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# Early and consecutive RT-PCR tests with both oropharyngeal swabs and sputum could improve testing yield for patients with COVID-19:

## An observation cohort study in China

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### ABSTRACT

**Objective:** The real-time polymerase chain reaction (RT-PCR) test is recommended for the diagnosis of COVID-19 and provides a powerful tool to identify new infections and facilitate contact tracing. In fact, as the prevalence of COVID-19 decreases, this RT-PCR testing remains as the main preventive measure to avoid rebound. However, inconsistent results can lead to misdiagnoses in the clinic. These inconsistencies are due to the variability in (1) the collection times of biological samples post infection, and (2) sampling procedures.

**Methods:** We applied the Kaplan–Meier method and multivariate logistic regression on RT-PCR results from 258 confirmed patients with COVID-19 to evaluate the factors associated with negative conversion. We also estimated the proportion (%) of negative conversion among patients who had tested twice or more, and compared the proportions arising from oropharyngeal swabs, sputum, and combined double testing, respectively.

**Main results:** The proportion of negative conversion was 6.7% on day 4, 16.4% on day 7, 41.0% at 2 weeks, and 61.0% at 3 weeks post-admission. We also found that 34.1% and 60.3% of subjects had at least one negative RT-PCR result on days 7 and 14 after the onset of symptoms, respectively. The proportion of negative conversions following sputum testing was higher than that from oropharyngeal swabs in the early stages but this declined after the onset of symptoms.

**Conclusion:** In the absence of effective treatments or vaccines, efficient testing strategies are critical if we are to control the COVID-19 epidemic. According to this study, early, consecutive and combined double testing, will be the key to identify infected patients, particularly for asymptomatic and mild symptomatic cases. These strategies will minimize misdiagnosis and the ineffective isolation of infected patients.

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### Introduction

The epidemic of novel coronavirus disease 2019 (COVID-19) continues to pose a serious risk to public health and was recognized as a pandemic by the World Health Organization (WHO) on March 11, 2020 (Baloch et al., 2020). Despite over 10 million infections and more than 2,000,000 deaths globally as of February 1, 2021 (China Global Television Network, 2020), the number of infected patients has dramatically decreased in some local areas and countries. In these regions, the prevention and disease control policy has shifted from the diagnosis of endemic

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infections to the identification of newly imported COVID-19 cases, with stringent contact tracing to prevent follow-up transmission. Therefore, early diagnosis, with low false negative rates to prevent misdiagnosis, are crucial if we are to successfully identify infected individuals for isolation and prevent rebound epidemics (Yi et al., 2020).

There have been several rebound epidemics in several hot-spot cities or provinces over recent times, thus reminding us that the battle is far from over, and is likely to remain a threat. COVID-19, with an  $R_0$  of 1.4–6.9 (Liberty et al., 2020), poses a significant challenge to complete and stringent patient identification, especially asymptomatic patients who can shed the virus and contribute to the transmission of SARS-CoV-2. Therefore, the identification of these viral carriers is extremely important, even if COVID-19 is well controlled in China, with most businesses and schools reopened nationwide. Notably, different from influenza viruses and community-acquired human coronaviruses, SARS-CoV-2 exhibits a high virus load during the early phases of illness, prior to efficient testing (To et al., 2020; Wölfel et al., 2020). The real-time polymerase chain reaction (RT-PCR) testing of respiratory specimens is recommended as the virologic criterion for diagnosis and the outcome evaluation of treatments (Zhao et al., 2020). However, RT-PCR cannot detect the SARS-CoV-2 virus in 30% of patients, and has demonstrated inconsistent test results due to biological sample variability in the timing of sample collection post-infection and sampling procedures (Zhang et al., 2020; Young et al., 2020; Wang et al., 2020). It is therefore crucial that we understand when and how patients should be tested by RT-PCR to identify their infection status with the SARS-CoV-2 virus. In the absence of effective specific antiviral therapies or vaccines, the rapid and efficient identification of new infections, contact tracing, and quarantine, are the most effective strategies against local rebound epidemics, such as in China, Singapore, and Korea.

