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SHORT COMMUNICATION

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Clearance and persistence of SARS-CoV-2 RNA in patients with COVID-19

Anália Carmo[1] | João Pereira-Vaz[1] | Vanda Mota[1] | Alexandra Mendes[1] | Célia Morais[1] | Andreia Coelho da Silva[1] | Elisabete Camilo[1] | Catarina Silva Pinto[2] | Elizabete Cunha[2] | Janet Pereira[2] | Margarida Coucelo[2,3] | Patrícia Martinho[2] | Lurdes Correia[1] | Gilberto Marques[1] | Lucília Araújo[1] | Fernando Rodrigues[1]

[1]Laboratory of Molecular Biology/Virology, Clinical Pathology Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

[2]Molecular Hematology Laboratory, Department of Clinical Hematology, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

[3]Group of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine of University of Coimbra (FMUC), Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra Institute for Clinical and Biomedical Research (iCBR), Coimbra, Portugal

Correspondence
Anália Carmo, Laboratory of Molecular Biology/Virology, Clinical Pathology Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra 3004-561, Portugal.
Email: 10919@chuc.min-saude.pt

Abstract

Patients with coronavirus disease-2019 may be discharged based on clinical resolution of symptoms, and evidence for viral RNA clearance from the upper respiratory tract. Understanding the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral clearance profile is crucial to establish a re-testing plan on discharge and ending isolation of patients. We aimed to evaluate the number of days that a patient needed to achieve undetectable levels of SARS-CoV-2 in upper respiratory tract specimens (nasopharyngeal swab and/or an oropharyngeal swab). The clearance and persistence of viral RNA was evaluated in two groups of positive patients: those who achieved two negative reverse transcription-polymerase chain reaction (RT-PCR) tests and those who kept testing positive. Patients were organized thereafter in two subgroups, mild illness patients discharged home and inpatients who had moderate to severe illness. Results from RT-PCR tests were then correlated with results from the evaluation of the immune response. The study evidenced that most patients tested positive for more than 2 weeks and that persistence of viral RNA is not necessarily associated with severe disease but may result from a weaker immune response instead.

KEYWORDS

COVID-19, clearance, RT-PCR, SARS-CoV-2

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus that crossed species barriers and caused the outbreak of coronavirus disease 2019 (COVID-19) in humans.[1-4]

The incubation period of SARS-CoV-2 ranges from 1 to 14 days, with a mean of 5 to 6 days. The first studies indicated that the viral load persisted up to 8 days after the onset of symptoms in mild cases and peaked in day 11 in more severe cases.[4,5] However, upon the resolution of clinical symptoms the clearance of SARS-CoV-2 from respiratory samples remains unclear, making the establishment of patient discharge and the ending isolation criteria difficult.[2,4,6]

According to recommendations, clinically recovered patients with COVID-19 may be discharged after two negative reverse transcriptase polymerase chain reaction (RT-PCR) tests from respiratory specimens within a 24 hours interval.[2,5] However, there are several reports of prolonged viral RNA detection requiring the consumption of numerous tests.[1,2,6]

COVID-19 outbreak had a major impact on the management of clinical virology laboratories and caused a reduced availability of laboratory consumables and reagents limiting the testing capacity. The need to repeat tests to detect viral RNA poses an additional challenge. Therefore, to achieve a rational use of human and laboratory resources it is important to establish the adequate time to determine viral clearance.

We aimed to evaluate the number of days that a patient needed to achieve undetectable levels of SARS-CoV-2 in upper respiratory tract specimens (nasopharyngeal swab and/or an oropharyngeal swab).

2 | METHODS

2.1 | Patients and samples

Retrospective and cross-sectional analysis of laboratory data obtained from nasopharyngeal/oropharyngeal swabs and serum samples received at the Clinical Pathology Unit of Centro Hospitalar e Universitário de Coimbra, Portugal. The samples were collected from symptomatic patients (fever, cough, chills and dyspnea) or from asymptomatic patients who had contact with infected patients. Swabs samples were used to detect SARS-CoV-2 RNA and serum samples were used to evaluate immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against SARS-CoV-2. Laboratory data and patient's characteristics, such as age, sex, and provenance (ward, emergency department, home) were collected anonymously from our laboratory database from 1 March to 30 April 2020.

