

Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019

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Summary: Combining RNA and antibody detections significantly improved the sensitivity of pathogenic diagnosis for COVID-19 in the early phase of infection. A higher titer of Ab was independently associated with a worse clinical classification.

Abstract

Background

The novel coronavirus SARS-CoV-2 is a newly emerging virus. The antibody response in infected patient remains largely unknown, and the clinical values of antibody testing have not been fully demonstrated.

Methods

A total of 173 patients with SARS-CoV-2 infection were enrolled. Their serial plasma samples (n=535) collected during the hospitalization were tested for total antibodies (Ab), IgM and IgG against SARS-CoV-2. The dynamics of antibodies with the disease progress was analyzed.

Results

Among 173 patients, the seroconversion rate for Ab, IgM and IgG was 93.1%, 82.7% and 64.7%, respectively. The reason for the negative antibody findings in 12 patients might due to the lack of blood samples at the later stage of illness. The median seroconversion time for Ab, IgM and then IgG were day-11, day-12 and day-14, separately. The presence of antibodies was <40% among patients within 1-week since onset, and rapidly increased to 100.0% (Ab), 94.3% (IgM) and 79.8% (IgG) since day-15 after onset. In contrast, RNA detectability decreased from 66.7% (58/87) in samples collected before day-7 to 45.5% (25/55) during day 15-39. Combining RNA and antibody detections significantly improved the sensitivity of pathogenic diagnosis for COVID-19 ($p < 0.001$), even in early phase of 1-week since onset ($p = 0.007$). Moreover, a higher titer of Ab was independently associated with a worse clinical classification ($p = 0.006$).

Conclusions

The antibody detection offers vital clinical information during the course of SARS-CoV-2 infection. The findings provide strong empirical support for the routine application of serological testing in the diagnosis and management of COVID-19 patients.

Keywords: antibody, SARS-CoV-2, COVID-19

Introduction

Since early December of 2019 and up to March 22, 2020, over 260,000 cases of coronavirus disease 2019 (COVID-19) caused by novel coronavirus (SARS-CoV-2) infection, with over 11,000 deaths have been reported in 184 countries.¹ The World Health Organization declared that COVID-19 can be characterized as a pandemic on March 11, 2020. According to recent reports, most of COVID-19 patients have an incubation period of 3 to 7 days.² Fever, cough and fatigue are the most common symptoms, whereas nasal congestion, runny and diarrhea are only noted in a small part of the patients.³ Severe cases might rapidly progress to acute respiratory distress syndrome (ARDS), septic shock and difficult-to-tackle metabolic acidosis and bleeding and coagulation dysfunction.⁴ It should be noted that some of COVID-19 patients only had mild atypical symptoms initially, even for severe and critical cases.⁵ The chest computed tomography of COVID-19 patients were characterized by the ground-glass opacity and bilateral patchy shadowing.⁶ For laboratory test, it was reported that most of patients had lymphopenia and elevated C-reactive protein.⁷ However, the above-mentioned clinical and laboratory characteristics are not easily distinguishable from pneumonia induced by infection with other common respiratory tract pathogens.

The timely and accurate diagnosis of the SARS-CoV-2 infection is the cornerstone of the efforts to provide appropriated treatment for patients, to limit further spread of the virus and ultimately to eliminate the virus from human society. Currently, PCR-based viral RNA detection is almost the only way to confirm the diagnosis of SARS-CoV-2 infection in practice. On the other hand, RNA testing based on throat or nasopharyngeal swabs brought out negligible false-negative risk.⁸ The reported positive rate varied for different swab specimens in COVID-19 patients.^{3,9} Many cases that were strongly epidemiologically linked to SARS-CoV-2 exposure and with typical lung radiological findings remained RNA negative in their upper respiratory tract samples. The performance of RT-PCR depends on many factors such as the sample types⁹, different stage of infection in patients,¹⁰ the skill of sample collection, and the quality and consistency of the PCR assays being used. These problems

lead to a noteworthy delay of early diagnosis and following management and propose serious challenge to providing timely life support treatment and preventive quarantine.

