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Differences of SARS-CoV-2 Shedding Duration in Sputum and Nasopharyngeal Swab Specimens among Adult Inpatients with COVID-19

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Summary conflict of interest statements

The authors declare no conflicts of interest.

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Abbreviation list

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

COVID-19: coronavirus disease 2019

CLD: chronic lung disease

M, IQR: Median (Inter Quartile Range)

Max, IQR: Max (Inter Quartile Range)

NPPV: noninvasive positive-pressure ventilation

HFNC: high-flow nasal cannula (HFNC) oxygen therapy

NPS: nasopharyngeal swab

SP: sputum specimen

HR (95%CI): hazard ratio (95% confidence interval).

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Abstract

Background

The viral shedding duration of SARS-CoV-2 has not been fully defined. Consecutive detection of SARS-CoV-2 RNA from respiratory tract specimens is essential for determining duration of virus shedding and providing evidence to optimize the clinical management of COVID-19.

Research Question

What are the shedding durations of SARS-CoV-2 RNA in upper and lower respiratory tract specimens respectively? What are their associated risk factors?

Study Design and Methods

A total of 68 patients with COVID-19 admitted to Wuhan Taikang Tongji Hospital and Huoshenshan Hospital from February 10, 2020 to March 20, 2020 were recruited. Consecutive SARS-CoV-2 RNA detection from paired specimens of nasopharyngeal swab (NPS) and sputum were carried out. The clinical characteristics of patients were recorded for further analysis.

Results

SARS-CoV-2 RNA was detected from NPS in 48 (70.6%) patients, and from sputum specimens in 30 (44.1%) patients. The median duration of viral shedding from sputum specimens (34 days, IQR 24-40 days) was significantly longer than from NPS (19 days, IQR 14-25 days; P<0.001). Elderly age was an independent factor associated with prolonged virus shedding time of SARS-CoV-2 (HR 1.71, 1.01-2.93). It was noteworthy that in 9 patients the viral RNA was detected in sputum after NPS turned negative. Chronic lung disease and steroids were associated with virus detection in sputum, and diabetes mellitus was associated with virus detection in both NPS and sputum.

Interpretation

These findings may impact a test based clearance discharge criteria given patients

with COVID-19 may shed virus longer in their lower respiratory tracts, with potential implication for prolonged transmission risk. In addition, more attention should be given to elderly patients who might have prolonged viral shedding duration.

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Introduction

Since December 2019, an outbreak of pneumonia started in Wuhan, China and gradually spread around world. The pathogen has been identified as a novel enveloped RNA beta-coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has a phylogenetic similarity to SARS-CoV, and has now been designated coronavirus disease 2019 (COVID-19) by the WHO[1]. The clinical manifestations of COVID-19 vary diversely from asymptomatic infection to mild upper respiratory tract infection and even acute respiratory distress syndrome [1-4]. Even though COVID-19 in China has been temporarily contained through proactive public health interventions including early detection and quarantine, it has rapidly spread to cause a pandemic around the world. Up to 9 June, 2020, the global number of laboratory-confirmed cases had been more than 7.0 million, highlighting that COVID-19 poses a substantial threat to the international health.

Characterizing the infectivity of SARS-CoV-2 is important for disease control and prevention. The duration of viral shedding, which has been recognized as a proxy measure of the infectious period for other respiratory viruses[5,6], is a current consideration with SARS-CoV-2. Hence, it is of urgent need to elucidate the viral shedding duration among patients with COVID-19 to optimize public health management policy.

COVID-19 is an infectious disease that transmitted mainly through respiratory tract. Therefore, consecutive detection of SARS-CoV-2 RNA from respiratory tract specimens using real-time reverse transcription–polymerase chain reaction (rRT-PCR) with approximate sensitivity of 70% and specificity of 95%[7], is crucial for defining virus shedding duration and may impact clinical decisions on a patient's discharge from the hospital and whether isolation and surveillance is required depending on infection control recommendations in a particular country. Nasopharyngeal swab (NPS) has been widely used for diagnosis and dynamic observation of COVID-19 patients on account of its ease of acquisition. Two consecutive negative detections of

SARS-CoV-2 RNA in NPS specimens have been recognized as criterion for discharge from hospital or release from quarantine[8-11]. Nevertheless, one limitation of NPS is the possibility of false negative results, raising the concern that persistence of viral shedding might be present in lower respiratory tract[12].

