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RESEARCH ARTICLE

CASE SERIES: SIGNIFICANCE OF POSITIVE SARS-COV-2 RT-PCR IN A CURED PATIENT: PERSISTENT NON-VIABLE VIRAL SHEDDING VERSUS TRUE INFECTION

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ABSTRACT

With the emergence of SARS-COV-2 as a cause of COVID 19 disease in December 2019 in Wuhan, China, and the spread of the infection later, down to its declaration a pandemic by the World Health Organization (WHO) in March 2020, there are still many questions that must be answered, among which the significance of an RT-PCR test turning positive after announcing a patient's recovery, and how should physicians act in the face of such a situation. In our case series, we describe the clinical characteristics of fourteen patients who had recovered from COVID-19 infection between March and May 2020, and who had retested positive after at least one week. Thirteen out of fourteen patients were asymptomatic upon retesting. Only one patient developed symptoms after being declared cured with positive RT-PCR. Upon reassessment, the patient had mild disease with no lower respiratory tract involvement. During the period between recovery and re-admission, the patient was self-isolating at home, so there was no contact tracing to see how contagious the disease was in this episode. We concluded that most of the available data on retesting positive after recovery suggest the presence of a non-viable viral genome segment, since the PCR only detects the genome and not the viable virus. However, in the presence of symptoms, the patient should be considered contagious until more solid scientific data come out.

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INTRODUCTION

Coronaviruses are enveloped viruses with positive sense ribonucleic acid (RNA) with a diameter ranging from 60 to 140 nm and a surface with spike like projections giving them a crown-like appearance under electron microscope (1). Generally, the viruses that circulate between humans are divided into four types, namely HKU1, NL63, 229E and OC43; they all cause mild respiratory symptoms in humans (Singhal, 2009). In December 2019, local hospitals in Wuhan started receiving patients presenting with severe pneumonia of unknown cause. Common exposure was traced back to the Huanan wholesale seafood market and on December 31st, China notified the outbreak to the World Health Organization (WHO) (1).The virus spread to the western hemisphere, and a pandemic was declared by the WHO on March 11, 2019(2).

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Clinical features of COVID-19 ranged from asymptomatic to acute respiratory distress syndrome state complicated by multi-organ dysfunction. Common symptoms include fever, cough, sore throat and other constitutional symptoms. These symptoms are indistinguishable from those of other viral infections. A subset of patients progressed to pneumonia within one week and even to death. The progression is associated with an extreme rise in inflammatory cytokines (Singhal, 2009). Shedding of the virus continues for a median duration of 20 days (Zhou, 2020). A higher viral load and a prolonged period of virus shedding (beyond 10 days) is observed in those patients with severe disease compared to those with mild illness (Liu, 2020). Serology tests and antibody response are being evaluated in patients with current or previous infections with detection of IgM and IgG after a median duration of 5 days and 14 days respectively (Guo, 2020; Zhao, 2020). Interestingly, some specific findings on chest computed tomography (CT) scan such as ground-glass opacities (most common), multifocal organizing pneumonia, or peripheral distribution of infiltrates are common among hospitalized patients and may precede

positive reverse transcription polymerase chain reaction (RT-PCR) assays by a median duration of 8 days (Wang, 2020). On January 13, WHO have declared protocol V for screening and diagnosis of SARS-COV-2, updated later on January 17 with the protocol V2, using the Real Time reverse transcription polymerase chain reaction (RT-PCR) techniques on biological samples, mainly nasopharyngeal and oropharyngeal swabs, lower respiratory specimen and other biological specimens (Corman, 2019; Corman, 2020). These assays included several RT-PCRs targeting two or three different SARS-CoV-2 gene regions, including RdRp (RNA-dependent RNA polymerase), N (nucleocapsid protein), and E (envelope protein). The E-gene assay is used as a screening test, while RdRp gene assay is used as confirmatory assay and N-gene assayas additional confirmatory assay (Corman, 2019; Corman, 2020). Some case reports and case series have discussed the possibility of reinfection or reactivation of the COVID-19 virus in patients who had the infection and then recovered with two documented negative PCR tests. Batisse *et al*. have raised two hypothesis in their case series of 11 patients: viral reinfection and viral reactivation from sanctuaries (10). Others have reported the possibility of false negative PCR result due to viral load fluctuation in infected patients (Chen, 2020). We conducted a case series report on 14 patients admitted to the Rafic Hariri University Hospital (RHUH) in Beirut, Lebanon, between March 1,2020 and May 15,2020 who met the current discharge criteria and retested positive again after 1 week or more. Our aim is to determine the demographic and clinical features of retested positive patients after recovery. We also raise the question about the possibility of the need to quarantine COVID-19 patients for prolonged period following discharge with necessary reexamination to evaluate their clinical outcomes and potential transmissibility.