Comparing to PCR, serological testing is advantageous with faster turn-around time, high-throughput and less workload. However, the clinical value of antibodies largely depends on the understanding of host antibody responses during infection. Given that SARS-CoV-2 is a newly emerging virus, the antibody response in COVID-19 patients remains largely unknown. This study investigates the dynamics of total antibody (Ab), IgM and IgG antibody against SARS-CoV-2 in serial blood samples collected from 173 confirmed COVID-19 patients and provides discussion on the clinical value of antibody testing.

Methods

Patients

A confirmed COVID-19 case and the clinical classification was defined based on the New Coronavirus Pneumonia Prevention and Control Program (4th edition) published by the National Health Commission of China. Briefly, patient with acute respiratory infection syndromes and/or abnormalities in chest CT images accompanied by detectable SARS-CoV-2 RNA in respiratory sample since illness onset for at least one time was confirmed as COVID-19 case. Patients were classified as in critical illness condition with ARDS or oxygen saturation <93% who required mechanical ventilation either invasively or non-invasively. This study enrolls a total of 173 cases of COVID-19, where all patients were admitted to the Shenzhen Third People's Hospital between Jan 11 and Feb 9, 2020, and were willing to donate their blood samples. All enrolled cases were confirmed to be infected with SARS-CoV-2 by use of real-time RT-PCR (rRT-PCR) on samples from the respiratory tract. For all enrolled patients, the date of illness onset, clinical classification, RNA testing results during the hospitalization period, and the personal demographic information were obtained from the clinical records. This study was reviewed and approved by the Medical Ethical Committee of

Shenzhen Third People's Hospital (2020-0018). Written informed consent was obtained from each enrolled patient.

Antibody measurement

The Ab, IgM antibody and IgG antibody against SARS-CoV-2 in plasma samples were tested using enzyme linked immunosorbent assay (ELISA) kits supplied by Beijing Wantai Biological Pharmacy Enterprise Co.,Ltd, according to the manufacturer's instructions. Briefly, the ELISA for total antibodies detection was developed based on double-antigens sandwich immunoassay (Ab-ELISA), using mammalian cell expressed recombinant antigens contained the receptor binding domain (RBD) of the spike protein of SARS-CoV-2 as the immobilized and HRP-conjugated antigen. The IgM μ -chain capture method (IgM-ELISA) was used to detect the IgM antibodies, using the same HRP-conjugate RBD antigen as the Ab-ELISA. The IgG antibodies were tested using indirect ELISA kit (IgG-ELISA) based on a recombinant nucleoprotein. The specificity of the assays for Ab, IgM and IgG was determined as 99.1% (211/213), 98.6% (210/213) and 99.0% (195/197) by testing of samples collected from healthy individuals before the outbreak of SARS-CoV-2.

Statistical analysis

For continue variables description, mean with standard deviation was used for normal distribution data and median with interquartile range (IQR) was used for non-normal distribution data. Cumulative seroconversion rates were calculated by Kaplan-Meier method. The association between antibody level and severity of illness were estimated by generalized estimating equations (GEE) model with logit link function. All statistical analysis was conducted by SAS 9.4 (SAS Institute, Cary, NC, USA).

Results

The characterization of patients

Among totally 368 COVID-19 patients admitted in the hospital (before Feb 9, 2020), 173 of them (47%) were enrolled in the study (Table 1). The median age of the studied patients was 48 years (IQR, 35-61) and 51.4% were females. There were 116 (67%) patients had clear epidemiological travel/residence history in Wuhan. Among them, 32 (18.5%) were in critical illness condition. By February 19, a total of 62 patients (35.8%) were recovered and discharged from hospital and 2 (1.1%) patients died with underlying chronic disease.