Sputum has been reported to be more sensitive than NPS in SARS-CoV-2 RNA detection since SARS-CoV-2 mainly bind with ACE2 receptor of lower respiratory tract[13-15]. However, the use of sputum specimen in clinical practice is quite limited because only a proportion of patients with COVID-19 produce sputum spontaneously. Induced sputum is a convenient option to get lower respiratory tract samples and Han et.al. proposed in a case report that SARS-CoV-2 RNA could be detected more readily in sputum specimen than in upper respiratory tract specimen[10]. The risk of medical staff exposure to COVID-19 is lower with sputum induction than with bronchoalveolar lavage methods, although bronchoalveolar lavage fluid exhibited the higher positive rate compared with the nasal and pharyngeal swabs samples[13,16]. However, the SARS-CoV-2 detection yield and distinct virus shedding duration between sputum and NPS remained unclear.

We conducted a prospective cohort study of 68 hospitalized patients with laboratory-confirmed COVID-19, by consecutively monitoring SARS-CoV-2 RNA detection from paired specimens of NPS and sputum aiming to identify viral shedding duration in upper and lower respiratory tract specimens respectively and to investigate possible factors associated with prolonged viral presence.

Methods

Data Collection

A cohort of 68 patients hospitalized (including intensive care unit [ICU] and non-ICU) in Wuhan Taikang Tongji Hospital and Huoshenshan Hospital were prospectively recruited from February 10 to March 20, 2020. They were all laboratory-confirmed COVID-19 patients according to the 7th version of 'Pneumonia diagnosis and

treatment for COVID-19 infection' with specific clinical symptoms and radiological abnormalities and two sequential positive SARS-CoV-2 RNA tests or specific serum IgM and IgG antibodies of SARS-CoV-2[11]. Demographic information, clinical indices, underlying diseases, treatment and outcome data were extracted from electronic medical records using a standardized data collection form. Study was approved by the Ethics Commission of Shanghai East Hospital, China and informed consent was obtained from participants.

CURB-65 score was determined on the day of admission according to clinical criteria (confusion; urea >7 mmol/l; respiratory rate≥30/min; either diastolic blood pressure ⩽60 mm Hg or systolic blood pressure <90 mm Hg; age≥65 years) defined by the British Thoracic Society (BTS)[17].

Real-Time Reverse Transcription Polymerase Chain Reaction Assay for SARS-CoV-2 in Respiratory Samples

Both nasopharyngeal swab and sputum specimens were collected every 1-2 days after admission for detection of the SARS-CoV-2 RNA using real-time reverse transcription–polymerase chain reaction (rRT-PCR) until two sequential negative results were obtained. Briefly, induced sputum was obtained after inhalation of 10 mL of 3% hypertonic saline through a mask with oxygen at a flow rate of 6L/min for 20 mins, if patients did not have sputum; tracheal aspirates sputum was collected through aspiration with a sterile catheter if patients were intubated. The SARS-CoV-2 rRT-PCR assay was developed by Master Biotechnology (China) with primers and probes targeting the N and Orf1b genes of SARS-COV-2 and applied in the laboratory of Taikang Tongji Hospital and Huoshenshan Hospital. Respiratory specimens with cycle threshold (Ct) values of <37 were considered positive for SARS-CoV-2, and those with Ct values of ≥37.0 underwent repeat testing. Upon repeated testing, respiratory specimens with Ct values of <40 were considered positive for SARS-CoV-2, and those with Ct values of ≥40 or with undetectable results were

considered negative. We defined the interval between symptom onset and the date of the first SARS-CoV-2 RNA negative result for respiratory samples including both nasopharyngeal swab and sputum specimens as the shedding duration.

Antibody Detection

Serum samples were detected for IgM/IgG antibodies against SARS-CoV-2 using the colloidal gold immunochromatography antibody detection kit (Innovita Biological Technology Co., Ltd., China). Briefly, the serum samples were firstly incubated at 56°C for 30 minutes to heat-inactivate viruses, and then added into the sample well of the testing plate. After addition of reaction buffer and incubation for 10-15 minutes at room temperature, testing result could be achieved and interpreted according to the instructions.

Statistical Analysis

The measurement data of normal distribution were presented as mean ± standard deviation (SD) and compared by t test or variance analysis. While the measurement data of non-normal distribution were expressed by median (M) and upper and lower quartile spacing (IQN) and compared by Wilcoxon or Kruskal-Wallis Rank Sum Test. The categoric variables were presented as numbers and percentages and were compared by chi-square or Fisher exact test. The analyses of risk factors associated with detecting SARS-CoV-2 RNA in NPS or sputum or both were conducted using one-way ANOVA or the chi-square test. To identify risk factors associated with the duration of SARS-CoV-2 RNA shedding, we used a Cox proportional hazards model that adjusted for baseline covariates. Outcome was defined as time interval from symptom onset to SARS-CoV-2 RNA negativity in both NPS and sputum specimens. For this analysis, we censored patients if they never cleared SARS-CoV-2 RNA or, if they were discharged alive or dead before they had cleared SARS-CoV-2 RNA. Potential variables for analysis of prolonged duration of SARS-CoV-2 RNA shedding were as follows: sex, age, comorbidities, lymphocyte counts, and treatment with steroids. A hazard ratio (HR) of >1 indicated prolonged viral RNA shedding. In

multivariable-adjusted Cox regression models, HR was further adjusted for covariates including age and sex. We performed Kaplan-Meier survival analysis to estimate the cumulative SARS-CoV-2 RNA-negativity rate among respiratory specimens and the stratified log-rank test to compare the difference of virus clearance between patients with age <65 years and ≥65 years. Statistical analyses were performed using STATA 15 and two-sided p value < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

