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The persistence and clearance of viral RNA in 2019 novel coronavirus disease survivors

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Abstract

Objective: To analyze the clearance time and the influencing factors of 2019-nCoV RNA in different samples from the patients with 2019 novel coronavirus disease (COVID-19), which may provide further evidence to improve the management of the patients during convalescence.

Methods: The clinical data and the laboratory test results of convalescent patients with COVID-19, who were admitted to Shanghai Public Health Clinical Center from January 20, 2020 to February 10, 2020, were collected retrospectively. The RT-PCR results for patients' oropharyngeal swab, stool, urine, and serum samples were collected and analyzed. The convalescent patients refer to the recovered non-fever cases without respiratory symptoms, and had two successive(upon 24h sampling interval) negative RT-PCR results of viral RNA for oropharyngeal swabs. The effects of CD4 + T lymphocytes, inflammatory indicators, and glucocorticoid treatment on viral nucleic acid clearance were analyzed.

Result:

In the 292 confirmed cases, 66 patients recovered after treatment, and were included in our study. Totally 28(42.4%) females and 38 males (57.6%) with a median age of 44.0 (34.0-62.0) year-old were analyzed. After in-hospital treatment, patients' inflammation indicators decreased with improved clinical condition. The median time from the onset of symptoms to first negative RT-PCR results for oropharyngeal swabs of convalescent patients was 9.5 (6.0-11.0) days. By the time of February 10, 2020, 11 convalescent cases (16.7%) still tested positive for viral RNA in stool specimens, and the other 55 patients' stool specimens turned negative for 2019-nCoV with median duration of 11.0（9.0-16.0）days. Among these 55 patients, 43 cases had longer duration for stool specimens detected negative for viral RNA than throat swabs, with median delay of 2.0 (1.0-4.0) days. The positivity of viral nucleic acid test for urine samples was low with only 4 (6.9%) positive results in 58 cases, viral RNA in 3 patients' urine specimens remained tested positive after throat swabs turned negative. Using multiple linear regression model, the analysis showed the CD4 + T lymphocyte counts may help predict the the duration of viral RNA detection in patients' stool ($P = 0.01$). The duration of viral RNA detection for oropharyngeal swabs and feces in the corticosteroid treatment group was longer than that in the non-corticosteroid treatment group, which were 15 days vs 8.0 days ($P = 0.013$) and 20 days vs 11 days ($P < 0.001$), respectively. There was no statistically significant difference on the inflammation indicators between patients with positive fecal viral RNA test results and the negative group ($P > 0.05$).

Conclusion:

In brief as the clearance of viral RNA in patients stool was delayed comparing to oropharyngeal swabs, it is important to detect the viral RNA in feces during the convalescence. As the delayed clearance of viral RNA in corticosteroid treatment group, corticosteroids is not recommended in the treatment of COVID-19, especially for the mild conditions cases. The duration of RNA detection may relate to the host cell immunity.

Keywords: 2019-nCoV; COVID-19; nucleic acid detection

In January 2020, a new coronavirus was confirmed as the cause of unexplained pneumonia in a group of patients from Wuhan, Hubei, and was subsequently named as "2019 novel coronavirus (2019-nCoV)" [1]. Due to more and more cases were reported out of China, the World Health Organization (WHO) announced on the evening of January 30 that the emerging new coronavirus pneumonia epidemic has constituted the "PHEIC" ("International Public health emergencies of concern ") [2]. Till February 11, 2020, there was 44,730 confirmed cases reported in China, and 395 cases were reported in other 24 countries [3-4]. The transmission capacity of 2019-nCoV is still underestimated in different studies. Initial studies showed its regeneration number R0 was 2.2-2.9 [5-7], meaning that each infector could transmit to another 2.2 to 2.9 cases. Recently a novel study revealed that the R0 of 2019-nCoV is 3.77 upon the clinical and epidemiological data of nearly 8866 patients in 30 provinces[8], which is higher than SARS-CoV (R0, 2-3) [9].

The patient's infectivity is determined by the presence of virus in different body fluids, secretions, and excreta. All the patients with positive viral RNA detection need to be isolated. As mentioned in the "Diagnosis and Treatment Scheme of New Coronavirus Infected Pneumonia" (trial version 5), only after the relieved symptoms and two successive(upon 24h sampling interval at least) negative viral nucleic acid results for respiratory specimens, the isolated cases can be released. However, it is not clear about the persistence and clearance of viral RNA in different specimens of COVID-19 patients. In this study, the viral RNA detection was applicated in throat swabs, stool, urine, and serum specimens, which was analyzed with different clinical conditions and lab results, in order to figure out the clearance time of virus and the influencing factors

Methods

Patient and Clinical Definitions:

From January 20, 2020 to February 10, 2020, all confirmed patients with COVID-19 in Shanghai region were admitted to the Shanghai Public Health Clinical Center. The convalescent patients refer to the recovered non-fever cases without respiratory symptoms, and had two successive(upon 24h sampling interval) negative RT-PCR results of viral RNA for oropharyngeal swabs. Patients in corticosteroid treatment group were ever treated with corticosteroids such as prednisolone and dexamethasone. Feces-positive group or feces-negative group were divided by the virus RNA detection results in .

