Am J Transl Res 2021;13(3):1197-1208
www.ajtr.org /ISSN:1943-8141/AJTR0116151

Original Article

Correlation of the ratio of IgM/IgG concentration to days after symptom onset (IgM/T or IgG/T) with disease severity and outcome in non-critical COVID-19 patients

Yan Rong[1], Fei Wang[2], Xiaoli Li[1], Xinhua Liang[3], Yang Zhou[1], Dandan Zhang[1], Jing Liu[4], Huadong Zeng[1], Jing Wang[5], Yi Shi[6]

[1]Department of Respiratory and Critical Care Medicine, Shenzhen Hospital, Southern Medical University, Shenzhen 518100, China; [2]Department of Orthopaedics, The University of Hong Kong - Shenzhen Hospital, Shenzhen 518053, China; [3]Department of Nephrology, Shenzhen Hospital, Southern Medical University, Shenzhen 518100, China; [4]Department of Health Management Center, Shenzhen Hospital, Southern Medical University, Shenzhen 518100, China; [5]Department of Radiology, Shenzhen Hospital, Southern Medical University, Shenzhen 518100, China; [6]Department of Respiratory and Critical Care Medicine, Jinling Hospital, The First School of Clinical Medicine, Southern Medical University, Nanjing 210002, China

Received June 12, 2020; Accepted December 18, 2020; Epub March 15, 2021; Published March 30, 2021

Abstract: Background: Correlation of SARS-CoV-2 serum antibodies with COVID-19 development and outcome has not been fully studied. Due to the time dynamic of antibodies, the antibody concentration of the same patient varies greatly at different times during the course of the disease. Therefore, our study used IgM/T or IgG/T (the ratio of serum antibody concentration to days after symptom onset) to reflect the patient's humoral immune status, and analyzed their correlation with COVID-19 development and outcome. Methods: Clinical data of 50 non-critical COVID-19 patients were retrospectively analyzed. Time-resolved fluorescence immunochromatography was used to quantitatively detect SARS-CoV-2 IgM and IgG. Correlation analysis was performed. Results: IgM antibody was positive on day 5 of symptom onset, increased within 2 weeks, and then gradually decreased. However, IgG antibody was positive on week 2 of symptom onset and continued to increase since. Additionally, IgM/T, but not IgG/T of recovery period (Spearman ρ=0.17; P=0.283), was negatively correlated with disease course in 2 weeks of symptom onset (Spearman ρ=-0.860; P=0.000). IgG/T of recovery period was positively correlated with clinical classification (Spearman ρ=0.432; P=0.004), number of involved lung lobes (Spearman ρ=0.343; P=0.026), and lung lesions (Spearman ρ=0.472; P=0.002). Conclusions: Within 2 weeks of symptom onset, higher IgM/T indicates faster recovery and shorter disease course. In recovery period, higher IgG/T suggests more serious disease. IgM/T or IgG/T may predict disease severity and outcome in non-critical COVID-19 patients.

Keywords: Correlation, IgM/T, IgG/T, disease severity, outcome, non-critical COVID-19

Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) threatens global public health. As of April 20, 2020, nearly 2.6 million people in 200 countries and regions have been confirmed with COVID-19. The spread rate of SARS-CoV-2 is much higher than MERS-CoV (Middle East respiratory syndrome-Coronavirus) and SARS-CoV (Severe Acute respiratory syndrome-Coronavirus). Strong humoral immune response is induced during SARS-CoV infection [1, 2], such as the production of IgM/IgG antibodies [3]. Similarly, the SARS-CoV-2 infection also induces the production of IgM/IgG antibodies [4]. In order to compensate for the false negative results of SARS-CoV-2 detection by RT-PCR [5, 6], researches on virus detection by specific IgM/IgG antibodies have been widely carried out. Serum specific antibodies are important effector molecules for the immune system to resist and eliminate viruses; and important for diagnosis of virus infection [5, 7, 8]. However, the role of specific IgM and IgG antibodies induced by SARS-CoV-2 in the development and outcome of COVID-19 has not been fully studied. Recently, the corre-

lation between SARS-CoV-2 serum antibody and disease condition of COVID-19 has been studied, but there are still some controversies. According to a cohort study from Hong Kong, there was no clear correlation between serum antibody and disease severity [9]. Another study on 173 cases of COVID-19 patients showed that the total antibody titer was associated with more serious disease, but this correlation was not found in IgM and IgG [5]. However, Zhang et al. analyzed 222 cases of COVID-19 patients and showed that IgG could predict disease severity and outcome [10]. The antibody concentration is closely related to the detection time (the number of days after symptom onset). The antibody concentration measured at different times during the course of the disease in the same patient may vary greatly. This may be the reason for controversies in the correlation of antibody with disease severity and outcome.

In this study, we proposed the concept of IgM/T or IgG/T, which was defined as the ratio of IgM/IgG antibody concentration to time (the days from symptom onset to antibody detection). This corrects the bias of antibody concentration affected by detection time, and may be a more objective indicator reflecting the level of humoral immune response than the absolute values of antibody concentration. The detailed clinical data of patients who were diagnosed as non-critical COVID-19 patients according to the latest diagnosis and treatment plan [11, 12] from the Hezheng Ward of Shenzhen Hospital of Southern Medical University were retrospectively collected and analyzed. The time dynamics of IgM and IgG was evaluated. The correlation of IgM/T or IgG/T with clinical condition and outcome of non-critical COVID-19 patients was analyzed and discussed.

Materials and methods

Study design and patients

This is a retrospective study. Non-critical COVID-19 patients who were hospitalized in Hezheng Ward of Shenzhen Hospital of Southern Medical University from 2020.2.3 to 2020.3.7 were included. All included patients underwent nucleic acid detection of SARS-CoV-2, antibody detection of specific IgM and IgG of SARS-CoV-2, and lung CT examination. The diagnosis and clinical classification criteria of all COVID-19 patients conform to the seventh edition of the COVID-19 diagnosis and treatment plan of the Expert Group of the National Health Commission [13] and the second edition of the COVID-19 diagnosis and treatment plan of the Expert Group of the Army [11].