Overall, a total of 68 patients with COVID-19 who underwent consecutive SARS-CoV-2 RNA detection from NPS and sputum specimens were included: 36 (52.9%) were men and 32 were (47.1%) women. The demographic and clinical characteristics of the patients are shown in **Table 1**. The median age of the patients was 67-year-old (interquartile range [IQR], 57 to 72). Fever was most commonly presented in 73.8% of the patients on admission (medium Tmax, °C [IQR], 38.5 [38.0-39.0]) followed by cough (45.6%). Dyspnea (33.8%) and fatigue (32.4%) were also frequently observed and diarrhea (10.3%) was less common. The median duration of fever, cough and diarrhea was 11.0 days (IQR 8.0-13.0), 20.0 days (11.0-26.0), and 4.0 days (2.0-5.0), respectively. Comorbidities were present in 39 (57.4%) patients, with chronic lung disease (17.6%) and diabetes mellitus (DM) (17.6%) being the most common underlying diseases, followed by cardiac disease (13.2%). Upon admission, 43 patients (63.2%) were diagnosed with COVID-19 based on positive NPS, while 25 patients (36.8%) based on positive serum IgM/IgG antibodies against SARS-CoV-2. During the hospitalization, the overall positive rate of serological test for IgM and IgG against SARS-CoV-2 were 76.5% (n=52) and 83.8% (n=57), respectively. As for treatment, 30 patients (44.2%) required mechanical ventilation. Among them, 5 were intubated and the rest 25 received noninvasive positive-pressure ventilation (NPPV). High-flow nasal cannula (HFNC) and

conventional oxygen support were used in 21 (30.9 %) and 18 (26.5%) patients respectively. Upon admission, the severity of patients was evaluated by CURB-65: 30 patients (44.1%) had score 1, 36 patients (52.9%) had score 2, and 2 patients reached score 3. Meanwhile, the overall mortality of all patients was 4.4%.

Distinct yields of SARS-CoV-2 RNA detection in nasopharyngeal swab and sputum specimens.

As shown in **Figure 1**, of all 68 patients with confirmed COVID-19, 72.1% (n=49) were identified with initial or follow-up positive NPS samples; 20.6% (n=14) patients with initial and follow-up negative NPS samples paired with follow-up positive sputum specimens; 7.4% (n=5) were diagnosed by serum IgM and IgG antibody assay while both NPS and sputum specimen remained negative during the hospitalization. Meanwhile, 16 patients were detected with SARS-CoV-2 RNA both in NPS and sputum specimens, among whom further analysis was carried out to characterize the time interval between the last time of NPS positive and the first time of sputum positive. As shown in **Figure 2**, 9 patients had positive testing for SARS-CoV-2 RNA in the sputum after NPS turned negative; 6 patients had positive sputum before NPS turned negative; 1 patient had positive sputum on the day when NPS turned negative. The time interval ranged from 6 days before to 16 days after the NPS turned negative.

Factors associated with viral RNA detection yields of nasopharyngeal swab and sputum specimens

We then explored the possible factors associated with the yields of NPS and sputum in detecting SARS-CoV-2 RNA respectively. The results showed chronic lung disease (CLD) and systemic steroids use were associated with SARS-CoV-2 RNA detection from sputum and diabetes mellitus was associated with viral RNA detection from NPS or sputum specimens. We futher performed a sensitivity analysis in patients without CLD in order to take into consideration of the possible effect of CLD on the association of systemic steroids with detection of SARS-CoV-2 RNA. There still existed a statistical difference in positive sputum rate between the steoids use group

and non-steroids use group (steroids use: 11/17; non-steroids use: 9/39; *P*=0.003), which was consistent with the previous results. Besides, chronic lung disease was associated with both NPS and sputum positive for SARS-CoV-2 RNA detection. (**Table 2**).

