Antibodies and antigen kinetics in patients with COVID-19

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Research Article

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Abstract

The antigen and antibody kinetics of SARS-CoV-2 infected patients remains unclear, and the clinical values of the serological test have not been completely elucidated. A total of 154 serum samples from 13 patients with COVID-19 infection were collected at about three-day intervals during hospitalization. Samples were screened for SARS-CoV-2-specific total antibodies (TAb), IgA, IgM, IgG, and antigen (Ag) using chemiluminescent microparticle immunoassays (CMIA). The overall seroconversion and/or four-fold increase rates of TAb, IgA, IgM, and IgG during hospitalization were 92.31%, 92.31%, 84.62%, and 92.31%, respectively. However, within a week of onset, antibodies were present in <50% of the patients. The combination of “Ag and/or TAb” maintained the positive rate at 81.82% during the first three days after symptom onset and quickly enhanced to 92.31% during 4–6 days after the symptom onset. The seropositive median day of Ag was two days after symptom onset. Among patients who underwent IgM and IgG seroconversion, the seroconversion median days of IgA, TAb, IgM, and IgG were 9.5 days, 10 days, 11 days, and 11.5 days after the symptom onset, respectively. Serological testing, especially virus-specific antigen testing, may be helpful for early identification of suspected patients and asymptomatic infections.

Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory infectious disease caused by SARS-CoV-2 . The incubation period lasts from 1–14 days, most commonly 3–7 days. The main manifestations include fever, dry cough, and fatigue. Patients could be classified into mild, moderate, severe, and critical [1]. Globally, as of November 23, 2021, there have been a total of more than 256 million confirmed cases of COVID-19, including more than 5.1 million deaths. “Early detection, early isolation, early diagnosis, and early treatment” are currently the most effective means to deal with COVID-19 . Accurate and rapid diagnosis of COVID-19 is essential to control the pandemic [2].

The confirmation of COVID-19 exclusively depends on the "gold standard" method, namely quantitative reverse transcription polymerase chain reaction (RT-qPCR) for virus nucleic acid detection. However, low level of viral load and virus mutation are the major cause of false-negative results. Serological testing could be applied as an important supplement in combination with the standard nucleic acid testing to improve detection sensitivity and accuracy, especially in cases with low viral load [3]. Antibody tests have been included in the COVID-19 diagnostic criteria and the exclusion criteria for suspected cases. However, the positive rates of the novel coronavirus-specific IgM antibody and IgG antibody tests within one week of symptom onset are relatively low [1]. Currently, most studies describing the serological diagnostic methods focused on the detection of SARS-CoV-2-specific total antibody (TAb), IgA, IgM, and IgG [3-5]. However, the kinetics of antigen (Ag) and antibodies of patients with COVID-19 remains insufficiently clear, especially in Asia.

Moreover, most Chinese people have been vaccinated, and it is difficult to find unvaccinated patients with COVID-19 in China. The antibodies and antigen levels of vaccinated patients might be different from those of unvaccinated patients. Studies on antigen and antibodies kinetics of unvaccinated patients could be helpful for serological diagnosis and seroepidemiological survey[6], especially in China.

To explore the SARS-CoV-2 Ag kinetics along with the changes of virus-specific TAb, IgA, IgM, and IgG, and to improve the accuracy of COVID-19 diagnosis, especially in the first week after symptom onset, the newly-developed chemiluminescence enzyme immunoassay kits were applied to detect SARS-CoV-2-specific Ag and antibodies in 154 serum samples from 13 admitted hospital patients with confirmed SARS-CoV-2 infection. The results indicated that virus-specific Ag and/or TAb could strongly enhance the detection rates of COVID-19, even in the early stage of the disease.

Methods

Patients and serum samples

A total of 154 serum samples from 13 patients with COVID-19 confirmed by RT-qPCR were collected at about three-day intervals during hospitalization. All of the enrolled patients were Chengdu residents and they were treated in Public Health Clinical Centre of Chengdu, one of the biggest designated hospitals for COVID-19 in Sichuan province. The study was approved by the Ethics Committee of Public Health Clinical Centre of Chengdu (ref. No. PJ-K2020-23-01). Due to the retrospective nature of the study, written informed consent was waived.