2.2 | Detection of severe acute respiratory syndrome coronavirus-2 RNA

Nasopharyngeal and oropharyngeal samples were collected using swabs immediately placed in standard viral transport medium. Viral RNA was extracted from 400 µL of respiratory samples and eluted in 50 µL of elution buffer using the EMAG automated nucleic acid extraction platform (Biomérieux). Detection of SARS-CoV-2 RNA was performed by an adapted previously described real-time RT-PCR assay targeting regions of the virus nucleocapsid (N) gene, and also targeting the human RNase P gene for sample quality control.[7] Purified genomic RNA from SARS-CoV-2 strain BetaCoV/Germany/BavPat1/2020 p.1, grown in cell culture and provided by the European Virus Archive, was used as positive template control.

2.3 | Assessment of immune response to SARS-CoV-2

The level of IgG and IgM against SARS-CoV-2, was determined in serum samples by chemiluminescent analytical system, using MAGLUMI analyzers (SNIBE: Shenzhen New Industries Biomedical Engineering Co, Ltd, Shenzhen, China) and MAGLUMI 2019-nCoV (SARS-CoV-2) IgM/IgG kits according with the manufacturer instructions.[8,9]

2.4 | Statistical analysis

Statistical analysis was performed using SPSS 25 (IBM). Quantitative variables were assessed for normality with the Shapiro Wilk test. Comparisons of quantitative variables between two groups were performed with the Student *t* or Mann-Whitney tests. Correlations between quantitative variables were assessed by computing Pearson or Spearman correlation coefficients, depending on whether normality requirements were met or not. The level of significance adopted was 0.05.

3 | RESULTS

From 1 March to 30 April 2020, 8892 respiratory samples from 7093 symptomatic patients or from contacts, were analyzed.

SARS-CoV-2 RNA was detected in 957 samples (10.7%), corresponding to 622 adults (8.8%) and to 21 children (2.9%). Among adults, women were 382 (61.4%), mean age of 55.8 ± 20.0 years (range, 20-99) and 240 were men (38.6%), mean age of 60.2 ± 19.9 years (range, 19-95), $P = .08$.

From the SARS-CoV-2 RNA positive patients only 210 patients (33.8%) were analyzed because all the remaining positives did not collect other sample. This cohort was divided in two groups: those who achieved two consecutive negative tests and those who maintained RT-PCR tests positive. The former comprised a total of 116 patients (55.2%) and the later comprised a total of 94 patients (44.8%).

In the group of patients with two consecutive negative tests, the first negative RT-PCR test was achieved 24.8 ± 9 days (range, 7-46 days) after the first positive test. In men, the first negative test took 24 ± 9 days (range, 7-46) and in women it took 25 ± 9 days (range, 9-44), $P > .05$, Table 1. The mean positive tests per patient before becoming negative was 2.1 (range, 1-7). These patients maintained positive tests for a mean of 22.3 ± 7 days. Only 30.2% of the patients (n = 35) achieved the first negative test within 20 days, after the first positive test, Table 1.

From these 116 patients that tested negatively twice, 69 (58.5%) were patients discharged home who had mild illness, and 47 (39.8%) were inpatients who had moderate to severe illness. In the patients discharged home, the number of days until the first negative test was 26.3 ± 8.5 and in the inpatient group it was 22.5 ± 9.3, $P = .027$. The inpatients aged over 65 took longer (23.9 ± 9.7 days) than those aged under 65 (18.3 ± 9.7 days), to reach the first negative test, $P = .026$, Table 1.

