The SARS-CoV-2 RNA with mild pulmonary consolidation lasts longer in non-severe COVID-19 patients: an observational study

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Research

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Abstract

Background: Since December 2019, COVID-19 has rapidly swept the world. It is particularly important to understand the dynamic changes of the whole disease course of non-severe patients from the onset to the follow-up after discharge.

Methods: On February 1, 2020, 18 cases of non-severe COVID-19 appeared in a hospital in Beijing. All patients were SARS-CoV-2 RNA positive by RT-PCR for pharyngeal swabs. We recorded the clinical information and viral dynamics of these patients from the onset of the disease to 2 months after discharge. According to the severity of lung consolidation, 18 patients were divided into two groups (mild pulmonary consolidation group [imaging score ≤10]; severe pulmonary consolidation group [imaging score >10]).

Results: Eighteen patients (median age 43) were included, including 14 females. Fever (11/18) and cough (8/18) were the most common symptoms. The duration of SARS-CoV-2 RNA positive in mild pulmonary consolidation group was significantly longer than severe pulmonary consolidation group (the median time was 30 days and 13 days, respectively, P= 0.0031). Two months after discharge, almost all patients were followed up for IgM antibody disappearance and IgG antibody production.

Conclusion: In non-severe COVID-19 patients, the positive duration of the SARS-CoV-2 RNA in patients with mild pulmonary consolidation was longer than the severe pulmonary consolidation. However, it is necessary for a large sample to verify our conclusions.

1. Introduction

Since December 2019, an unexplained pneumonia occurred in Wuhan, China, which was quickly identified and named COVID-19 (caused by SARS-CoV-2), and then quickly spread all over the world[1]. As of September 23, 2020, more than 30 million people have been infected and nearly 1 million people have died in the world[2].

COVID-19 has a variety of ways of transmission, including respiratory transmission, digestive tract transmission and so on[1, 3, 4]. According to the definition of COVID-19 's diagnosis and treatment guidelines, whether in China or other countries, the majority of COVID-19 's patients were non-severe patients(Meet any of the following can be identified as severe or critical case: finger oxygen saturation ≤ 93%; $PaO_2/Fi0_2$ ≤ 300mmHg; respiratory failure and mechanical ventilation was needed; shock; admitted to ICU, etc.)[5]. Although the mortality rate of non-severe patients is not high, they are indeed an important reason for COVID-19 's wide spread. According to Sutton D et al. research[6], the proportion of asymptomatic infections was as high as 88%. Therefore, understanding the clinical course and viral dynamics changes of mild patients will help us to prevent and control the spread of this disease.

Unfortunately, as far as we know, there were no detailed reports on the diagnosis, treatment and follow-up of mild patients. Therefore, we will share a cases series of clustered nosocomial infection in a hospital and fully record their diagnosis, treatment and follow-up results.

2. Methods

2.1 Patients

On February 1, 2020, a SARS-CoV-2 RNA positive case occurred in a hospital in Beijing. Subsequently, an epidemiological investigation was carried out by the Beijing Center for Disease Control and Prevention, and a number of confirmed cases were found one after another. Eventually, 18 confirmed cases have been admitted to Beijing Hui Hospital. This is a case series report that collected 18 COVID-19 patients with mild illness from this hospital (according to China's policy at that time, the rest of the severe patients were transferred to other designated hospitals for centralized treatment).

This study has been approved by the Ethics Committee of Peking University First Hospital (2020-032) and has been registered in Chinese clinical trial registry (ChiCTR2000030096). All patients signed the informed consent form.

2.2 Data collection and laboratory examination

After admission, respiratory, serum and fecal samples were collected as far as possible, and the content of SARS-CoV-2 RNA was determined by reverse transcription PCR (RT-PCR). In order to avoid being mistakenly taken as saliva, sputum samples were taken from the respiratory tract samples of the patient after deep cough. What's more, to avoid cross-infection between patients, all patients wear N95 masks in separate wards (especially when taking throat swabs or sputum samples).

RT-PCR was performed with primers and probes for ORF1ab and N genes and a positive reference gene. This result is considered to be positive only when the cycle threshold (Ct) value of the reference gene is < 38[7-9].

Chest imaging score: the main abnormalities of lung parenchyma were ground glass shadow and consolidation. According to the degree of lesion in chest imaging, the score ranges from 0 to 72, where 0 is normal[10-12]. According to the severity of pulmonary lesions, the patients were divided into two groups with a cut-off value of 10. Two imaging experts were blindly graded, and if the scores were different, the third expert would evaluate and discuss them.