Population description: On May 15, 2020, RHUH had a total of 619 confirmed cases of COVID-19 infections, of which 246 had been cured. Following two negative PCR results, 70 of those recovered patients were retested after one week. This retest began after noting that a small number of patients who repeated their test for personal preferences after recovery had positive PCR results. This couldn't be done for all recovered patients since many of them have lost follow-up. 14 out of the 70 patients (20%) were retested positive. Our case series describes those 14 patients who were retested positive for COVID-19 by RT-PCR in their nasopharyngeal secretions after testing negative and being discharged from the hospital. They have all recovered from moderate or severe illness. The mean age of our sample was 39.9 years, males representing 57.14% and females 42.86% of the population. One patient had a history of coronary artery disease (CAD), hypertension (HTN) and type 2 diabetes mellitus (DM). Two other patients had hypertension and one patient had coronary artery disease. None of the patients had any chronic lung or kidney disease. The results are shown in (Table 1). Symptoms upon admission were as follows: fever (57.14%), dry cough (57.14 %), myalgia and arthralgia (57.14%), headache (35.7%), sore throat (35.7%), dyspnea (28.5%), anosmia (28.5%), diarrhea (7.14%) and productive cough (7.14%). One of the patients had severe disease; however, none of them needed intensive care unit (ICU) admission. The white blood cell count (WBC count) ranged from 1200 to 8900 cell/µL(mean 5326 cell/µL), hemoglobin levels (Hb) ranged from 10 to 16 g/dL(mean 13.56 g/dL), and platelets counts varied between 107 and 261 x1000/µL (mean 210,000/µL).Serum creatinine was between 0.5 and 0.9 mg/dL(mean 0.735 mg/dL). C-reactive protein (CRP) levels ranged from0.3 to 265 mg/L(mean of 34.55 mg/L). Absolute neutrophils count (ANC) ranged from 989 to 4500 cells/µL (mean of 2476 cells/µL). Absolute lymphocyte count (ALC) ranged from 156 to 3800 cells/µL (mean of 2127 cells/µL). The results are presented in (Table 2). CT scans and chest x-rays findings were normal in 57.1% of the patients. Unilateral multiple patchy ground glass opacities (GGO) were observed in 14.3% of the patients, and bilateral multiple patchy ground glass opacities were seen in 28.6% of them. The results are shown in (Table 3). All patients were treated only with supportive care except for the severe case, that was treated with lopinavir-ritonavir for 10 days. Serology testing was performed on only 2 patients (patients 13 and 14) due to lack of testing during March and April; It was done one week after their hospitalization and showed positive total antibodies. The average duration to symptoms resolution after admission was 9.14 days (range 3 to 20 days). The median duration to the first negative PCR result was 14 days (range 5-30 days). All patients were tested negative again after 24hours. They were discharged from the hospital without symptoms, and were retested again within 7 to 10 days. At this time, all of them had a positive PCR result. These results are shown in (Table 4).

Only one patient (patient 11) was re-admitted to the hospital after being retested positive. He complained of a dry cough and sore throat. This patient is a 28 year-old man who had mild illness on his first admission with only a sore throat and normal chest CT scan. His symptoms resolved after 5 days and PCR was then negative 12 days later. Six days after the hospital discharge, the patient started to complain of the same symptoms in addition to mild dry cough. At that time, an RT-PCR test for COVID-19 from nasopharyngeal sample was requested and turned out to be positive. He was re-admitted for re-evaluation. His laboratories upon re-admission were as follows: white blood cells 7400/µL, 59% neutrophils and 30% lymphocytes, platelets of 250,000/µL and a hemoglobin level of 15 g/dL. The serology test on re-admission showed positive IgG and IgM antibodies. He had a smooth course in the hospital. Symptoms resolved 3 days after admission, and he was discharged after 14 days. He took him 17 days to retest negative again. The average duration of retesting negative in our sample was 10.7 days (range 2-20 days). The patients were advised to remain in quarantine for 14 days after their first discharge. No close contactbecame infected although the patients were retested positive after few days.