Herein, we carried out a cohort study among confirmed patients in Beijing Ditan Hospital. Our goal was to demonstrate the impact of sample collection timing and procedures for RT-PCR testing and the diagnosis of COVID-19 patients.

## Methods

### Clinical characteristics and laboratory procedures

Our study included all patients with confirmed COVID-19 that were admitted to the Beijing Ditan Hospital with symptoms between January 20 and April 17, 2020. In total, we enrolled 258 confirmed cases, corresponding to 44% of the confirmed patients in Beijing.

Clinical samples, including oropharyngeal swabs or sputum specimens, were collected at multiple timepoints for RT-PCR testing in accordance with the Chinese guidance on infection prevention and control in healthcare settings (World Health Organization, 2020). A cycle threshold value less than or equal to 38 in at least one gene was interpreted as positive (Chan et al., 2020; Zhu et al., 2020).

### Statistical analysis

Continuous variables are expressed as medians and interquartile ranges (IQR) as appropriate. Categorical variables are summarized as the numbers and corresponding percentages in each category. The Kaplan–Meier method was used to plot and estimate the proportion of negative results conversion during the study period. This study started on the date of the initial symptom in each patient and proceeded until the date of negative conversion, defined as the day when both oropharyngeal swab and sputum samples appeared negative and never reverted in subsequent tests

until patient discharge or follow-up date. We also applied multivariate logistic regression to evaluate the potential factors associated with the duration of negative conversion among discharged patients (>14 days vs. ≤14 days). We also provided odds ratio (OR) and corresponding 95% confidence intervals (95% CIs).

It is known that some COVID-19 patients produce inconsistent laboratory testing results prior to negative conversion, particularly from oropharyngeal swabs. Therefore, we estimated the proportion of patients with a negative RT-PCR test; this was defined as the number of patients with a negative RT-PCR result divided by the total number of patients who were tested twice during the study period.

To evaluate the performance of RT-PCR with regards to oropharyngeal swabs and sputum, we estimated the proportions of the RT-PCR testing results by oropharyngeal swab, sputum specimens, and combined double testing, within the 21 days of each patient from the 1st day (the 2nd day of time origin). This was because only a subset of patients was independently tested by oropharyngeal swabs or sputum specimens each day. To estimate the proportions of the RT-PCR testing results, we normalized the data to the total number of patients tested each day and assumed identical proportions for the results. The positive result of combined double testing was defined as either test (oropharyngeal swab or the sputum specimen) being positive within 24 h. True negative results were defined as recovered patients who converted to be negative and remained negative on all subsequent tests.

All analyses were conducted with R software version 3.6.2 (R Foundation for Statistical Computing).

## Results

A total of 258 patients with confirmed COVID-19 were enrolled in our study (Table 1). Only 146 of these patients (57%) presented with fever. Forty-three patients (17%) had severe COVID-19 and required oxygen supplementation, while 154 (60%) had mild disease; 61 (23%) experienced an uncomplicated illness. No specific antiviral drugs, such as lopinavir and remdesivir, were administered. Frequent RT-PCR testing was conducted on oropharyngeal swabs (2223 samples) and sputum (2001 samples), at least

**Table 1**  
General information relating to 258 COVID-19 patients in Beijing Ditan Hospital.

Characteristics	Patient numbers
Total no. (%)	258 (100)
Age groups (years)	
<18	21 (8)
18–39	124 (48)
40–65	82 (32)
>65	31 (12)
Sex no. (%)	
Male	128 (50)
Female	130 (50)
Classification no. (%)	
Uncomplicated and mild illness	215 (83)
Severe and critical illness	43 (17)
Initial symptoms-no. (%)	
No symptoms	4 (2)
1–2 symptoms	191 (74)
More than 2 symptoms	63 (24)
Coexisting complications-no. (%)	
Hypertension	34 (13)
Diabetes	17 (7)
COPD	13 (5)
Cardiovascular diseases	10 (4)
Renal diseases	6 (2)
Autoimmune disorders	5 (2)
Others	6 (2)
Any one of above	41 (16)

once for every 2–3 days, over the first 14 days upon hospital admission, and then at least once for 5 days until discharge. The median testing time was 3.2 days (IQR: 2.3–5.0).