In addition, we also followed up the results of SARS-CoV-2 RNA and antibodies (IgM and IgG) re-examined in the outpatient clinic about 2 months after discharge. Sandwich enzyme-linked immunosorbent assays for serum anti-SARS-CoV-2 (IgM and IgG) level were performed[13].

2.3 Statistical analysis

Quantitative variables and categorical variables are represented by median (IQR) and numbers (percentage), respectively. We used the Mann-Whitney U test or Fisher's exact test to compare differences between patients who mild pulmonary lesion and serious pulmonary lesion. We used Spearman's correlation to assess the relation between viral Ct value and other parameters. Statistical analyses were performed using SPSS ver. 25.0 (SPSS, Chicago, IL, USA). A P value of <0.05 was considered significant.

3. Results

3.1 The clinical characteristics at onset

According to the classification of COVID-19 prevention and treatment guidelines in China(Table S1), the 18 patients included in this study were all non-severe type. Table 1 shows the clinical characteristics of 18 patients with confirmed non-severe COVID-19: 11 with mild pulmonary lesion (imaging score ≤10) and 7 with severe pulmonary lesion (imaging score >10). The median age of 18 patients enrolled in this study was 43 years old (ranging from 27 to 60 years old), including 14 females. Fever and cough were the most common clinical symptoms, accounting for 61.1% (11/18) and 44.4% (8/18), respectively. Most of the patients were in good health and had no chronic diseases, except that #10 and #15 had high blood pressure and #11 had diabetes. Most patients (13/18) were treated with interferon and thymosin.

In the laboratory examination, the white blood cell (WBC) count was lower than the lower limit of the normal value (reference 3.5-9.5×10^9/L) in 5 cases, nobody is above the normal value. Four patients' (#1, #9, #12, #18) lymphocyte count was lower than the normal value (reference 1.1-3.2×10^9/L), but the lymphocyte percentage (reference 20%-50%) was lower than the normal value in only 1 case and higher than the normal value in 2 cases. C-reactive protein (reference <8 mg/L) was higher than normal in 5 patients. During hospitalization, all patients underwent at least 3 chest X-ray or CT examinations. The median score of the first image was 4(range 0-24). The first imaging score of most patients (15/18) was the highest, and only 3 patients (#12, #17 and #18) showed progress.

3.2 Relationship between the degree of pulmonary lesion and the duration of virus

As shown in Figure 1, patients #1 to #11 are patients with mild lung lesions (imaging score≤10, named mild pulmonary consolidation group), and patients #12 to #18 are patients with severe lung lesions (imaging score>10, named severe pulmonary consolidation group). The SARS-CoV-2 RNA positive duration (total duration) of mild pulmonary consolidation group was significantly longer than severe pulmonary consolidation group, and the difference was statistically significant (P= 0.0031, Figure 1A and 2A). We noted that all patients in mild pulmonary consolidation group were positive for more than 14 days, of which 6 patients were positive for more than 1 month. In contrast, only 3 people in severe pulmonary consolidation group lasted more than 14 days, and none of them lasted more than 1 month (Figure 1A).

Then we discussed the difference of virus duration in different samples. As shown in Figures 1B and Figure 2B, there was no significant difference in the positive time of throat swabs between the two groups (P= 0.2010). In most sputum samples, the positive time of mild pulmonary consolidation group was longer than that of severe pulmonary consolidation group, but the difference was not statistically significant (Figure 1C and Figure 2C, P=0.6376). It is worth noting that no one in severe pulmonary consolidation group was positive, 4 in mild pulmonary consolidation group were positive, and 3 were positive for more than 14 days, but there was no significant difference between the two groups, in stool sample (Figure 1D and Figure 2D, P=0.0923).

3.3 Changes in viral dynamics in all patients

Figure 3 shows the quantitative results of SARS-CoV-2 RNA of different types of specimens collected by all patients at each time point. Most of the patients did not re-positive after testing negative for SARS-CoV-2 RNA. Of course, there are exceptions, such as patients #1 and #8 in throat swabs, patients #1, #6 and #7 in sputum samples, and patients #1, #3, #6 and #8 in stool samples.

