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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

Rui Liu[a,1], Huan Han[a,1], Fang Liu[b,c], Zhihua Lv[a], Kailang Wu[b], Yingle Liu[b], Yong Feng[d,*], Chengliang Zhu[a,*]

[a] Dept. of Clinical Laboratory, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei, China
[b] Dept. State Key Laboratory of Virology (Wuhan University), College of Life Sciences of Wuhan University, Wuhan 430072, Hubei, China
[c] Dept. Wuhan Institute of Biotechnology, Wuhan 430075, Hubei, China
[d] School of Basic Medical Sciences, Wuhan University, Wuhan 430071, Hubei, China

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ABSTRACT

Background: There's an outbreak of a novel coronavirus (SARS-CoV-2) infection since December 2019, first in China, and currently with more than 80 thousand confirmed infection globally in 29 countries till March 2, 2020. Identification, isolation and caring for patients early are essential to limit human-to-human transmission including reducing secondary infections among close contacts and health care workers, preventing transmission amplification events. The RT-PCR detection of viral nucleic acid test (NAT) was one of the most quickly established laboratory diagnosis method in a novel viral pandemic, just as in this COVID-19 outbreak.

Methods: 4880 cases that had respiratory infection symptoms or close contact with COVID-19 patients in hospital in Wuhan, China, were tested for SARS-CoV-2 infection by use of quantitative RT-PCR (qRT-PCR) on samples from the respiratory tract. Positive rates were calculated in groups divided by genders or ages.

Results: The positive rate was about 38% for the total 4880 specimens. Male and older population had a significant higher positive rates. However, 57% was positive among the specimens from the Fever Clinics. Binary logistic regression analysis showed that age, not gender, was the risk factor for SARS-CoV-2 infection in fever clinics.

Conclusions: Therefore, we concluded that viral NAT played an important role in identifying SARS-CoV-2 infection.

1. Introduction

Since December 2019, an epidemic Coronavirus disease (COVID-19) caused by novel coronavirus (SARS-CoV-2) infection has occurred unexpectedly in China. Till March 2, 2020, more than 80 thousand confirmed cases have been reported in China. Of these cases, 49 thousand were identified in Wuhan City. Identification, isolation and caring for patients early are essential to limit human-to-human transmission including reducing secondary infections among close contacts and health care workers, preventing transmission amplification events. Following the national recommendations for diagnosis and treatment of pneumonia caused by 2019-nCoV (the 5th edition) and current status of clinical practice in Hubei Province, RT-PCR analysis was used to detect the causative virus from respiratory secretions. However, some investigators and clinical doctors argued that CT imaging should be served to identify the SARS-CoV-2 infection instead, since quite a bit cases showed progressive multiple peripheral ground-glass opacities in both lungs even the RT-PCR results were negative [1,2].

Since RT-PCR was one of the most quickly established laboratory diagnosis method in a novel viral pandemic, just as this COVID-19, it served efficiently to confirm a viral infection within 2 h. During the early Feb, the need of detect increased dramatically up to 10 thousands of samples per day in Wuhan City. But to what extend was this RT-PCR based viral nucleic acid test (NAT) could reflect the real SARS-CoV-2 infection?

Therefore in this study, we retrospectively analysed NAT test of 4880 cases from Jan 22 to Feb 14, 2020 in Renmin Hospital of Wuhan University. All the cases were suspected of SARS-CoV-2 infection because of, (1) typical respiratory infection symptoms such as fever, cough and dyspnoea, or (2) close contact with a COVID-19 patient. RT-

* Corresponding authors.
E-mail addresses: yongfeng@whu.edu.cn (Y. Feng), xinchengzhu@163.com (C. Zhu).
[1] These authors contribute equally to this study.

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Table 1
SARS-CoV-2 NAT positive rate of 4880 cases with their respiratory specimens by RT-PCR.