RT-qPCR assay

RT-qPCR was applied for viral RNA detection every 2–3 days after patients’ admission. Briefly, nasopharyngeal samples were collected from suspected patients and placed into collection tubes with virus preservation solution. Viral RNA samples were extracted on the Automatic Nucleic Acid Extraction System (NP968C, Tianlong Science & Technology Co., Ltd, Xi’an, China) using the Virus DNA/RNA Isolation Kit (Tianlong Science & Technology Co., Ltd, Xi’an, China) in line with manufacturer’s instructions. A commercial RT-qPCR kit, 2019 Novel Coronavirus (2019-nCoV) RNA detection kit (Daan Gene Co., Ltd. of Sun Yat-Sen University, Guangzhou, China, registration no. 20203400063) was used for the detection of SARS-CoV-2 open reading frame 1 ab (ORF1ab) and nucleocapsid protein (N) genes [7, 8]. Patients with one positive RT-qPCR result were recognized as SARS-CoV-2 positive. After symptom resolution, patients with two consecutive negative RT-qPCR results with a sampling time interval of at least 24 hours were defined as SARS-CoV-2 negative [1, 9].

SARS-CoV-2-specific antibodies and antigen detection

The chemiluminescent microparticle immunoassays (CMIA) were applied for the virus-specific TAb, IgG, IgM, IgA, and Ag detection following the manufacturer’s instructions (Innodx Biotech Co. Ltd., Xiamen, China, Chinese national medical devices registration approval numbers 20203400198 (TAb)).

For TAb detection, the double antigen sandwich CMIA strategy was applied. In the first step, each sample was mixed with the magnetic microparticles coated with recombinant SARS-CoV-2 antigen (M_Ag1). The specific TAb in the sample interacted with the recombinant antigen coated on the magnetic microparticles, and the ingredients not bound to the magnetic microparticles were washed and removed. In the second step, recombinant antigen-labeled acridinium ester (Ag2_AE) was added to form a "M_Ag1–TAb–Ag2_AE" complex. After washing, the substrate solution was added. The chemiluminescence reaction signal was measured and expressed as relative luminescence unit (RLU). RLU positively correlated with the specific antibodies or antigen levels in the serum sample.

For IgG detection, the indirect CMIA strategy was applied. In the first step, each sample was mixed with the magnetic microparticles coated with recombinant SARS-CoV-2 antigen (M_Ag1). The specific IgG in the sample interacted with the recombinant antigen coated on the magnetic microparticles, and the ingredients not bound to the magnetic microparticles were washed and removed. In the second step, anti-human IgG labeled acridinium ester (antiIgG_AE) was added to form a "M_Ag1–IgG–antiIgG_AE" complex. After washing, the substrate solution was added. The chemiluminescence reaction signal was measured and expressed as RLU.

For IgM detection, the capture CMIA strategy was applied. In the first step, each sample was mixed with the magnetic microparticles coated with anti-human IgM monoclonal antibody (M_antiIgM), where the specific IgM in the sample interacted with the anti-human IgM monoclonal antibody coated on the magnetic microparticles, and the ingredients not bound to the magnetic microparticles were washed and removed. In the second step, recombinant SARS-CoV-2 antigen-labeled acridinium ester (Ag2_AE) was added to form a "M_antiIgM–IgM–Ag2_AE" complex. After washing, the substrate solution was added. The chemiluminescence reaction signal was measured and expressed as RLU.

For IgA detection, the indirect CMIA strategy was applied. In the first step, each sample was mixed with the magnetic microparticles coated with recombinant SARS-CoV-2 antigen (M_Ag1), where the specific IgA in the sample interacted with the recombinant antigen coated on the magnetic microparticles, and the ingredients not bound to the magnetic microparticles were washed and removed. In the second step, anti-human IgA labeled acridinium ester (antiIgA_AE) was added to form a "M_Ag1–IgA–antiIgA_AE" complex. After washing, the substrate solution was added. The chemiluminescence reaction signal was measured and expressed as RLU.

For Ag detection, the direct CMIA strategy was applied. In the first step, each sample was mixed with lysate and the magnetic microparticles coated with SARS-CoV-2-specific antibody (M_Ab1). The virus particles in the sample were lysed to expose the internal virus nucleocapsid protein (NP) antigen. The NP antigen interacted with the specific antibody coated on the magnetic microparticles. After incubation, SARS-CoV-2-specific antibody-labeled acridinium ester (Ab2_AE) was added to form a "M_Ab1–Ag–Ab2_AE" complex. The ingredients not bound to the magnetic microparticles were washed and removed. Then, the substrate solution was added. The chemiluminescence reaction signal was measured and expressed as RLU.