Patients who met one or more of the following criteria were diagnosed as COVID-19: 1) SARS-CoV-2 nucleic acid was positive as tested by RT-PCR; 2) The sequence of the isolated virus was highly homologous to SARS-CoV-2; 3) Serum IgM or IgG antibody specific to SARS-CoV-2 was positive. The clinical classifications of no-critical COVID-19 patients were: 1) mild COVID-19: the symptoms included fever and mild respiratory symptoms, but lung imaging did not show features of pneumonia; 2) common COVID-19: the symptoms included fever and cough and other respiratory symptoms, and lung imaging showed features of viral pneumonia. However, the patients' vital signs were stable, and the blood oxygen saturation was >93% when there was no oxygen support. Finally, a total of 50 eligible no-critical COVID-19 patients were included in this study. Written informed consent was obtained from every patient and the study was approved by the Ethics Committee of Shenzhen Hospital of Southern Medical University (Approval number: NYSZYYEC20200013).

Data collection

The epidemiology, demographics, and lung imaging data were obtained from the electronic medical record system. All data were independently reviewed by two investigators (RY and LXH) to verify the accuracy of the data.

The nasal and pharyngeal swabs of patients before admission were tested for SARS-CoV-2 by kits provided by 6 companies (DAAN, Sansure Biotech, BGI, Shanghai ZJ Biotech, Geneodx, Biogerm). All positive results were reviewed and confirmed at the Shenzhen Center for Disease Control and Prevention. After admission, all patients underwent nasal and pharyngeal swab nucleic acid detection every 3-5 days, and anal swab nucleic acid detection in later stage.

Blood samples were collected from all patients during 5 to 38 days after the onset of symptoms. A total of 60 blood samples were collect-

ed from 50 non-critical COVID-19 patients for IgM/IgG antibody detection. Among them, 9 samples were collected from 3 patients (3 samples each), 8 samples were collected from 4 patients (2 samples each), and the remaining 43 samples were from each of 43 patients. Serum antibody level was detected with time-resolved fluorescence immunochromatography. In detail, the SARS-CoV-2 specific IgM/IgG antibody detection kit (Beijing Digret Biotechnology Co., Ltd., Beijing, China) was used. The fluorescence intensity (Flu) reflects the antibody concentration. The Flu cutoff values of IgM and IgG were 0.88 and 1.02, respectively.

The CT images of all patients were reviewed and evaluated by a radiologist (WJ) in a blinded manner. The following four aspects were evaluated: 1) the affected site and lobe of the lung; 2) the score of the lesion range in the affected lung lobe; 3) the severity of lung lesions according to the number of affected lung lobe and the range of lesions of each involved lobe; 4) the lung imaging manifestations of the lesions. The scoring criteria for involved lung lobes were based on the lesion range of each lobe, which were graded as 0 (none), 1 (diameter <1 cm), 2 (diameter 1 to <3 cm), 3 (diameter 3 cm to <50% of the lobe) or 4 (50%-100% of the lobe).

Statistical analysis

All data were statistically analyzed using SPSS 25.0. Continuous variables were presented as median (interquartile range 25%, 75%) if they were non-normal distribution and categorical variables as count (%). Chi-square or Fisher exact tests were used to compare categorical variables. Mann-Whitney U test (for 2 samples) or Kruskal-Wallis test (for k samples) was performed to compare continuous variables. Spearman correlation was used to evaluate the correlation between different factors. A *P* value <0.05 was considered statistically significant.

Results

Clinical characteristics of non-critical COVID-19 patients

The clinical data of patients were shown in **Table 1**. The 50 non-critical COVID-19 patients included in this study had a median age of 34 (25-42) years. Among them, 15 patients (30%) had chronic underlying diseases, of which chronic lung disease (4 cases; 8%) and viral hepatitis (4 cases; 8%) were most common. The clinical symptoms were mainly fever 33 (66%) and cough 35 (70%), and the median time from symptom onset to hospital visit was 2 days (1, 4). The course of disease was defined as from the onset of symptoms to clinical cure. The median course of disease was 12.5 days (10, 16), with a range of 5 days to 30 days. For clinical classification, there were 10 cases with mild COVID-19 and 40 cases with common COVID-19. The mild cases had significantly less number of patients with symptoms of cough and sore throat (P=0.004, and P=0.046, respectively) than common cases. However, they were not significantly different in other aspects.

Characteristics of lung imaging of common non-critical COVID-19 patients

As shown in **Table 2**, the patients with two affected lobes were most common, accounting for 30%. The lobes with a lesion range score of 2 were most common, accounting for 46%. Comprehensive evaluation showed that the median severity of lung lesions was 4 points (3, 6). The main features of lung imaging were ground glass opacity, interlobular interstitial thickening accompanied with ground glass opacity and consolidation accompanied with ground glass opacity. Only 2 cases were with pleural thickening or a small amount of pleural effusion. No case had lymphadenopathy.

Time-dynamics of specific IgM/IgG antibodies in non-critical COVID-19 patients

The sample collection time was from 5 days to 38 days after the onset of symptoms, of which 7 samples were collected within 7 days, 22 samples were collected from 8-14 days, 18 samples were collected from 15-27 days, and the remaining 13 samples were collected after 28 days. Five of the 60 samples had abnormally high IgM/IgG and were analyzed separately. The remaining 55 samples were included in the time-dynamics analysis of IgM and IgG.

The median IgM within 7 days of symptom onset was 1.03 (0.89, 1.08) Flu, which rose to 1.12 (0.94, 1.44) Flu in 8-14 days, then gradually decreased to 1.01 (0.90, 1.13) Flu in 15-27 days, and decreased to 0.95 (0.33, 1.07) Flu after 28 days (**Figure 1**). There was significant difference in IgM among different time periods

