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Characteristics and diagnosis rate of 5630 subjects receiving SARS-CoV-2 nucleic acid tests from Wuhan, China

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Clinical Medicine   **COVID-19**   **Infectious disease**

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a novel viral pneumonia (COVID-19), which is rapidly spreading throughout the world. The positive result of nucleic acid test is a golden criterion to confirm SARS-CoV-2 infection, but the detection features remain unclear.

We performed a retrospective analysis in 5630 high-risk individuals receiving SARS-CoV-2 nucleic acid tests in Wuhan, China, and investigated their characteristics and diagnosis rates.

The overall diagnosis rate was 34.7% (1952/5630). Male (P = 0.025) and older populations (P = 2.525 × 10^{-39}) were at significantly higher risk of SARS-CoV-2 infection. People were generally susceptible, and most cases concentrated in people of 30–79 years. Furthermore, we investigated the association between diagnosis rate and the amount of testing in 501 subjects. Results revealed a 1.27-fold improvement (from 27.9% to 35.5%) of diagnosis rate from testing once to twice (P = 5.847 × 10^{-9}) and a 1.43-fold improvement (from 27.9% to 39.9%) from testing once to 3 times P = 7.797 × 10^{-14}). More than 3 testing administrations was not helpful for further improvement. However, this improvement was not observed in subjects with pneumonia (P = 0.097).

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Characteristics and diagnosis rate of 5630 subjects receiving SARS-CoV-2 nucleic acid tests from Wuhan, China

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BACKGROUND. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a novel viral pneumonia (COVID-19), which is rapidly spreading throughout the world. The positive result of nucleic acid test is a golden criterion to confirm SARS-CoV-2 infection, but the detection features remain unclear.

METHODS. We performed a retrospective analysis in 5630 high-risk individuals receiving SARS-CoV-2 nucleic acid tests in Wuhan, China, and investigated their characteristics and diagnosis rates.

RESULTS. The overall diagnosis rate was 34.7% (1952/5630). Male (P = 0.025) and older populations ($P = 2.525 \times 10^{-39}$) were at significantly higher risk of SARS-CoV-2 infection. People were generally susceptible, and most cases concentrated in people of 30–79 years. Furthermore, we investigated the association between diagnosis rate and the amount of testing in 501 subjects. Results revealed a 1.27-fold improvement (from 27.9% to 35.5%) of diagnosis rate from testing once to twice ($P = 5.847 \times 10^{-9}$) and a 1.43-fold improvement (from 27.9% to 39.9%) from testing once to 3 times ($P = 7.797 \times 10^{-14}$). More than 3 testing administrations was not helpful for further improvement. However, this improvement was not observed in subjects with pneumonia ($P = 0.097$).

CONCLUSION. All populations are susceptible to SARS-CoV-2 infection, and male and older-aged populations are at significantly higher risk. Increasing the amount of testing could significantly improve diagnosis rates, except for subjects with pneumonia. It is recommended to test twice in those high-risk individuals whose results are negative the first time, and performing 3 tests is better, if possible.

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Introduction

An ongoing outbreak of a novel viral pneumonia, formally called the coronavirus disease 2019 (COVID-19), has rapidly spread throughout the world and raised urgent global concerns (1–3). The causative pathogen is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), isolated by Chinese scientists on January 7, 2020 (4, 5). By April 7, 2020, there are 83,095 cumulative confirmed cases in China and more than 1.33 million cumulative confirmed cases in the world (data were available at https://ncov.dxy.cn/ncovh5/view/pneumonia).

Accumulated evidence has confirmed that SARS-CoV-2 infection shows sustained human-to-human transmission (6–8). Although SARS-CoV-2 causes a lower death rate than SARS, its transmission between individuals is much more prevalent. Besides typically infected patients, asymptomatic and mildly symptomatic infectors are becoming important infectious sources, which are not reported in SARS previously (9–12). More notably, the average incubation period of SARS-CoV-2 infection was 5.2 days, but some individuals may have a long incubation period of more than 14 days (13–15).