#### Proportion of negative conversions over time since symptom onset

The median duration of patients with SARS-CoV-2 was 17 days (range: 1–60 days). In total, 195 of the 258 patients eventually recovered from COVID-19 disease with several consecutive negative test results and were discharged. The proportion of negative result conversion was 6.7% on the 4th day, 16.4% on the 7th day, 41.0% at 2 weeks, and 61.0% at 3 weeks after the onset of symptoms and hospital admittance, as demonstrated by the Kaplan–Meier method (Figure 1). The most drastic reduction in the proportion of negative conversion was observed on the 9th to 12th days after hospital admission (5.1% each; 10 patients). However, we also noticed that 66 (25.6%) of the patients still had the SARS-CoV-2 virus over 30 days. Moreover, multivariable logistic regression (Table 2) revealed that older patients were associated with a longer duration to negative conversion. In particular, we found that elderly patients (aged >65 years) were associated with a significantly longer time to negative conversion when compared with younger patients (adjusted odds ratio (aOR): 6.38; 95% confidence interval: 1.31, 31.11). No significant differences were identified with regards to sex, clinical classification, complications, and initial symptoms.

#### Inconsistent RT-PCR testing results prior to negative conversion and recovery

Of the 258 study patients, 222 had at least two positive RT-PCR results from oropharyngeal samples prior to negative conversion. All of these patients recovered and were discharged. In total, 1674 oropharyngeal swabs tests were performed prior to negative conversion; of these, 733 (43.8%) provided negative RT-PCR results. Theoretically, all RT-PCR tests before negative conversion should be positive. However, we still observed that 81.1% (180/222) patients had at least one negative RT-PCR result prior to negative conversion and clinical discharge (Figure 2). Further analysis of positive rates and disease progression showed that 34.1% (30 of 88) and 60.3% (79 of 131) of patients with at least one negative RT-PCR result were identified on days 7 and 14 after the onset of symptoms. These percentages expose the inconsistency of the testing results and the necessity for multiple testing before final

clinical discharge. These findings also indicate that an early diagnosis can reduce false negative rates.

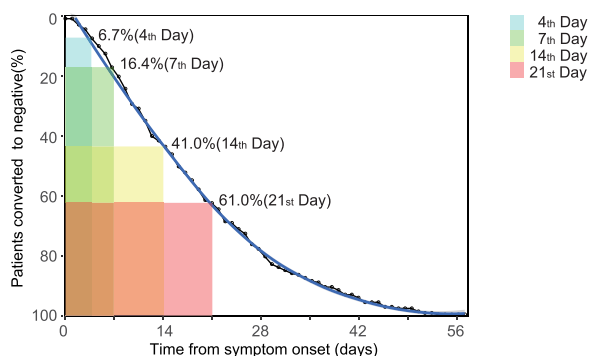
#### Proportions of negative conversion for RT-PCR testing by oropharyngeal swabs, sputum specimens, and combined double testing

RT-PCR test results by oropharyngeal swabs, sputum specimens, and combined double testing, are shown in Figure 3. The median number of patients tested by RT-PCR from oropharyngeal swab samples in the first 21 days was 59% (range: 35–73), while those from sputum samples was 47% (range: 10–62). The proportion of negative results in sputum samples was higher than that in the oropharyngeal swabs in the early stages after symptoms (1st day to 5th day). In comparison, these proportions became lower or equal in the sputum and oropharyngeal swabs between days 5 and 21. Notably, the negative proportions for combined double testing were much lower than those for each test individually. For example, as early as the 1st day after symptom onset, the proportion of negative conversions from oropharyngeal swabs was 18.4%, while that for sputum was 30.0%. Combined double testing results with both samples reduced the proportion of negative conversions of SARS-CoV-2 to 4.6%.