Table 1. The clinical data of non-critical COVID-19 patients

	Total COVID-19 patients (n=50)	Mild COVID-19 patients (n=10)	Common COVID-19 patients (n=40)	*P* value
Age (years)	34 (25-42)	34 (22-36.5)	34.5 (25-47)	0.451
Sex				1
Male	17 (34%)	3 (30%)	14 (35%)	
Female	33 (66%)	7 (70%)	26 (65%)	
Exposure history				1
Yes	36 (72%)	7 (70%)	29 (72.5%)	
No	14 (28%)	3 (30%)	11 (27.5%)	
Chronic comorbidities				
Chronic lung disease	4 (8%)	1 (10%)	3 (7.5%)	
High blood pressure	1 (2%)	0 (0%)	1 (2.5%)	
Postoperative tumor	2 (4%)	0 (0%)	2 (5%)	
Diabetes	1 (2%)	0 (0%)	1 (2.5%)	
Chronic kidney disease	1 (2%)	0 (0%)	1 (2.5%)	
Allergic rhinitis	2 (4%)	0 (0%)	2 (5%)	
Viral hepatitis	4 (8%)	0 (0%)	4 (10%)	
Hyperlipidemia	1 (2%)	0 (0%)	1 (2.5%)	
No comorbidities	35 (70%)	9 (90%)	26 (65%)	0.246
Signs and symptoms				
Fever	33 (66%)	5 (50%)	29 (72.5%)	0.277
Cough	35 (70%)	3 (30%)	32 (80%)	0.004
Expectoration	4 (8%)	1 (10%)	3 (7.5%)	0.603
Sore throat	13 (26%)	0 (0%)	13 (32.5%)	0.046
Chest pain, Chest distress, breathlessness	11 (22%)	1 (10%)	10 (25%)	0.424
Muscle aches	5 (10%)	1 (10%)	4 (10%)	1
Fatigue	3 (6%)	1 (10%)	2 (5%)	0.496
Gastrointestinal symptoms	4 (8%)	1 (10%)	3 (7.5%)	1
Headache and dizziness	4 (8%)	1 (10%)	3 (7.5%)	1
Chills	5 (10%)	0 (0%)	5 (12.5%)	0.569
Runny nose	3 (6%)	1 (10%)	2 (5%)	0.496
Time interval from symptom onset to first visit (days)	2 (1, 4)	1 (1, 2)	2 (1, 4)	0.309
Course of disease (days)	12.5 (10, 16)	8.5 (7, 18.3)	14 (10, 19.5)	0.734

Table 2. Lung imaging evaluation of common non-critical COVID-19 patients

Number of affected lobes	Number of patients with lung lobe involvement (n=40)	Lesion range score (points)	Lung lobe involvement score (n=100 points)
1	10 (25%)	1	29 (29%)
2	12 (30%)	2	46 (46%)
3	9 (22.5%)	3	23 (23%)
4	6 (15%)	4	2 (2%)
5	3 (7.5%)		

(P=0.039). In contrast, the median IgG in 7 days was 0.96 (0.76, 1.05) Flu, which was lower than the cutoff value. The IgG gradually increased to a median value of 1.16 (0.89, 2.94) Flu in 8-14 days. After that, the IgG continued to increase until it reached 3.49 (0.98, 16.93) Flu after 28 days (**Figure 1**). There was significant difference in IgG among different time periods (P=0.02).

In addition, there were 11 patients with anal swabs positive for SARS-CoV-2 nucleic acid. These patients all showed negative pharyngeal swab and positive anal swab nucleic acid results during the recovery period, and had

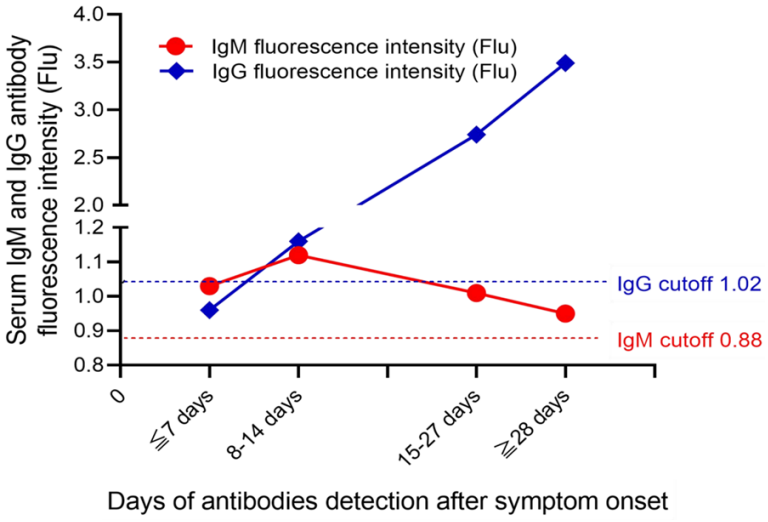

Figure 1. Time-dynamics of IgG and IgM. Serum IgG/IgM Flu were continuous variables with non-normal distribution and were expressed as median (interquartile range 25%, 75%). The picture was plotted based on the median of IgG and IgM. IgM antibody was detected as early as 5 days after symptoms onset, which was higher than the cut-off value. It gradually increased within 2 weeks after symptoms onset, and then began to decline. In contrast, IgG was below the cut-off value within 7 days after symptom onset, and gradually increased from 8 days after symptom onset until the end of the observation period (38 days after symptom onset).

no obvious clinical symptoms. For these patients, IgM and IgG antibodies were detected within 2-3 days after anal swab test. The results showed that two of the patients had abnormally elevated IgM and IgG levels on the 28th and 29th day of symptom onset. The 2 samples from these 2 patients were excluded from the time-dynamics analysis. The remaining 9 patients were not significantly different from non-anal swab positive patients during the same period in IgM and IgG levels (**Figure 2** and **Table 3**).

IgM/T and IgG/T analysis

Our study showed that the concentration of IgM antibodies continued to rise within 2 weeks after the onset of symptoms, while the concentration of IgG antibodies increased from 8 days after the onset of symptoms to the end of the observation period. In order to study the relationship between humoral immunity and disease development and outcome, we analyzed the correlation of IgM/T (within 2 weeks after the onset of symptoms) and IgG/T (from 8 days after the onset of symptoms to the end of the observation period) with clinical condition and outcome of non-critical COVID-19 patients. The concentration of IgG antibodies was lower than the cut-off value within 8 days after symptom onset, which had no clinical significance. Thus, the IgG/T within 8 days after symptom onset was not included.

IgM/T negatively correlates with the course of disease within 2 weeks

In our study, there were 6 patients with course of disease within 7 days, 7 patients within 8-10 days, 11 patients within 10-14 days, 16 patients within 15-20 days, and 10 patients more than 20 days. The median IgM/T was 0.17 (0.15, 0.20) in patients with course of disease within 7 days, 0.14 (0.11, 0.16) within 8-10 days, and 0.08 (0.07, 0.10) within 11-14 days. The higher the IgM/T was, the shorter the course of disease was. There was significant difference in IgM/T among the groups (P<0.001). Spearman correlation analysis showed that IgM/T was significantly negatively correlated with the course of disease within 2 weeks (Spearman ρ=-0.860; P<0.001) (**Figure 3**).