Table 1. Demographic characteristics of all included subjects

	All subjects (n = 5630)	SARS-CoV-2 (n = 1952)	Non–SARS-CoV-2 (n = 3678)	P value[A]
Sex, n (%)				0.016
Male	2631 (46.7)	955 (48.9)	1676 (45.6)	
Female	2999 (53.3)	997 (51.1)	2002 (54.4)	
Age				
Median (IQR), range, years	51 (36–63), 0–95	56 (42–66), 0–95	47 (34–60), 0–92	5.752×10^{-52}
Age groups, years, N (%)				2.297×10^{-42}
0–14	66 (1.2)	9 (0.5)	57 (1.5)	
15–49	2634 (46.8)	710 (36.4)	1924 (52.3)	
50–64	1706 (30.3)	642 (32.9)	1064 (28.9)	
≥ 65	1224 (21.7)	591 (30.3)	633 (17.2)	

[A]P value was calculated by Mann-Whitney U test.

These features determine that rapid and accurate diagnosis of SARS-CoV-2 in high-risk populations such as suspected cases and close contacts is crucial to control the pandemic in the community and hospitals. The Chinese center for disease and prevention recommends a specific real-time reverse transcription PCR (RT-PCR) method for diagnosis of SARS-CoV-2, by detecting its 2 target genes, *open reading frame 1ab* (*ORF1ab*) and *nucleocapsid protein* (*N*).

Several factors possibly affect detection results of SARS-CoV-2, including patient condition, viral load, sampling quality, specimen delivery time, and the testing process. Delineating features of SARS-CoV-2 in a large population is necessary to provide valuable information for clinical treatment and pandemic control. Here, we performed a large retrospective study to investigate the characteristics and diagnosis rate of 5630 subjects receiving SARS-CoV-2 nucleic acid tests from Wuhan, China.

Results

Characteristics of included subjects and confirmed cases of SARS-CoV-2 infection. Demographic characteristics of 5630 subjects are shown in Table 1. A total of 34.7% (1952 of 5630) of subjects were positive for SARS-CoV-2 nucleic acid test. More males were observed in the SARS-CoV-2 group than Non–SARS-CoV-2 group (48.9% vs. 45.6%, $P = 0.016$). The ages of the SARS-CoV-2 group were also older than ages of Non–SARS-CoV-2 group (all $P < 0.05$). Binary logistic regression analysis showed that males had a 1.14-fold increased risk of SARS-CoV-2 infection than females (OR = 1.14, 95% CI = 1.02–1.27, $P = 0.025$). Older age was another risk factor, and people older than 65 years had a 6.04-fold increased risk of SARS-CoV-2 infection (OR = 6.04, 95% CI = 2.96–12.31, $P = 7.545 \times 10^{-7}$). More details are shown in Table 2.

Moreover, we summarize characteristics of subjects of the SARS-CoV-2 group based on sex and age (10 years in each group) in Figure 1. In these 1952 confirmed cases of SARS-CoV-2 infection, the proportion of males (48.9% [955 of 1952]) was close to that of females (51.1% [997 of 1952]). The age of confirmed cases was in approximately normal distribution, regardless of whether subjects were male or female. The cases mainly concentrated in ages of 30–79 years, with a highest proportion in the age group of 50–69 years. These results suggested that all populations were susceptible to SARS-CoV-2 infection, especially in the middle-aged and elderly people.

Association between diagnosis rates and the amount of testing. The overall diagnosis rate of 5630 subjects was 34.7% (1952 of 5630). Because some subjects were tested multiple times due to medical considerations (detailed in Methods), we further compared the initial and overall diagnosis rates in all included subjects in order to examine whether increasing the amount of testing improve diagnosis rate. As shown in Table 3, results revealed that the overall diagnosis rate was significantly improved compared with the diagnosis rate of testing only once (34.7% vs. 30.6%, $P = 9.828 \times 10^{-52}$). The improvements were also demonstrated in males, females, different age groups (age ≥ 15 years), subjects from fever clinic, and subjects with pneumonia (all $P < 0.05$).