We noticed that most of the patients in mild pulmonary consolidation group were still virus positive after the first reexamination, while in severe pulmonary consolidation group, all the patients quickly turned negative, and all kinds of samples were negative for the first reexamination and subsequent repeated reexaminations. In mild pulmonary consolidation group, 7 sputum samples were positive, and 6 patients remained positive in the first reexamination.

3.4 Correlation between viral load and other indicators

In order to explore the potential relationship between virus Ct value and other indexes, the correlations between ORF1ab gene and N gene and other indexes were analyzed (Figure 4, Figure S1, Figure S2, Figure S3). We know that the lower the Ct value, the higher the viral load. Figure 4D shows that there is a significant positive correlation between the patient's maximum viral load and the percentage of peripheral blood lymphocytes (R=0.49, P=0.039). In addition, there was also a good correlation between the first viral load and the percentage of peripheral blood lymphocytes (Figure S1D, R=0.46 P= 0.053). We also analyzed age, WBC, HBG, PLT, etc., and there was no significant correlation (Figure S1, Figure S2, Figure S3).

3.5 Follow up results one month after discharge

In order to prevent the virus from returning to positive after discharge, all patients were followed up for one month (Table 2). Most of the patients were followed up twice, and only 4 patients were followed up once. Throat swabs were collected from all patients and all patients were negative. In addition, in order to observe the production of antibodies in patients, both IgM and IgG antibodies were detected. IgM antibodies were positive in patients 10, 12 and 17, but then turned negative. All patients were positive for IgG antibody at the first reexamination. In the second reexamination, only 2 patients (patients #2 and #5) changed from positive to negative. Pulmonary CT also suggested that the lesions of all patients were completely or obviously absorbed. (information not shown)

4. Discussion

We recorded and followed up the complete pathogenesis of 18 patients with non-severe COVID-19 infection in a hospital in Beijing, and recorded the dynamic changes of a total of 235 virus results. Although they develop the disease together, their clinical processes were quite different. It was noteworthy that in these non-severe patients, the negative conversion of the virus with mild lung lesions were slower. This played an important role in preventing and controlling the spread of COVID-19, that was, patients with mild pulmonary consolidation might be quarantined for longer.

Borremans et al. study[14] (with 516 individuals) showed that the virus persists in the lower respiratory tract and digestive tract for the longest time. With the development of the disease, the viral load of all patients decreased in waves. However, there was a great difference in the rate of decline, in which the sample of the upper respiratory tract decreased the fastest, followed by the lower respiratory tract and gastrointestinal tract. This was consistent with our conclusion that the throat swabs shown in Figure 1 were positive for no more than 20 days, while both sputum and feces are more than 20 days. In addition, we also found that the total duration of the virus in these patients who tested positive in stool was very long, and all of them lasted for more than a month. Interestingly, the lesion in the lungs of these patients were mild. Wu et al. study[15] of 74 people showed that the positive duration of fecal samples was significantly longer than that of respiratory samples (16.7 days vs. 27.9 days; $P<0.05$). In addition, the presence of gastrointestinal symptoms was not associated with fecal sample viral RNA positivity ($P=0.45$). He et al. study[16] believed that high detection rate and a long positive duration of SARS-CoV-2 in sputum samples (detection rate 95% [19/20]; mean time 42.8 day), while the detection rate of fecal samples is only 55%, with an average duration of 22.3 days.

There were 2 asymptomatic patients (#1 and #7), and their virus positive duration was more than 1 month, which was longer than most symptomatic patients. Long et al. study (74 patients, 37 were asymptomatic)[17] showed that asymptomatic patients had a longer viral shedding time than symptom patients (median time 19 days vs. 14 days; $P=0.028$; HR 1.69 [95% CI 1.06–2.70]).However, there was no significant difference in initial median Ct values between 37 asymptomatic patients and 37 symptomatic patients(ORF1b 32.8 vs. 31.7, $P=0.336$; N 32.6 vs. 33.5, $P = 0.126$). Interestingly, a South Korean study came to a different conclusion. A study of 303 patients (63.7% were symptomatic at the time of isolation; 36.3% asymptomatic patients; 19.1% developed symptoms during isolation). The negative conversion rate of asymptomatic patients on the 14th day and 21st day was slightly higher than symptomatic patients (14 days: 33.7% vs. 29.6%; 21 days: 75.2% and 69.9%). They also showed that there was similar in virus clearance time between asymptomatic and symptomatic patients (median time 17 days and 19.5 days for asymptomatic and symptomatic patients, respectively; $P =0.07$)[18].