## Discussion

In this study, we retrospectively analyzed RT-PCR results from 258 patients with COVID-19 in Beijing Ditan Hospital, and explored the impacts of different sampling times and clinical procedures on the RT-PCR test results. In the absence of useful specific antiviral therapy, we found that more than 60.0% of patients eventually converted to negative test results (clinical recovery) within 21 days of the initial onsets of symptoms. Younger patients were more likely to achieve clinical recovery and negative conversion within a shorter time; 81.1% of study patients presented at least one inconsistent negative test result prior to clinical recovery, negative conversion, and discharge, thus suggesting that a single RT-PCR test can still produce a high false-negative rate in infected patients by virtue of the natural history of the virus, even though we excluded the potential impacts of technical bias.

Many of those who have died from COVID-19 may not have received RT-PCR testing in time. Considering the same sample collection protocols and testing approaches used in this study, we speculated that the decreasing trend in positive testing rates over time might also be representative of the dynamics of virus infection. In particular, an increased viral load in the oropharyngeal areas has been observed with the MERS virus, which belongs to the same coronavirus family as COVID-19 (Poissy et al., 2014). COVID-19 is a self-limiting disease in approximately 80% of patients with mild disease (Xu et al., 2020) who might be reluctant to visit a doctor during the early stages of infection, and only present later at the clinics if symptoms worsen. In addition, asymptomatic patients with SARS-CoV-2 have been extensively reported (Chan et al., 2020; Zhou et al., 2020). Under such circumstances, these patients might have already contributed to massive transmission in the community and could also be misdiagnosed at clinics before oropharyngeal swab-based RT-PCR testing commenced for case detection. Recent models suggest that asymptomatic and pre-symptomatic transmission, along with delays in case recognition, can significantly reduce the efficacy of contact tracing (Adam et al., 2020). The present study also showed that a certain number of patients can rapidly convert to a negative result and thus create a hurdle for the identification and quarantine of infected patients. Thus, delays in RT-PCR testing can lead to misdiagnosis and the non-identification of infectious patients prior to negative conversion, thus preventing effective quarantine, contact tracing, and the

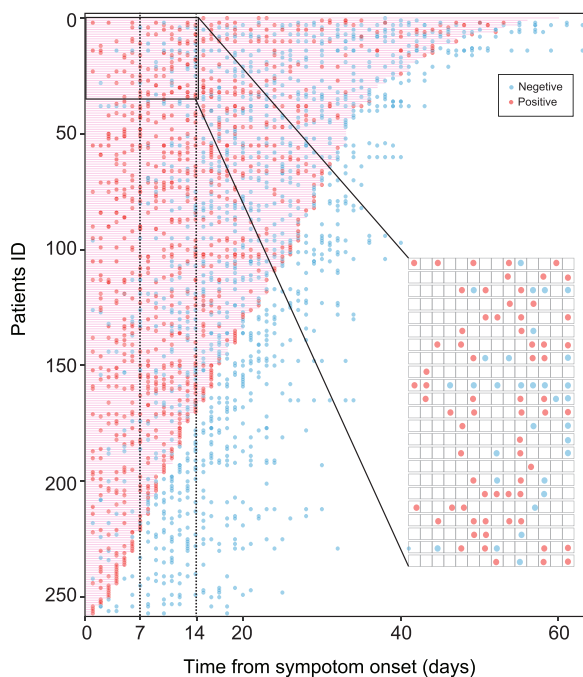


**Figure 1.** Estimated proportion of negative conversions in COVID-19 patients over time from the onset of initial symptoms.

The black dots and lines were calculated by actual proportions while the dark blue curve was estimated by lowest smoothing. The x-axis represents the number of days after the onset of symptom, where day 0 represents the day of symptom onset. The y-axis represents the proportion of patients converting to negative on the 4th day (blue area), 7th day (green area), 14th day (yellow area) and 21st day (pink area) are highlighted.