DISCUSSION

The world's early and preliminary knowledge about the possibility of re-infection or re-activation of COVID-19 is one of the current major challenges, which if proven true would make major changes in our approach to the pandemic. Our case series describes14 patients who tested positive for an average of 10 days after testing negative twice. The double test was performed to reduce any chances of false negative results. However, the double test can represent false negative results since the overall RT-PCR positivity for the virus is between 30 and 40% (Wang, 2020). The sampling procedure can affect the positivity of the test and many factors interfere here: tube quality, storage time, temperature,

Table 1 Description of the population

Patient	Gender	Age	Location	Comorbidities
Patient 1	Male	27	Mount Lebanon	None
Patient 2	Male	25	Mount Lebanon	None
Patient 3	Female	47	Baalbeck	None
Patient 4	Male	56	Beirut	HTN, CAD
Patient 5	Male	27	Beirut	None
Patient 6	Female	25	Mount Lebanon	None
Patient 7	Female	43	Beirut	None
Patient 8	Male	41	Beirut	None
Patient 9	Female	29	Nabatieh	None
Patient 10	Male	34	Akkar	None
Patient 11	Male	28	Mount Lebanon	None
Patient 12	Male	80	North Lebanon	HTN, CAD, DM
Patient 13	Female	52	Beirut	None
Patient 14	Female	45	Mount Lebanon	HTN

Table 2 Lab results of the patients

Patient	White cells	Hemoglobin	Platelets	Creatinine	CRP	ANC	ALC
Patient 1	7660	14.3	254	0.76	1.8	3440	3200
Patient 2	5500	15.0	209	0.80	0.8	1500	3135
Patient 3	2300	13.0	134	0.76	2.7	989	966
Patient 4	4300	16.0	107	0.77	17.8	2400	1240
Patient 5	5200	15.0	201	0.80	2.3	2200	2100
Patient 6	8900	13.0	182	0.70	1.2	4500	3500
Patient 7	6400	13.0	228	0.60	0.6	2900	2800
Patient 8	6700	15.0	261	0.90	0.3	1876	3800
Patient 9	3800	13.0	245	0.50	3.0	1060	2100
Patient 10	4600	13.0	184	0.90	155	3300	782
Patient 11	8500	15.0	259	0.80	13	4100	3300
Patient 12	1200	10.0	256	0.60	265	900	156
Patient 13	6300	11.0	233	0.70	12.4	4000	1500
Patient 14	3200	13.6	192	0.70	7.9	1500	1200

Table 3 Radiographic findings of the patients

Patient	Modality	Result
Patient 1	CT	Normal
Patient 2	CXR	Normal
Patient 3	CXR	Normal
Patient 4	CXR	Normal
Patient 5	CT	Diffuse unilateral GGO
Patient 6	CT	Diffuse unilateral GGO
Patient 7	CXR	Normal
Patient 8	CT	Normal
Patient 9	CT	Diffuse bilateral GGO
Patient 10	CT	Diffuse bilateral GGO
Patient 11	CT	Normal
Patient 12	CT	Diffuse bilateral GGO
Patient 13	CT	Diffuse bilateral GGO
Patient 14	CT	Normal

Table 4: PCR E-gene results

	Upon admission					After discharge		
Patient	1st PCR	2nd PCR	3rd PCR	4th PCR	5th PCR	1st PCR	2nd PCR	3rd PCR
Patient 1	32.5	ND	ND			35.6	37.0	ND
Patient 2	24.89	38.0	39.9	ND	ND	33.8	31.9	ND
Patient 3	29.7	39.0	35.0	ND	ND	37.6	38.0	ND
Patient 4	19.9	34.2	33.6	ND	ND	37.0	39.0	ND
Patient 5	27.7	37.0	ND	ND		34	ND	
Patient 6	34.0	ND	ND			34.7	ND	
Patient 7	24.1	38.0	ND	ND		38.2	ND	
Patient 8	18.4	29.0	ND	ND		37.7	ND	
Patient 9	17.7	21.0	ND	ND		38.7	36.0	ND
Patient 10	34.8	ND	ND			38.0	38.7	ND
Patient 11	22.18	ND	ND			32.0	34.9	39.5
Patient 12	31.18	38.0	ND	ND		36.8	ND	
Patient 13	37.6	ND	ND			38.2	ND	
Patient 14	20.0	29.0	ND	ND		35.0	37.0	ND

