free without fever-reducing medications for 3 consecutive days; (2) improvement in any symptoms related to COVID-19, including reduced coughing and shortness of breath; (3) ≥7 days since the onset of the first symptom related to COVID-19; and (4) testing negative for the SARS-CoV-2 virus twice (at least 24 hours apart) with nucleic acid RT-PCR. If the clinical characteristics and testing conditions are met, both the WHO and U.S. Centers for Disease Control and Prevention consider the patient officially recovered from COVID-19.[3]

Between April 21 and May 21, 2020, a total of 137 individuals who officially recovered from COVID-19 were enrolled in this study. A total of 6 patients (4 men and 2 women) refused to participate for personal reasons; as a consequence, a sample of 131 patients was considered. This outpatient service is currently ongoing, and further details about the post-acute outpatient service and evaluation of the patients have been described elsewhere.[12]

Measures

All patients who agreed to be screened underwent individual assessment. Even though the patients were formally considered recovered from COVID-19, a new RT-PCR test was repeated at the time of post-acute care admission. Demographic information, medical and medication history, laboratory findings, and radiological features were collected. A multidisciplinary approach, including internal medicine, geriatric, ophthalmologic, otolaryngologic, pneumologic, cardiologic, neurologic, immunologic, and rheumatologic evaluations, has been put in place for a comprehensive assessment of all the possible damage caused by the SARS-CoV-2 virus.[11,12] In particular, during the first visit, a specific focus is paid to collecting information and data about the persistence of signs and symptoms related to COVID-19: cough, fatigue, diarrhea, headache, smell disorders, dysgeusia, red eyes, joint pain, shortness of breath, loss of appetite, sore throat, and rhinitis. Smoking was categorized as current or never/former smoker. Body weight was measured through an analog medical scale. Body height was measured using a standard stadiometer. BMI was defined as weight (kilograms) divided by the square of height (meters).

Statistical Analysis

Continuous variables were expressed as mean (SD), and categorical variables were expressed as frequencies by absolute value and percentage of the total. Descriptive statistics were used to describe demographic and key clinical characteristics of the study population according to COVID-19 nucleic acid RT-PCR test results. The differences in proportions and means of covariates between patients who tested positive and those who tested negative for the nasopharyngeal swab were assessed using Fisher's exact test and *t*-test statistics, respectively. Cox models with robust variance estimates assessed the association between clinical characteristics and persistent positive RT-PCR tests. Candidate variables to be included in the Cox model were selected on the basis of biological and clinical plausibility as a potential risk factor for persistent positive RT-PCR tests. To identify the factors independently associated with positive nasopharyngeal swab tests, crude prevalence rate ratios and 95% CIs—controlling for age and sex—were first estimated. A multivariable Cox model was computed including all the variables that were associated with the outcome at an α level of 0.1, after adjustment for age and sex. All analyses were performed in June 2020 using SPSS, version 11.0.

RESULTS

Of the 131 patients admitted to the follow-up protocol who repeated a nasopharyngeal swab, 22 patients

Table 1. Clinical Characteristics of Participants According to Nasopharyngeal Swab Test for SARS-CoV-2