**Table 2**  
Factors that exert impact on the time of negative conversion  $\geq 14$  days among 195 discharged COVID-19 patients.

Characteristics	Discharged patients N (%)	Time of conversion to negative (days)		Univariate analysis		Multivariate analysis	
		<14 N (%)	$\geq 14$ N (%)	OR (95%CI)	P	aOR (95%CI)	P
Total	195 (100)	80 (41)	115 (59)				
Age groups (years)							
<18	15 (8)	10 (12)	5 (4)	ref		ref	
18–39	87 (45)	38 (48)	49 (43)	2.58 (0.81–8.18)	0.108	2.47 (0.77–7.96)	0.129
40–65	67 (34)	29 (36)	38 (33)	2.62 (0.81–8.51)	0.109	3.10 (0.89–10.82)	0.075
>65	26 (13)	3 (4)	23 (20)	15.33 (3.06–76.90)	0.001	14.98 (2.59–86.53)	0.003
Sex							
Male	99 (51)	36 (45)	63 (55)	ref		ref	
Female	96 (49)	44 (55)	52 (45)	0.68 (0.38–1.20)	0.180	0.72 (0.39–1.33)	0.290
Classification							
Uncomplicated and mild illness	164 (84)	73 (91)	91 (79)	ref		ref	
Severe and critical illness	31 (16)	7 (9)	24 (21)	2.75 (1.12–6.74)	0.027	1.86 (0.61–5.72)	0.276
Initial symptoms							
No symptoms	27 (14)	10 (12)	17 (15)	ref		ref	
1–2 symptoms	123 (63)	50 (63)	73 (63)	0.86 (0.36–2.03)	0.729	0.72 (0.29–1.77)	0.478
More than 2 symptoms	45 (23)	20 (25)	25 (22)	0.74 (0.28–1.96)	0.538	0.44 (0.15–1.30)	0.138
Coexisting complications							
No complications	149 (76)	64(80)	85 (74)	ref		ref	
1 or more complications	46 (24)	16 (20)	30 (26)	1.41 (0.71–2.81)	0.326	0.65 (0.26–1.63)	0.363



**Figure 2.** Results of multiple RT-PCR testing in COVID-19 patients. The x-axis represents the number of days after the onset of symptoms. Each row represents the detection point for each patient. The pink bar indicates the time to recovery and negative conversion for SARS-CoV-2 patients as defined in the methods section. Red and blue point represent positive and negative results in nucleic acid tests, respectively.

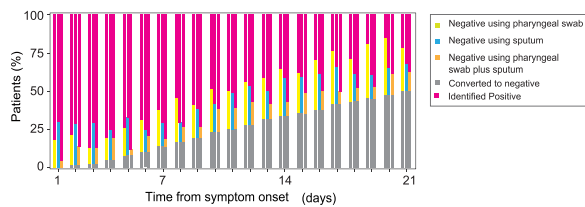
reduction of disease transmission. Compared to other diagnostic approaches, such as chest CT, and ELISA kits for the detection of immunoglobulin (Ig) M and IgG antibodies, RT-PCR can detect the virus early and provide better opportunities for infection control. Surveillance methods based on RT-PCR should therefore be performed as early as possible.

In the present study, 81.1% of patients presented with at least one inconsistent negative test result prior to clinical recovery, negative conversion, and discharge. Similarly, a previous study of confirmed COVID-19 patients in Singapore also observed inconsistent RT-PCR results prior to complete clinical recovery (Young

et al., 2020). Consequently, single negative results cannot completely preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions. Patients still shed the virus even with mild symptoms or even after becoming asymptomatic (afebrile after treatment) (Chang et al., 2020). Furthermore, at the early stages of illness, we observed a number of patients with negative RT-PCR results. Thus, although RT-PCR is recommended as a powerful tool for early diagnosis, negative results should be followed by multiple testing and clinical observations, patient histories, and epidemiological information.

