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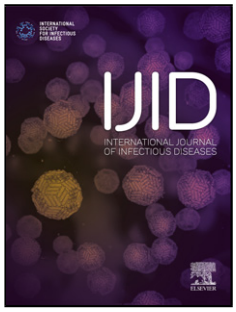

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Comparison of Different Samples for 2019 Novel Coronavirus Detection by Nucleic Acid Amplification Tests

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Abstract

A severe respiratory ongoing outbreak of pneumonia associated with 2019 novel coronavirus was recently emerged in China. Here, we reported the epidemiological, clinical, laboratory and radiological characteristics of 19 suspect cases. We compared

the positive ratio of 2019-nCoV nucleic acid amplification test from different samples including oropharyngeal swab, blood, urine and stool with 3different Fluorescent RT-PCR kits. Nine out of the 19 patients were detected 2019-nCoV infection using oropharyngeal swab samples, and the virus nucleic acid was also detected in eight of these nine patients using stool samples. None of positive results was identified in the blood and urine samples. Thses three different kits got the same result for each sample and the positive ratio of nucleic acid detection for 2019-nCoV was only 47.4% in the suspect patients. Therefore, it is possible that the really infected patients have been missed by using nucleic acid detection only. It might be better to make a diagnosis combining the Computed Tomography scans and the nucleic acid detection together.

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Most human coronavirus infections in the past 20 years were not regarded as highly pathogenic to human beingsuntil the outbreak of severe acute respiratory syndrome and Middle East respiratory syndrome coronavirus (Zhong et al., 2003; Drosten et al., 2003; Fouchier et al., 2003). Although coronaviruses are broadly distributed in humans and animals, knowledge of non-segmented positive sense RNA viruses is limited (Cui et al., 2019; Woo et al., 2012).At the end of 2019, the China country office of the World Health Organization(WHO) reported a cluster of pneumonia cases in Wuhan City, China, and the causative pathogen was identified one week later as a novel coronavirus (2019-nCoV) (Wu et al., 2020; Zhou et al., 2020). China's National Health Commission provided guidance to laboratories and WHO has named this disease *COVID-19* (Wang et al., 2020).

A total of 19 suspected cases were collected at Sichuan Provincial People's Hospital (ten patients) and Sichuan Mianyang 404 Hospital (nine patients). All study procedures conformed with the Declaration of Helsinki, and the protocol was accepted by the Institutional Review Board and the Ethics Committee of Sichuan Provincial People's Hospital. Each participant participated in the study voluntarily and provided signed informed consent. We collected four kinds of sample: oropharyngeal swabs, blood, urine, and stool sample-from the 19 cases for nucleic acid detection. We reviewed all patients' medical histories, clinical charts, nursing records, physical findings, and computed tomography (CT) scans, and the hematological, biochemical, radiological, and microbiological investigation results were recorded and analyzed.

Almost all the suspected patients had symptoms of respiratory disease and two had diarrhea. Oropharyngeal swab specimens were obtained and sent for detection of viral respiratory pathogens by nucleic acid amplification testing (NAAT). All of the 19 cases were reported as negative for all other known pathogens tested, including influenza A and B, parainfluenza, respiratory syncytial virus, rhinovirus, adenovirus, and four common coronavirus strains known to cause illness in humans (HKU1, NL63, 229E, and OC43). Stool samples from the two diarrhea cases were tested for common diarrheal pathogens to rule out other causes (To et al., 2019).

Viral nucleic acid was extracted from the specimens following a common workflow and stored at -80°C.Two highly conserved sequence regions (ORF1b and N) in rotavirus were selected for primers and probes design. Three different 2019-NCoV Fluorescent RT-PCR Kits with different manufacturers but almost the same detection efficiency was used for real-time-PCR assay, including GeneoDx (GZ-TRM2, China), Maccura (Sichuan, China) and Liferiver (W-RR-0479-02, China).

As Table1 shown, the median age was 33 years, and 57.89%were women. According to the results of the oropharyngeal swab NAAT, nine patients were confirmed to be infected with 2019-nCoV, and the other ten cases were negative for 2019-nCoV based on the nucleic acid test results. We found that all nine confirmed patients and five out of the ten negative cases showed bilateral distribution of patchy shadows and patchy ground-glass opacities in CT scans (Figure 1). To avoid false negative results, we recollected oropharyngeal swab specimens for these negative cases and reconducted

the 2019-nCoV nucleic acid tests for three consecutive days. However, the results all remained negative.