IgM/T is not significantly affected by host factors

However, IgM/T were not significantly different among patients of different age groups (<19 years old, 19-40 years old, >40 years old: 0.11 (0.05, 0.20) vs 0.12 (0.08, 0.16) vs 0.12 (0.09, 0.16), P=0.885), sex (male and female: 0.1 (0.07, 0.18) vs 0.12 (0.08, 0.15, P=0.887), and with presence or absence of chronic underlying disease (0.12 (0.11, 0.16) vs 0.10 (0.07, 0.16), P=0.276) (**Figure 4**). Although there were also no significant differences in IgM/T among patients with different clinical classification (mild and common COVID-19: 0.14 (0.08, 0.17) vs 0.11 (0.08, 0.15), P=0.555), number of lung lobes involved (<2 lobes and ≥2 lobes: 0.13 (0.09, 0.18) vs 0.10 (0.07, 0.14), P=0.168), comprehensive score of lung lesions (0 points, 1-3 points and >3 points: 0.13 (0.08, 0.15) vs 0.12 (0.07, 0.18) vs 0.105 (0.09, 0.16),

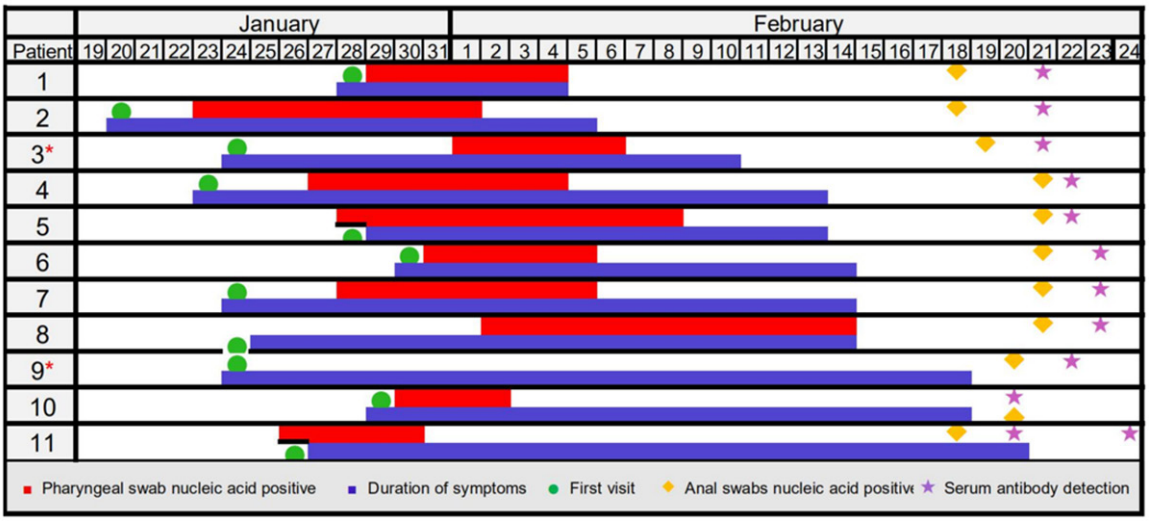

Figure 2. Time course of anal swab positive patients. This chart records the date of first visit, the duration of symptoms, the date of positive pharyngeal swab and the duration of pharyngeal swab positive, the date of pharyngeal swab turning negative, the date of positive anal swab and the date of serum antibody test of 11 patients with positive anal swab. Among them, the antibody levels of patients 3 and 9 increased abnormally.

Table 3. IgM and IgG antibodies in patients with anal swab positive

Patient NO.	Interval from symptom onset to antibody test (days)	IgM (Flu)	IgG (Flu)
1	24	1.10	8.04
2	32	1.29	8.47
3*	28	5.58	21.45
4	30	0.98	1.17
5	25	1.01	2.74
6	24	0.38	28.25
7	29	0.26	3.56
8	30	0.39	3.49
9*	29	9.52	21.61
10	22	0.55	1.79
11	25	1.38	3.34
	29	0.37	3.87

Note: *The anal swab-positive patients with abnormal antibody levels.

P=0.168), we observed that the lower the median IgM/T, the more severe clinical classification, the more involved lobes and the higher comprehensive score of lung lesions (**Figure 4**).

IgG/T after 8 days of symptom onset is not related to the course of disease

The IgG/T of patients with course of disease within 7 days, 8-10 days, 11-14 days and more than 15 days were 0.09 (0.08, 0.22), 0.125 (0.068, 0.255), 0.09 (0.05, 0.28) and 0.125 (0.112, 0.548), respectively. There was no significant difference in the median IgM/T between the course of disease (P=0.421). Spearman correlation analysis showed that IgG/T after 8 days of symptom onset was not

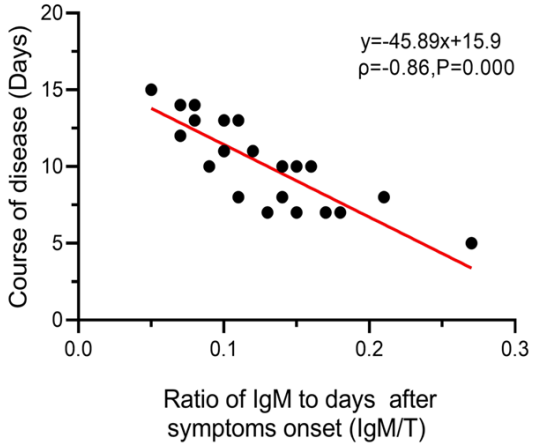

Figure 3. Correlation of IgM/T (within 2 weeks after symptom onset) with the course of disease. Within two weeks after the symptoms onset, a total of 27 blood samples from 24 patients were tested for IgM, including 21 blood samples from 21 patients each and 6 blood samples from 3 patients (2 samples each patient). If the patient has two or more samples, the average IgM value is used to calculate the IgM/T. Spearman correlation was used to analyze the correlation of IgM/T with course of disease (Spearman ρ=-0.860; P=0.000).