There was some controversy about the duration of antibodies in COVID-19 patients. After two months follow-up, 11.1% (2/18) of patients became seronegative for SARS-CoV-2-IgG. Long et al. study[17] showed that in the early convalescent phase (about 8 weeks after discharge), 26.3% (16/61) of patients turned seronegative of IgG (40%[12/30] in asymptomatic patients and 12.9% [4/31] in symptomatic patients). This may be related to the length of our follow-up. Another study by this team showed that 93.3% (28/30) of patients had a significant decrease in neutralizing antibody titers during follow-up two months after discharge, with a median decrease of 34.8%, of which 4 patients had neutralizing antibody titers by more than 50%[19]. However, another study showed that in the four months after COVID-19 was diagnosed, the level of antibody did not decrease. Antibody levels in 91.1% (1107/1215) of COVID-19 patients began to rise after infection and then remained stable for up to 4 months[20].

This study also has some inevitable limitations. First of all, the number of cases included is relatively small, but we have made up for some defects in some aspects through detailed course records and follow-up. Secondly, as COVID-19 is an emerging infectious disease, and medical supplies are relatively scarce, we are unable to quantitatively detect the virus changes of patients every day.

5. Conclusion

In conclusion, in non-severe COVID-19 patients, the positive duration of the SARS-CoV-2 in patients with mild pulmonary consolidation was longer than that in patients with severe pulmonary consolidation. However, it is necessary for a large sample of clinical trials to verify our conclusions.

Abbreviations

Ct: cycle threshold; RT-PCR: reverse transcription polymerase chain reaction;

Declarations

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Authors' contributions: Chi Zhang and Jiawen Li drafted the manuscript; Jing Mu, Yunv Jin, Yan Han, Haiyang Li, Chunxiao Zhang and Peng Yu participated in the collection and arrangement of clinical cases; Chi Zhang and Jiawen Li participated in the creation of figures and tables; Daitao Zhang, Xiangfeng Dou and Yanhui Chu participated in the detection and review of SARS-CoV-2 RNA; He Wang and Rui Guo participated in imaging diagnosis and imaging scoring; Zhao Wu, Xiaoqin Dong and Chi Zhang participated in the proofreading of this paper; Guiqiang Wang and Hong Zhao provided the overall principle and direction of the study. All authors read and approved the final manuscript.

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Availability of data and materials: All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Ethical Approval and Consent to participate: This study has been approved by the Ethics Committee of Peking University First Hospital (2020-032) and has been registered in Chinese clinical trial registry (ChiCTR2000030096). All patients signed the informed consent form.

Consent for publication: Not applicable.

Competing interests: All authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Tables

Table 1 Baseline characteristics of the COVID-19 patients

	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16
Sex	Female	Female	Female	Female	Female	Male	Female	Female	Female	Male	Female	Male	Female	Female	Male	Female
Age (year)*	30-50	<30	30-50	30-50	30-50	30-50	30-50	<30	30-50	>50	30-50	>50	30-50	30-50	30-50	>50
Clinical symptom																
Fever	×	×	×	√	√	√	×	√	√	√	×	√	√	√	√	×
Cough	×	×	×	√	×	√	×	√	√	×	×	√	×	√	√	√
Sore throat	×	√	×	×	×	×	×	×	×	√	×	×	×	√	×	×
Fatigure	×	×	×	×	×	×	×	×	√	×	√	√	√	×	×	√
Muscle soreness	×	×	×	×	×	×	×	×	×	×	√	×	×	×	×	×
Diarrhoea	×	√	√	×	×	×	×	×	√	×	×	×	×	×	×	×
Blood routine and CRP																
WBC (×109/L)	3.1	7.1	4.3	3.5	4.2	3.3	5.7	4.5	2.3	6.3	4.1	2.3	4.0	6.1	5.3	4.7
Neutrophil (%)	54.2	75.4	47.8	48.7	51	26.8	56.7	64.4	53.6	64	58.5	57.6	37.1	64.3	57.2	64
Lymphocyte (%)	29.8	20	37.1	41.6	34.9	63.5	34.3	27.3	35.8	19.5	30.1	36.5	53.8	26.9	40.7	28.8
Lymphocyte count (×109/L)	0.9	1.4	1.6	1.4	1.5	2.1	1.9	1.2	0.8	1.2	1.2	0.8	2.2	1.6	2.5	1.4
HBG (g/L)	142	136	122	85	141	155	130	144	146	147	129	143	134	137	148	110
PLT (×109/L)	136	277	108	257	195	174	164	134	129	189	222	109	171	233	190	195
CRP (mg/L)	6.07	0.09	0.22	1.26	2.01	0.45	3.65	0.91	3.95	0.73	21.46	17.97		3.17	7.6	9.91
Image scoring																
First imaging score	0	0	0	0	0	0	2	2	4	4	10	12	12	12	13	13
Highest imaging score	0	0	0	0	0	0	2	2	4	4	10	14	12	12	13	13
SARS-CoV-2 PCR test																
First Ct value (ORF1ab gene)	22.0	34.0	23.0	38.0	38.0	29.0	25.0	22.0	27.0	22.0	23.0	35.0	27.0	20.0	34.0	28.4
First Ct value (N gene)	22.0	38.0	24.0	36.0	33.0	29.0	26.0	38.0	28.0	23.0	24.0	34.0	30.0	22.0	38.0	30.3
Lowest Ct value (ORF1ab gene)	22.0	28.0	23.0	38.0	20.0	29.0	22.0	22.0	27.0	22.0	23.0	35.0	27.0	20.0	34.0	28.4
Lowest Ct value (N gene)	22.0	28.0	24.0	34.0	21.0	29.0	23.0	38.0	28.0	23.0	24.0	34.0	30.0	22.0	38.0	30.3