Seroconversion of antibodies against SARS-CoV-2 in COVID-19 patients

A total of 535 plasma samples collected during the hospitalization period of the 173 patients were tested for antibodies against SARS-CoV-2. The seroconversion rate for Ab, IgM and IgG was 93.1% (161/173), 82.7% (143/173) and 64.7% (112/173), respectively (Table 1). Twelve patients who remained seronegative for Ab testing possibly due to that their samples involved were all collected at the early stage of illness (10 earlier than day-10, the other two on day-11 and-13 after onset). The cumulative seroconversion curve showed that the rate for Ab and IgM reached 100% around 1-month after onset. The seroconversion was sequential appeared for Ab, IgM and then IgG (Figure 1A). The median time to Ab, IgM and IgG seroconversion was 11-, 12- and 14-day, separately. One of two patients tested on the onset day was seropositive. Overall, the seroconversion of Ab was significantly quicker than that of IgM ($p = 0.012$) and IgG ($p < 0.001$), that possibly attributed to the double-antigen sandwich form of the assay used which usually show much higher sensitivity than capture assay (IgM) and indirect assay (IgG). Moreover, all isotypes of viral specific antibodies, including IgM, IgA and IgG, can be detected by double-sandwich assay, which may also contribute to the superior performance of Ab test. In comparisons of seroconversion rates of antibodies between critical and non-critical patients, none of the three markers showed significant difference (data not shown).

The diagnosis value of antibody assays for patients in different time after onset

In the early phase of illness within 7-day since onset, the RNA test had the highest sensitivity of 66.7%, whereas the antibody assays only presented a positive rate of 38.3% (Table 2). However, the sensitivity of Ab overtook that of RNA test since day 8 after onset and reached over 90% across day 12 after onset (Figure 1B). In samples from patients during day 8-14 after onset, the sensitivities of Ab (89.6%), IgM (73.3%) and IgG (54.1%) were all higher than that of RNA test (54.0%, Table 2). Among samples from patients in later phase (day 15-39 since onset), the sensitivities of Ab, IgM and IgG were 100.0%, 94.3% and 79.8%, respectively. In contrast, RNA was only detectable in 45.5% of samples of day 15-39. Further analyses demonstrated that in patients with undetectable RNA in their respiratory tract samples collected during day 1-3, day 4-7, day 8-14 and day 15-39 since onset, there were 28.6% (2/7), 53.6% (15/28), 98.2% (56/57) and 100% (30/30) had detectable antibody in total Ab assay, respectively (Table S1). Whatever, combined use of the tests of RNA and Ab improved markedly the sensitivities of pathogenic-diagnosis for COVID-19 patients in different phases (Table 2).

The dynamics of antibody levels with the progress and severity of disease

To investigate the dynamics of antibody level according disease course, the antibody levels were expressed using the relative binding signals compared to the cutoff value of each assay (S/CO). The longitudinal changes of antibody and RNA in 9 representative patients, including 6 in non-critical group (Figure 2A) and 3 in critical group (Figure 2B), were presented in Figure 3. The first positive time point of RNA tests appeared earlier than that of Ab in 7 of 9 patients, except for the case 185 (Ab was detectable 2 days earlier than RNA) and the case 111 (at the same day). It should be noted that the risings of antibodies were not always accompanied by RNA clearance, particularly in the 3 critical patients. This finding suggested that antibodies may not be sufficient to clear the virus. In the pooled analyses on all involved patients, the average antibody levels showed a marked increase since about 1-

week after onset and continuously elevated during the next 2 weeks (Figure 3A). Further analyses suggested that there was no significant difference on the average S/CO value of Ab tests between critical and non-critical patients before day 12 after onset (Figure 3B). However, critical patients showed significantly higher Ab S/CO values than non-critical cases in about 2-week after onset ($p=0.02$) and this association was not significant in either IgM or IgG tests (data not shown). For further exploration, we determined the relative Ab titer of these samples (expressed as relative optical density, rOD) by serial dilution measurements of each sample. The quantitative data of Ab titers also revealed a significant difference ($p=0.004$) between patients in critical and non-critical groups (Figure 3C). Multivariate longitudinal GEE analyses suggested that age ($\beta=0.139$, $p < 0.001$), gender ($\beta=1.415$, $p=0.006$) and Ab titer ($\beta=0.336$, $p=0.006$) were the independent factors strongly associated with the clinical classification based on the severity (Table S2).