SARS-CoV-2 shedding duration and risk factors of prolonged viral presence

The median duration of viral shedding from NPS and from sputum specimens was 19 days (IQR 14-25 days) and 34 days (IQR 24-40 days), respectively (*P*<0.001), and by pooling together, the median duration of SARS-CoV-2 RNA shedding from either NPS or sputum specimens was 21 days (IQR, 16-31 days). Of 63 patients with rRT-PCR confirmed SARS-CoV-2 infection, only 4 patients (6.3%) had undetectable virus RNA within 8 days, 18 patients (28.6%) tested negative within 14 days, and 41 patients (65.1%) tested negative within 28 days after illness onset (**Figure 3**).

We further explored SARS-CoV-2 shedding duration and potential risk factors. In a multivariable model, elderly age (≥65 years) was identified as an independent factor associated with the viral shedding time in hospitalized patients (**Table 3**). SARS-CoV-2 RNA clearance was significantly delayed in patients aged ≥65 years compared with those aged <65 years after onset of illness (HR, 1.71 [95%CI, 1.12-2.93]; p<0.01; **Figure 3B**).

Recurrent positive detections of viral RNA from nasopharyngeal swab specimens in 2 cases

We found 2 patients who had recurrent positive detection of SARS-CoV-2 RNA from NPS specimens (**Figure 4**) after serially negative tests. Case 1 is a 68-year-old woman with a history of diabetes mellitus for 20 years. After 9 consecutive negative NPS tests, SARS-CoV-2 RNA was detected again in NPS at day 29 after illness onset while the SP were tested positive serially for 6 times from day 16 to day 29. Case 2 is a 55-year-old man with hypertension and cardiac disease. From day 9 to day 25 after illness onset, the patient had 11 consecutive negative NPS test and 7 consecutive positive SP tests, and then he had recurrent positive detection of virus RNA in NPS at

day 25. These two cases continued to receive isolation and surveillance in hospital until NPS test turned negative. When these two cases converted to NPS positive, they remained clinically stable without recurrence of symptoms and substantial changes in laboratory examinations.

Discussion

In the present study, we have found the median duration of SARS-CoV-2 shedding from either NPS or sputum specimens was 21 days and the median duration of viral shedding from sputum was significantly longer than from NPS. Age was identified as an independent risk factor of prolonged viral shedding time. Meanwhile, a combination of NPS and sputum specimens for detecting viral RNA could improve the diagnostic sensitivity. Chronic lung disease and steroids use are associated with the detection of virus RNA from NPS, and DM is associated with the detection of virus RNA from both NPS and sputum specimens. In addition, it was noteworthy that in 9 of 16 hospitalized patients where SARS-CoV-2 RNA was detected both in NPS and sputum specimens, virus RNA could be detected in sputum specimen after the NPS specimen turned negative.

Since coronavirus RNA detection is more sensitive than virus isolation by culture, most studies have used viral RNA tests as a potential marker to assess the potential transmission risk and to inform decisions regarding patients' isolation. For SARS-and MERS-COV, the duration of viral RNA detection in respiratory specimens was about 3-4 weeks after illness onset[18-20]. Recently, Cao et.al reported that SARS-COV-2 RNA persisted for a median of 20 days in survivors and that is consistent with the findings from our present study[21]. Additionally, we have found that age was an independent factor associated with prolonged SARS-COV-2 RNA shedding. Previously, it has been suggested that increased age was associated with mortality in SARS and MERS and may lead to death in COVID-19 patients[22,23]. One possible reason for this is the age-dependent dysfunction of lymphocyte and the overproduction of type 2

cytokines[24]. This could further result on slower viral clearance and prolonged shedding time[21].

According to the Chinese guideline for COVD-19[11], the criteria for discharge were absence of fever for at least 3 days, substantial improvement in both lungs in chest CT, clinical remission of respiratory symptoms, and two throat-swab samples negative for SARS-CoV-2 RNA obtained at least 24 h apart[25]. However, there is growing evidence showing that a certain amount of discharged patients have tested positive during follow-up[9]. In the present study, we describe two patients in detail who had a recurrence of detection of SARS CoV-2 virus RNA from NPS after previously converting to negative testing. The possible reasons for the relapse are multifold. First, COVID-19 is a novel coronaviral infectious disease, so the clinical features and course has not been fully understood. The pathogen of the disease is an RNA beta-coronavirus named SARS-COV-2 and mutation may occur during transmission which could lead to ineffective antibodies produced by the recovered patients. If the discharged patient is re-infected by the mutated virus, the nucleic acid test may be positive again. Negative results may also occur if a patient still has very low levels of viral shedding, but their viral load is below the lower threshold of assay detection.

