Retesting Positive for SARS-CoV-2 RNA in Recovered COVID-19 Patients Reveals Low Levels of Non-Replicating Virus

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Short Report

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Abstract

Background: The follow-up of COVID-19 recovered patients is especially important to assess their infectivity and/or transmissibility statuses in order to maximize the COVID-19 management and containment. The aim of this study was to determine both total (genomic) and replicative (sub-genomic) SARS-CoV-2 RNA levels in nasal/oropharyngeal swab (NOS) samples from patients at follow-up times after COVID-19 recovering.

Materials/methods: We tested 176 NOS samples of COVID-19 recovered patients who were followed up at the Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome from 21 April to 18 June 2020, according to our COVID-19 care protocol. The RT-PCR tests were performed using the Allplex™ 2019-nCoV and the Quanty COVID-19 assays (for total RNA detection and quantification, respectively) and an in-house assay (for replicative RNA detection).

Results: Of 176 NOS samples studied, 32 (18.2%) tested positive for total RNA, with C_T values ranging from 29.3 to 38.8 for E, RdRP, and N genes (9 samples), 32.2 to 39.3 for RdRP and N genes (7 samples) or 35.8 to 39.8 for the N gene (16 samples). Consistently, viral loads ranged from 1.6×10^1 to 1.3×10^4 RNA copies/mL. Interestingly, we found replicative RNA in only one of 32 positive samples based on the presence of E-gene sub-genomic RNA (C_T value of 39.1). The C_T value (29.3) of E-gene genomic RNA in this sample was the lowest among the C_T values of all 9 samples in which the E gene was detected. Testing samples obtained from the 32 patients at the time of COVID-19 diagnosis showed that the C_T values ranged from 17.1 to 38.1 for E, RdRP, and N genes. Of note, the mean C_T value of E-gene sub-genomic RNA (34.9) in these samples differed of 9.0 ± 2.8 from the mean C_T value of E-gene genomic RNA (25.9). Finally, all but one of the 32 patients had positive serology results against SARS-CoV-2.

Conclusions: Our findings show that at least a proportion of COVID-19 recovered patients were still positive for SARS-CoV-2 RNA, despite to a lower extent, and that only a minority of them was likely to have actively replicating virus in the upper respiratory tract.

Background

Real-time PCR (RT-PCR) retesting positive for SARS-CoV-2 RNA in recovered COVID-19 patients^{1,2} poses not only questions regarding the SARS-CoV-2 infection course but, most importantly, the COVID-19 patients' infectivity status.³ Assessing this status may be crucial to optimize measures for COVID-19 management and containment. We investigated RT-PCR retested positive respiratory samples from

recovered COVID-19 patients for the presence of SARS-CoV-2 replicative RNA to assess active virus replication.⁴

Methods

We studied 176 recovered COVID-19 patients who were admitted to the post-acute outpatient service of the Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome (Italy), from 21 April to 18 June 2020, for COVID-19 follow-up as previously described.^{5,6} Before inclusion in the follow-up, patients had been discontinued of quarantine (e.g., had two negative RT-PCR results for SARS-CoV-2 RNA detection 24 hours apart) according to World Health Organization established criteria.⁵

Therefore, nasal/oropharyngeal swab (NOS) samples obtained from patients at the time of follow-up were analyzed for both total (genomic) and replicative (subgenomic) SARS-CoV-2 RNAs. RT-PCR testing was performed using the Seegene Allplex™ 2019-nCoV and the Clonit Quanty COVID-19 assays for total RNA detection and quantification, respectively; replicative (E-gene) RNA was detected by an in-house RT-PCR assay.⁴ Results were expressed as C_T values (<40 for positive detection) or quantified as RNA (N gene) copies/mL. Only for patients with positive results for total RNA, samples previously obtained at the time of COVID-19 diagnosis—and kept at -80°C until testing—were also tested for replicative RNA. Additionally, serological testing was performed using SARS-CoV-2 IgG/IgA Euroimmun enzyme-linked immunoassays (reference IgG/IgA values ≥ 1.1 index for positive detection).