Characteristics	Total sample (N=131)	Negative (*n*=109)	Positive (*n*=22)	*p*-value
General and clinical characteristics				
Age, years	55.8 ± 14.8	55.7 ± 14.7	56.4 ± 15.7	0.84
Sex, female	51 (38.9)	41 (37.6)	10 (45.4)	0.41
Education, years	14.4 ± 7.8	14.9 ± 8.2	12.4 ± 4.3	0.21
Smoking habit	11 (8.3)	9 (8.2)	2 (9.0)	0.33
Influenza vaccination	23 (17.5)	17 (15.5)	6 (27.2)	0.20
Hypertension	38 (29.0)	32 (29.3)	6 (27.2)	0.53
Heart failure	8 (6.1)	6 (5.5)	2 (9.0)	0.40
Diabetes	7 (5.3)	5 (4.5)	2 (9.0)	0.33
Renal failure	4 (3.0)	3 (2.7)	1 (4.5)	0.52
COPD	12 (9.1)	10 (9.1)	2 (9.0)	0.67
BMI (kg/m^2)	26.2 ± 4.2	25.9 ± 4.3	27.6 ± 3.2	0.10
Symptoms related to COVID-19				
Cough	22 (16.7)	16 (14.6)	6 (27.2)	0.13
Fatigue	67 (51.1)	56 (51.3)	11 (50.0)	0.54
Diarrhea	5 (3.8)	4 (3.6)	1 (4.5)	0.60
Headache	14 (10–6)	11 (10.0)	3 (13.6)	0.42
Smell disorders	18 (13.7)	16 (14.6)	2 (9.0)	0.38
Dysgeusia	15 (11.4)	11 (10.0)	4 (18.1)	0.22
Red eyes	21 (16.0)	16 (14.6)	5 (22.7)	0.42
Joint pain	33 (25.1)	28 (25.6)	5 (22.7)	0.51
Short of breath	58 (44.2)	50 (45.8)	8 (36.3)	0.28
Loss of appetite	13 (9.9)	11 (10.0)	2 (9.0)	0.62
Sore throat	9 (6.8)	5 (4.5)	4 (18.1)	**0.04**
Rhinitis	19 (14.5)	13 (11.9)	6 (27.2)	**0.05**
Follow-up				
Days from COVID-19 onset	55.8 ± 10.8	56.5 ± 11.1	52.6 ± 8.8	0.26
Days from first positive test	47.1 ± 10.6	47.4 ± 10.8	45.5 ± 9.3	0.46

Note: Boldface indicates statistical significance ($p<0.05$).
Data are given as means ±SD for age, education, BMI, and follow-up days. Number (%) for all the other variables are reported.
COPD, chronic obstructive pulmonary disorder.

(16.7%) tested positive again. Characteristics of the study population according to RT-PCR test results are summarized in Table 1. The mean age of the 131 patients participating in the GAC19-PAC study protocol was 55.8 (SD=14.8, range=19–89) years, and 51 (39%) were women, without a significant difference between patients with positive and those with negative test results. Compared with participants showing negative RT-PCR tests, those who tested positive after being declared recovered from COVID-19 had marginally significantly higher BMI (27.6 [SD=3.2] vs 25.9 [SD=4.3]). Conversely, no significant differences were observed for the other clinical characteristics and diseases.

During the first follow-up visit, the persistence of the symptoms most frequently associated with COVID-19 was assessed (Table 1). None of the patients had fever, and all reported global improvement in their overall clinical condition. However, some symptoms such as fatigue (51%), dyspnea (44%), and coughing (17%) were still present in a significant percentage of the patients. However, for most of these symptoms, no difference was observed between patients with a negative test and those with a positive test. The only 2 symptoms that showed a higher and significant prevalence in patients with a positive test were sore throat (18% vs 4%, p=0.04) and signs of rhinitis (27% vs 12%, p=0.05).

The mean time from the onset of SARS-CoV-2 infection symptoms to the first follow-up visit was 55.8 (SD=10.8) days (range =37–87 days), without a significant difference between patients with a positive test and those with a negative test. The mean time from the first positive RT-PCR test to the first follow-up visit was 47.1 (SD=10.6) days (range =11–70 days), without a significant difference between patients with a positive test and those with a negative test. Furthermore, the mean length of hospital stay

Table 2. COVID-19 Treatment of Study Participants According to Nasopharyngeal Swab Test for SARS-CoV-2

Characteristics	Total sample (N=131)	Negative (*n*=109)	Positive (*n*=22)	*p*-value
Medication				
Lopinavir/ritonavir	41 (31.2)	36 (33.0)	5 (22.7)	0.24
Darunavir/ritonavir	63 (48.0)	51 (46.7)	12 (54.5)	0.33
Hydroxychloroquine	95 (72.5)	78 (71.5)	17 (77.2)	0.39
Anti–interleukin-6 receptor	32 (24.4)	26 (23.8)	6 (27.2)	0.46
Azithromycin	47 (35.8)	37 (33.9)	10 (45.5)	0.21
Enoxaparin	54 (41.2)	44 (40.3)	10 (45.5)	0.41
Corticosteroids	7 (5.3)	5 (4.5)	2 (9.0)	0.33
Oxygen therapy				
Oxygen support	66 (50.3)	55 (50.4)	11 (50.0)	0.57
NIV or CPAP	11 (8.3)	11 (10.0)	0 (0.0)	0.20
Invasive ventilation	4 (3.0)	4 (3.6)	0 (0.0)	0.47