Discussion

The present data demonstrated that typical antibody responses to acute viral infection are widely induced in COVID-19 patients. To be expected, the total antibody was first detected, followed by IgM and IgG. The seroconversion rate and the antibody levels increased rapidly during the first two weeks, the cumulative seropositive rate reached 50% on the 11th-day and 100% on the 39th-day. The seroconversion time of Ab, IgM and IgG antibodies appeared consequently ($p < 0.05$) with a median seroconversion day of 11, 12 and 14, respectively. Due to the lack of blood samples collected from patients in the later stage of illness, how long the antibodies could last remain unknown. Our results demonstrated an excellent sensitivity of Ab test in detections of patient's samples since 1-week after onset. Notably, even in the early stages of the illness within 1-week, some patients with undetectable RNA could be screened out through Ab testing. Combining RNA and antibody tests significantly raised the sensitivity for detecting patients ($p < 0.001$). These findings indicate that serological test be an important supplement to RNA detection during the illness course.

Up to date, the confirm diagnosis of SARS-CoV-2 infection entirely depend on the viral RNA testing. Even though with high analytical sensitivity, the real-world performance of RNA test is unsatisfied. Many suspected patients had to be tested for several days with multiple samples before confirm diagnosis were made, and during the waiting time they might have not enough priority to receive relevant treatments and quarantine managements.³ These problems make the timely diagnosis of SARS-CoV-2 infection one of the bottlenecks for adapting relevant actions to limit the damage of current outbreak. Our study provided robust evidences that: 1) the acute antibody response in SARS-CoV-2 infection patient is very similar to many other acute viral infections; 2) the serological testing can be a powerful approach in achieving timely diagnosis; and 3) the total antibody is more sensitive than IgM and IgG for detecting SARS-CoV-2 infection.

Thus, the antibody testing might play vital roles in the following settings: 1) for the suspected patients under the initial visit or clinically diagnosed patients has not been confirmed by RNA testing, the positive result of antibody increases the confidence to make a COVID-19 diagnosis; 2) for healthy close contact who is in the quarantine period, he/she should be deemed as a probable carriers if antibody positive, then the RNA should be tested more frequently and the close contacts of him/her should be observed; 3) for the RNA confirmed patient, seropositive indicates that the specific antibodies had been induced. Besides, epidemiological studies could be conducted using immunoassays. Additional, it can play an important role in searching potential animal hosts for SARS-CoV-2 using Ab-ELISA because the double-sandwich method makes it free from species restriction. It has been less than three months since the SARS-CoV-2 first invaded humans, and the prevalence of antibody against SARS-CoV-2 is nearly zero. Therefore, at least during the current outbreak which is likely to continue to May or June 2020, seropositive individuals could be a probably preceding infector. During this short-period, the total antibody could be considered as a recent infection marker similar as IgM. As the higher sensitivity than IgM and IgG, the Ab detection should be given high priority to be implemented in current clinical and public health

practice. If, unfortunately, SARS-CoV-2 become a common respiratory transmission pathogen lasting in humans, like influenzas or other less-pathogenic coronaviruses, rather than be completely eradicated as SARS-CoV-1, the serological diagnosis of acute SARS-CoV-2 infection will more depend on the detection of IgM in post-epidemic areas in the subsequent epidemic seasons. The Ab and IgG could be used to understand the epidemiology of SARS-CoV-2 infection and to assist in determining the level of humoral immunity in patients. Even then, the Ab will be a more sensitive marker for sentinel monitoring of imported cases in naive community.

In addition to the diagnosis value, our study revealed a strong positive correlation between clinical severity and Ab titer since 2-week after illness onset, for the first time in COVID-19 patients. The results suggested that a high Ab titer may be considered as a risk factor of critical illness, independently from older age, male gender and comorbidities (Table S2). Although it was still unclear how the causal relation between hormonal response and illness severity, the results raise a possible usage of the high Ab titer as a surrogate marker for worse clinical prognosis. Furthermore, it might be an evidence for the possibility of antibody-dependent disease enhancement effects, which was commonly found in SARS-CoV-1 patients.¹¹⁻¹³ Whatsoever, our finding suggested that the clinical meanings of the level of antibody against SARS-CoV-2 during the acute phase of infection warrant further study.