The Ethics Committee of our Institution approved the study (reference number 18656/20) and informed consent was obtained from each patient before including his/her samples in the study.

Results

As shown in the **Table**, 32 (18.2%) of 176 NOS samples tested positive for total SARS-CoV-2 RNA, with C_T values ranging from 29.3 to 38.8 for E, RdRP, and N genes (9 samples), 32.2 to 39.3 for RdRP and N genes (7 samples), or 35.8 to 39.8 for the N gene (16 samples). The corresponding viral loads ranged from 1.6×10^1 to 1.3×10^4 SARS-CoV-2 RNA copies/mL. Interestingly, replicative SARS-CoV-2 RNA was found in only one (0.03%) of 32 positive samples, which displayed a subgenomic E gene C_T value of 39.1 (**Table**). In this sample, the genomic E gene C_T value was 29.3, which was the lowest value among those of nine samples with genomic E gene detections.

To confirm these findings, samples obtained from the 32 patients at the time of COVID-19 diagnosis were also tested (**Table**). As expected, all samples were positive for the subgenomic E gene, with C_T values ranging from 30.1 to 39.8. Of note, the mean C_T value for the genomic E gene (25.9) differed by 9.0 ± 2.8 from the mean C_T value of the subgenomic E gene (34.9). Furthermore, all but one of 32 patients had positive serology (i.e., IgG and/or IgA) against SARS-CoV-2 (data not shown), as well as 139 of remaining

144 patients (data not shown). The mean ($\pm$ standard deviation) time from COVID-19 diagnosis to follow-up was 48.6 ($\pm$ 13.1) in 32 patients and 57.7 ($\pm$ 16.9) in 144 patients.

Discussion

We showed that around 18% of COVID-19 patients in our center became RT-PCR positive for SARS-CoV-2 RNA after clinical recovery and previous negative results. Excluding contamination to potentially explaining post-negative, positive RT-PCR findings,³ the positivity observed by us, which was similar to that reported elsewhere,² was suggestive but not necessarily a reflection of viral carriage in our patients. While virus isolation success depends on viral load, RT-PCR does not discriminate between non-infectious RNA and infectious (or viable) virus in NOS samples.⁴ Our samples contained less than 10^6 copies/mL of SARS-CoV-2 RNA (the highest value observed was 1.3×10^4), which is the threshold estimated to yield an isolate.⁴ Therefore, we used SARS-CoV-2 replicative RNA detection as a reliable surrogate of virus replication in Vero/E6 cell line culture.⁴ It was worthy to noting that only one of 32 patients who retested RT-PCR positive had an again replicating virus in his/her NOS sample (**Table**). We did not exclude the possibility for SARS-CoV-2 re-infection in this patient as well as in other 31 RT-PCR positive patients—no whole-genome sequencing and phylogenetic analyses were performed.³ However, the patient became positive 15 days after COVID-19 recovery and, notably, his/her RT-PCR profile was more similar to those observed in the 32 patients at COVID-19 diagnosis (**Table**). Ultimately, antibody response and clinical evaluation at the follow-up completed our meaningful patients' assessment, showing that 31 of 32 patients were positive for anti-SARS-CoV-2 specific IgG/IgA immunoglobulins and remained asymptomatic after COVID-19 recovery.

In conclusion, this study highlights that at least a proportion of COVID-19 recovered patients may be still positive (albeit at lower levels) for SARS-CoV-2 RNA, but that only a minority of the patients may carry a replicating SARS-CoV-2 in the upper respiratory tract. Further studies are necessary to define the virological recovery in COVID-19

Declarations

Author Contributions: Drs Sanguinetti and Cattani had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Liotti and Menchinelli contributed equally to the study. Drs Sanguinetti and Cattani contributed equally as senior authors.

