Medical Hypotheses 144 (2020) 109980

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Occult SARS-CoV-2 infection; a possible hypothesis for viral relapse

A B S T R A C T

Coronavirus disease 2019 (COVID-19) has emerged as a global public health emergency, which is characterized by high infection rate and fatal course. Recent data reported that the test for Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) RNA might become positive again after one or two consecutively negative tests. Many researchers are currently evaluating the clinical characteristics of the SARS-CoV-2 reactivation. In this letter, we proposed a possible mechanism of SARS-CoV-2 reactivation or relapse after negative nasopharyngeal swabs PCR.

To the Editor,

The number of total SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) infected cases has reached more than 7,000,000 globally, as the pandemic continues its spread in different areas in the world. However, the latest data show that there were more than 3,236,000 active cases, and more than 3,476,000 patients have recovered from the disease [1].

The relapse of COVID-19 patients has dazed scientists around the world, who have to decide whether the patients who tested positive after negative tests were reinfected or the SARS-CoV-2 was relapsed or reactivated.

Recent data from China reported that the test for SARS-CoV-2 RNA might become positive again after one or two consecutively negative tests [2–5]. Many researchers are currently evaluating the clinical characteristics of the SARS-CoV-2 reactivation. The Chinese study by Liu et al., included five SARS-CoV-2 reactivated patients. Of them, one patient had progressive lymphopenia and progressive neutrophilia, indicating the potential value of leukocyte counts on COVID-19 reactivation [5]. This progressive lymphopenia could be explained by the ability of SARS-CoV-2 to infect T lymphocytes through receptor-dependent S protein-mediated membrane fusion but does not replicate, and then the viral RNA degrade [6].

Chen and colleagues published a case report of a patient whose oropharyngeal swab test for SARS-CoV-2 became positive again after two consecutively negative results. The respiratory symptoms of the patients had improved without fever. After three days of the positive results, the patients returned negative [2]. This patient was capable of transmitting the virus to healthy people if she had been discharged after the second negative test, as per most of the published guidelines. Another study by Li et al. [7] including 13 discharged COVID-19 patients who were quarantined for 4-week at home after negative PCR. Of them, the sputum samples of four (31%) patients returned positive 5–14 days after discharge, although the respiratory symptoms, as well as the body temperature, were normal.

Although the mechanism of relapse of SARS-CoV-2 has not determined yet, monitoring the disease prognosis and effective control of the relapse of the epidemic become the next focus of research.

In this letter, we proposed a possible mechanism of SARS-CoV-2 reactivation or relapse after negative nasopharyngeal swabs PCR.

The Middle East respiratory syndrome coronavirus (MERS-CoV) closely resembles SARS-CoV. It is previously confirmed that MERS-CoV has the ability to infect different human immune cell lines, including monocytes and dendritic cells as well [8].

Large numbers of SARS-CoV particles and genomic sequences were detected within the peripheral blood mononuclear cells (PBMCs; monocytes and lymphocytes), epithelial cells of the respiratory tract, the intestinal mucosa, and brain cells [9,10].

Yilla et al. examined whether human PBMCs can support SARS-CoV replication, which might participate in the progression or reactivation of SARS disease. They reported evidence of SARS-CoV entry to PBMCs, but limited replication and minimal production of infectious virus [11]. On the other hand, Li and colleagues reported that SARS-CoV can not only infect but also can replicate within PBMCs [12].

Bioinformatics modeling, as well as the *in vitro* experiments revealed that SARS-CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) as a receptor for entry into human cells. ACE2 receptor serves as a human cell-binding site for the spike protein (S- protein) of SARS-CoV-2 [13,14].

ACE2 receptor is expressed in different human tissues, such as renal, cardiovascular, gastrointestinal organs, lung alveolar epithelial cells, arterial and venous endothelial cells, and arterial smooth muscle cells [15,16]. ACE2 is also expressed on the surface of monocytes, macrophages, and smooth muscle cells [17].

ACE2 has been reported to be the entry receptor for SARS-CoV and SARS-CoV-2 into the human cells, including the monocytes [18,19]. Human monocytes contain large amounts of angiotensin II and small amounts of angiotensin I, while the human polymorphonuclear leukocytes contain small amounts of angiotensin I and II [20].

Regarding the lymphocytes, Wang and colleagues showed that SARS-CoV-2 has the ability to infect T lymphocytes (MT-2 cell line) through receptor-dependent S protein-mediated membrane fusion, but does not replicate [6]. Besides, lymphocytes have a much higher concentration of SARS-CoV RNA than plasma [21]. In addition, it was proved that SARS-CoV can infect and replicate within PBMCs in a self-limited manner [22,23].