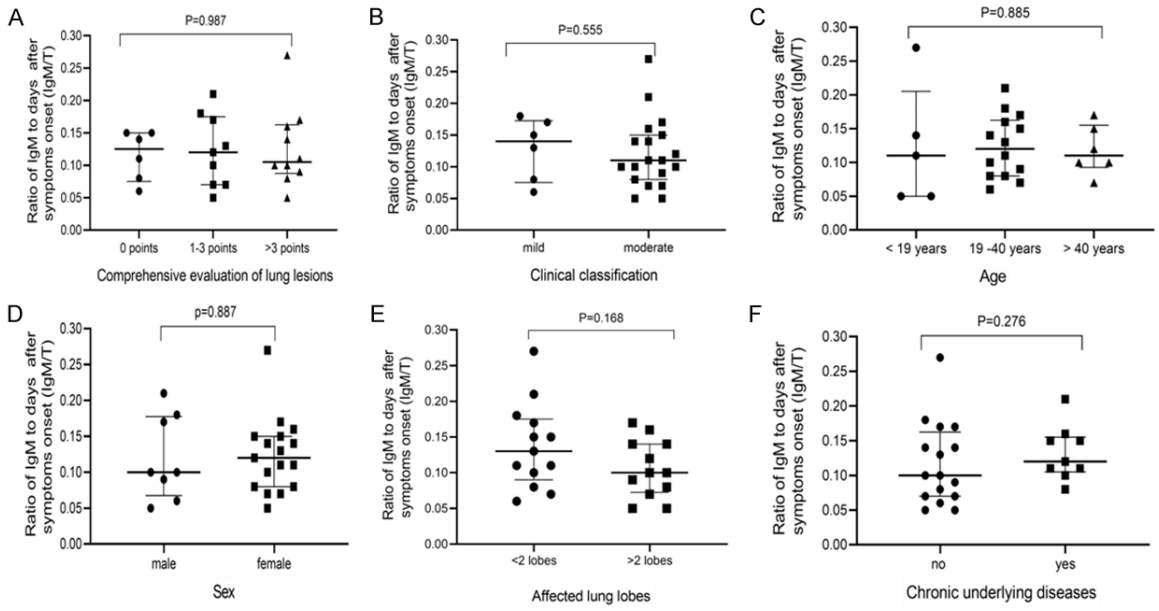

Figure 4. Analysis of host factors affecting IgM/T. There were no significant differences in IgM/T in terms of comprehensive score of lung damage (A), clinical classification (B), age (C), sex (D), number of lung lobes involved (E), and the presence or absence of chronic underlying disease (F).

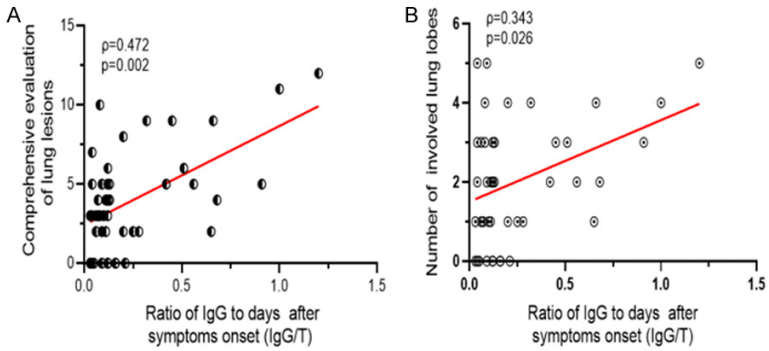

Figure 5. Correlation of IgG/T (from 8 days after symptom onset until the end of the observation period) with clinical characteristics. From 8 days after the symptoms appeared to the end of the observation period, a total of 53 blood samples from 42 patients were detected for IgG. Among them, 34 samples were from 34 patients, 10 samples were from 5 patients (2 samples each), and 9 samples were from 3 patients (3 samples each). If the patient has two or more samples, the average IgG value is used to calculate the IgG/T. Spearman correlation was used to evaluate the correlation of IgG/T with comprehensive score of lung lesions (Spearman ρ=0.472; P=0.002) (A) and number of involved lung lobes (Spearman ρ=0.343; P=0.026) (B).

related to the course of disease (Spearman ρ=0.17; P=0.283).

IgG/T positively correlates with the number of involved lung lobes and the comprehensive score of lung lesions

However, IgG/T was positively related to the number of involved lung lobes (Spearman ρ=0.343; P=0.026) and the comprehensive score of lung lesions (Spearman ρ=0.472; P=0.002) (**Figure 5**). Additionally, IgG/T was higher in common COVID-19 patients than mild patients (0.12 (0.078, 0.043) vs 0.085 (0.042, 0.12), P=0.039) (**Figure 6A**), in patients with ≥2 lung lobes involved than <2 lung lobes involved (0.125 (0.09, 0.495) vs 0.08 (0.048, 0.14), P=0.026) (**Figure 6B**), and, in patients with a comprehensive score of 0, 1-3, 4-6, and ≥7 points (0.07 (0.038, 0.12) vs 0.085 (0.06, 0.18) vs 0.125 (0.113, 0.488) vs 0.385 (0.11, 0.915), P=0.018) (**Figure 6C**). The higher the comprehensive score, the higher the IgG/T. Further analysis showed that patients of different age groups (<19 years old, 19-40 years old, >40 years old: 0.125 (0.08, 0.365) vs 0.115 (0.055, 0.168) vs 0.165 (0.09, 0.675), P=0.262) (**Figure 6D**), sex (male and female: 0.165 (0.09, 0.585) vs 0.115 (0.053, 0.243), P=0.054) (**Figure 6E**), and with presence or absence of chronic underlying diseases (0.09 (0.04, 0.665) vs 0.12 (0.075, 0.30),