Nuclear Acid	Sputum (n = 57)		Bronchoalveolar Lavage Fluid (n = 5)		Nasal and Pharyngeal Swabs (n = 4818)		Total	Total Positive Rate
	n	Positive Rate	n	Positive Rate	n	Positive Rate		
NP	28	49.12%	4	80.00%	1910	39.64%	1942	39.80%
ORF1ab	29	50.88%	5	100.00%	1966	40.81%	2000	40.98%
Double positive	28	49.12%	4	80.00%	1843	38.25%	1875	38.42%

Table 2
SARS-CoV-2 NAT positive rate in Male and Female groups.

Nuclear Acid	Male (n = 2251)		Female (n = 2629)		χ^2	p value
	n	Positive Rate	n	Positive Rate		
NP	943	41.89%	999	38.00%	7.672	0.006
ORF1ab	967	42.96%	1033	39.29%	6.739	0.009
Double positive	910	40.43%	965	36.71%	7.095	0.008

PCR were performed to detect ORF1ab and NP genes fragments from respiratory specimens, including nasal and pharyngeal swabs (NPS), bronchoalveolar lavage fluid (BLF) and sputum.

2. Materials and methods

2.1. Data collection

4880 cases from Jan 22 to Feb 14 were tested for SARS-CoV-2 infection in Renmin Hospital of Wuhan University, who were suspected or at high risk of infection because of, (1) typical respiratory infection symptoms such as fever, cough and hard breath, or (2) close contact with a SARS-CoV-2 patients. Among these cases, 2251 were man (46.13%), 2629 were women (53.87%). The Median Age was 50 years (IQR = 27). Groups based on age: 18–29 (n = 482), 30–39 (n = 1097), 40–49 (n = 841), 50–59 (n = 1011), 60–69 (n = 886), > 70 (n = 563). This study was approved by the Ethics Committee of the Renmin Hospital of Wuhan University (WDRM2020-K066) and the need for informed consent was waived.

3. RT-PCR detection

The presence of SARS-CoV-2 in respiratory specimens was detected by real-time RT-PCR amplification of SARS-CoV-2 open reading frame 1ab (ORF1ab), nucleocapsid protein (NP) genes fragments using kits provided by Shanghai Huirui Biotechnology Co., Ltd. Conditions for amplifications were 50 °C for 15 min, 95 °C for 3 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 30 s. As previously described [3], when two targets (ORF1ab, NP) tested positive by specific real-time RT-PCR, the case would be considered to be laboratory-confirmed. A cycle threshold value (Ct-value) less than 37 was defined as a positive test, and a Ct-value of 40 or more was defined as a negative test. A medium load, defined as a Ct-value of 37 to less than 40, required confirmation by retesting.

Table 4
Risks of SARS-CoV-2 positive rate upon Gender and Age.

Risk factors	Regression coefficient[a]	p value	odds ratio	95% CI for coefficient
Gender	0.147	0.016	1.158	1.028–1.305
Age	0.032	< 0.001	1.033	1.029–1.037

[a] These are regression coefficients adjusted for gender and age. CI = confidence interval.

3.1. Statistical analysis

Statistical analyses were done by using the SPSS25.0 software. Chi-square test was used to compare inter-group differences, and binary logistic regression analysis was performed to analyze the risk factors for SARS-CoV-2 prevalence. Statistical significance was defined as $P < 0.05$.

4. Results

4.1. Nasal and pharyngeal swabs showed poor positive rate in 4880 cases

1875 out of 4880 (38.42%) were positive by RT-PCR-based NAT test with their respiratory specimens. Among this, 39.80% were positive for SARS-CoV-2-NP and 40.98% for SARS-CoV-2-ORF1ab (Table 1). We could see that the bronchoalveolar lavage fluid (BLF), exhibited the most highest positive rate of 100% for SARS-CoV-2 ORF1ab gene (n = 5). The nasal and pharyngeal swabs (NPS) samples (n = 4818) showed a poor positive rate of 38.25%. The Sputum exhibited a 49.12% positive rate.