In the present study, we found that viral RNA could be detected in sputum specimen after the NPS specimen turned negative, which was consistent with previous report describing 22 patients with COVID-19 who had positive rRT-PCR results for SARS–CoV-2 in the sputum or feces after negative conversion of pharyngeal swabs[26]. We also found that the duration of viral shedding in sputum specimens was longer than that in NPS. These findings may impact a test based clearance discharge criteria given patients with COVID-19 may shed virus longer in their respiratory tracts, with potential implication for prolonged transmission risk. Additionally, although not routinely recommended for initial diagnostic testing for SARS-CoV-2[27], induced sputum should be considered as an alternative for testing SARS–CoV-2 RNA when individuals are highly suspected of COVID-19 but nasopharyngeal or oropharyngeal

consecutively negative.

There are still several limitations of the present study. First, the interpretation of our findings might be limited by its small sample size. Second, NPS specimens were obtained by different clinicians and this could have an impact on its detecting sensitivity. Third, lymphocyte subtypes and serum IgM/IgG antibody titers test were not performed. It was therefore not possible to determine the relationship between antiviral response and prolonged SARS-CoV-2 shedding. At last, another limitation is that we detected virus by rRT-PCR instead of by virus isolation by culture. It is becoming more widely accepted that prolonged viral shedding may not indicate infectivity, because rRT-PCR does not distinguish between infectious virus and non-infectious nucleic acid.[28] In spite of this, relative cautious management strategies are still warranted for optimal transmission prevention, especially among vulnerable populations and healthcare staffs. Further studies are needed to determine whether individuals with prolonged positive NPS or sputum are infectious or not.

Interpretation

In patients hospitalized with COVID-19, the median duration of viral shedding from sputum specimens was significantly longer than from NPS. Elderly age was independently associated with prolonged SARS-CoV-2 shedding in the respiratory specimens. Viral RNA could be detected in sputum specimen after the nasopharyngeal swabs became negative in some patients. These findings may impact a test based clearance discharge criteria given patients with COVID-19 may shed virus longer in lower respiratory tracts, with potential implication for prolonged transmission risk. In addition, more attention should be given to elderly patients who might have prolonged viral shedding period. Besides, more studies are needed to determine whether prolonged viral shedding indicates infectivity of patients.

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Acknowledgments

Xiaodong Wu is responsible for the content of the manuscript, including the data and analysis. Ting Shi, Qiang Li and Xiaodong Wu conceived and designed the study. Xin Zhang, Jiaxing Sun, Feilong Wang and Jing Hua coordinated to collect the data with technical guidance from Xiaodong Wu. Kun Wang, Xiaodong Wu, Huayu Zhang and Ting Shi analyzed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript. This work was supported by National Natural Science Foundation of China (grant number:81700006), National Key R&D Program (2018YFC1313700), National Natural Science Foundation of China (grant number: 81870064) and the "Gaoyuan" project of Pudong Health and Family Planning Commission (PWYgy2018-06).

Reference

1. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med.* 2020.
2. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497-506.
3. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA.* 2020.
4. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet.* 2020;395(10223):507-513.
5. Wang Y, Guo Q, Yan Z, et al. Factors Associated With Prolonged Viral Shedding in Patients With Avian Influenza A(H7N9) Virus Infection. *J Infect Dis.* 2018;217(11):1708-1717.
6. Tsang TK, Cowling BJ, Fang VJ, et al. Influenza A Virus Shedding and Infectivity in Households. *J Infect Dis.* 2015;212(9):1420-1428.
7. Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. *BMJ.* 2020;369:m1808.
8. Xie X, Zhong Z, Zhao W, Zheng C, Wang F, Liu J. Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing. *Radiology.* 2020:200343.
9. Lan L, Xu D, Ye G, et al. Positive RT-PCR Test Results in Patients Recovered From COVID-19. *JAMA.* 2020.

10. Han H, Luo Q, Mo F, Long L, Zheng W. SARS-CoV-2 RNA more readily detected in induced sputum than in throat swabs of convalescent COVID-19 patients. *The Lancet Infectious Diseases.* 2020.
11. Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition).2020, Mar. *http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml.*
12. Winichakoon P, Chaiwarith R, Liwsrisakun C, et al. Negative Nasopharyngeal and Oropharyngeal Swabs Do Not Rule Out COVID-19. *J Clin Microbiol.* 2020;58(5).
13. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *Jama.* 2020.
14. Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol.* 2020;94(7).
15. Yang Y, Yang M, Shen C, et al. Evaluating the accuracy of different respiratory specimens in the laboratory diagnosis and monitoring the viral shedding of 2019-nCoV infections. *medRxiv.* 2020.
16. Liu R, Han H, Liu F, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Clin Chim Acta.* 2020;505:172-175.
17. Lim WS, van der Eerden MM, Laing R, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. *Thorax.* 2003;58(5):377-382.
18. Xu D, Zhang Z, Jin L, et al. Persistent shedding of viable SARS-CoV in urine and stool of SARS patients during the convalescent phase. *Eur J Clin Microbiol Infect Dis.* 2005;24(3):165-171.
19. Oh MD, Park WB, Choe PG, et al. Viral Load Kinetics of MERS Coronavirus Infection. *N Engl J Med.* 2016;375(13):1303-1305.
20. Lau LLH, Cowling BJ, Fang VJ, et al. Viral Shedding and Clinical Illness in Naturally Acquired Influenza Virus Infections. *Journal of Infectious Diseases.* 2010(10):10.
21. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020.
22. Choi KW, Chau TN, Tsang O, et al. Outcomes and prognostic factors in 267 patients with severe acute respiratory syndrome in Hong Kong. *Ann Intern Med.* 2003;139(9):715-723.
23. Hong KH, Choi JP, Hong SH, et al. Predictors of mortality in Middle East respiratory syndrome (MERS). *Thorax.* 2018;73(3):286-289.
24. Opal SM, Girard TD, Ely EW. The immunopathogenesis of sepsis in elderly patients. *Clin Infect Dis.* 2005;41 Suppl 7:S504-512.