Note: Data are given as number (percent) for all the variables.
CPAP, continuous positive airway pressure; NIV, noninvasive ventilation.

for the acute phase of COVID-19 was similar between patients with a negative test and those with a positive test (12.1 [SD=7.9] days vs 10.0 [SD=6.5] days, p=0.25). Similarly, no difference was observed for the mean number of days from the hospital discharge and the first follow-up visit (30.0 [SD=14.2] days vs 32.1 [SD=14.3] days, p=0.53). Finally, no difference was observed for the mean number of days from the second negative swab and the first follow-up visit (17.1 [SD=8.0] days vs 17.3 [SD=5.8] days, p=0.95).

Table 2 shows the treatments received during the acute phase of COVID-19. Regarding the prevalence of pharmacological treatments (antiviral drugs, hydroxychloroquine, anti–interleukin-6 receptor drugs, antibiotics, enoxaparin, corticosteroids) and oxygen therapy, no significant differences were observed between patients with positive and those with negative RT-PCR tests.

Finally, in the unadjusted model, there was a direct association between sore throat and a positive RT-PCR test (prevalence ratio [PR]=5.43, 95% CI=1.23, 24.0) (Table 3). After adjusting for age and sex, this association remained statistically significant. In the fully adjusted model, the likelihood of testing positive for SARS-CoV-2 infection was significantly higher among participants with a persistent sore throat (PR=6.50, 95% CI=1.38, 30.6) and symptoms of rhinitis (PR=3.72, 95% CI=1.10, 12.5).

DISCUSSION

A total of 22 of 131 patients (16.7%) affected by COVID-19 who fully met the criteria for discontinuation of quarantine (no significant acute clinical symptoms and 2 consecutive negative RT-PCR tests) presented a new positive RT-PCR test at a follow-up visit after at least 2 weeks. These findings indicate that a noteworthy rate of recovered patients with COVID-19 could still be asymptomatic carriers of the virus. Even in the absence of specific guidelines, the 22 patients who tested positive for COVID-19 again were suggested to quarantine for a second time. In

Table 3. Unadjusted and Adjusted Association (PR and 95% CI) Between Potential Risk Factors and the Positive RT-PCR for SARS-CoV-2 Test

Characteristic	Unadjusted, PR (95% CI)	Age-sex adjusted, PR (95% CI)	Fully adjusted, PR (95% CI)
Age, years[a]	1.00 (0.97, 1.03)	—	0.99 (0.96, 1.02)
Sex (female)	0.80 (0.30, 2.12)	—	0.85 (0.31, 2.38)
Cough	2.17 (0.71, 6.55)	2.30 (0.76, 6.96)	1.93 (0.54, 6.80)
Sore throat	5.43 (1.23, 24.0)	4.44 (1.07, 18.3)	6.50 (1.38, 30.6)
Rhinitis	3.03 (0.89, 10.3)	2.82 (0.93, 8.54)	3.72 (1.10, 12.5)
BMI	1.09 (0.98, 1.21)	1.06 (0.96, 1.18)	1.10 (0.99, 1.23)

[a]PR per year increase.
PR, prevalence ratio; RT-PCR, reverse transcription–polymerase chain reaction.

addition, no other positive cases emerged within their families and close contacts. All patients had observed social distancing measures and worn face masks indoors. In the light of these observations, it is very difficult to affirm whether these patients were really contagious.

To date, few studies conducted during the SARS-CoV-2 outbreak in China described some similar data. One study showed 4 cases of healthcare professionals who tested positive after hospital discharge and discontinuation of quarantine,[8] whereas another study described a single-case patient who tested positive 70 days after the onset of COVID-19.[9] However, there is only 1 study indicating the rate of recovered patients testing positive again after the quarantine period. Mei et al.[4] documented that 23 of 651 patients (3%) tested positive on a retest for SARS-CoV-2 by RT-PCR assay in a routine health check. At the same time, there is no evidence suggesting whether persistent signs or symptoms related to COVID-19 could help identify those patients with a long-term positive test.

