

## PCR Assays Turned Positive in 25 Discharged COVID-19 Patients

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**Abstract:**

We report the observation that 14.5% of COVID-19 patients had positive RT-PCR testing again after discharge. We describe correlations between laboratory parameters and treatment duration ( $r = -0.637$ ;  $p = 0.002$ ) and time to virus recrudescence ( $r = 0.52$ ;  $p = 0.008$ ) respectively, suggesting the need for additional measures to confirm illness resolution in COVID-19 patients.

**Keywords:** coronavirus, COVID-19, discharged patients, recurrent viral activity, D-dimer level.

## Introduction

Since Dec 8<sup>th</sup> 2019, many cases has been reported by investigators who described the clinical characteristics of hospitalized patients with COVID-19 infection<sup>1-3</sup>. We noticed the recent report<sup>4</sup> from Lan *et al.* that 4 medical staff were still virus carriers after recovery from COVID-19 infection. Here we gave a report on 25 discharged patients with their medical record review and further analysis.

## Methods

Enrolled from Jan 23<sup>th</sup> 2020 to Feb 21<sup>th</sup> 2020, total 172 COVID-19 infected patients were discharged from Shenzhen Third People's Hospital. They all met the following criteria of hospital discharge in China: (1) Normal body temperature for more than 3 consecutive days. (2) Significant reduction of respiratory symptoms evaluated by following indicators: cough and expectoration disappeared, normal ranges for inflammatory markers IL-6 and CRP, as well as oxygenation index  $\geq 350$ . (3) Substantial improvement over conventional chest radiography detection. (4) At least two consecutively negative results of RT-PCR testing separated by at least 24-hour interval. Considering the risk of reinfection, all discharged patients were required another 14 days of self-segregating at home for further observation. The cloacal swab and nasopharyngeal swab samples were both collected from these discharged patients each three days for RT-PCR detection of COVID-19 in the same way as they were in hospital. Among them, 25 discharged patients were identified with positive results again. COVID-19 RNA Detection Kits (Real-time fluorescent PCR method) approved by the National Medical Products Administration were used for virus testing as described previously<sup>5</sup>. Conditions for the amplifications include reverse transcription at 50°C for 15min, pre-denaturation at 95°C for 15 min, followed by 45 cycles of 94°C for 15 s and 55°C for 45 s for fluoresce detection. The receiver operating characteristic (ROC) curve analysis was used to determine the optimal threshold cut-off value. And a cycle threshold (Ct) value  $\leq 40$  was defined as a positive test. Other demographic, clinical, radiologic and laboratory findings were extracted from the electronic medical records of the patients. The anti-viral compound ritonavir/lopinavir (Kaletra®) combined with interferon alpha (IFN- $\alpha$ ) was used as the potential antiviral therapy for all these patients during their

first hospitalization. Ritonavir/lopinavir table was administrated with 500 mg once daily while 50  $\mu$ g IFN- $\alpha$  was aerosolized 2 times a day. Besides, Chinese herbal medicines were widely recommended by the Chinese Clinical Guideline for the Diagnosis and Treatment of Novel Coronavirus Pneumonia (On Trials) issued by National Health Commission of China. The herbal formula of Lung Cleansing and Detoxifying Decoction was used for these 25 patients during their second hospital stay. The study was approved by Shenzhen Third People's Hospital Ethics Committee and the informed consent was waived.

## Results

The study population included 172 discharged COVID-19 patients from Jan 23th 2020 to Feb 21th 2020. These were 25 of discharged patients (total 14.5%) sent to hospital again because of the positive RT-PCR results on virus again. With the median age of 28 years (IQR: 16.25-42), 17 of them were females, including 6 children under 12 years old. According to their previous medical records, these patients had experienced an average of  $15.36 \pm 3.81$  days of hospital stay, as well as  $13.33 \pm 3.93$  days of potential antiviral therapy with ritonavir/lopinavir and IFN- $\alpha$ , no obvious differences with other discharged patients. Before discharging from the hospital, these patients all showed improvements on chest computed tomography (CT) evidence and exhibited with two consecutive negative results (24 hours of interval) on virus mRNA by RT-PCR assays.

After leaving the hospital to self-quarantine at home, the discharged patients hadn't taken drug any longer but just monitored at home by RT-PCR detection of COVID-19 with both cloacal swab and nasopharyngeal swab samples in each three days. Unexpectedly, cloacal swab samples of 14 patients turned positive on virus mRNA and another 11 patients showed positive results of nasopharyngeal swab sample testing. Overall, the mean duration from their final negative PCR result to hospital discharge was  $2.71 \pm 1.88$  days. While the average period from positive again to previous discharge lasted for  $5.23 \pm 4.13$  days. Thereby, these 25 patients experienced an average of  $7.32 \pm 3.86$  days from their last negative RT-PCR result to turning positive again.

Notably, these patients once represented with the common symptoms of fever (68%) and cough (60%) and 24 of them were non-severe types at the first onset. At this time of hospital readmission, only 8 patients (32%) had mild cough. Besides, CT scan results indicated that 12 of them were characterized by improvement of original lesions compared with images before leaving the hospital, while another 8 patients showed no worsening than previous results. When they readmitted to hospital, the formula of Lung Cleansing and Detoxifying Decoction was used for these 25 patients. Within an average of 2.73 days of hospital stay, the RT-PCR results of virus mRNA detection were all turning to negative in both nasopharyngeal swab and cloacal swab samples. These patient were all stayed for a prolonged observation.