*The combination of age and gender information with other identifiers may compromise patient anonymity, so we provide ages as age-ranges

Table 2 is not available with this version of the manuscript.

Figures

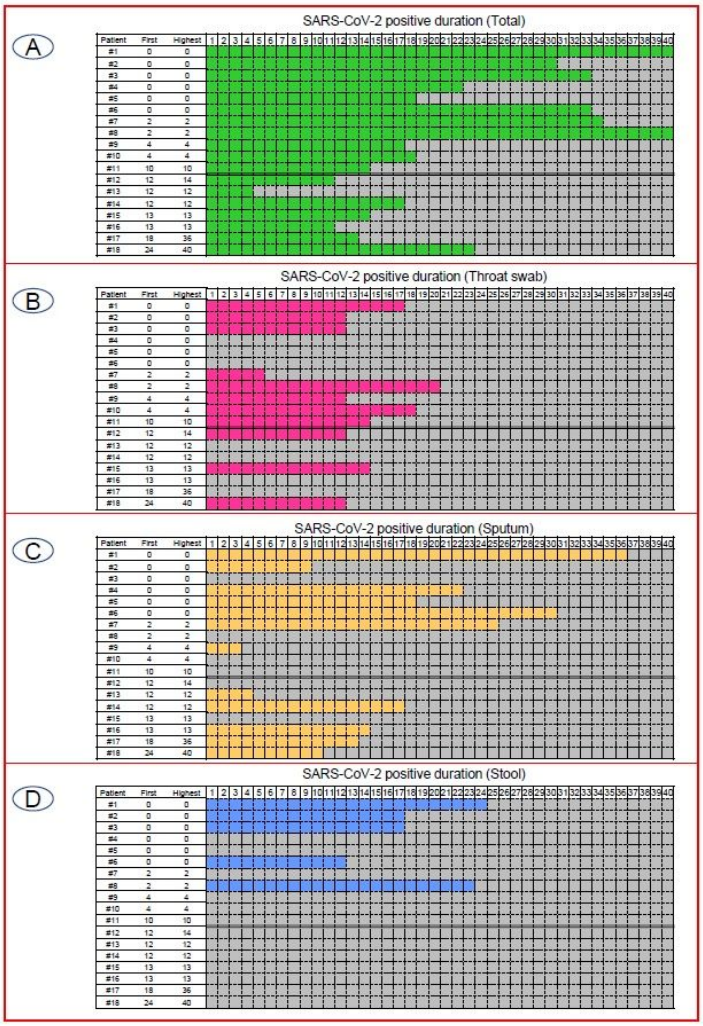

Figure 1

Duration of SARS-CoV-2 in all patients. (A) Total duration, (B) Throat swab sample, (C) Sputum sample, (D) Stool sample. The detection tool of SARS-CoV-2 was RT-PCR.