This study provides a given rate of patients (16.7%) who still have a positive RT-PCR test for SARS-CoV-2 nucleic acid after recovering from COVID-19. According to the WHO guidelines, these 22 patients were eligible to be considered recovered from COVID-19 and thus be discontinued from quarantine.[13] These data suggest that some symptoms continue to be present—in a milder form than in the acute phase of the disease—in a high rate of patients but without substantial differences between patients with negative RT-PCR test results and those who still test positive. The only 2 symptoms that seem to correlate with the persistence of a positive test are sore throat and signs of rhinitis. Consequently, the persistence of these 2 symptoms should not be underestimated and should be adequately assessed in all patients considered recovered from COVID-19.

It is important to highlight that patients with COVID-19 testing positive after recovery represent an important public health problem. As an emerging infectious disease, the clinical and virologic course of SARS-CoV-2 infection requires further study. Although it is not possible to draw definitive conclusions for public health actions on the basis of these data and other reported evidence,[14] these results emphasize relevant clinical characteristics that are important to evaluate, including the extensive clinical course, the persistence of signs and symptoms related to COVID-19, the presence of viral RNA fragments after disease recovery, and the potential failure of the viral antibody for the clearance of the virus.[15]

Limitations

Despite this study dealing with a highly relevant issue, some limitations should be noted. These include the lack of information on symptom history before acute COVID-19 infection and the lack of details on symptom severity. Furthermore, this is a single-center study with a relatively small number of patients, without a control group of patients discharged from hospital for other acute illnesses. For example, patients with pneumonia can also suffer from persistent symptoms,[16] suggesting that these findings could be not exclusive to COVID-19. In particular, sore throat and rhinitis are subjective symptoms rather than objective parameters, which may have bias among the patients and are not easy to analyze quantitatively. However, the clinical characteristics of the participants make it possible to exclude that other acute illnesses were present at the time of evaluation. Unfortunately, objective laboratory parameters—such as the peak level of viral RNA, the first duration of viral RNA positivity, and the viral antibodies yielded when the viral RNA becomes negative—are not available to minimize subjectivity and aid in quantitative assessment. Another important limitation of the study is the methodology used to diagnose SARS-CoV-2 infection. Recent data report the risk of eliciting false-negative and false-positive results with RT-PCR in diagnosing COVID-19. It is well known that results from RT-PCR using primers for various genes can be affected by the variation of viral RNA sequences. In fact, genetic diversity and a possible rapid evolution of this novel coronavirus have been observed in various studies.[17,18] Finally, these data should be considered preliminary and require validation from larger data samples.

CONCLUSIONS

Apart from these limitations, this study offers a unique opportunity to investigate the clinical sequelae of COVID-19. In particular, the criteria for discontinuation of quarantine and assessment of patients who recovered from COVID-19 with specific follow-up protocols need to be implemented.[19] To contain the spread of the virus, it is extremely important to better evaluate all patients who recovered from COVID-19 but still test positive for the virus.[20] This will be a crucial contribution to a better understanding of both the natural history of COVID-19 as well as the public health implications of viral shedding. The main question for the containment of the SARS-CoV-2 pandemic infection that still needs to be answered is whether the persistent presence of virus fragments means that the patient is still contagious. Tests are conducted using RT-PCR, which looks for small fragments of viral RNA. A positive nasopharyngeal swab test can reveal whether a patient is still shedding viral fragments but is not able to discern whether they are still infectious.[7,8] It is important to highlight that

also in other viral diseases, such as Zika, it has been documented that specific RNA can be identified long after the clearance of the virus.[21] RT-PCR is unable to differentiate between an infectious virus and noninfectious RNA.[22] In patients with clinical improvement who are completely asymptomatic,[23] a postnegative positive RT-PCR test result does not necessarily reflect reinfection or viral carriage.[24]

Evidence on SARS-CoV-2 infection and COVID-19 increases each day, and new guidelines about the recovery criteria will continue to change. In clinical practice, it is urgent to have criteria to identify patients who, even if declared recovered, continue to test positive. If patients continue to have symptoms potentially related to COVID-19, such as sore throat or rhinitis, it is reasonable to be cautious in avoiding close contact, wearing a face mask, and possibly repeating a nasopharyngeal swab.

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