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Negative viral nucleic acid test of induced sputum: an additional criteria for COVID-19 patient's discharge

S.-L. ZHENG[1], W.-L. SUN[2], L.-N. SUN[3], X.-H. ZENG[4], Q.-X. WANG[5]

[1]Department of General Medicine, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, P.R. China
[2]Department of Laboratory, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, P.R. China
[3]Department of Anesthesiology, Weifang Medical University, Weifang, Shandong, P.R. China
[4]Department of Lung Centre, Weifang Yangguang Ronghe Hospital, Shandong, P.R. China
[5]Department of Infection Management, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, P.R. China

Abstract. – OBJECTIVE: Concerns have been raised that patients with Coronavirus Disease 2019 (COVID-19) are still infectious with a re-positive nucleic acid test of the pharyngeal swab after hospital discharge. The aim of this study was to investigate the clinical relevance of induced sputum as an additional indicator for the current clinical discharge criteria of COVID-19 patients to prevent virus recurrence.

PATIENTS AND METHODS: Twenty-one COVID-19 patients who met the national clinical discharge criteria were discharged from the hospital and tested daily for the presence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) nucleic acid in their pharyngeal swabs and every other day for the presence of SARS-CoV-2 in their induced sputum. Once the patient's induced sputum was negative after two consecutive tests, testing was discontinued.

RESULTS: Among 21 discharged patients from COVID-19, the first pharyngeal swab and induced sputum tests for viral nucleic acid were positive in 3 (14.3%) and 8 (38.1%) patients respectively. Induced sputum was significantly more positive than pharyngeal swab ($p < 0.05$). In our cohort, all pharyngeal swabs became negative at day 7, and all induced sputa turned negative at day 11 after discharge. Interestingly, patients with negative pharyngeal swabs experienced viral relapse, whereas patients with negative induced sputum did not revert to positivity.

CONCLUSIONS: The detection rate of positive viral nucleic acid in induced sputum was high. Patients with negative induced sputum nucleic acid tests did not have a relapse of SARS-COV-2, indicating that viral nucleic acid testing of induced sputum should be used as an additional criterion for patients with national clinical discharge criteria COVID-19.

Key Words:
COVID-19, Nucleic acid, Induced sputum, Pharyngeal swab, Discharge criteria.

Introduction

On 7 January 2020, a novel coronavirus was identified by the Chinese Center for Disease Control and Prevention in the pharyngeal swab samples from patients. The virus has been named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the World Health Organization and its associated disease is termed Coronavirus Disease 2019 (COVID-19)[1]. The diagnosis of COVID-19 requires sputum, pharyngeal swab, lower respiratory tract secretion, and other samples to test positive for SARS-CoV-2 nucleic acid by Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)[2]. Pharyngeal swabs are often used as a substitute since sputum is not always present in all patients, which leads to false negative results in some patients and requires repeated testing for confirmation. When patients are clinically cured, no sputum is present, and it is common practice to use the pharyngeal swab to check for viral nucleic acid prior to discharge. As more and more patients are discharged, up to 14% of these patients test positive and they are at risk of continuing to infect others. Induced sputum for research has been widely used in chronic respiratory diseases[3,4]. Therefore, we investigated the value of induced sputum to test the presence of virus in COVID-19 patients who met the current