The viral RNA detection of COVID-19

A magnetic bead method nucleic acid extraction kit was applied in a fully automated nucleic acid extraction instrument (Master Biotechnology, China). The total RNA was extracted from 200ul sample and dual fluorescence PCR was performed according to the instructions from the company. The clinical conditions and lab results of these case together with the viral RNA results in different specimens at different time were collected retrosepctively.

Statistical Analysis:

Continuous variables were summarized as the means and standard deviations or medians and interquartile ranges (IQR) as appropriate. Categorical variables were expressed as counts and percentages in each category. T-tests or Wilcoxon rank-sum tests were applied to continuous variables, Fisher's exact tests or chi-square tests were used for categorical variables. Multiple linear regression was applied to determine the relationship between outcomes and the exploratory factor. $P < 0.05$ was considered significant.

Results

The demographics and the laboratory exanimation results

From January 20, 2020 to February 10, 2020, 292 patients with COVID-19 were admitted to the Shanghai Public Health Clinical Center. 66 convalescent patients were included in our study. Totally 28(42.4%) females and 38 males (57.6%) with a median age of 44.0 (34.0-62.0) year-old were analyzed. The oldest patient was 78 years old and the youngest was 16 years old. There was no difference with or without corticosteroid treatment (P> 0.05). On admission, the high level of red blood cell sedimentation rate of 70.0 (25.5-90.0) mm/h, a high-sensitivity C-reactive protein of 8.4 (1.6-20.3) mg /L, and a procalcitonin of 0.03 (0.02-0.05) ng/ml was found, which was decreased to 44.0 (29.5-81.3) mm/h, 0.5 (0.5-2.1) mg/L, 0.02 (0.02-0.02) ng/ml respectively upon treatment. See Table 1 and Table2.

The virus RNA detection of COVID-19 in deferent samples

The median time from the onset of symptoms to first negative RT-PCR results for oropharyngeal swabs of convalescent patients was 9.5 (6.0-11.0) days with improved symptoms such as fever, cough, and dyspnea. The time is viability different in patients from 2 to 22 days. The RT-RCR of viral RNA was performed for stool, urine, and blood specimens during the convalescence. Till the end of the observation(February 10, 2020), 11 convalescent cases (16.7%) still tested positive for viral RNA in stool specimens. The other 55 patients' stool specimens turned negative for 2019-nCoV with median duration of 11.0（9.0-16.0）days. 12 patients (21.8%)'s viral RNA in oropharyngeal swabs or feces turned negative at the same time. 78.2%(43/55) cases had longer duration for stool specimens detected negative for viral RNA than throat swabs, with median delay of 2.0 (1.0-4.0) days. The positivity of viral nucleic acid test for urine samples was low with only 4 (6.9%) positive results in 58 cases, viral RNA in 3 patients' urine specimens remained tested positive after throat swabs turned negative.14 serum specimens were tested for 2019-nCoV, none of them show positive results. SeeTable 2.

The factors related to virus clearance

The analysis was applied on the correlation between the absolute values of CD4 + T lymphocytes, C-reactive protein, red blood cell sedimentation rate, procalcitonin and the time of detoxification of feces in patients during convalescence. Using multiple linear regression model(F = 2.669, P = 0.044, and adjusted R2 = 0.122), the analysis showed the CD4 + T lymphocyte counts may help predict the the duration of viral RNA detection in patients' stool (***P*** = 0.01). See Table 3.

During hospitalization, five patients received corticosteroid treatment. The duration of viral RNA detection for throat swabs and feces in the corticosteroid treatment group was longer than that in the non- corticosteroid treatment group, which were 15 days vs 8.0 days (***P*** = 0.013) and 20 days vs 11 days (***P*** <0.001), respectively. We further analyzed the differences in the results of the last inflammatory indicators tests upon the virus RNA results in fecal. There was no statistically significant difference on the inflammation indicators between patients with positive fecal viral RNA test results and the negative group (***P***> 0.05).See Table 4.