Testing the same population with the same amount of tests could provide an accurate comparison of improvement of diagnosis rates. Due to actual factors, only a small part of subjects were eligible for analysis if they met the following criteria: (a) subjects were suspected cases or close contacts of confirmed

Table 2. Risk factors of SARS-CoV-2 infection in all included subjects

	OR	95% CI	P value[A]
Sex			0.025
Female	Reference		
Male	1.14	1.02–1.27	
Age groups, years			2.525×10^{-39}
0–14	Reference		
15–49	2.40	1.18–4.87	0.016
50–64	3.93	1.93–7.99	1.610×10^{-4}
≥ 65	6.04	2.96–12.31	7.545×10^{-7}

[A]ORs, 95% CIs, and P values were adjusted by sex and age. Reference, 1.

cases of SARS-CoV-2; (b) subjects could be continuously followed in our hospital after the first testing; and (c) subjects received 3 or more SARS-CoV-2 nucleic acid tests. A total of 501 subjects fulfilled the inclusion criteria. Table 4 demonstrates the association between diagnosis rate and the amount of testing in these subjects. Results revealed that increasing the amount of testing could significantly improve the overall diagnosis rates ($P_{overall} = 9.851 \times 10^{-21}$). There was a 1.27-fold improvement from testing once to twice (from 27.9% to 35.5%) and a 1.43-fold improvement from testing once to 3 times (from 27.9% to 39.9%). Multiple between-group comparisons further supported the result ($P_{t = 1 \, vs. \, t = 2} = 5.847 \times 10^{-9}$, $P_{t = 1 \, vs. \, t = 3} = 7.797 \times 10^{-14}$, $P_{t = 2 \, vs. \, t = 3} = 0.002$). Similar trends were also observed in most subgroup analysis, but it was notable that increasing the amount of testing did not improve the diagnosis rate of subjects with pneumonia ($P_{overall} = 0.097$). In subjects older than 14 years and without pneumonia, diagnosis rates were significantly improved when the amount of testing increased from 1 to 2, except for subjects older than 65 years, and improved more when the amount of testing increased from 1 to 3 in all subgroups (all $P_{t = 1 \, vs. \, t = 3} < 0.05$). However, when the amount of testing increased from 2 to 3, the improvement was not significant in some subgroups. In addition, there were 154 subjects who received 4 administrations of SARS-CoV-2 nucleic acid tests. Thus, we also compared the diagnosis rates when the amount of testing increased from 3 to 4, and we identified that the overall diagnosis rate was not improved ($P = 1.000$). These results suggest that increasing the amount of testing could significantly improve the overall diagnosis rate of SARS-CoV-2, and 2 or 3 may be suitable.
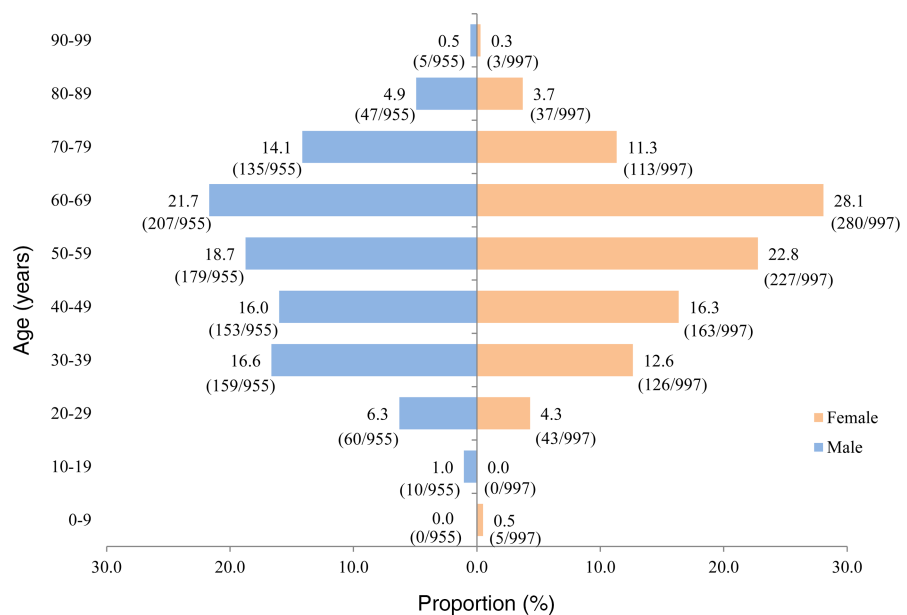

Figure 1. Characteristics of SARS-CoV-2 cases based on sex and age.