It should be noted that there were some limitations of this study. First, for most of RNA tests of the patients were based on upper respiratory tract specimens, the positive rate may be higher in detection using lower respiratory tract specimens, such as bronchoalveolar lavage fluid and deep tracheal aspirates, may yield higher sensitivity for RNA tests. Second, all the patients enrolled in this study were based on the positive findings of RT-PCR using respiratory samples. Untypical patients with lower respiratory viral load might be missed. Besides, the performance of RT-PCR depends on many factors such as the quality and consistency of the PCR assays being used, the skill of sample collection, and the sample types. Also, RT-PCR cannot distinguish the viable replicating virus and simply residual RNA,

which may affect the results. Third, we cannot evaluate the persistence of antibodies because samples were collected during the acute phase of patients. Fourth, although it had shown good specificity in healthy people, the cross-reactivity of the assay to other coronaviruses should be further assessed.

In conclusion, the findings demonstrate that antibody tests have important diagnosis value in addition to RNA tests. These findings provide strong evidence for the routine application of serological antibody assays in the diagnosis and clinical management of COVID-19 patients.

NOTES

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Conflict of interests: We declare no competing interests.

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Table 1. Demographics and clinical characteristics of patients and sample cohort with COVID-19 in this study.

	Total	Non-critical	Critical
Number	173	141	32
Gender, n(%)			
Male	84 (49)	63 (45)	21 (66)
Female	89 (51)	78 (55)	11 (34)
Age, median(IQR)	48 (35-61)	41 (33-56)	64 (58-66)
Epidemiological exposure (1 month)			
Been to Wuhan	116 (67)	92 (65)	24 (75)
Unclear or others	57 (33)	49 (35)	8 (25)
Comorbidities, n(%)*	41 (24)	26 (18)	15 (47)
Clinical outcome, n(%)			
Recovery	62 (36)	54 (38)	8 (25)
Still in hospital	109 (63)	89 (62)	22 (69)
Death	2 (1.2)	0	2 (6.3)
RNA confirmed time since onset, days, median(IQR) †	4 (3-6)	4 (3-6)	6 (4-10)
Days since onset of 1 st sample for antibody test, median(IQR) ‡	7 (5-10)	7 (5-9)	10 (6-16)
RNA (TS/NS) at the involved 1 st sample			
positive, n(%)	89 (51)	73 (52)	16 (50)
negative, n(%)	65 (38)	55 (39)	10 (31)
no data, n(%)	19 (11)	13 (9.2)	6 (19)
rRT-PCR CT, median(IQR)	29 (25-31)	29 (24-32)	29 (28-31)
No. of antibody tested samples			
Of each case, median(IQR)	3 (2-4)	3 (2-4)	4 (3-5)
Total	535	404	131

Data are presented as medians (interquartile ranges, IQR) and n(%). TS, throat swabs; NS, nasal swabs. * The comorbidities included hypertension (n=20), diabetes (n=11), coronary heart disease (n=3), chronic hepatitis B infection (n=5), tumors (n=2), obstructive sleep apnea syndrome (n=1), chronic bronchitis (n=1), hyperlipidemia (n=1), renal insufficiency (n=1), tuberculosis (cured, n=1) and fatty liver disease (n=1). † The data indicated the time of confirmation for positive for 2019-nCoV infection by using rRT-PCR on respiratory sample since illness onset. ‡ The data indicated the time since illness onset of the first plasma sample of patients involved for serological test in this study.

Table 2. Performance of different detections in samples at different time since onset of patients.

Days after onset	n	RNA		Ab		IgM		IgG		RNA+Ab	
		n(+)	Sensitivity (% , 95%CI)	n(+)	Sensitivity (% , 95%CI)	n(+)	Sensitivity (% , 95%CI)	n(+)	Sensitivity (% , 95%CI)	n(+)	Sensitivity (% , 95%CI)
Total	173	112 [§]	67.1 (59.4, 74.1)	161	93.1 (88.2, 96.4)	143	82.7 (76.2, 88)	112	64.7 (57.1, 71.8)	172	99.4 (96.8, 100.0)
1-7	94	58 [§]	66.7 (55.7, 76.4)	36	38.3 (28.5, 48.9)	27	28.7 (19.9, 39.0)	18	19.1 (11.8, 28.6)	74	78.7 (69.1, 86.5)
8-14	135	67 [§]	54.0 (44.8, 63.0)	121	89.6 (83.2, 94.2)	99	73.3 (65.0, 80.6)	73	54.1 (45.3, 62.7)	131	97.0 (92.6, 99.2)
15-39	90	25 [§]	45.5 (32.0, 59.5)	90	100.0 (96.0, 100.0)	83 [*]	94.3 (87.2, 98.1)	71 [#]	79.8 (69.9, 87.6)	90	100.0 (96.0, 100.0)

* Two patients missed IgM tests due to inadequate plasma samples. # One patient missed IgG tests due to inadequate plasma samples. § There were 7, 11 and 35 patients had not been performed RNA testing during the 1-7 onset day, 8-14 onset day and 15-39 onset day, respectively.