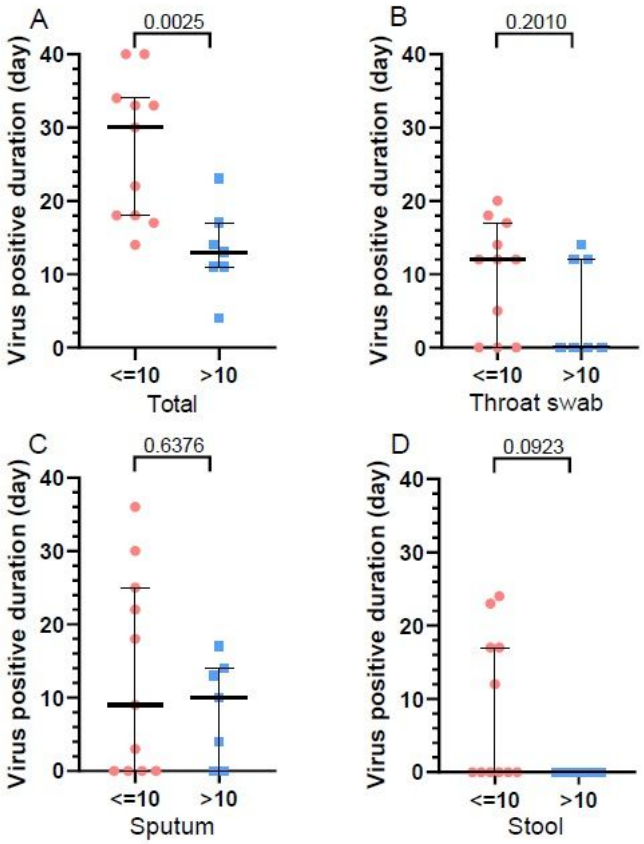

Figure 2

Duration of virus with different degrees of pulmonary inflammation (imaging score ≥10 or <10). (A) Total duration, (B) Throat swab sample, (C) Sputum sample, (D) Stool sample

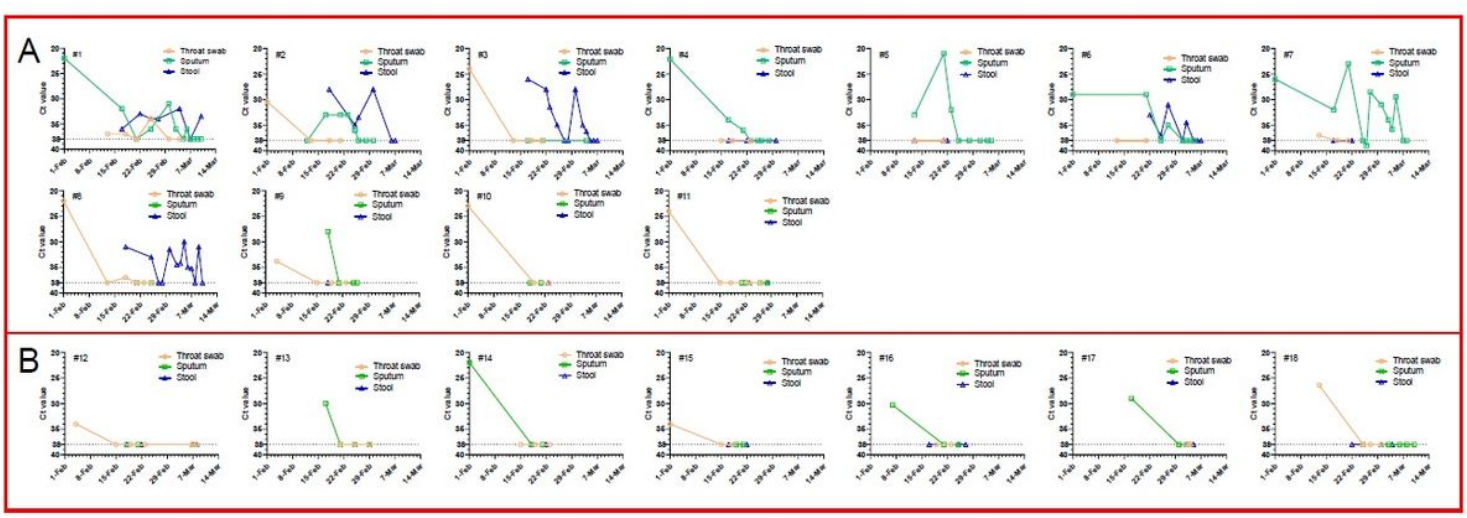

Figure 3

The changes of SARS-CoV-2 dynamics in all patients. (A) mild pulmonary inflammation (imaging score ≤10), (B) severe pulmonary inflammation (imaging score >10)