4.2. Male had a higher positive rate than female in the total 4880 cases

The male patients are 2251, female are 2629. There is no obvious gender difference during sample collection. But for male, 40.43% were positive while for female, 36.71% were positive (Table 2). The Positive Rate were significantly higher in Male than in Female cases ($p < 0.01$).

Table 3
SARS-CoV-2 NAT positive rate in groups according to age.

Nuclear Acid	18–29 (n = 482)	30–39 (n = 1097)	40–49 (n = 841)	50–59 (n = 1011)	60–69 (n = 886)	≥70 (n = 563)	χ^2	p value
Gender rate (M/F)	229/253	494/603	389/452	451/560	417/469	271/292	3.071	0.689
nCov-NP	129	280	287	446	439	361	320.802	< 0.001
nCovORF1ab	124	286	296	461	466	367	353.547	< 0.001
Double Positive	120	271	278	434	424	348	306.946	< 0.001
Double Positive Rate	24.90%	24.70%	33.06%	42.93%	47.86%	61.81%		

Table 5
SARS-CoV-2 NAT positive rate of 1707 cases from Fever Clinics.

Nuclear Acid	Sputum (n = 14)		Bronchoalveolar Lavage Fluid (n = 3)		Nasal and Pharyngeal Swabs (n = 1690)		Total	Total Positive Rate
	n	Positive Rate	n	Positive Rate	n	Positive Rate		
NP	11	78.57%	2	66.67%	980	57.99%	993	58.17%
ORF1ab	12	85.71%	3	100.00%	1031	61.01%	1046	61.28%
Double positive	11	78.57%	2	66.67%	960	56.80%	973	57.00%

Table 6
SARS-CoV-2 NAT positive rate in Fever Clinics grouped according to age.

Nuclear Acid	18–29 (n = 103)	30–39 (n = 241)	40–49 (n = 292)	50–59 (n = 385)	60–69 (n = 450)	≥70 (n = 236)	χ^2	p value
Gender rate (M/F)	58/45	126/115	152/140	185/200	224/226	120/116	2.988	0.702
nCov-NP	41	119	161	222	264	186	64.456	< 0.001
nCovORF1ab	41	127	168	234	289	187	62.98	< 0.001
Double Positive	40	118	157	219	258	181	58.835	< 0.001
Double Positive Rate	38.83%	48.96%	53.77%	56.88%	57.33%	76.69%		

Table 7
SARS-CoV-2 NAT positive rate in Fever Clinics in different date periods.

Nuclear Acid	22–29th Jan (n = 286)		30th Jan–6th Feb (n = 988)		7th–14th Feb (n = 433)		χ^2	p value
	n	Positive Rate	n	Positive Rate	n	Positive Rate		
NP	152	53.15%	648	65.59%	193	44.57%	58.205	< 0.001
ORF1ab	169	59.09%	668	67.61%	209	48.27%	48.166	< 0.001
Double positive	152	53.15%	631	63.87%	190	43.88%	51.148	< 0.001

4.3. The positive rate increased in older cases

When we analyzed the positive rate according to age (Table 3), and we could see that positive rate increased from 24.90% (age 18–30) to 61.81% (age > 70).

4.4. Gender and age are two risk factors for SARS-CoV-2 infection

Binary logistic regression analysis showed that gender and age were two risk factor for SARS-CoV-2 infection (Table 4). Male and older people were more sensitive to the this novel virus infection ($p < 0.05$).

4.5. Specimens from Fever Clinics exhibited significant higher positive rate

Why the positive rate in this 4880 cases were this low? Or did the viral NAT test fail to serve for the diagnosis in this pneumonia epidemic? Since the 4880 specimens conclude all cases during the period in the hospital, such as fever, cough and hard breath, or close contact with COVID-19 patient, we further analyzed data from adult fever clinics (n = 1707, Table 5). Notably, the positive rate of patients in fever clinics (57.00%) was significant higher than the rate of the total (38.42%).