A recent study by Zhang and colleagues investigated whether monocytes express ACE2, and therefore SARS-CoV-2 could infect monocytes. They used the flow cytometry staining for ACE2 on monocytic cell lines and confirmed that all monocytes are ACE2 positive. Furthermore, they reported that the expression level of ACE2 on the monocytes in COVID-19 patients is significantly lower than healthy

https://doi.org/10.1016/j.mehy.2020.109980
Received 17 May 2020; Accepted 5 June 2020

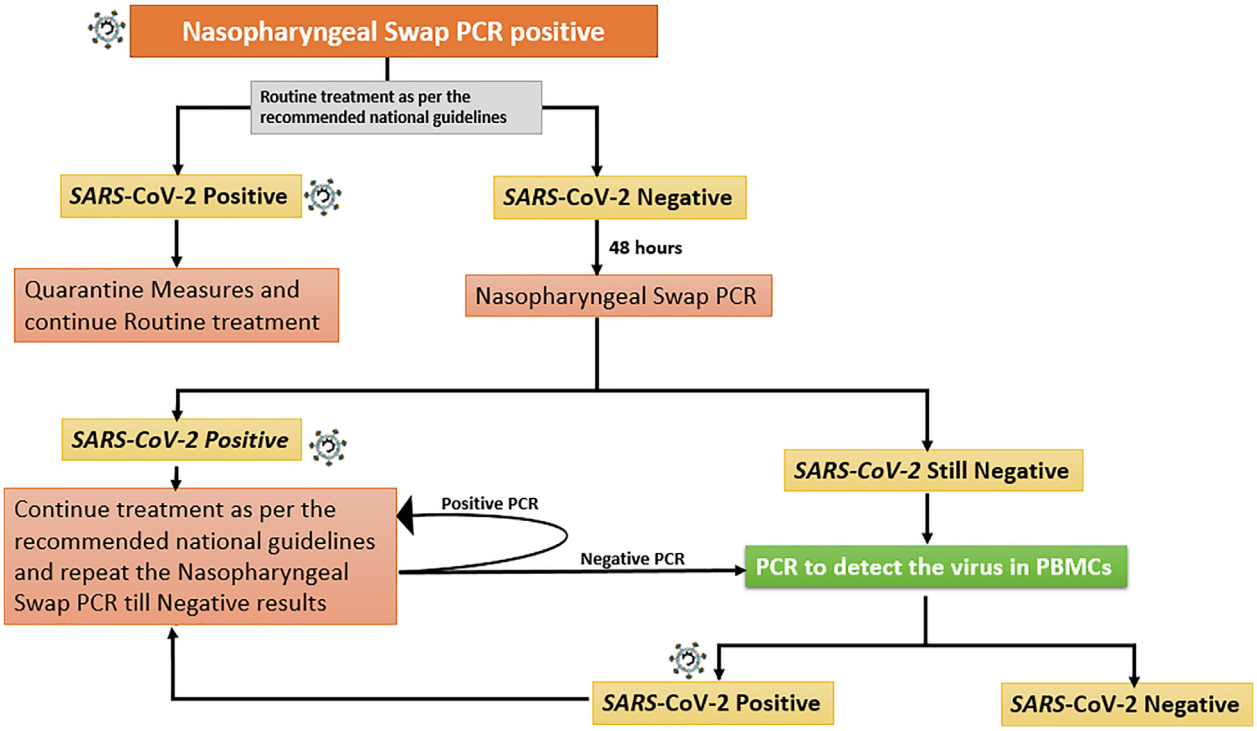

Fig. 1. How to deal with a patient with positive or negative nasopharangeal swap SARS-CoV-2 RNA PCR test.

patients [24].

Therefore, we can hypothesize that occult SARS-CoV-2 might exist in the human PBMCs (monocytes mainly) and cause viral relapse after negative PCR on samples from the respiratory tract.

This hypothesis showed important issues regarding the problems of infection control, diagnosis, and management of COVID-19. Accommodating confirmed COVID-19 patients and patients mislabeled as having COVID-19 in the same facility may be disastrous. Due to the possibility of SARS-CoV-2 reactivation that might increase the risk of transmitting the virus to other people if the patient had been discharged, we suggest the following: (A) quantitative RT-PCR on samples from the respiratory tract (both oropharyngeal and nasopharyngeal swabs should be performed). (B) RT-PCR on blood samples is recommended to exclude the Occult SARS-CoV-2 infection in the peripheral blood mononuclear cells (Fig. 1), especially the monocyte by the following proposed methodology:

1. A peripheral blood sample will be collected from the patient into sterile EDTA tubes; then, peripheral blood mononuclear cells will be isolated by gradient centrifugation.
2. Monocytes will be isolated using the commercial reagent Percoll PLUS.
3. Total RNA will be extracted from the isolated monocytes using the RNeasy Mini kit.
4. cDNA will be synthesized with a cDNA synthesis kit.
5. Sars Cov 2 viral RNA copy number could be quantified using qRT-PCR via targeting the spike and ORF1a genes using the SYBR Green kit.

(C) patients who had been discharged after negative PCR should be quarantined and do self-isolation at home for more than two weeks, monitor themselves for possible relapse, and retested after two weeks of discharge or if the symptoms appeared again.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.109980.