Table 3. The initial and overall diagnosis rates of the 5630 subjects

	Diagnosis rate, % (n/N)[A]		
	Initial (testing for the first time)	Overall	P value[B]
All subjects	30.6 (1721/5630)	34.7 (1952/5630)	9.828×10^{-52}
Sex			
Male	31.9 (840/2631)	36.3 (955/2631)	2.148×10^{-26}
Female	29.4 (881/2999)	33.2 (997/2999)	1.297×10^{-26}
Age groups, years, N (%)			
0–14	10.6 (7/66)	13.6 (9/66)	0.500
15–49	23.7 (623/2634)	27.0 (710/2634)	2.966×10^{-20}
50–64	33.6 (573/1706)	37.6 (642/1706)	2.695×10^{-16}
≥ 65	42.3 (518/1224)	48.3 (591/1224)	3.548×10^{-17}
Subjects from fever clinic	36.6 (1347/3685)	40.6 (1497/3685)	4.728×10^{-34}
Subjects with pneumonia	31.5 (108/343)	37.0 (127/343)	3.815×10^{-6}

[A]Initial diagnosis rate referred to the proportion of subjects with a positive result of SARS-CoV-2 at the first testing time. Overall diagnosis rate referred to the proportion of subjects with any positive result of SARS-CoV-2. [B]P value was calculated by McNemar's test.

Discussion

Speeding up the diagnosis of suspected cases and close contacts is the key to win the battle of the SARS-CoV-2 pandemic. In this retrospective study, we included 5630 subjects receiving a SARS-CoV-2 nucleic acid test from Wuhan, China. By analyzing the big data, we answered several crucial issues as follows: (a) the overall diagnosis rate of high-risk populations was 34.7%, and being male and of an older age were risk factors of SARS-CoV-2 infection; (b) all populations were susceptible to SARS-CoV-2 infection, especially people 50–69 years old; and (c) increasing the amount of testing could significantly improve the overall diagnosis rate of SARS-CoV-2, and 2 or 3 times may be suitable.

The positive result of a nucleic acid test is a golden criterion to confirm SARS-CoV-2 infection. Although reverse transcription PCR (RT-PCR) is an excellent recommended method for SARS-CoV-2 detection, the results are in risk of bias by viral load, specimen type, sample collection, and quality control of the testing process (16). Our study had distinct advantages to ensure the accuracy of testing results, including specimen collection by experienced clinicians, quick specimen transportation maintained in viral-transport medium, and timely detection under strict quality control in a laboratory certificated by College of American Pathologists (CAP).

Sex differences are quite common in human diseases. We identified that being male was a significant risk factor of SARS-CoV-2 infection, which could be supported by previous evidence. Sex differences were confirmed and considered a feature of SARS-CoV infection (17, 18). Recently, a systematic review and meta-analysis from arXiv preprint also found that males were more susceptible and vulnerable to SARS-CoV-2 than females (19). The mechanism of sex difference in SARS-CoV-2 is unclear, but potential explanations include smoking condition, different levels of ACE2 expression, and distinct immune responses between males and females (20–22). Aging is another crucial risk factor of human diseases. Results from our study also found that older people were more likely to be infected by SARS-CoV-2. In addition, we observed that SARS-CoV-2–infected cases occurred in all ages and presented in an approximately normal distribution. This finding was consistent with previous studies (14, 23). The smaller numbers of SARS-CoV-2 cases from children and quite older people are possibly due to less outdoor activities and, thus, less exposure to this virus.