Discussion

The novel coronavirus was firstly identified in respiratory specimens from patients with COVID-19 [10], and viral nucleic acids were subsequently detected in patients' stool, urine, and gastrointestinal mucosa [11, 12]. Recently a neonatal infection was reported, indicating the possible of fecal-oral and vertical transmission from mother to child other than the currently confirmed droplet transmission and direct contact transmission. In this study we found that the viral RNA can be detected in the stool of 81.8% (54/66) cases even with the

negative results for throat swabs. The continuous detection of viral nucleic acids in feces suggests that the virus may be transmitted through the digestive tract or re-transmitted through aerosols containing viruses. Therefore, it is necessary urgently to standardize the stool transport process of COVID-19 patients to reduce the risk. On the other hand, the viral RNA detection for feces should be applied regularly in patients with COVID-19 even in recovery period. The transmission by urine or blood maybe less frequently for the low positive rate in patients.

Corticosteroids have been widely used in the treatment of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), which are now also used in conjunction with other drugs to treat 2019-nCoV infected patient. However, in the published clinical management opinion for the new coronavirus, the application of corticosteroids is not recommended unless there are other indications[13-14]. The use of Corticosteroid may delay the clearance of viral nucleic acids in patients and should be avoided during viral replication. There were some bias exist in our study that the patients in the corticosteroid treatment group were more severe and with lower CD4+ T lymphocytes counts. Our point is that the mild patients are not recommended with corticosteroids treatment, which may delay the virus clearance. The randomized controlled double-blind experiments with expand sample sizes will help to clarify. The T cell immunity may play an important role during 2019-nCoV infection. The absolute values of CD4 + T lymphocytes, C-reactive protein, erythrocyte sedimentation rate, and procalcitonin measured upon admission were analyzed with virus clearance. The lower the absolute value of CD4 + T lymphocytes before treatment, the longer duration of virus clearance. The relationship between the fecal viral RNA results and the inflammation indicators of the patients was analyzed without statistical difference on erythrocyte sedimentation rate, C-reactive protein, and procalcitonin during rehabilitation.

In brief as the clearance of viral RNA in patients stool was delayed comparing to oropharyngeal swabs, it is important to detect the viral RNA in feces during the convalescence. As the delayed clearance of viral RNA in corticosteroid treatment group, corticosteroids is not recommended in the treatment of COVID-19, especially for the mild conditions cases. The duration of RNA detection may relate to the host cell immunity.

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Table 1 The demographics and laboratory findings of convalescent COVI-19 patients

	All patients (n=66)
Sex Female	28
Male	38
Age (yrs, IQR)	44 (34.0-62.0)
CD4 + T lymphocyte value (cell/ul, IQR)	417 (272.3-599.3)
Erythrocyte sedimentation rate,ESR (mm/h, IQR)	70 (25.5-90.0)
C-reactive protein,CRP (mg/L, IQR)	8.4 (1.6-20.3)
Procalcitonin (ng/ml, IQR)	0.03 (0.02-0.05)

Table 2 The clearance time of viral RNA with or without the corticosteroid treatment

	All patients (n=66)	**GC treatment (n=5)**	**no GC treatment (n=61)**	***P* value**
Sex Female	38	4	34	*0.559*
Male	28	1	27	
Age(yrs, IQR)	44.0 (34.0-62.0)	51.0 (44.0-68.8)	41.0 (34.0-61.3)	*0.294*
Pharyngeal swab virus nucleic acid negative time	9.5 (6.0-11.0)	15 (9.8-16.8)	8 (6.0-11.0)	***0.013***
Fecal virus nucleic acid negative time	11 (9.0-16.0)	20 (17.5-22.5)	11 (9.0-14.0)	***<0.001***
Feces behind throat swab virus nucleic acid negative time	2 (1.0-4.0)	8 (2.25-11.0)	2 (1.0-3.0)	0.115

Table 3 Multiple linear regression analysis of immune and inflammation parameters on the virus clearance

Model	B	Std. Error	Beta	t	P value
Constant	15.883	2.281		6.965	
CD4 + T lymphocyte value	-0.009	0.003	-0.445	-2.699	***0.010***
Eythrocyte sedimentation rate,ESR	0.019	0.019	0.153	1.006	0.320
C-reactive protein,CRP	-0.027	0.045	-0.102	-0.589	0.559
Procalcitonin,PCT	-6.278	15.607	-0.064	-0.402	0.689

Table 4 The analysis of viral RNA in stool with the inflammation indicators of patients

	Stool test positive（n=11）	**Stool test negative（n=55）**	***P* value**
Last erythrocyte Sedimentation rate,ESR	82.0（36.8-90.8）	40.5（28.0-79.0）	0.210
C-reactive protein,CRP	1.02（0.5-2.9）	0.5（0.5-1.2）	0.305
Procalcitonin,PCT	0.02（0.02-0.02）	0.02（0.02-0.02）	0.940