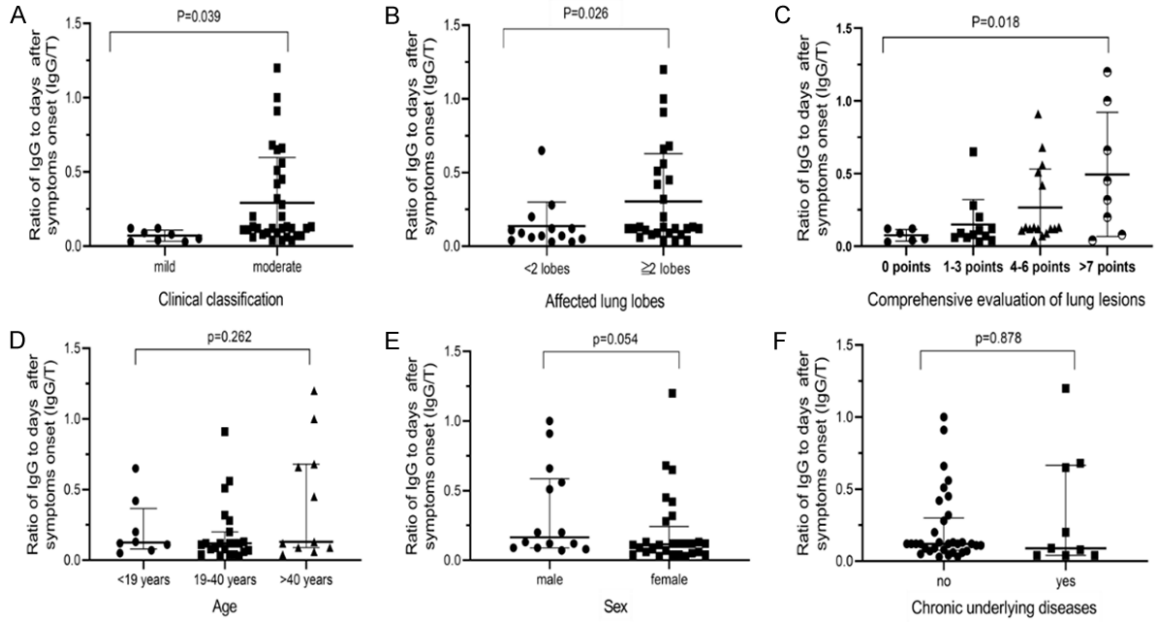

Figure 6. Analysis of host factors affecting IgG/T. There were significant differences in IgG/T among patients of different clinical classifications (A), numbers of lung lobes involved (B), and comprehensive score of lung lesion (C). However, IgG/T showed no significant differences in age (D), sex (E) and chronic underlying diseases (F).

P=0.878) (**Figure 6F**) were not significantly different in IgG/T.

Data of one representative case

In this study, the three other samples with abnormal antibody levels were all from the same patient. The patient was a 28-year-old female from Hubei, whose mother was diagnosed with COVID-19. This patient had consecutive negative results for pharyngeal swabs, nasal swabs, and anal swabs. Within 8-16 days after the onset of symptoms, three consecutive IgG/IgM antibody tests showed abnormal elevations. Among them, IgM/T in 2 weeks was 1.22, higher than the average level of 0.12. Moreover, IgG/T was 1.98, which was also higher than the average level of 0.24. The patient also had chronic hepatitis C virus, showing an increased HCV-RNA titer, accompanied by fever, cough and other symptoms. During the course of the disease, the patient had significant secondary leukopenia, but the patient recovered well, with a total disease course of 12 days (**Figure 7A**). The representative CT images were shown in **Figure 7B**.

Discussion

In this study, the earliest time for SARS-CoV-2 specific antibody testing was the fifth day after the onset of symptoms, and the IgM antibody was already positive. However, the median IgG antibody level was positive from the second week. Lee NY et al. [12] continuously tested IgG antibodies in a patient with COVID-19, and the results showed that the patient showed IgG positive on the 9th day after the onset of symptoms. However, they did not test IgM. Our results showed that IgM increased within 2 weeks of the onset of symptoms and then began to decline, while IgG antibody levels continued to increase until the end of the observation period. However, due to the lack of longer-term samples, we cannot evaluate the trend of IgG antibodies over a longer period of time after disease recovery. Du et al. [14] found that IgM and IgG antibody titers showed a downward trend between 6-7 weeks and 7-8 weeks after the onset of symptoms. Guo et al. [8] studied the time dynamics of IgM and IgG in 135 patients, including non-critical and critical COVID-19 patients. In their results, the IgM antibody time dynamic curve was similar to ours, but unlike us, their results showed that IgG increased within 3 weeks, and was with no longer increase thereafter. This may be related to the fact that the clinical classification of patients is different from ours. To KK et al. [9] collected 108 blood samples from 23 patients and analyzed the difference between IgM and

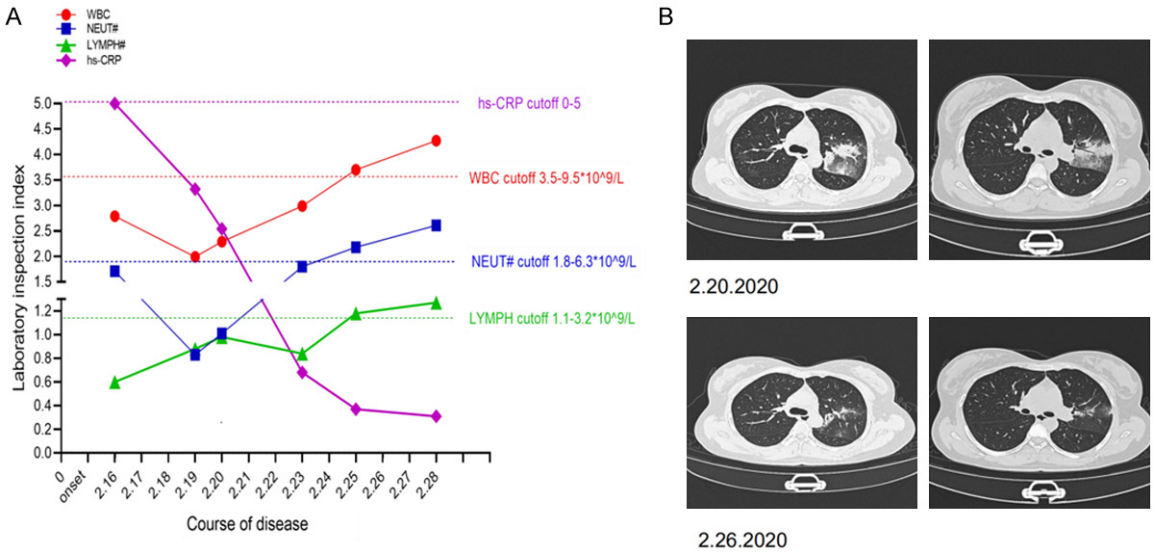

Figure 7. Blood routine and lung imaging changes during the patient's course. The patient was diagnosed as COVID-19 and had chronic hepatitis C. At the onset of the disease, the patient was in a serious condition with obvious secondary leukopenia. A. The patient began to have fever and cough on February 12, 2020. On February 16, we found that the patient's white blood cells, neutrophils, and lymphocytes all declined, and hs-CRP was at the upper limit of normal. On February 19, white blood cells and neutrophils continued to decline. After the patient was hospitalized and received antiviral treatment, the patient's white blood cells, granulocytes, and lymphocytes gradually increased, and hs-CRP gradually decreased to the lower limit. On February 25, the blood routine results returned to normal. The patients underwent three antibody tests on February 20, February 23, and February 28, and two lung CT scans on February 20 and February 26. B. CT results of lungs on admission and follow-up. February 20: There was a large consolidation and ground glass opacity in the upper lobe of the left lung, and interstitial thickening, showing a "paving stone" sign. There was also ground glass opacity in the upper lobe of the right lung, with blurred boundaries. February 26: The lesions in the left and right upper lobes were completely absorbed.