In the group of patients that kept RT-PCR tests positive (94 out of 210 patients) (44.8%) a total of 4.2 ± 1.9 tests were done per patient (range, 2-11). The number of days between the first and the last positive tests was 32.55 ± 9.6 days (range, 12-51 days). In this group of long-lasting positives, women remained positive for longer than men (34.2 ± 8.9 vs 28.7 ± 10.2 days), $P = .017$, Table 2. Interestingly, 24.4% (23 out of 94) despite being positive, already tested negative at least once. These 23 patients kept positive for longer than the other 71 patients that never had a negative test (39.87 ± 7.4 days vs 30.18 ± 9.0 days), $P < .001$. We hypothesized that these patients were false negatives due to a deficient collection of material and/or to a low viral load.

TABLE 1 Characteristics of patients with two negative reveree transcription-polymerase chain reaction tests

Number of patients	116				
Sex	**Women**		**Men**		***P* value**
	75 (64.7%)		41 (35.3%)		
Age, y	56 ± 19 (26-95)		57.9 ± 19 (26-95)		*P* > .05
Mean number of days to achieve the first negative test	25 ± 9 (9-44)		24 ± 9 (7-46)		*P* > .05
Patients according disease severity	**Inpatients**		**Patients discharged home**		***P* value**
Number of patients	47 (39.8%)		69 (58.5%)		
Mean number of days to achieve the first negative test	22.5 ± 9.3 (9-41)		26.3 ± 8.5 (7-46)		*P* = .027
	Women	Men	Women	Men	
Number of patients	30	17	45	24	
Age	75 ± 14 (45-93)	76.1 ± 11 (56-91)	43.8 ± 11.3 (25-61)	44.2 ± 10.8 (26-64)	*P* < .001

Note: Data are reported as mean ± standard deviation, (minimum-maximum). There was a significant difference between the mean number of days to achieve the first negative test in the inpatients and in the patients discharged home, *P* = .027. The inpatients were older than the patients discharged home, *P* < .001.

Regarding disease severity, 58 out of 94 (61.2%) were mild illness patients discharged home and 36 out of 94 (38.3%) were inpatients with moderate to severe illness. In patients discharged home the number of days that they kept testing positive is significantly higher than what it was observed in inpatients (35.38 ± 8.0 days, range, 19-51 days vs 28 ± 10.11 days; range, 12-49 days; *P* < .001), Table 2.

TABLE 2 Characteristics of patients that maintain positive RT-PCR tests

Number of patients	94		
Sex	**Women**	**Men**	***P* value**
	66 (70.2%)	28 (29.8%)	
Age, y	49 ± 19 (25-95)	63 ± 18 (39-92)	*P* < .05
Mean number of days from first positive test	34.2 ± 8.9 (12-49)	28.7 ± 10.2 (13-51)	*P* = .017
Patients according disease severity	**Inpatients**	**Patients discharged home**	
Number of patients	36 (38.3%)	58 (61.2%)	
Mean number of days from first positive test	28.0 ± 10.11 (12-49)	35.4 ± 8.0 (19-51)	*P* < .001

Note: Data are reported as mean ± standard deviation, (minimum-maximum). There was a significant difference between the mean number of days from first positive test considering sex, *P* = .017. The comparison of the mean number of days from the first positive test between inpatients and patients discharged home, was statistically significant *P* < .001.
Abbreviation: RT-PCR, reverse transcription-polymerase chain reaction.

In an attempt to understand why some patients maintained positive tests for longer, we correlated the detection of SARS-CoV-2 RNA with the host immune response to virus infection. Therefore, we analyzed the production of specific IgM and IgG antibodies.

In patients with two negative tests 79 out of 116 (68.1%) performed quantification of specific antibodies. On the day of the first negative RT-PCR test, 13 out of 79 (16.5%) were positive for IgM and IgG, and 45 out of 79 (56.9%) were positive for IgG only. Therefore, a total of 58 patients (73.4%) were positive for IgG. From the IgG positive patients, 37 (63.8%) were patients discharged home and 21 (36.2%) were inpatients. The comparison of IgG levels between the two patients subgroups showed that the production of IgG was significantly lower in mild illness patients than in the inpatients (7.2 ± 8.9 vs 14.7 ± 12.3 AU/mL), *P* = .006.