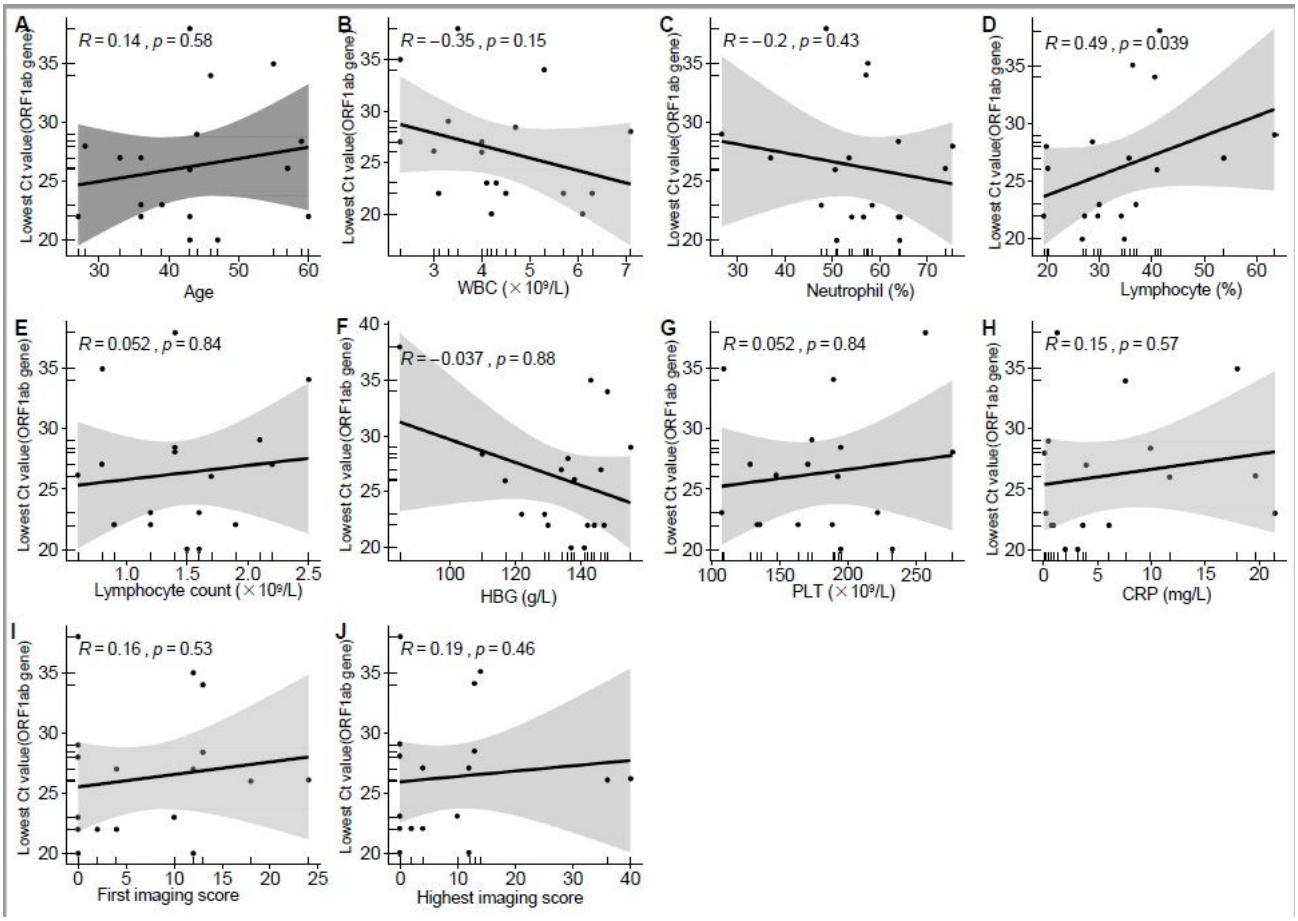

Figure 4

The correlation between the lowest SARS-CoV-2 Ct value (ORF1ab gene) and other clinical indicators in the course of the disease.

Supplementary Files

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