Figure Legends

Figure 1. Cumulative incidence of seroconversion of antibodies against SARS-CoV-2 among COVID-19 patients during the acute phase since illness onset. (A) Cumulative incidence of seroconversion of Ab, IgM and IgG among 173 patients of this study. P values were determined by Log-Rank test to compare different markers. (B) Profiling of sensitivity performances of RNA, Ab, IgM and IgG in time series since illness onset. A heat-map of detection of SARS-CoV-2 infection according to the time (days) since onset by a single RNA or antibody test.

Figure 2. Dynamic profiling of viral RNA and antibodies in representative COVID-19 patients since onset of disease. The changes of the levels of RNA in upper respiratory specimens (nasal and/or throat swabs) and antibodies (total Ab, IgM and IgG) in plasma of 9 patients were presented. Among these cases, 6 were in normal to moderate illness condition (A) and 3 were in critical condition (B). The cutoff values for antibody tests were $S/CO=1$ (plotted to left-Y axis) and was $CT=40$ for RNA detection (plotted to right-Y axis). RNA negative samples are denoted with a CT of 45. The blue area indicated the antibody seronegative zone, whereas the purple area indicated undetectable RNA zone. Meanwhile, a purple broken line was used to indicate the first time point with detectable RNA and a red broken line was used to indicate the first antibody seroconversion (total Ab) time point.

Figure 3. The average levels of antibodies against SARS-CoV-2 among COVID-19 patients since illness onset. (A) Comparison of the average S/CO value of in between total Ab, IgG and IgM. Comparison of the average S/CO value (B) and relative quantitative titer (C) of Ab test between critical and non-critical patients. The medians of antibody detection value (S/CO for tests of Ab, IgM and IgG, for panel A and B) and of total Ab titer (rOD, for panel C) of samples at the same time point since onset was used to plot the graph. Patient's samples collected from day 1-3, 4-6, 7-9,

10-12, 13-15, 16-18, 19-21, 22-39 since illness onset were pooled for analysis. Four parameter logistic (4PL) fitting curves were used to show the rising trend of antibodies (total Ab, IgG, IgM).