Concept and design: Liotti, Menchinelli, Sanguinetti, Cattani.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Liotti, Menchinelli, Sanguinetti, Cattani.

Critical revision of the manuscript for important intellectual content: All authors.

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Supervision: Posteraro, Landi.

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Additional Information: The members of the Gemelli Against COVID-19 Post-Acute Care Study Group are listed in reference 6.

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Tables

Table. Positive SARS-CoV-2 RNA Detection Results for NOS Samples Obtained At COVID-19 Diagnosis or After COVID-19 Recovery in 32 Study Patients

COVID-19 diagnosis samples tested by RT-PCR for					COVID-19 recovery samples tested by RT-PCR for				
Genomic RNA				Subgenomic RNA	Genomic RNA			Subgenomic RNA	RNA load (copies/mL)
le	E gene	RdRP gene	N gene	E gene	E gene	RdRP gene	N gene	E gene	N gene
	31.6	31.3	31.2	34.5	29.3	30.7	31.2	39.1	1.2×10^4
	27.0	26.9	30.0	36.0	30.0	30.5	31.2	-	8.9×10^3
	19.3	20.8	22.1	35.2	31.5	34.7	32.8	-	3.3×10^3
	21.6	22.0	22.9	36.4	31.8	31.4	32.3	-	5.5×10^3
	30.0	32.8	38.1	30.2	31.8	34.3	34.5	-	3.2×10^3
	20.8	20.9	22.3	37.3	32.2	32.8	34.1	-	5.3×10^3
	27.3	29.9	31.3	36.9	32.3	30.9	32.7	-	6.4×10^3
	26.9	27.0	31.2	38.1	35.0	34.4	36.1	-	4.0×10^2
	22.5	23.7	24.9	31.0	38.8	33.65	33.9	-	2.6×10^3
	21.3	21.4	28.9	38.9	-	32.2	33.4	-	1.2×10^4
	26.6	26.9	28.1	33.0	-	32.8	33.2	-	1.3×10^4
	22.8	24.2	25.3	31.0	-	34.2	33.7	-	6.9×10^3
	25.8	25.8	26.1	39.8	-	34.8	39.1	-	3.0×10^2
	20.8	20.4	21.1	32.0	-	35.0	35.1	-	1.9×10^3
	29.4	30.1	32.2	37.0	-	36.5	39.2	-	3.2×10^3
	27.9	29.13	31.1	32.0	-	38.1	39.3	-	1.6×10^1
	30.6	29.9	31.8	32.1	-	-	35.7	-	5.4×10^3
	28.5	29.1	30.8	36.8	-	-	36.8	-	2.9×10^3
	26.9	22.2	26.1	30.1	-	-	37.5	-	1.1×10^3
	25.7	25.2	28.9	38.0	-	-	37.9	-	2.6×10^3
	27.0	29.0	30.2	32.3	-	-	38.1	-	1.9×10^3
	28.5	29.4	30.0	32.3	-	-	38.4	-	4.9×10^1
	27.1	28.6	29.3	36.1	-	-	38.9	-	4.5×10^2
	25.4	22.9	24.1	34.8	-	-	39.0	-	5.6×10^1
	28.7	29.5	31.4	37.3	-	-	39.1	-	5.4×10^3
	27.1	27.7	29.2	37.1	-	-	39.1	-	1.9×10^3
	26.7	27.7	29.6	39.2	-	-	39.2	-	2.0×10^3
	17.1	19.1	19.9	33.0	-	-	39.2	-	8.5×10^2
	27.0	28.9	30.0	32.1	-	-	39.3	-	5.0×10^1
	22.9	23.8	25.8	37.1	-	-	39.4	-	1.6×10^2
	28.6	30.4	30.9	33.0	-	-	39.6	-	5.3×10^2
	29.1	28.0	30.9	36.2	-	-	39.8	-	3.4×10^2

Abbreviations: RT-PCR, real-time polymerase chain reaction; E, envelope; RdRP, RNA-dependent RNA polymerase; N, nucleocapsid.