Therefore, we extracted RNA from the blood, urine, and stool of all 19cases to determine whether the 2019-nCoV could be detected by NAAT (Table 1). In the nine confirmed patients, eight stool samples showed positive results for 2019-nCoV; interestingly, the virus could still be detected in stool samples from patients without diarrhea symptoms. However, the other ten cases showed negative results for 2019-nCoV in stool samples, and all of the 19 cases showed negative results for 2019-nCoV in both blood and urine samples. To avoid false results, we used three different kits to test samples and got the same result for each sample.

Although no nucleic acid positives were detected in serum, we cannot say that the virus will not enter the blood; it might be at a low concentration. None of the patients assessed in this study were diagnosed with viremia, but it has previously been reported that viruses have been detected in the sera of patients with viremia who were infected with other coronaviruses (Wang et al., 2020). The number of positive oropharyngeal swab samples was very close to the number of positive stool samples, and eight stool samples tested positive in nine patients who were confirmed using the oropharyngeal swab NAAT. This may indicate that feces may be capable of transmitting infection (further study is needed to determine whether the whole virus is found in feces or just pieces of nucleic acid) even if the patient does not have diarrhea.

In this study, we compared the positive ratio of 2019-nCoV NAAT from different samples, and the positive ratio of nucleic acid detection for 2019-nCoV was only 47.4%. Therefore, precise diagnosis of COVID-19 seemed very difficult by relying on nucleic acid detection alone. It might be better to reach a diagnosis by combining CT scans and NAAT results, and this may be very important for the prevention and control of COVID-19.

Statements

This work has no conflict of interest. The protocol was accepted by the Institutional Review Board and the Ethics Committee of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. This work was supported by Sichuan Science and Technology Program (No. to ZL Yang). No funding organization played role in the study design or conduct.

Conflict of Interest Statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company.

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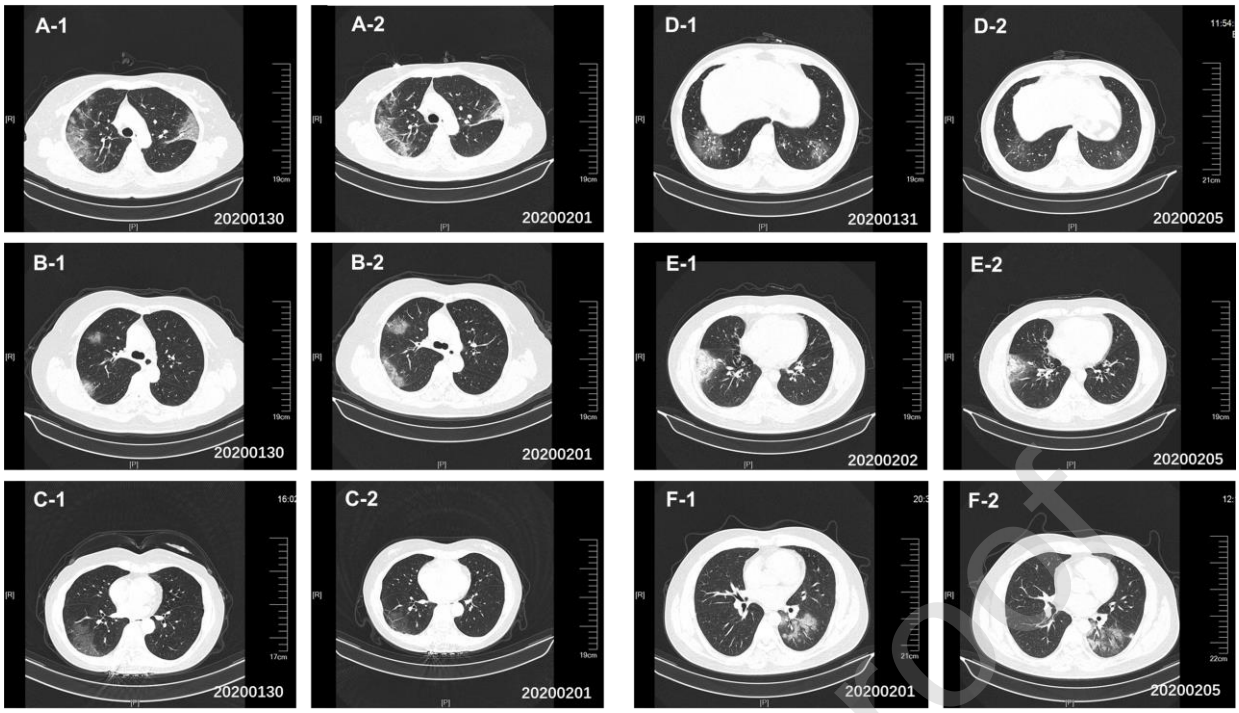