IgG in critical and non-critical patients. The IgM and IgG antibody titers of non-critical patients showed a linear increase after being positive during the observation period (25 days), while the IgM and IgG antibody titers of critical patients showed an inflection point until 2-3 weeks, and then remained stable or decreased. Abbasi et al. [15] proposed that quantitative antibody levels were essential for studying the immune response to viruses. Here, we detected IgM and IgG antibody levels with the time-resolved fluorescence immunochromatography, which can quantitatively detect IgM and IgG antibody levels.

In this study, two patients with positive anal swabs had abnormally elevated IgM and IgG. IgM, as an antibody in the acute phase of infection, generally begins to decline gradually two weeks after the onset of symptoms [8]. However, in these 2 patients, the IgM antibody was still abnormally increased 28 and 29 days after the symptom onset. Therefore, we consider that they may have uncontrolled SARS-CoV-2 infection, relapse or re-infection. There is still no evidence of SARS-CoV-2 re-infection. The results of Bao et al. [16] showed that rhesus monkeys infected with SARS-CoV-2 developed protective immunity less than a month after the initial infection. However, the results of Wu et al. [17] showed that not everyone produced neutralizing antibodies. Of the 175 COVID-19 patients in recovery phase, neutralizing antibodies were not detected in 10 patients. Moreover, it is still unclear whether these people who have been infected with SARS-CoV-2 have protective immunity [4]. In addition, whether the patients with negative pharyngeal swabs and positive anal swabs are contagious during the recovery period is an important issue of social concern [18-20]. Our results showed that some patients may be contagious. Further studies with more cases are needed to verify this result.

The humoral immune status of each patient is different. The cross-sectional antibody level is related to the days of antibody detection after symptom onset and the host immune response level, and cannot objectively reflect the humor-

al immune status of the patient [8]. Therefore, we used the ratio of antibody concentration to the days of antibody detection after symptom onset, that is, IgM/T and IgG/T, as an indicator to approximately reflect the humoral immune status of the host, eliminating the effect of time of detection. We found that IgM/T, which reflects the humoral immune level of the host during the acute phase of infection, was significantly negatively correlated with the course of the disease. At the same time, we observed that the lower the median IgM/T, the more severe the corresponding clinical condition. Although the difference was not statistically significant, this result is worthy of our attention. Antibody-mediated humoral immunity plays an important role in removing viruses [21]. Higher IgM/T indicate that the host has a strong ability to clear viruses, which is conducive to disease recovery. Zheng et al. [22] found that the recovery of COVID-19 depended largely on the immune status. In addition, our research showed that IgG/T in recovery period was positively correlated with clinical classification, the number of lobes involved and the severity of lung lesions. The higher the IgG/T, the more severe the clinical classification, the more the lung lobes are involved, and the higher the comprehensive score of lung lesions. These results indicate that higher IgG/T in recovery period may indicate a more serious condition of non-critical COVID-19 patients. Zhao et al. [5] also found that total antibody level was related to the severity of the disease. However, unlike us, they used antibody titers as evaluation indicators and did not remove the effect of antibody detection time on antibody levels. In addition, the study population in their studies included both critical and non-critical COVID-19 patients. Furthermore, we found that neither IgM/T nor IgG/T were affected by the patient's age, sex, or chronic underlying disease. To KK et al. [9] also found that IgG in patients with COVID-19 did not associate with the age of the host and comorbidities. However, these results are inconsistent with the fact that immune aging can damage the host's innate and adaptive immune response [23, 24]. This inconsistency may be related to the age of subjects in our study. Further studies are needed.

One COVID-19 patient with chronic hepatitis C had significant secondary leukopenia during the course of the disease. We believe that it may be related to the suppression of cellular immune function characterized by CD8+ T cell failure, which is caused by hepatitis C virus infection [25] and in turn leads to further increase of cellular immune suppression after SARS-CoV-2 infection. In addition, the patient's abnormally elevated IgM and IgG may represent the enhanced humoral immunity. Cellular immunity and humoral immunity play a synergistic role in viral infection. In SARS-CoV infection, cellular immune function immunosuppression [26] is also accompanied with strong humoral immunity [1]. The patient recovered well, which may be related to the patient's increased IgM/T within 2 weeks. Meanwhile, the patient's significantly increased IgG/T also indicates the severity of the patient's condition. Therefore, the combined analysis of antibodies and clinical conditions is more helpful in explaining the condition and understanding COVID-19.

However, this study still has some limitations. First, the sample size was small. Second, the SARS-CoV-2 antibody result was horizontal. Longitudinal studies may better explore the time dynamics of individual SARS-CoV-2 antibodies. Further longitudinal studies with larger sample sizes are needed.

In conclusion, this study has shed light on the role and predictive potential of antibody time-dynamics, IgM/T and IgG/T, in COVID-19. Combined analysis of clinical characteristics and antibody dynamics is very important for a more comprehensive understanding of COVID-19, and also provides a scientific basis for the prevention and control of COVID-19.

Acknowledgements

This work was supported by National Natural Science Fundation of China (No. 81470206) and Shenzhen Baoan District Science and Technology Innovation Bureau Project (No. 2017JD170).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yi Shi, Department of Respiratory and Critical Care Medicine, Jinling Hospital, The First School of Clinical Medicine, Southern Medical University, No. 305 East Zhongshan Road, Nanjing 210002, China. Tel: +86-139517-08712; E-mail: yishi_202011@163.com; Dr. Jing

Wang, Department of Radiology, Shenzhen Hospital, Southern Medical University, No. 1333, Xinhu Road, Baoan District, Shenzhen 518100, China. Tel: +86-17727819156; E-mail: wjing7997@163.com

References

[1] Li G, Chen X and Xu A. Profile of specific antibodies to the SARS-associated coronavirus. N Engl J Med 2003; 349: 508-509.

[2] Cheng M, Chan CW, Cheung RC, Bikkavilli RK, Zhao Q, Au SW, Chan PK, Lee SS, Cheng G, Ho WK and Cheung WT. Cross-reactivity of antibody against SARS-coronavirus nucleocapsid protein with IL-11. Biochem Biophys Res Commun 2005; 338: 1654-1660.