References

[1] COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU).
[2] Chen D, et al. Recurrence of positive SARS-CoV-2 RNA in COVID-19: A case report. Int J Infect Dis 2020;93:297–9. https://doi.org/10.1016/j.ijid.2020.03.003.
[3] Y. Li et al., Positive result of Sars-Cov-2 in faeces and sputum from discharged patient with COVID-19 in Yiwu, China, J. Med. Virol., p. jmv.25905, 2020, doi: 10.1002/jmv.25905.
[4] Zhang J, Yan K, Ye H, Lin J, Zheng J, Cai T. SARS-CoV-2 turned positive in a discharged patient with COVID-19 arouses concern regarding the present standard for discharge. Int J Infect Dis 2020. https://doi.org/10.1016/j.ijid.2020.03.007.
[5] Ye G, et al. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. J Infect May 2020;80(5):e14–7. https://doi.org/10.1016/j.jinf.2020.03.001.
[6] Wang X, et al. SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. Cell Mol Immunol 2020. https://doi.org/10.1038/s41423-020-0424-9.
[7] Y. Li et al., Positive result of Sars-Cov-2 in faeces and sputum from discharged patient with COVID-19 in Yiwu, China, J. Med. Virol., p. jmv.25905, 2020, doi: 10.1002/jmv.25905.
[8] Chu H, et al. Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response. Virology 2014;454–455:197–205. https://doi.org/10.1016/j.virol.2014.02.018.
[9] Liu J, et al. Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARS-CoV, MERS-CoV, and 2019-nCoV. J Med Virol May 2020;92(5):491–4. https://doi.org/10.1002/jmv.25709.
[10] Gu J, et al. Multiple organ infection and the pathogenesis of SARS. J Exp Med 2005;202(3):415–24. https://doi.org/10.1084/jem.20050828.
[11] Yilla M, et al. SARS-coronavirus replication in human peripheral monocytes/macrophages. Virus Res 2005;107(1):93–101. https://doi.org/10.1016/j.virusres.2004.09.004.
[12] Li L, et al. SARS-coronavirus replicates in mononuclear cells of peripheral blood (PBMCs) from SARS patients. J Clin Virol 2003;28(3):239–44. https://doi.org/10.1016/S1386-6532(03)00195-1.
[13] Zhou P, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579. https://doi.org/10.1038/s41586-020-2012-7.

[14] N. Dong et al., Genomic and protein structure modelling analysis depicts the origin and infectivity of 2019-nCoV, a new coronavirus which caused a pneumonia outbreak in Wuhan, China, bioRxiv, p. 2020.01.20.913368, 2020, doi: 10.1101/2020.01.20.913368.
[15] Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett 2002;532(1–2):107–10. https://doi.org/10.1016/S0014-5793(02)03640-2.
[16] Hamming I, Timens W, Bulthuis M, Lely A, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004;203(2):631–7. https://doi.org/10.1002/path.1570.
[17] B. Trojanowicz et al., Monocytic angiotensin-converting enzyme 2 relates to atherosclerosis in patients with chronic kidney disease, Nephrol. Dial. Transplant., p. gfw206, May 2016, doi: 10.1093/ndt/gfw206.
[18] Kuba K, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nat Med 2005;11(8):875–9. https://doi.org/10.1038/nm1267.
[19] Hoffmann M, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and Is blocked by a clinically proven protease inhibitor. Cell 2020;181(2):271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052.
[20] Kitazono T, Padgett RC, Armstrong ML, Tompkins PK, Heistad DD. Evidence That Angiotensin II Is Present in Human Monocytes. Circulation 1995;91(4):1129–34. https://doi.org/10.1161/01.CIR.91.4.1129.
[21] Wang H, et al. Detection and monitoring of SARS coronavirus in the plasma and peripheral blood lymphocytes of patients with severe acute respiratory syndrome. Clin Chem 2004;50(7):1237–40. https://doi.org/10.1373/clinchem.2004.031237.
[22] Yilla M, et al. SARS-coronavirus replication in human peripheral monocytes/macrophages. Virus Res 2005;107(1):93–101. https://doi.org/10.1016/j.virusres.2004.09.004.
[23] Li L, et al. SARS-coronavirus replicates in mononuclear cells of peripheral blood (PBMCs) from SARS patients. J Clin Virol 2003;28(3):239–44. https://doi.org/10.1016/S1386-6532(03)00195-1.
[24] D. Zhang et al., COVID-19 infection induces readily detectable morphological and inflammation-related phenotypic changes in peripheral blood monocytes, the severity of which correlate with patient outcome, medRxiv, p. 2020.03.24.20042655, 2020, doi: 10.1101/2020.03.24.20042655.

Mostafa H. Elberry[a], Hussien Ahmed[b,c,*]
[a] *Virology and Immunology Unit, Cancer Biology Department, National Cancer Institute, Cairo University, Egypt*
[b] *Clinical Research Management Program, Edson College, Arizona State University, USA*
[c] *Faculty of Medicine, Zagazig University, Sharkia, Egypt*
E-mail addresses: hoseen011232@medicine.zu.edu.eg, habdelg1@asu.edu (H. Ahmed).

[*] Corresponding author at: Clinical Research Management Program, Edson College, Arizona State University, USA. Tel.: +201006037334.