Antibody response among COVID-19 patient with and without re-detectable positive RT-PCR results in convalescent phases

Jing Peng

Shenzhen Sami Medical Center

Zhi-Yong Liu

Shenzhen Sami Medical Center

Xiaojuan YU

xiamen university

Xiao-Yan Chen

Shenzhen Sami Medical Center

Kai Zhang

Shenzhen Sami Medical Center

Yi Liu

Shenzhen Sami Medical Center

Ying-Ying Su (✉ yingyingsu@xmu.edu.cn)

xiamen university <https://orcid.org/0000-0003-3883-357X>

Chang Qing Sun

Shenzhen Sami Medical Center

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Abstract

Objective: To analyze the dynamic of total, IgA, IgM and IgG antibody of the confirmed COVID-19 patients during convalescent phases to understand the kinetics of antibody response among recovered patients.

Methods: From March 4 to April 29, 2020, a total of 143 recovered COVID-19 patients with clear date of illness onset available were enrolled in this study. Nasopharyngeal and anal swabs were collected for SARS-CoV-2 RNA testing. Blood samples were collected for antibodies testing.

Results: A total of 275 blood samples up to 96 days after illness onset were collected from 143 recovered patients. High titers of total and IgG antibodies continued to persist for over 3 months, with 100% and 99.3% patients remaining positive for total and IgG antibody. IgM antibody declined rapidly with a median time to seronegative at 67 (95%CI: 59, 75) days after illness onset. Around 25% patients were seronegative for IgA antibody at month 3 after illness onset. No statistical significance difference was founded in the antibody kinetics between patients with and without re-detectable positive RT-PCR results during in convalescent phases.

Conclusion: Similar high antibody titers of total and IgG antibody continued to persist for over 3 months among recovered COVID-19 patients with and without re-detectable positive RT-PCR results.

Introduction

Coronavirus disease 2019 (COVID-19) is now pandemic globally which spreads rapidly to over 200 countries, with over 88 million confirmed cases and 1.9 million deaths up to 12th January 2021[1, 2]. Some studies reported that a proportion of recovered patients had re-detectable positive of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA with real-time reverse transcriptase-polymerase chain reaction (RT-PCR) tests [3-5]. Young and mild COVID-19 patients seemed to have higher risk of re-detectable positive RT-PCR results during convalescent phases [3, 6]. The antibody level tend to be similar among re-positive and non-re-positive groups [3, 6]. However, these studies included only one antibody test of each patient and did not adjusted onset time which was significantly correlated with antibody level. It is still unclear whether the dynamic of antibody responses in patients who have been re-positive after discharge differ from those who have not.

This study retrospectively analyzed the dynamic of total, IgA, IgM and IgG antibody of the confirmed COVID-19 patients during convalescent phases to understand the kinetics of antibody response among recovered patients.

Method

Study design and participants

In Shenzhen, COVID-19 patients who met the criteria of the hospital discharged were continued to be quarantined in the Shenzhen Sami Medical Center for 14 days of medical observation. The criteria for the hospital discharged including: a) normal body temperature for more than 3 days; b) significant improvement in respiratory symptoms; c) significant improvement in absorption of acute exudative lesions on lung imaging; and d) negative nucleic acid testing of two consecutive respiratory specimens (sampling interval of at least 1 day). During the 14 days quarantined period, all recovered patients would receive the SARS-CoV-2 RNA and antibody tests. After discharge from medical center, all patients were also invited to participate semimonthly or monthly follow-up to donor blood sample for antibody testing. All participants provided written informed consents.

This study was approved by Medical Ethical Committee of Shenzhen Sami Medical Center.

Laboratory examination

Nucleic acid detection

Nasopharyngeal and anal swabs were collected at 7th and 14th days in the medical center and tested by commercial RT-PCR assay target ORF1ab and N genes of SARS-CoV-2 as pervious described[6]. Briefly, nucleic acids were extracted by High Pure Viral RNA Kit (Roche, Mannheim, Germany). Nucleic acid amplification and identification by commercial RT-PCR test kits approved by National Medical Products Administration of China (Bio-Germ, Shanghai, China).