Moreover, we identified that increasing testing times significantly improved diagnosis rate, with a 1.27-fold improvement from testing once to twice (from 27.9% to 35.5%) and a 1.43-fold improvement from testing once to 3 times (from 27.9% to 39.9%). More than 3 testing times seemed not to be helpful for further improvement. Increasing the amount of testing could effectively reduce biases from several actual factors, such as sample collection, viral load of different disease process, and patients' conditions. However, increasing testing times did not help improve diagnosis rate in people with pneumonia. A probable explanation is that SARS-CoV-2 cases with pneumonia have a high viral load and could be detected at the first testing. Based on these results, we recommend that 2 testing times should be performed in those high-risk people when the testing result is negative for the first testing, and 3 testing times is better, if available.

Table 4. Diagnosis rates in 501 subjects with 3 testing times

| | Diagnosis rate based on different testing times, % (n/N)[A] | | | | P values[B] | | |
	t = 1	t = 2	t = 3	Overall	t = 1 vs. t = 2	t = 1 vs. t = 3	t = 2 vs. t = 3
All subjects	27.9 (140/501)	35.5 (178/501)	39.9 (200/501)	9.851×10^{-21}	5.847×10^{-9}	7.797×10^{-14}	0.002
Sex							
Male	29.3 (65/222)	35.6 (79/222)	42.3 (94/222)	3.551×10^{-10}	0.004	5.997×10^{-7}	0.002
Female	26.9 (75/279)	35.5 (99/279)	38.0 (106/279)	7.771×10^{-12}	3.576×10^{-7}	2.135×10^{-7}	0.371
Age groups, years, n (%)							
0–14	37.5 (3/8)	37.5 (3/8)	37.5 (3/8)	1.000	–	–	–
15–49	28.4 (66/232)	38.4 (89/232)	42.2 (98/232)	8.164×10^{-12}	7.152×10^{-7}	1.275×10^{-7}	0.154
50–64	24.1 (41/170)	29.4 (50/170)	32.9 (56/170)	1.120×10^{-5}	0.013	1.831×10^{-4}	0.173
≥ 65	33.0 (30/91)	39.6 (36/91)	47.3 (43/91)	5.718×10^{-5}	0.125	7.323×10^{-4}	0.052
Subjects from fever clinic	18.4 (44/239)	23.4 (56/239)	29.7 (71/239)	1.477×10^{-9}	0.014	1.687×10^{-6}	0.001
Subjects with pneumonia	23.5 (8/34)	26.5 (9/34)	32.4 (11/34)	0.097	–	–	–

[A]t refers to the first testing times. Diagnosis rate when t = 1 refers to the proportion of subjects with a positive result of SARS-CoV-2 at the first testing time, diagnosis rate when t = 2 refers to the proportion of subjects with any positive result of SARS-CoV-2 at the first 2 testing times, and so on. [B]$P_{overall}$ values were calculated by Cochran's Q test, and P values of comparisons between groups were calculated by McNemar's test and further adjusted by Bonferroni correction.

A main limitation of this study was that only throat swabs were evaluated. Compared with specimens from the lower respiratory tract, including bronchoalveolar lavage fluid and sputum, nasopharyngeal swabs and throat swabs are more convenient and suitable for universal screening of SARS-CoV-2 cases in high-risk populations, such as suspected cases or those with close contact with suspected cases. A recent study found that positive rate of nasal swabs (5 of 8) was higher than that of throat swabs (126 of 398), but the result was possibly biased by the quite smaller number of nasal swabs (24). Another study reported a diagnosis rate of 38.25% in 4818 subjects for nasopharyngeal swabs (25). It seemed a litter higher than our diagnosis rate of 34.7% in 5630 subjects. In spite of this, biases from sampling, testing, or patients' conditions could be also reduced by increasing the amount of testing. This strategy is theoretically suitable to improve diagnosis rate of nasopharyngeal swabs.

In summary, the overall diagnosis rate of SARS-CoV-2 was 34.7% in 5630 subjects from Wuhan, China. All populations were susceptible, and being male and of an older age were risk factors of SARS-CoV-2 infection. Increasing the amount of testing could significantly improve the overall diagnosis rate of SARS-CoV-2, except for suspected cases with pneumonia. We recommend that 2 testing times should be performed in those high-risk people when the testing result is negative for the first testing, and 3 testing times is better, if available. To the best of our knowledge, this is the first study to delineate characteristics and testing strategy of SARS-CoV-2 in a large population. This work facilitates a better understanding of SARS-CoV-2 detection and provides directive and feasible information to combat the SARS-CoV-2 pandemic.