(ND= not detected)

transportation processes and the quality of the kits used (Kang, 2020). None of the contacts of the patients became infected in our study. A positive RNA signal could represent inactivated virus or viral gene fragments without active replication (Kang, 2020). A follow-up of 13 patients who re-tested positive in the Guangdong Province found no new cases in 104 close contacts of these patients (Kang, 2020). Lan *et al*. described four healthcare workers who re-tested positive for the virus after recovery (5 to 13 days after the two negative tests); they were asymptomatic and none of them had any infected contact or family member (Lan, 2020). However, the fact that one of ourpatients, during quarantine, had symptomsfor7 days, raises the theory of virus reactivation versus re-infection. Ye *et al*. found evidence of COVID-19 reactivation in patients without specific clinical characteristics (Ye, 2020). Ravioli *et al*. have reported two cases of patients with COVID-19 pneumonia confirmed by a positive RT-PCR testand which they developed after testing negative twice by nasopharyngeal samples taken 24 hours apart; this also raises the possibility of reactivation (16).Another case report presented a patient who, after recovering along with two negative PCR tests, re-tested positive after 14 days while he was asymptomatic (Zhang, 2020). The possibility of reinfection seems to be less likely in our cases because all patients have self-isolated for at least 14 days after testing twice as negative. However, it may be possible that COVID-19 RNA is mutating rapidly and causing respiratory infection again. So far, little is understood about the immune system and its response to COVID- 19 infection.

Conclusion

In conclusion, the significance of a positive RT-PCR test in a cured COVID-19 patientis still not well understood, and whether a person who is retested positive should be isolated or not is still to be clarified. But what can be suggested is that any patient who re-develops symptoms should be isolated. In this case we need to stay vigilant, especially if symptoms recur, awaiting more clinical data to come out.

REFERENCES

Singhal T. A Review of Coronavirus Disease-2019 (COVID-19). Indian J Pediatr. 2020 Apr;87(4):281–6.

WHO. Coronavirus disease (COVID-19) pandemic [Internet]. World Health Organization. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019?gclid=CjwKCAjwmMX4BRAAEiwA-zM4JlSO9ROHxoBjlylE9jR5WKPQcEmtKEUatIde8YTdDlA1nE4BRrLZwhoCcLYQAvD_BwE

Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet. 2020 Mar;395(10229):1054–62.

Liu Y, Yan L-M, Wan L, Xiang T-X, Le A, Liu J-M, *et al*. Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis. 2020;20(6):656–7.

Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, *et al*. Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19). Clin Infect Dis Off Publ Infect Dis Soc Am. 2020 Mar 21;

Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, *et al*. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis. 2020 Mar 28;ciaa344.

Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7;

Corman V, Landt O, Kaiser M, Molenkamp R, *et al*. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill.2020 Jan 23; 25(3): 2000045.

Corman V, Bleicker T, Brünink S, Drosten C.Diagnostic detection of Wuhan coronavirus 2019 by real-time RTPCR -Protocol and preliminary evaluation as of Jan 13, 2020- Berlin, 13.01.2020

Batisse D, Benech N, Botelho-Nevers E, Bouiller K, Collarino R, Conrad A, *et al*. Clinical recurrences of COVID-19 symptoms after recovery: viral relapse, reinfection or inflammatory rebound? J Infect. 2020 Jun;S0163445320304540.

Chen D, Xu W, Lei Z, Huang Z, Liu J, Gao Z, *et al*. Recurrence of positive SARS-CoV-2 RNA in COVID-19: A case report. Int J Infect Dis. 2020 Apr;93:297–9.

Wang Y, Kang H, Liu X, Tong Z. Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. J Med Virol. 2020;92(6):538–9.

Kang H, Wang Y, Tong Z, Liu X. Retest positive for SARS-CoV-2 RNA of "recovered" patients with COVID-19: Persistence, sampling issues, or re-infection? J Med Virol. 2020 Jun 3;

Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, *et al*. Positive RT-PCR Test Results in Patients Recovered From COVID-19. JAMA. 2020 Feb 27;

Ye G, Pan Z, Pan Y, Deng Q, Chen L, Li J, *et al*. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. J Infect. 2020;80(5):e14–7.

Ravioli S, Ochsner H, Lindner G. Reactivation of COVID-19 pneumonia: A report of two cases. J Infect. 2020 May;S0163445320302796.

Zhang J-F, Yan K, Ye H-H, Lin J, Zheng J-J, Cai T. SARS-CoV-2 turned positive in a discharged patient with COVID-19 arouses concern regarding the present standards for discharge. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. 2020 Mar 19;97:212–4.

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