Corresponding Author: Qingxiu Wang MS; e-mail: fywqx@wfmc.edu.cn

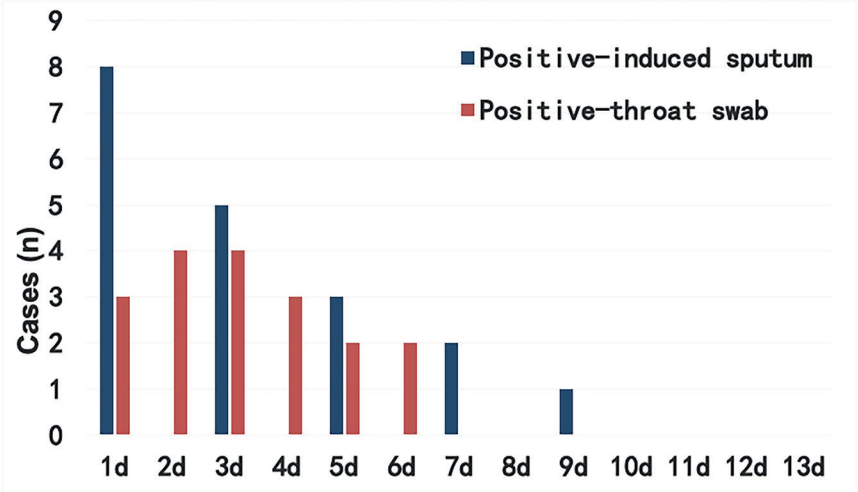

Figure 1. Changes of pharyngeal test and sputum nucleic acid after meeting clinical discharge criteria.

discharge criteria to properly evaluate clinical status of patients and prevent further infections.

Patients and Methods

Objects of Study

A total of 21 patients with COVID-19 admitted to a hospital in Weifang, China, from 23 January 2020 to 9 March 2020 were selected. The patients all met the diagnostic standards of the "COVID-19 Diagnosis and Treatment Program (Trial Fifth Edition)" issued by the National Health and Family Planning Commission of China. The Diagnosis of COVID-19 was based on epidemiological data, clinical symptoms, laboratory and imaging findings, and RT-PCR results of pharyngeal swabs or sputum SARS-COV-2. We included patients who (1) were between 20 and 60 years of age, (2) met the current clinical discharge criteria, i.e., normal temperature for more than 3 days, significant improvement in respiratory symptoms, and significant inflammatory uptake on lung imaging and (3) had no chronic respiratory, cerebrovascular, hepatic, or renal disease. Patients were excluded if they had (1) a (family) history of bronchial asthma, allergic rhinitis, or other allergies, (2) a history of mental illness, (3) inability to cooperate with the study, or (4) dyspnoea. Informed consent was obtained from all participants in this study.

Research Methods

SARS-CoV-2 nucleic acid testing of pharyngeal swabs and induced sputum was performed in all COVID-19 patients on the first day of meeting clinical discharge criteria. Pharyngeal swabs were performed daily, and sputum was induced every other day. Sputum and pharyngeal swab tests were discontinued in patients with two consecutive negative sputum test results.

Pharyngeal Swab Collection

To avoid vomiting due to irritation of the laryngeal wall, specimens were collected two hours after ingestion. Swabs were preserved in sterile saline and were avoided by placing in a viral pre-

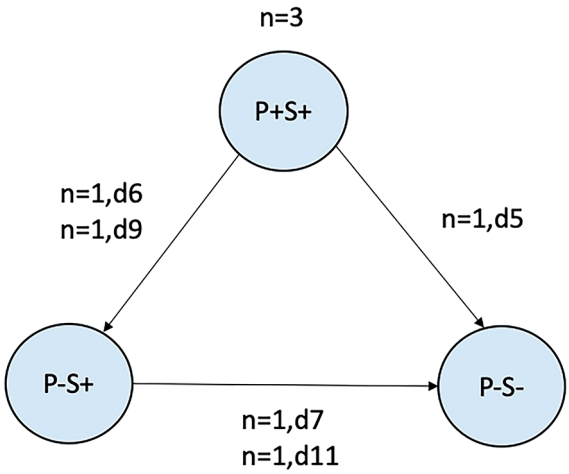

Figure 2. Results of three patients with positive pharyngeal swabs and induced sputum. P+S+:the nucleic acids of the pharyngeal swab and induced sputum were positive;P-S+:the nucleic acids of pharyngeal swab were negative, but the induced sputum was positive;P-S-:The nucleic acids of the pharyngeal swab and induced sputum were negative.

serving solution to avoid antibiotic-induced allergy. Patients were asked to rinse their mouths with normal saline, tilt their heads slightly back, and open their mouths wide, accompanied by an "ah" sound. We swabbed across the base of the tongue and gently wiped the pharyngeal tonsils on both sides, as well as the posterior pharyngeal wall at least three times. The swab head was immersed in a tube containing 2 to 3 ml of virus preservation solution (isotonic saline, tissue culture media, or phosphate buffer saline), the tail was discarded, and the tube was immediately covered tightly.