Finally, we compared the proportions of negative conversions because of RT-PCT testing by oropharyngeal swabs, sputum specimens, and combined double testing within 21 days of the onset of symptoms. In general, the proportions of negative conversions were lower for the combined double testing of oropharyngeal swabs and sputum than each test independently, thus indicating that multiple specimen-based testing might be more sensitive for the detection of infections. In our previous study, we showed that some patients who were negative in oropharyngeal swabs but positive in sputum, thus suggesting that the virus might persist in the sputum for a longer time period (Chen et al., 2020). Interestingly, in the early stages (before the 5th day), the oropharyngeal swabs show a higher performance with regards to identifying infections, thus suggesting that many infected patients with COVID-19 require a lag time to produce virus in the sputum. Furthermore, many patients infected with COVID-19 have no sputum during the early stages. Dynamic changes in viral loads have also been reported in different biological samples (Zou et al., 2020). However, there is still no gold standard for how and when these biological samples should be tested for COVID-19. Therefore, considering our findings, we strongly recommended that multiple biological specimens should be collected and tested from suspected cases to minimize the misdiagnosis of infectious patients. Furthermore, we should ensure that patients stay in hospital as long as possible until they gave recovered completely.

This study has some limitations that need to be considered, including the rapid emergence of COVID-19, the limited numbers of patients, and the associated lack of data for analysis. However, given the urgency of our response to COVID-19, we performed analysis in as much data as possible and acquired a significant amount of clinical information for epidemiological investigation.



**Figure 3.** Multiple RT-PCR test results within 21 days of the initial symptoms. The deep pink bar represents the estimated positive proportion of COVID-19 patients. The yellow bar represents the estimated negative proportion of COVID-19 patients using oropharyngeal swabs alone. The deep blue bar represents the estimated negative proportion of COVID-19 patients using sputum alone. The orange bar represents the estimated negative proportion of COVID-19 patients by RT-PCR tests using combined double testing with both oropharyngeal swabs and sputum. The gray bar represents the estimated negative proportion of COVID-19 patients due to viral negative conversion.

Furthermore, to minimize recall sampling bias, this epidemiological study was subjected to an estimated model with an approximate calculation. More frequent and paired specimen collection may improve the accuracy of our estimation.

In summary, until we have effective treatments and an appropriate vaccine, one of the most effective preventive strategies that we can use to reduce transmission is efficient early testing to identify, isolate, and trace, infected patients so that we can prevent local epidemic rebound. Considering the high transmission potential of SARS-CoV-2, misdiagnoses can readily lead to a new local epidemic. Hence, according to our current findings, it is important that we perform early, consecutive, and combined double testing, if we are to identify all COVID-19 patients, particularly those that are asymptomatic or mild symptomatic, and therefore minimize misdiagnosis. Only through the isolation of virtually all infected patients can we prevent a further COVID-19 local epidemic.

### Contributors

FZ and HZ conceptualized and designed the study and had full access to all data; these authors were also responsible for data integrity, accuracy, and analysis. FZ, YS, and CC contributed to manuscript writing. GG, SW, DY, RS, LW and WX contributed to diagnosis, treatment, and sample acquisition. YS and CC contributed to data collection, data analysis, and data interpretation. MC contributed to the statistical analysis. FY, LY and YW contributed to sample testing. All authors reviewed and approved the final version of the manuscript.

### Ethical approval

This study was approved by the Ethics Committee of Beijing Ditan Hospital, Capital Medical University (Reference number: KT2020-006-01).