Figure 1

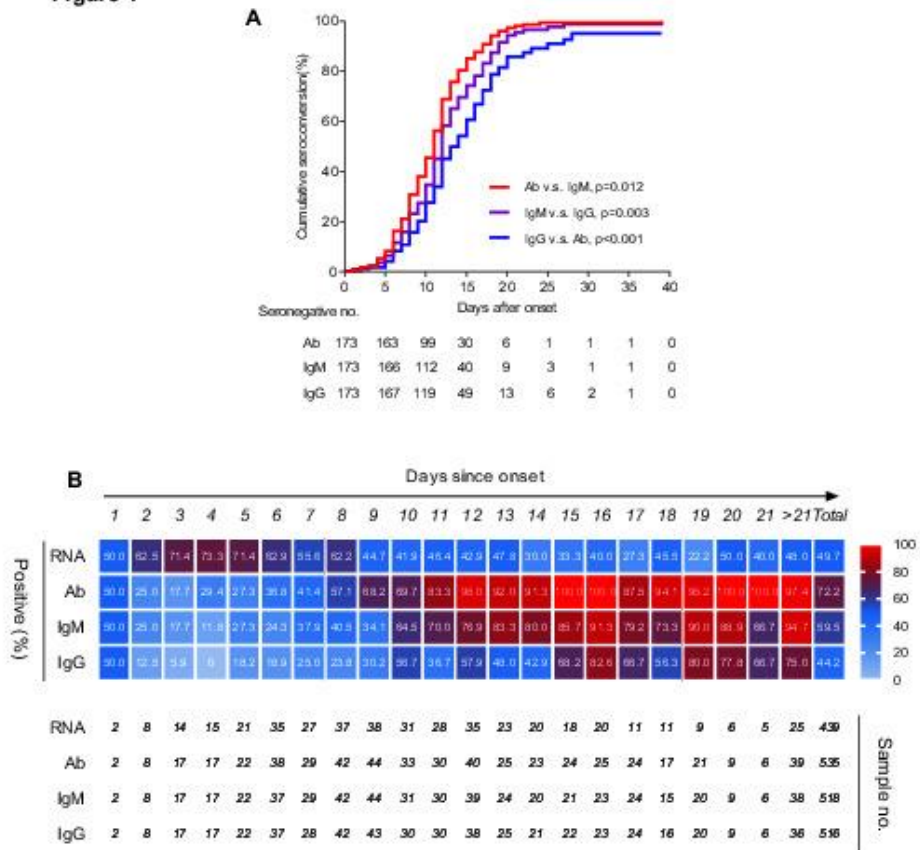


Figure 2

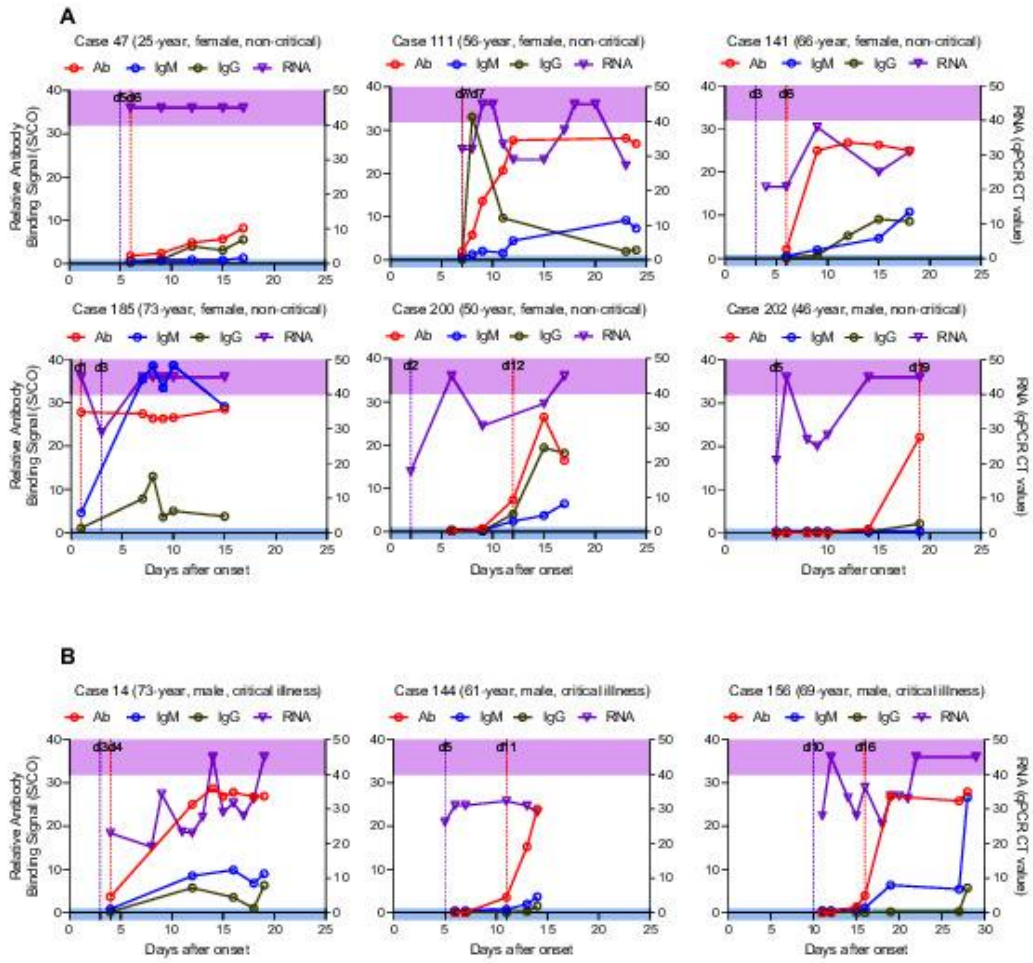


Figure 3