Induced Sputum Collection

Patients inhaled 400 μg of salbutamol before sputum induction and rinsed their mouths with clear water and blew their noses after 10 minutes. Ultrasonic nebulised 3% hypertonic saline was then inhaled for 15 min and the patients vigorously coughed up sputum into a collection cup. If the patient produced insufficient sputum, the inhaled solution was changed to 4% hypertonic saline for 7 min. If necessary, the concentration was increased to 5%, and the sputum induction was discontinued after 7 minutes when there is insufficient sputum. Sputum was immediately processed after collection[5].

SARS-CoV-2 Nucleic Acid Tests

Pharyngeal swabs and induced sputum were preserved with a viral preservation solution and sent to the Laboratory of Weifang Center for Disease Control and Prevention. Ribonucleic Acid (RNA) was extracted from the patient samples and RT-PCR was performed to detect the presence of SARS-CoV-2. If the signal was positive, the detection reagents were refilled, and the RT-PCR was repeated to verify the test results.

Statistical Analysis

The data were compared using Fisher's exact test, and the distribution of the data was expressed as a range. All data were analysed using SPSS version 26.0. $p < 0.05$ was considered statistically significant.

Results

Changes of SARS-CoV-2 Nucleic Acid Tests After Meeting Clinical Discharge Criteria

We included 11 males and 10 females COVID-19 patients who met national clinical discharge criteria with an average age of 40.5 (24-56) years. We observed that 3 (14.3%) patients were positive for nucleic acid in their first pharyngeal swab and 8 (38.1%) patients tested positive in induced sputum. The detection rate of SARS-CoV-2 in induced sputum was significantly higher than that of the pharyngeal swab ($p < 0.05$). On the third day, 4 (19.0%) pharyngeal swabs and 5 induced sputum (23.8%) were positive, which decreased to 2 (9.5%) and 3 (14.3%), on day 5, respectively. On the seventh day, induced sputum was positive in two (9.5%) patients, while all pharyngeal swabs were negative. All pharyngeal swabs and induced sputum were negative by day 11 (Figure 1). Virus clearance in induced sputum took longer than that in the pharyngeal swab (11 *vs.* 7 days).

Outcomes of Patients With Positive Pharyngeal Swabs and Induced Sputum

Three patients with COVID-19 tested positive for SARS-CoV-2 in the pharyngeal swab and induced sputum on days 1 and 3 after meeting clinical discharge criteria, with one patient had negative tests in pharyngeal swab and induced sputum on the 4th and 5th day respectively. Another had negative tests on the 7th and 9th day respectively, and the third patient on the 7th and 11th day respectively (Figure 2).

Viral Relapse

The pharyngeal swabs SARS-COV-2 were positive in 3 patients on the first day and increased to 4 patients on the 2nd and the 3rd day. One patient who tested positive for SARS-CoV-2 in induced sputum showed a negative pharyngeal swab for three consecutive days. In contrast, we did not observe the presence of virus in both pharyngeal swabs and induced sputum of the patients with negative induced sputum.

Discussion

COVID-19 is characterized by fever, fatigue, and dry cough. In most patients, ground-glass opacity and patchy opacity appear on chest Computed Tomography (CT) at the beginning of the illness. Respiratory droplets and direct contact are the main routes of transmission, suggesting that further transmission of SARS-COV-2 after discharge depends mainly on the presence of SARS-COV-2 in the respiratory tract. Therefore, it is desirable that there is no residual virus in the

respiratory tract when the patient is discharged from hospital. With active treatment, fever and cough improved quickly in most patients and the lesions seen by chest CT were absorbed. Subsequently, the amount of virus in the alveoli was significantly reduced and less virus remained in the larynx, but it may not be completely eradicated. In this study, the results showed that the detection rate of SARS-CoV-2 in induced sputum after meeting discharge criteria was as high as 38.1%, and much higher than that in the pharyngeal swab. This indicates that patients are still carrying viruses and that nucleic acid tests of the pharyngeal swab alone cannot reliably detect the presence of virus in the body. In addition, our data stated that even two consecutively negative pharyngeal swabs may not discharge the patient from the hospital. Therefore, we suggest that there is a need to improve testing for residual virus and to adopt more stringent discharge criteria.