25. China. NHCotPsRo. Pneumonia diagnosis and treatment for COVID-19 infection (trial version 7 revised version) [EB/OL]. 2020.
26. Chen C, Gao G, Xu Y, et al. SARS-CoV-2-Positive Sputum and Feces After Conversion of Pharyngeal Samples in Patients With COVID-19. *Ann Intern Med.* 2020.
27. Interim Guidelines for Collecting H, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019. Centers for Disease Control and Prevention, United States.(COVID-19).https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html.
28. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. *Clin Infect Dis.* 2020.

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1 **Table 1. Demographic and Clinical Characteristics of 68 COVID-19 Patients.**

Demographic and clinical characteristics	Patients (n=68)
Age	
Yrs (M, IQR)	67 (57-72)
≥65 years — no. (%)	40 (58.8)
Male — no. (%)	36 (52.9)
Underlying diseases — no. (%)	
Chronic lung disease	12 (17.6)
Diabetes mellitus	12 (17.6)
Cardiac disease	9 (13.2)
Malignant tumor	3 (4.4)
Clinical features — no. (%)	
Fever	50 (73.5)
T, °C (M, IQR)	38.5 (38-39)
Cough	31 (45.6)
Dyspnea	23 (33.8)
Fatigue	22 (32.4)
Diarrhea	7 (10.3)
Patients diagnosed with COVID-19 at admission— no. (%)	
by NPS (+)	43 (63.2)
by IgM/IgG (+)	25 (36.8)
IgM/IgG against SARS-CoV-2 during hospitalization— no. (%)	
IgM positive	52 (76.5)
IgG positive	57 (83.8)
Respiratory support— no. (%)	
NPPV	25 (36.8)

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HFNC	21 (30.9)
Conventional oxygen therapy	18 (26.5)
Intubation	5 (7.4)
CURB-65— no. (%)	
1	30 (44.1)
2	36 (52.9)
3	2 (2.9)
Duration of different symptoms in survivors, days (M, IQR)	
Fever	11.0 (8.0-13.0)
Cough	20.0 (11.0-26.0)
Diarrhea	4.0 (2.0-5.0)
Mortality—no.(%)	3 (4.4)

M, IQR: Median, Inter Quartile Range. NPPV: noninvasive positive-pressure ventilation. NPS, nasopharyngeal swab. HFNC: high-flow nasal cannula oxygen therapy. SP, sputum. Yrs (M, IQR): Years, (Median, Inter Quartile Range).

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Table 2. Factors associated with SARS-CoV-2 RNA detection yields in nasopharyngeal swab and sputum specimens during the hospitalization.

Characteristics	NPS(+)	NPS(-)	P value	SP[b](+)	SP(-)	P value	NPS(+)&SP(+)	Others	P value
	(N=49)	(N=19)		(N=30)	(N=38)		(N=16)	(N=52)	
Chronic lung disease	8	4	0.646	10	2	0.003	6	6	0.017
Diabetes mellitus	5	7	0.010	9	3	0.018	3	9	0.895
Fever	35	15	0.398	25	25	0.103	14	36	0.147
Cough	23	7	0.452	13	17	0.908	7	23	0.973
Fatigue	16	6	0.932	9	13	0.712	6	16	0.615
Diarrhea	6	1	0.395	4	3	0.464	3	4	0.203
Steroids	12	8	0.153	13	7	0.025	7	13	0.150
Lymphocyte numbers	0.88±0.47	1.13±0.75	0.721	0.99±0. 47	1.14±0.89	0.135	1.00±0.20	1.00±0.06	0.517

NPS: nasopharyngeal swab. SP: sputum specimen.