4.6. The age, not the gender, was the risk factor for SARS-CoV-2 infection in Fever Clinics

When we analyzed the positive rate between Male and Female cases from Fever Clinics, we did not observed significant difference. There were 499 positive male (57.69%) and 474 female (56.29%) from 1707 total Fever Clinics specimens.

When we analyzed data in age-based groups in Fever Clinics, we could see the older had higher positive rate (Table 6). Positive rate in each group were 38.83%, 48.96%, 53.77%, 56.88%, 57.33% and 76.69%, which were significantly higher than the rate in the total 4880 cases.

Binary logistic regression analysis showed that the age, not the gender, was the risk factor for SARS-CoV-2 infection in Fever Clinics.

There was a quite special period that a large amount of patients rushed to health care centres or fever clinics because of panic in early Feb in Wuhan City, when a bigger amount of patients far beyond the capacity of laboratory testing. We analyzed positive percentage for SARS-CoV-2 infection in each week during 22th Jan to 14th Feb from Fever Clinics in Renmin Hospital. 63.87% (631 out of 988) were double positive for viral genes testing, which indicated a real high number of SARS-CoV-2 infection among this panic suspect population (see Table 7).

5. Discussion

For the 2019 novel coronavirus disease (COVID-19), patients can be afebrile in the early stages of infection, with only chills and respiratory symptoms, but not always high temperature [4–6]. Elevated C-reactive protein (CRP) and lymphopenia are important factors. More and more evidence had shown distinct and complicated performance of COVID-19 as compared to SARS or MERS, which provided typical clinical symptoms for diagnosis [5]. Therefore, diagnosis of suspected SARS-CoV-2 caused pneumonia in Wuhan was based on clinical characteristics, chest imaging [1,7], and the ruling out of common bacterial and viral pathogens that cause pneumonia as suggested by the latest National recommendations for diagnosis and treatment of pneumonia caused by 2019-nCoV (the 6th edition).

Here in this study, we analyzed recent 4880 cases by laboratory diagnosis of suspect SARS-CoV-2 infection in one hospital in Wuhan City, showing 38.42% positive percentage for total, but 57.00% positive

in Fever Clinics. Relatively increased positive percentage in Fever Clinics suggested that the fever associated clinical symptoms were linked with viral NAT, at some degree. Interestingly, total positive percentage were associated with gender and age, while positive percentage in Fever Clinics was only associated with age, not gender. Therefore, consistent with other reports, we could conclude that for suspect SARS-CoV-2 infection, positive percentage would be higher in Male and Old, but in Fever Clinics, gender was not a risk factor.

The epidemic seems to be spreading rapidly worldwide, especially in Korea, Italy and Iran. Based on the reasons mentioned above, we suggest that viral NAT is a rapid, easy conducted, and widely used laboratory diagnosis for SARS-CoV-2 infection, especially for Fever Clinics. Clinical characteristics, chest imaging and etiology testing based on viral genes RT-PCR should be combined to tell a confirmation [4,8]. Since there exists infection with atypical symptoms, RT-PCR should be taken for several times just in case of fake negative results.

For those countries who are facing an increased number of SARS-CoV-2 infection, we strongly suggest to prepare more reagents for SARS-CoV-2 NAT. Also, more laboratories and facilities should be trained and prepared for RT-PCR detection under a Biological Safety Protection 3-Level, just in case the horrible increasing requirements for detection that happened in early Feb in Wuhan, China.

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Author contributions

Y.F and C.L.Z designed the study and analyzed the data. R.L and H.H collected and analyzed the data. F.L., Z.H.L., Y.L.L., and K.L.W wrote the paper. Y.F and C.L.Z read and approved the final manuscript.

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