Figure 1. CT scans of the 2019-nCoV nucleic acid–detected positive patients. Increasing and multifocal ground-glass changes were visible. A: patient 1, January 30, 2020 (hospital day 2, illness day 5, A-1); February 1, 2020 (hospital day 4, illness day 7, A-2). B: patient 2, January 30, 2020 (hospital day 2, illness day 5, B-1); February 1, 2020 (hospital day 4, illness day 7, B-2). C: patient 3, January 30, 2020 (hospital day 1, illness day 6, C-1); February 1, 2020 (hospital day 3, illness day 8, C-2). D: suspected case 1, January 31, 2020 (hospital day 1, illness day 4, D-1); February 1, 2020 (hospital day 6, illness day 9, D-2). E: suspected case 3, February 1, 2020 (hospital day 1, illness day 6, E-1); February 5, 2020 (hospital day 5, illness day 10, E-2). F: suspected case 4, January 31, 2020 (hospital day 1, illness day 2, F-1); February 1, 2020 (hospital day 4, illness day 5, F-2).

Table1. 2019-nCoV nucleic acid detection results of the 19 cases in different samples and Characteristics index of these cases.

	Age (years)	Sex	CT scan results are abnormal	Presenting symptoms and signs				Nucleic acid test of 2019-nCoV				Several Laboratory Plasma data					
				Fever	Cough	Fatigue	Diarrhea	Throat swabs	Stool sample	Urine sample	Blood sample	Lymphocyte count(cells/L) (1.0–3.2 × 10^9)	Hematocrit (0.35-0.45)	Activated partial thromboplastin time (s);(23.3-32.5)	Fibrinogen (g/dL); (1.80-3.50)	C-reactive protein (mg/L); (0.0–5.0)	Urea (mmol/L); (2.8–8.1)
Patient 1	62	Female	+	-	+	+	+	+	+	–	–	1.74	0.355	27	4.04(↑)	9.56(↑)	4.19
Patient 2	45	Female	+	+	+	+	–	+	+	–	–	0.901(↓)	0.371	34.6(↑)	4.33(↑)	22.56(↑)	2.70(↓)
Patient 3	59	Female	+	+	+	–	–	+	+	–	–	1.065(↓)	0.331(↓)	34.2(↑)	4.75(↑)	24.6(↑)	2.86(↓)
Patient 4	33	Female	+	+	–	–	–	+	+	–	–	1.52	0.308(↓)	33.2	2.49	37.13	2.7
Patient 5	34	Male	+	+	–	–	–	+	-	–	–	NA	NA	39.0(↑)	3.59(↑)	NA	3.89
Patient 6	43	Male	+	+	+	+	-	+	+	–	–	NA	NA	29.1	4.03(↑)	9.46(↑)	3.0(↓)
Patient 7	26	Male	+	+	+	+	-	+	+	–	–	0.900(↓)	0.137(↓)	37.7	3.69(↑)	20.24(↑)	3.0(↓)
Patient 8	18	Female	+	+	-	-	-	+	+	–	–	1.97	0.36	30.5	2.34	0.94	4.1
Patient 9	25	Male	-	+	+	-	-	+	+	–	–	0.490(↓)	0.380(↓)	33.4	3.91	22.66(↑)	3.9
Suspect cases1	31	Male	+	+	+	+	-	–	–	–	–	1.117	0.359(↓)	34.1(↑)	2.5	146.64(↑)	2.78(↓)
Suspect cases2	33	Male	+	+	-	-	+	–	–	–	–	1.712	0.507(↑)	33.9(↑)	1.20(↓)	19.60(↑)	3.82
Suspect cases3	33	Male	+	+	+	-	-	–	–	–	–	1.564	0.464	34.5(↑)	0.90(↓)	105.93(↑)	5.35
Suspect cases4	39	Male	+	-	-	+	-	–	–	–	–	1.444	0.489	31.3	4.48(↑)	8.27(↑)	2.49(↓)
Suspect cases5	50	Female	+	-	+	+	-	–	–	–	–	1.766	0.37	31.3	2.27	2.3	2.9
Suspect cases6	38	Female	+	+	+	+	-	–	–	–	–	1.07	0.371	32.1	3.80(↑)	38.95(↑)	3.71
Suspect cases7	31	Female	+	+	+	-	-	–	–	–	–	1.588	0.304(↓)	31.3	2.96	4.78	3.14

Suspect cases8	34	Female	+	-	-	-	-		-	-	-	-		1.597	0.416	30.2	0.50(↓)	1.25	2.98
Suspect cases9	23	Female	+	-	+	+	+		-	-	-	-		1.534	0.443	29.7	3.96(↑)	2.11	2.97
Suspect cases10	8	Female	-	+	+	-	-		-	-	-	-		2.846	0.378	NA	NA	20.52(↑)	NA

+ =positive. – =negative. ↑=above normal range. ↓=below normal range.