1 **Table 3. Multivariable analyses of risk factors associated with duration of**

2 **SARS-CoV-2 RNA detection in hospitalized patients.**

Characteristics	Unadjusted HR (95% CI)	P value	Adjusted HR* (95% CI)	P value
Age≥65yrs	1.66 (0.99- 2.82)	0.06	**1.71 (1.01-2.93)**	0.04
Sex, male	1.04 (0.63-1.73)	0.867	1.21 (0.69-2.13)	0.50
Diabetes mellitus	0.57 (0.30-1.08)	0.18	0.64 (0.31-1.29)	0.21
Chronic lung diseases	0.72 (0.38-1.36)	0.30	0.88 (0.40-1.97)	0.76
Lymphocyte counts	1.01 (.083-1.23)	0.91	0.98 (0.78-1.21)	0.83
Systemic steroids	0.74 (0.41-1.32)	0.30	1.08 (0.51-2.24)	0.84
Cardiac diseases	0.59 (0.29-1.20)	0.12	1.00 (0.45-2.27)	0.99
Hypertension	0.61 (0.34-1.10)	0.09	0.55 (0.26-1.16)	0.76
Malignant tumor	0.23 (0.30-1.70)	0.07	0.15 (0.16-1.49)	0.11

3 HR (95%CI): hazard ratio (95% confidence interval). Yrs: years

4 * Adjusted for age and sex.

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Figure Legends

Figure 1. **Detection of SARS-CoV-2 RNA in nasopharyngeal swab and sputum specimen from COVID-19 patients during the hospitalization.**

NPS (+): nasopharyngeal swab specimen positive. NPS (-): nasopharyngeal swab specimen negative. SP (+): sputum specimen positive. SP (-): sputum specimen negative.

Figure 2. **Results of SARS-CoV-2 RNA detection in 16 patients with both NPS and SP samples positive, by timing of first positive testing for SARS-CoV-2 RNA.**

Day 0 is the day of first positive testing for SARS-CoV-2 RNA in each patient.

NPS: nasopharyngeal swab. SP: sputum.

Figure 3. Cumulative proportion of patients who had detectable SARS-CoV-2 RNA by days after onset of illness. (A) From both NPS and SP specimens; (B) from NPS and SP separately; (C) with age <65 years versus ≥65 years, respectively.

NPS: nasopharyngeal swab. SP: sputum.

Figure 4. Illustrated information about 2 cases that patients had recurrent positive detection of SARS-COV-2 RNA from nasopharyngeal swab.

NPS (+): nasopharyngeal swab specimen positive. NPS (-): nasopharyngeal swab specimen negative. SP (+): sputum specimen positive.

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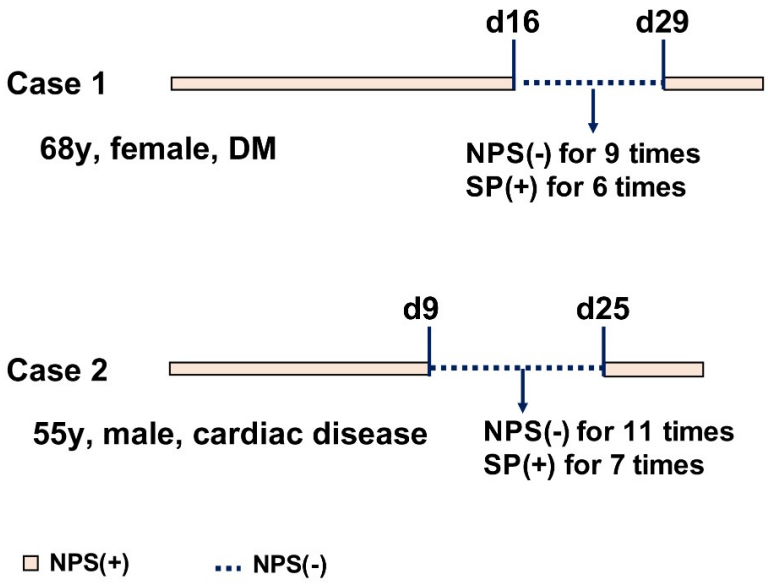
d16
d29
Case 1
68y, female, DM
NPS(-) for 9 times
SP(+) for 6 times
d9
d25
Case 2
55y, male, cardiac disease
NPS(-) for 11 times
SP(+) for 7 times
NPS(+)
NPS(-)