Clinical studies[6-8] of induced sputum are mainly used for bronchial asthma, chronic obstructive pulmonary disease, and pulmonary hypertension to study local inflammatory cells and cytokines. In recent years, some scholars[7,9] have conducted research on paediatric tuberculosis and mycoplasma pneumoniae by induced sputum. Therefore, we used induced sputum to determine the presence of SARS-CoV-2 and to investigate its use in determining whether COVID-19 patients who completed treatment and had no clinical symptoms were discharged from the hospital. The results showed that patients who were eligible for discharge had a high rate of viral detection in the induced sputum. In addition, the total clearance time of the virus was very long, up to 11 days. This indicates that the long residence time of the virus in the alveoli necessitates the use of induced sputum for viral testing in cases where the current discharge criteria are met.

SARS-CoV-2 can enter host cells through the cell receptor Angiotensin-Converting Enzyme 2 (ACE2). It has been shown that approximately 0.64% of human lung cells express ACE2, of which 83% cells are Alveolar Type 2 cells (AT2)[10]. Additionally, about 1.4% of all AT2 cells express ACE2. This suggests that AT2 are likely the target cells of SARS-COV-2. It is possible that the virus does not primarily attack the nasopharynx, but rather targets the terminal airways and the alveoli. Therefore, viral nucleic acid testing of induced sputum could more accurately detect residual virus, and discharged patients still carry the virus.

The optimal environment for SARS-CoV-2 is the lungs,where the number and concentration of viral particles are greatest. Pan et al[11] showed that the viral load in pharyngeal swabs and sputum samples from patients with SARS-COV-2 peaked around 5-6 days after onset and that the viral load in sputum samples from patients who died 8 days after onset remained very high. Generally, sputum samples have higher viral loads than pharyngeal swab samples. Therefore, viral nucleic acid testing in induced sputum is useful for early diagnosis and determination of patient's discharge.

The physician raised the issue of viral recurrence in the discharged patient. According to the "COVID-19 Diagnosis and Treatment Program (Trial Fifth Edition)". The discharge criteria call for two consecutive negative viral nucleic acid tests on alternate days. The most common and simplest way of sampling is to take a pharyngeal swab. However, the amount of pharyngeal virus is relatively small and variable. Our findings further demonstrate that the detection of viral nucleic acid in the pharyngeal swab was unreliable and might result in the virus escaping detection and reappearing in the patient. Residual viruses in the alveoli are shed intermittently and may contribute to further disease transmission. No SARS-COV-2 in pharyngeal swabs and induced sputum were found after nucleic acid test in induced sputum turned negative, indicating that the virus is no longer present in the body and there is no possibility of recurrence.

This study is the first to suggest the need to detect the presence of SARS-CoV-2 in patient-induced sputum if clinical discharge criteria are met, but the small sample size in this study may affect the accuracy of the results. Due to the rapid decrease in the number of new cases in our country, we were unable to recruit more patients for further study.

Conclusions

In summary, induced sputum is more reliable than pharyngeal swab for the detection of residual SARS-CoV-2 in patients who met the current discharge criteria,and COVID-19 may not recur in patients with a negative induced sputum test; therefore, we strongly recommend further examination of sputum for the presence of viral nucleic acid prior to discharge.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Availability of Data and Materials
The analysed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' Contributions
Dr. Shiliang Zheng and Dr. Qingxiu Wang conceived the idea and designed the study. All authors performed all of the experiments and reviewed the manuscript and read and approved the final manuscript.

Ethical Statement
This study was approved by the institutional Ethics Board of Affiliated Hospital of Weifang Medical College. Written informed consent was obtained from all patients for publication of this study.

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