Antibody detection

Blood samples were collected at least one time during 14 days quarantine period in medical center, and then collected semimonthly or monthly after discharge. Total, IgA, IgM and IgG antibody against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein in the sera of COVID-19 patients were tested with a commercial chemiluminescence microparticle immunoassay (CMIA, Xiamen InnoDx Biotech Co., Ltd., China) as previous describe [7]. Briefly, total antibody detection was based on the double-antigen sandwich method. The capture method was applied to detect the IgM antibody. For IgG and IgA antibody detection, indirect method was used. The test procedure of all assays were performed in accordance with the manufacturer's instructions. The antibody levels were expressed by the relative binding signals compared to the cutoff value of each assay (S/CO). The sensitivity of total, IgM, IgG and IgA antibody were 96.3%, 86.3%, 99.6% and 91.9% respectively, and the specificity of total, IgM, IgG and IgA antibody were 99.3%, 99.3%, 99.0% and 98.6%, respectively (unpublished data for IgG and IgA) [7].

Statistical analysis

Geometric mean titers (GMT) for Total, IgA, IgM and IgG antibody with 95% confidence interval were calculated by days after illness onset. Multivariable log-binomial regression models with generalized estimating equations were used to compare the antibody level between re-positive and non-re-positive groups, which adjusted for age and days after illness onset. The probability of maintaining seropositive

and median time to seronegative were calculated by the Kaplan-Meier method, and compared by log-rank tests. All analyses were conducted by using SAS software (version 9.4), and p value less than 0.05 considered as statistical significance.

Results

Baseline Characteristics of patients

From March 4 to April 29, 2020, a total of 306 recovered COVID-19 patients receiving medical observation in the Shenzhen Sami Medical Center. Among them, 143 (46.7%) with clear date of illness onset available were enrolled in this study. There were 23(16.1%) recovered patients who had re-positive RT-PCR results during the period of the medical observation. As shown in table 1, the median age (IQR) of re-positive patients and non-re-positive patients were 50 (34, 58) and 50 (37, 61) respectively. Sixty-seven patients (46.9%) were male. The median duration of follow-up were 45 days (IQR: 40-70, range:26-96) post illness onset for all patients. Patients with re-positive RT-PCR result during 14-days medial observation followed longer than patients without (72 days vs 44 days post illness onset).

Antibody kinetics

A total of 275 blood samples were collected from 143 patients, with median 4 and 1 samples for each patient in re-positive and non-re-positive group separately. All recovered patients were seropositive for total antibody in their first sample during the medical center. As shown in figure 1, the levels of total antibody were decreased slowly and remain at high S/CO value at around 3 months after illness onset (GMT=162.5, 95%CI: 64.7-408.0), with none patients seronegative in their last samples. For IgG antibody, the levels remain at a plateau from 1 month to 3 months after illness onset. Only one patient was negative for IgG antibody at 53 days after illness onset. The levels of IgM antibody declined fastest compared with other antibodies, with median time to seronegative at 67 (95%CI: 59, 75) days after illness onset. IgA antibody declined at a rate between IgM and IgG. Around 25% patients were seronegative for IgA antibody at month 3 after illness onset.

As shown in figure 2 and figure 3, the antibody levels and the prevalence of seropositive for total, IgG, IgM and IgA were similar among patients with and without re-detectable positive RT-PCR results (all p values higher than 0.05).

Discussion

This study shows that high antibody titers of total and IgG antibody among recovered COVID-19 patients continued to persist for over 3 months. IgM antibody declined rapidly with a median time to seronegative at around 2 months. IgA antibody declined slower than IgM, but faster than IgG. However, no statistical significance difference was founded in the antibody kinetics among patients with and without re-detectable positive RT-PCR results during in convalescent phases.

In line with the current studies[8, 9], our study confirmed that IgG antibodies remained stable over 3 months. Antibody response of SARS-CoV-2 infection may be similar to SARS-CoV-1 or other viral infection[10]. IgM antibodies response is fleeting, and among half of patients, IgM antibodies would be undetectable around 2 months. Thus similar to other viral infections, IgM antibody against SARS-CoV-2 could be a marker of acute infection with SARS-CoV-2[11].