[3] Woo PC, Lau SK, Wong BH, Chan KH, Chu CM, Tsoi HW, Huang Y, Peiris JS and Yuen KY. Longitudinal profile of immunoglobulin G (IgG), IgM, and IgA antibodies against the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in patients with pneumonia due to the SARS coronavirus. Clin Diagn Lab Immunol 2004; 11: 665-668.

[4] Mallapaty S. Will antibody tests for the coronavirus really change everything? Nature 2020; 580: 571-572.

[5] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, Wang X, Yuan J, Li T, Li J, Tian S, Hong C, Wang F, Liu Y, Wang Z, He Q, Li Z, He B and Zhang Z. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 2020; 71: 2027-2034.

[6] Wang Y, Kang H, Liu X and Tong Z. Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. J Med Virol 2020; 92: 538-539.

[7] Xiang F, Wang X, He X, Peng Z, Yang B, Zhang J, Zhou Q, Ye H, Ma Y, Li H, Wei X, Cai P and Ma WL. Antibody detection and dynamic characteristics in patients with COVID-19. Clin Infect Dis 2020; 71: 1930-1934.

[8] Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, Dela Cruz C, Wang Y, Wu C, Xiao Y, Zhang L, Han L, Dang S, Xu Y, Yang Q, Xu S, Zhu H, Xu Y, Jin Q and Wang J. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis 2020; 71: 778-785.

[9] To K, Tsang O, Leung WS, Tam A, Wu TC, Lung D, Yip C, Cai JP, Chan J, Chik T, Lau D, Choi C, Chen LL, Chan WM, Chan KH, Ip J, Ng A, Poon R, Luo CT and Yuen KY. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020; 20: 565-574.

[10] Zhang B, Zhou X, Zhu C, Feng F, Qiu Y, Feng J, Jia Q, Song Q, Zhu B and Wang J. Immune phenotyping based on neutrophil-to-lymphocyte ratio and IgG predicts disease severity and outcome for patients with COVID-19. Front Mol Biosci 2020; 7: 157.

[11] Army Egot. COVID-19 diagnosis and treatment plan (trial second version). Chinese Journal of Tuberculosis and Respiratory Diseases 2020; 43.

[12] Lee NY, Li CW, Tsai HP, Chen P, Syue LS, Li MC, Tsai CS, Lo CL, Hsueh PR and Ko WC. A case of COVID-19 and pneumonia returning from Macau in Taiwan: clinical course and anti-SARS-CoV-2 IgG dynamic. J Microbiol Immunol Infect 2020; 53: 485-487.

[13] National Health Commission of the People's Republic of China. COVID-19 Diagnosis and Treatment Program (Trial 7th Edition).

[14] Du Z, Zhu F, Guo F, Yang B and Wang T. Detection of antibodies against SARS-CoV-2 in patients with COVID-19. J Med Virol 2020; 92: 1735-1738.

[15] Abbasi J. The promise and peril of antibody testing for COVID-19. JAMA 2020; 323: 1881-1883.

[16] Bao L, Deng W, Gao H, Xiao C, Liu J, Xue J, Lv Q, Liu J, Yu P, Xu Y, Qi F, Qu Y, Li F, Xiang Z, Yu H, Gong S, Liu M, Wang G, Wang S, Song Z, Liu Y, Zhao W, Han Y, Zhao L, Liu X, Wei Q and Qin C. Lack of reinfection in rhesus macaques infected with SARS-CoV-2. bioRxiv 2020. 03.13.990226; https://www.biorxiv.org/content/10.1101/2020.03.13.990226v2.

[17] Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, Ling Y, Zhang Y, Xun J, Lu L, Jiang S, Lu H, Wen Y and Huang J. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv 2020.03.30.20047365. https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2.

[18] Cheung K, Hung I, Chan P, Lung KC, Tso E, Liu R, Ng YY, Chu M, Chung T, Tam A, Yip C, Leung KH, Fung A, Zhang R, Lin Y, Cheng H, Zhang A, To K, Chan KH and Leung W. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from the Hong Kong cohort and systematic review and meta-analysis. Gastroenterology 2020; 159: 81-95.

[19] Wu Y, Guo C, Tang L, Hong Z, Zhou J, Dong X, Yin H, Xiao Q, Tang Y, Qu X, Kuang L, Fang X, Mishra N, Lu J, Shan H, Jiang G and Huang X. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. Lancet Gastroenterol Hepatol 2020; 5: 434-435.

[20] Zhang T, Cui X, Zhao X, Wang J, Zheng J, Zheng G, Guo W, Cai C, He S and Xu Y. Detectable SARS-CoV-2 viral RNA in feces of three children

during recovery period of COVID-19 pneumonia. J Med Virol 2020; 92: 909-914.
[21] Zhou G and Zhao Q. Perspectives on therapeutic neutralizing antibodies against the novel coronavirus SARS-CoV-2. Int J Biol Sci 2020; 16: 1718-1723.
[22] Zheng C, Wang J, Guo H, Lu Z, Ma Y, Zhu Y, Xia D, Wang Y, He H, Zhou J, Wang Y, Fei M, Yin Y, Zheng M and Xu Y. Risk-adapted treatment strategy for COVID-19 patients. Int J Infect Dis 2020; 94: 74-77.
[23] van Werkhoven CH, Huijts SM, Bolkenbaas M, Grobbee DE and Bonten MJ. The impact of age on the efficacy of 13-valent pneumococcal conjugate vaccine in elderly. Clin Infect Dis 2015; 61: 1835-1838.
[24] Weinberger B, Herndler-Brandstetter D, Schwanninger A, Weiskopf D and Grubeck-Loebenstein B. Biology of immune responses to vaccines in elderly persons. Clin Infect Dis 2008; 46: 1078-1084.
[25] Barili V, Fisicaro P, Montanini B, Acerbi G, Filippi A, Forleo G, Romualdi C, Ferracin M, Guerrieri F, Pedrazzi G, Boni C, Rossi M, Vecchi A, Penna A, Zecca A, Mori C, Orlandini A, Negri E, Pesci M and Ferrari C. Targeting p53 and histone methyltransferases restores exhausted CD8+ T cells in HCV infection. Nat Commun 2020; 11: 604.
[26] Panesar NS. Lymphopenia in SARS. Lancet 2003; 361: 1985.