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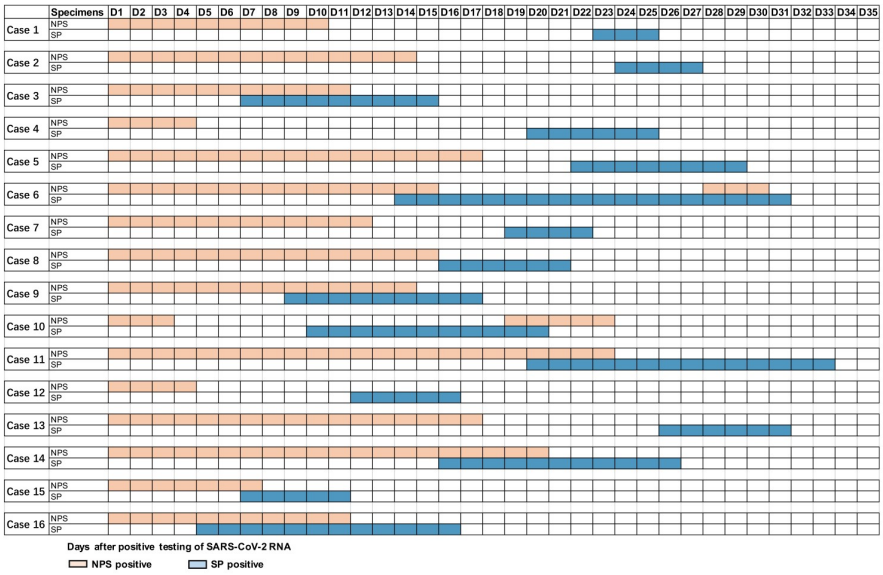

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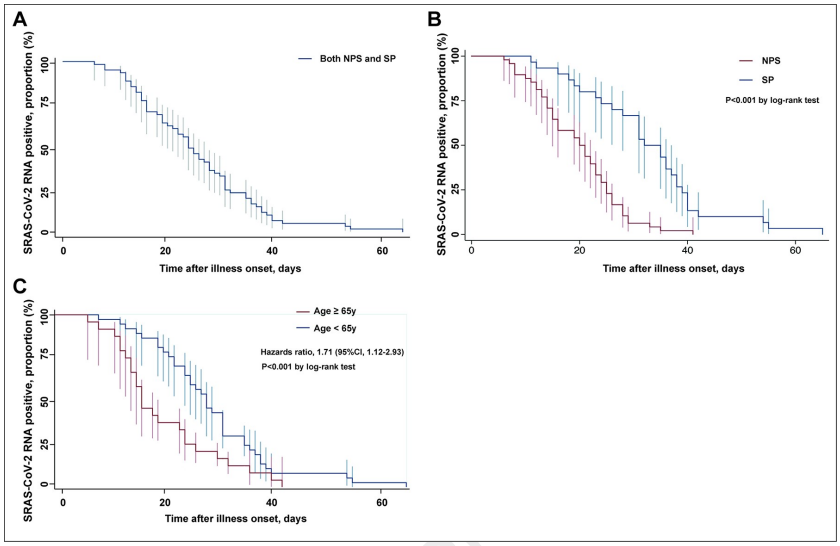

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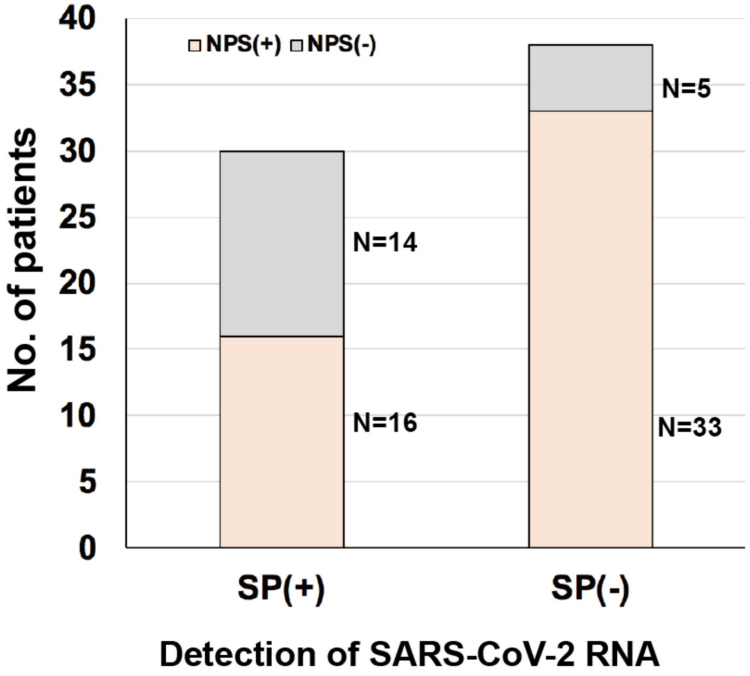
NPS(+)
NPS(-)
No. of patients
40
35
30
25
20
15
10
5
0
N=14
N=16
N=5
N=33
SP(+)
SP(-)
Detection of SARS-CoV-2 RNA

Journal