The antibody levels and the prevalence of seropositive for total, IgG, IgM and IgA were similar among patients with and without re-detectable positive RT-PCR results. There are some preprint researches raised possibilities which thought to be related to the re-positive, such as virology, sample collecting methods and immunology. There were studies indicated that the SARS-CoV-2 nucleic acid could remain in the lower respiratory tract specimens and feces longer than respiratory tract for up to 50 days[12, 5]. In addition, the samples quality and the sensitivity and specificity of the commercial detection kits may result in the false positive result which on some extent account for the happening of the re-positive patients[3]. There was a study showing that the levels of IgG and IgM were similar in the re-positive and non-re-positive patients, which was similar to this study[3]. Another preprint study suggested that the titer levels of IgG and IgM were not correlated with the clinical course, therefore the antibody levels could not be predictors of the disease process.

Some limitations should be noticed in this study. First, the convenient collection of blood samples led to unequally distributed sample sizes over time. Second, majority of patients provided only one blood sample during convalescent phases. Third, the duration of follow-up was relatively short.

In conclusion, this study confirmed that high antibody titers of total and IgG antibody among recovered COVID-19 patients continued to persist for over 3 months. The antibody response among patients with and without re-detectable positive RT-PCR results was similar during in convalescent phases.

Declarations

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest: The authors have declared that no conflict of interest exists.

Ethics approval: This study was approved by Medical Ethical Committee of Shenzhen Sami Medical Center.

Availability of data and material: The data will be available beginning 9 months and ending 36 months following article publication. The data will be shared with investigators whose proposed use of the data has been approved by an independent review committee identified for individual participant data meta-analysis. Proposals should be directed to yingyingsu@xmu.edu.cn or sunchangqing@ssmc-sz.com. To gain access, data requestors will need to sign a data access agreement.

Code availability: software application.

Authors' contributions Conceptualization was performed by Jing Peng and Chang-Qing Sun. Investigation was performed by Jing Peng, Zhi-Yong Liu, Xiao-Yan Chen, Kai Zhang and Yi Liu. Data curation was performed by Zhi-Yong Liu, Xiao-juan YU, Xiao-Yan Chen, Kai Zhang, Yi Liu and Chang-Qing Sun. Methodology was performed by Zhi-Yong Liu, Ying-Ying Su and Chang-Qing Sun. Formal analysis was performed by Xiao-juan YU and Ying-Ying Su. Writing-original draft was performed by Jing Peng, Zhi-Yong Liu, Xiao-juan YU and Ying-Ying Su. Writing-review & editing was performed by Ying-Ying Su and Chang-Qing Sun. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Baseline Characteristics of patients

Variables	Re-positive	Non-re-positive	Total
Number	23	120	143
Age(median, IQR)	50(34, 58)	50(37, 61)	50(37, 61)
Gender(%)			
Male	7(30.4)	60(50.0)	67(46.9)
Female	16(69.6)	60(50.0)	76(53.1)
No. of samples collected			
Of each case, median(IQR)	4(2, 5)	1(1, 1)	1(1, 3)
Total	85	190	275
Duration of follow up(days after illness onset)			
Median(IQR)	72(62, 80)	44(39.5, 62)	45(40, 70)
Range	40, 91	26, 96	26, 96

Figures

Figure 1

Antibody kinetics of COVID-19 patient in convalescent phases. A) The changes levels of total, IgG, IgM and IgA antibodies; B) Kaplan-Meier estimates of the probability of seropositive for total, IgG, IgM and IgA antibodies.

Figure 2

Comparison of antibody level of COVID-19 patient with and without re-detectable positive RT-PCR results. A) Total antibody; B) IgG antibody; C) IgM antibody; D) IgA antibody.

A. Total antibody

B. IgG

C. IgM

D. IgA

Figure 3

Kaplan–Meier curves comparing the prevalence of seropositive between COVID-19 patient with and without re-detectable positive RT-PCR results. A) Total antibody; B) IgG antibody; C) IgM antibody; D) IgA antibody.