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### Conflict of interests

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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### References

- Adam Dillon C, Wu Peng, Wong Jessica Y, Lau Eric HY, Tsang Tim K, Cauchemez Simon, et al. Clustering and superspreading potential of SARS-CoV-2 infections in Hong Kong. *Nat Med* 2020;26(11):1714–9.
- Baloch Saira, Baloch Mohsin Ali, Zheng Tianli, Pei Xiaofang. The coronavirus disease 2019 (COVID-19) pandemic. *Tohoku J Exp Med* 2020;250(4):271–8.
- Chan Jasper Fuk-Woo, Yuan Shuofeng, Kok Kin-Hang, To Kelvin Kai-Wang, Chu Hin, Yang Jin, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020;395:514–23.
- Chang De, Mo Guoxin, Yuan Xin, Yi Tao, Peng Xiaohua, Wang Fu-Sheng, et al. Time kinetics of viral clearance and resolution of symptoms in novel coronavirus infection. *Am J Respir Crit Care Med* 2020;201(9):1150–2.
- Chen, Gao Guiju, Yanli Xu, Lin Pu, Qi Wang, Wang Liming, et al. SARS-CoV-2-positive sputum and feces after conversion of oropharyngeal samples in patients with COVID-19. *Ann Intern Med* 2020;172(12):832–4.
- China Global Television Network. Global coronavirus cases top 5.4 million. Johns Hopkins University; 2020. . . [Accessed 16 April 2020] <https://www.cgtn.com/special/Battling/the/novel/coronavirus/What/we/know/so/fa>.
- Liberty Iche Andriyani, Fadilah Mariatul, Josef Hari Kusnanto. Scientific prediction of the Covid-19 pandemic and what should society do. *Rev Prim Care Pract Educ* 2020;3:22–6.
- Poissy J, Goffard A, Parmentier-Decrucq E, Favory R, Kauh M, Kipnis E, et al. Kinetics and pattern of viral excretion in biological specimens of two MERS-CoV cases. *J Clin Virol* 2014;61:275–8.
- To Kelvin Kai-Wang, Tsang Owen Tak-Yin, Leung Wai-Shing, Tam Anthony Raymond, Wu Tak-Chiu, Lung David Christopher, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020;20:565–74.
- Wang Wenling, Xu Yanli, Gao Ruqin, Lu Roujian, Han Kai, Wu Guizhen, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* 2020;323(18):1843–4.
- Wölfel Roman, Corman Victor M, Guggemos Wolfgang, Seilmaier Michael, Zange Sabine, Müller Marcel A, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;581(7809):465–9.
- World Health Organization. Report of the WHO-China joint mission on coronavirus disease 2019 (COVID-19). 2020. . . [Accessed 16–24 February 2020] <https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report>.
- Xu Xiao-Wei, Wu Xiao-Xin, Jiang Xian-Gao, Xu Kai-Jin, Ying Ling-Jun, Ma Chun-Lian, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-CoV-2) outside of Wuhan, China: retrospective case series. *BMJ* 2020;368:m606.
- Yi Ye, Lagniton Philip NP, Ye Sen, Li Enqin, Xu Ren-He. COVID-19: what has been learned and to be learned about the novel coronavirus disease. *Int J Biol Sci* 2020;16:17534.
- Young Barnaby Edward, Ong Sean Wei Xiang, Kalimuddin Shirin, Low Jenny G, Tan Seow Yen, Loh Jiashen, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA* 2020;323(15):1488–94.
- Zhang Wei, Du Rong-Hui, Li Bei, Zheng Xiao-Shuang, Yang Xing-Lou, Hu Ben, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *Emerg Microbes Infect* 2020;9:386–9.
- Zhao Jing-Ya, Yan Jia-Yang, Qu Jie-Ming. Interpretations of “diagnosis and treatment protocol for novel coronavirus pneumonia (trial version 7)”. *Chin Med J* 2020;133(11):1347–9.
- Zhou Xian, Li Yang, Li Tao, Zhang Wenhong. Follow-up of asymptomatic patients with SARS-CoV-2 infection. *Clin Microbiol Infect* 2020;26:957–9.
- Zhu Na, Zhang Dingyu, Wang Wenling, Li Xingwang, Yang Bo, Song Jingdong, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727–33.
- Zou Lirong, Ruan Feng, Huang Mingxing, Liang Lijun, Huang Huitao, Hong Zhongsi, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med* 2020;382:1177–9.