On the other hand, previous clinical and laboratory outcomes were compared between these 25 patients (with conversion of RT-PCR results from negative to positive after being discharged from the hospital) and rest 147 patients (continuously represented negative for the virus after discharge). They showed no distinguished differences on the levels of laboratory parameters before leaving the hospital. Subsequently, correlation analysis indicated that there was a significant inverse correlation existed between serum D-Dimer level before discharging and the duration of treatment in these 25 patients ( $r=-0.637$ ,  $p=0.002$ ), instead of the rest 147 patients. Furthermore, lymphocyte concentrations before these 25 patient leaving the hospital were significantly positively correlated ( $r=0.52$ ,  $p=0.008$ ) with the time interval for virus reappearing.

## **Discussion**

These 25 patients with COVID-19 infection all met the criteria for hospital release from quarantine, while the RT-PCR testing then conversed to positive without aggravation on symptoms after 2 to 13 days. It seems that there was a fluctuating period between of seeming improvement in clinics and full recovery from virus. These less symptomatic carriers brought more challenges to the management and control of COVID-19 epidemic in China and any other affected countries. According to our study, it is probably that two negative RT-PCR tests 24 hours apart may not be sufficient for viral clearance evaluation. Repeated viral RT-PCR testing separated by prolonged duration like 48 hours is essential

to assure that virus has actually cleared and the discharged patients no longer transmitted the virus. On the other hand, we suggested that some immunological parameters such as D-dimer and absolute lymphocyte count, and even antibody test should be combined with RT-PCR negative test as additional measures to assure that infected patients have completely recovered and can be released from quarantine. Besides, the RT-PCR results then turning to negative within an average of 2.73 days of hospital stay. Even the Chinese herbal medicine was used for these readmitted patients, it is hard to assess the effect on virus clearance. Further case-control study and cohort study will be needed to pursue that.

**Author Contributions:** Dr. Yanchao Pan and Lei Liu had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Jing Yuan and Shanglong Kou contributed equally to this work. Acquisition, analysis, or interpretation of data: Jing Yuan, Yanhua Liang, Jianfeng Zeng. Concept and design: Jing Yuan, Lei Liu. Drafting of the manuscript: Yanchao Pan, Shanglong Kou. Statistical analysis: Yanchao Pan, Shanglong Kou.

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**Conflict of Interest Disclosures:** All authors declare that they have no conflict of interest exists.

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Table. Baseline Characteristics of 25 Discharged Patients with positive results of RT-PCR again.

Groups by age range, case number	≤14, N=6	20-40, N=13	41-60, N=6	Total, N=25	P value
Sex (numbers) (male, female)	1, 5	4, 9	3, 3	8, 17	NS
<b>Symptom of first admission to hospital</b>					
Fever (number (percentage))	3 (50%)	9 (69.23%)	5 (83.33%)	17 (68%)	NS
Cough (number percentage)	2 (33.33)	9 (69.23%)	4 (66.67%)	15 (60%)	NS
<b>Symptom of second admission to hospital</b>					
Mild cough (number (percentage))	2 (33.33%)	3 (18.75%)	3 (50%)	8 (32%)	
<b>Laboratory parameters before discharge (mean, (SD))</b>					
lymphocyte count (10 <sup>9</sup> /L)	3.61 (1.52)	1.62 (0.46)	1.51 (0.43)	2.07 (1.19)	0.001
LDH level (U/L)	218.40 (49.85)	156.50 (29.74)	188 (40.42)	180.24 (44.5)	0.025
CRP level (mg/L)	0.84 (0.85)	6.77 (5.05)	5.30 (4.78)	4.90 (4.79)	NS
IL-6 level (pg/ml)	2.49 (0.93)	3.3.0 (1.94)	3.41 (0.81)	3.19 (1.40)	NS
D-Dimer level (μg/ml)	0.29 (0.14)	0.36 (0.29)	0.43 (0.27)	0.37 (0.26)	NS
<b>Clinical features (mean, (SD)) (days)</b>					
Length of first hospital stay	15 (2.83)	15.23 (3.35)	16 (5.83)	15.36 (3.81)	NS
antivirus treatment durations	13.33 (3.93)	13.46 (4.31)	13.5 (4.46)	13.44 (4.08)	NS
Time from negative PCR test to discharge	3.67 (2.50)	2.17 (1.53)	2.83 (1.72)	2.71 (1.88)	NS
Time from positive again to last negative	8.33 (5.61)	6.77 (3.40)	7.5 (3.21)	7.32 (3.86)	NS

Time from positive again to last discharge	4.67 (4.23)	5.92 (4.72)	4.67 (2.94)	5.32 (4.13)	NS
Time from positive again to second hospitalization	1.33 (1.51)	1.85 (2.12)	2.67 (2.07)	1.92 (1.96)	NS
Time from readmission to negative again	1 (0)	2 .69 (2.06)	3.67 (2.07)	2.73 (2.03)	NS

Data are given as mean  $\pm$  standard deviation (SD). Determined using one-way analysis of variance.

Abbreviations: LDH, lactate dehydrogenase; CRP, C-reactive protein; IL-6, interleukin-6.

Figure. Correlation analysis for serum D-Dimer level and duration of treatment, as well as lymphocyte count and time interval for virus reappearing.

Correlation analysis on serum D-Dimer level and the duration of treatment ( $r=-0.637$ ,  $p=0.002$ ) (A), as well as correlation between lymphocyte concentration and the time interval for virus reappearing ( $r=0.52$ ,  $p=0.008$ ) (B). The D-Dimer values and lymphocyte counts were collected from the last measurements before their first hospital discharge. The duration of treatment was derived from their previous records. The time interval for virus reappearing was calculated from last negative before previously leaving the hospital to turning positive again of RT-PCR testing on COVID-19.

Figure 1