Methods

Data collection. Data were collected from January 22 to February 18, 2020, at Tongji Hospital in Wuhan, China. A total of 5630 subjects who were at high risk of SARS-CoV-2 infection were included. These subjects met one of the following criteria: (a) subjects presented fever and/or typical respiratory symptoms; (b) subjects presented radiological characteristics, including bilateral pneumonia, unilateral pneumonia, or ground-glass opacity; and (c) subjects had potential epidemiological exposure history of COVID-19. Throat swabs were collected from each subject by clinicians; they were maintained in viral-transport medium and transferred to the Department of Laboratory Medicine (Tongji Hospital) for SARS-CoV-2 testing as soon as possible. Testing results were reported within 24 hours of sample collection. Characteristics of subjects were obtained from medical records.

Detection of SARS-CoV-2. Viral RNA extraction was conducted using a Magnet Viral Nucleic Acid Extraction Kit on PAN9600 Automated Nucleic Acid Extraction System (Tianlong). The SARS-CoV-2 nucleic acid detection kit (Shanghai Huirui Biotechnology Co. Ltd) was approved by National Health

Commission of China, which was provided by Wuhan Center for Disease Control and Prevention. In the early outbreak of SARS-CoV-2, Shanghai Huirui Biotechnology Co. Ltd was one of the first recommended companies providing SARS-CoV-2 nucleic acid detection kits by the National Health Commission of China. Two target genes, *ORF1ab* and *N*, were simultaneously amplified using the real-time RT-PCR assay. If the Ct value was lower than 35, the result was considered as positive. If the Ct value was greater than or equal to 39.2, the result was considered as negative. If the Ct value was between 35 and 39.2, the result required retesting for confirmation. Three negative controls and 1 positive control were set in each run of the experiment to ensure the quality-of-detection process. All procedures were performed according to the manufacturer's protocol.

SARS-CoV-2 is a new virus leading to a rapid outbreak of novel viral pneumonia, and many features of this disease were unclear during its early outbreak in Wuhan. To better admit SARS-CoV-2 patients, some doctors suggested testing subjects more than once based on their medical condition. Usually, a subject was tested multiple times due to the following reasons: (a) a suspected case presented typical clinical symptoms (e.g., fever, cough) and/or abnormal imaging of chest radiography/CT, but the result of nucleic acid test of SARS-CoV-2 was negative or inconclusive; (b) a positive case did not show typical clinical symptoms, but the result of the nucleic acid test of SARS-CoV-2 was close to cutoff value of the diagnostic kit; and (c) a positive case had an improved condition after receiving clinical treatments for several days and was then checked for the status of SARS-CoV-2. When testing for SARS-CoV-2 multiple times, the time interval between 2 tests was at least 2 days.

Statistics. Continuous variables are expressed as median and interquartile range (IQR). Categorical variables are expressed as frequency or percentage. Mann-Whitney *U* test was used to compare the difference of independent samples between 2 groups. McNemar's test or Cochran's Q test was used to examine the difference of related samples between 2 or multiple groups, respectively. Binary logistic regression analysis was performed to estimate the ORs and 95% CI of factors associated with the diagnosis rate of SARS-CoV-2 infection. *P* values of multiple between-group comparisons were adjusted by Bonferroni correction. Statistical analyses were performed using IBM SPSS Statistics version 20.0 and GraphPad Prism 8.0.2. A 2-sided *P* value below 0·05 was considered as statistically significant.

Study approval. This retrospective study was approved by the Ethics Committee of Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology (TJ-IRB20200201). Because of the rapid emergence of this infectious disease, and retrospective and anonymous analysis, written informed consent was waived.

Author contributions

YL, LC, and ZS designed the study and supervised the research. NS, YZ, XW, and JP performed the assays and collected data. NS, WL, and FW performed statistical analyses. All authors contributed to the writing and editing of the manuscript and approved the manuscript

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