Regarding the patients that kept RT-PCR tests positive, IgM and IgG was evaluated in 84 out of 94 patients (89.4%). On the day of the last positive RT-PCR test, 18 out of 84 patients (21.4%) were positive for IgM and IgG, and 39 out of 84 (46.4%) were positive for IgG only. Therefore, a total of 57 out of 84 patients (67.9%) were positive for IgG. Among IgG positive patients, 33 (61.1%) were patients discharged home and 24 (44.4%) were inpatients. The comparison of the IgG levels between the two subgroups of patients showed that the production of IgG was significantly lower in the mild illness patients than in the inpatients (4.55 ± 5.8 vs 19.6 ± 12.7 AU/mL), *P* < .001.

4 | DISCUSSION

Patients with COVID-19 may be discharged based on clinical resolution of symptoms, and evidence for viral RNA clearance from the

upper respiratory tract. Therefore, understanding the SARS-CoV-2 viral clearance profile is crucial to establish a re-testing plan on discharge and ending isolation of patients.

We analyzed the presence of viral RNA in two groups of patients: those who achieved two negative RT-PCR tests and those who kept testing positive. Patients were organized thereafter in two subgroups, mild illness patients discharged home and inpatients who had moderate to severe illness.

The results evidenced that most patients from either group tested positive for more than 2 weeks. In fact, 69.8% of the patients that tested negative twice needed more than 20 days to achieve the first negative test. Moreover, 34% of patients that kept RT-PCR tests positive at the end of our study, tested positive for 40 days. These results may indicate that in a situation of lack of testing resources, patients might be re-tested only after the 20th day from the first positive test and beyond.

Surprisingly, mild illness patients discharged home took longer to become negative than inpatients. Our results are in accordance with the results from Lan et al[10] but in disagreement with several studies pointing that delayed viral clearance is associated with severe illness.[1,5,11]

In previous studies the correlation between virus persistence and severe disease was based on the detection of viral RNA in non-survivors until death.[8,11-13] It is important to emphasize that RT-PCR does not evaluate the infectious capacity of the detected RNA. In fact, no live virus was isolated from sample cultures obtained 8 days after the onset of symptoms.[5,14] Therefore, the persistence of viral RNA may not be associated with disease severity but may indicate that the immune response is unable to promote the virus RNA clearance.

To evaluate the host immune response, we determined SARS-CoV-2 specific IgM and IgG in the same day of viral RNA detection. In accordance with previous studies, patients maintain RT-PCR tests positive even after seroconversion.[5,14]

Our results also showed that the patients discharged home from both groups presented a significantly lower IgG titer than the inpatients, which is probably associated with the disease severity. In accordance with other studies, moderate/severe illness patients appear to have higher antibody titers than those with milder disease. Although increased titers of specific antibody may induce the expression of proinflammatory factors they also contribute to the inactivation and clearance of virus.[12,15-17]

Our study evidenced that patients discharged home were younger, had mild disease, presented low IgG titer and maintained viral RNA for a longer period of time. On the other hand, patients in the inpatient group were older, moderately to severely ill, presented a higher titer of IgG and had a better capability to achieve viral RNA clearance.

Since mild illness is associated with a low viral load,[4,18,19] we hypothesize that the exposition of patients discharged home to SARS-CoV-2 did not elicit an effective immune response, which may explain the milder disease and the need of more time to viral RNA clearance.

The lack of information regarding persistence of virus RNA and infectivity, disease severity and immune response, supports the current guidance of viral clearance confirmation before patient transference out of dedicated COVID-19 wards and of ending isolation in mild illness patients.

In conclusion, our study highlights that viral RNA may persist for a long period of time in respiratory samples; mild illness patients present a weak immune response; viral RNA may remain even after the rise of IgG titer; and persistence of viral RNA is not necessarily associated with severe disease but may result from a weaker immune response instead.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTION

All authors contribute to the article.

ORCID

Anália Carmo http://orcid.org/0000-0002-7704-4736
João Pereira-Vaz http://orcid.org/0000-0002-1444-4097

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