All the tests were performed under rigorous biosafety conditions using an automated magnetic chemiluminescence analyzer (WANTAI BioPharm Caris200, Innodx Biotech Co. Ltd) in line with the manufacturer's guidelines. The titer of antibodies or antigen was tested once per serum sample. The signal-to-cutoff (S/CO) ratio was calculated with S/CO < 1.0 being recognized as negative and S/CO ≥ 1.0 being recognized as positive. Seroconversion was interpreted as the SARS-CoV-2-specific TAb, IgG, IgM, IgA, or Ag detection results changing from negative to positive in sequential samples.

Statistical analyses

Continuous variables were presented as the median (interquartile range, IQR) and analyzed with Wilcoxon rank-sum test. Categorical variables were shown as counts (%) and analyzed using Fisher's exact probability test. Correlation analysis was conducted using Spearman's test. A *P* value <0.05 was recognized as statistically significant. Data were analyzed with Rstudio (version 3.6.1) and Statistical Product and Service Solutions (SPSS Inc. IBM Corp., Chicago, IL, version 25).

Results

Demographic and patient characteristics

The median age of the enrolled patients was 69 years (IQR, 54–71 years) and 61.5% (8/13) were women. Of the 13 patients, 1 patient had asymptomatic infection, one was classified as severe, and the remaining 11 patients were classified as mild or moderate according to the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial 8th edition)[1]. The characteristics of these patients are summarized in Supplementary Table 1 and Supplementary Table 2.

Performance of CMIA for virus-specific antibodies and antigen

We used the CMIA for virus-specific antibodies and antigen detection. In accordance with the manufacturer's instruction, clinical tests for evaluation of specificity and sensitivity of the TAb detection kit were conducted at three institutions. A total of 386 suspected cases were enrolled, including 222 COVID-19 confirmed patients and 155 excluded cases. The results demonstrated that the specificity was 98.06% (95% confidence interval [CI], 94.46%–99.34%). The overall sensitivity was 80.29%, which gradually increased with the disease progress. The sensitivity was 41.54% (95% CI, 30.36%–53.66%) during the first week from the disease onset, 85.51% (95% CI, 78.67%–90.42%) during the second week, and 92.52% (95% CI, 87.10%–95.77%) after two weeks from onset. To test the diagnostic value of SARS-CoV-2-specific IgG and IgM, 1000 people with SARS-CoV-2 RT-qPCR detection results were enrolled and received IgG and IgM tests. The sensitivities for IgG and IgM were 86.71% (95% CI, 82.29%–90.16%) and 89.16% (95% CI, 85.03%–92.26%), respectively. The specificities for IgG and IgM were 99.75% (95% CI, 98.59%–99.96%) and 99.25% (95% CI, 97.81%–99.74%), respectively. To determine the diagnostic value of the kits for

SARS-CoV-2-specific Ag, we enrolled 30 donors without COVID-19-related symptoms, who tested negative on RT-qPCR. Among these samples, none tested Ag positive, resulting in a specificity of 100% for SARS-CoV-2-specific Ag detection.

The overall seroconversion and/or four-fold elevation rates of TAb, IgA, IgM, and IgG during hospitalization were 92.3% (12/13), 92.3% (12/13), 84.6% (11/13), and 92.3% (12/13), respectively (Supplementary Figure 1a). In addition, 92.3% (12/13) of the patients displayed seropositivity of virus-specific Ag (Supplementary Figure 1a). The only asymptomatic patient (patient ID 5) also demonstrated seroconversion and four-fold elevation in the titers of TAb, IgG, IgM, and IgA (Supplementary Figure 1b), indicating that being asymptomatic might not affect serological dynamics of patients with COVID-19.

To objectively define the disease stage, the initial day of symptom onset was recognized as the starting time point. We found that the 10th–12th days after the initial onset of symptoms could be recognized as the key time period when most patients yielded antibodies (Figure 1a). In contrast, before the 10th–12th days, the antigen test was important for confirmation of viral infection. Within three days after symptom onset, 92.3% of patients tested positive for Ag, while all became negative within 18 days (Figure 1a). The combination of antigen test and total antibody (Ag and/or TAb) could greatly improve the diagnostic efficacy (Figure 1b, P>0.05, Fisher's exact probability test).

Regarding seroconversion pattern analysis among 13 patients with confirmed COVID-19, 10 patients underwent IgM and IgG seroconversions within 18 days after the symptom onset. Three types of seroconversion were observed: 1) IgG seroconversion earlier than that of IgM (three patients); 2) synchronous seroconversion of IgG and IgM (three patients); and 3) IgG seroconversion following that of IgM (four patients). The seroconversion median days of IgA, TAb, IgM, and IgG were 9.5 days, 10 days, 11 days, and 11.5 days after symptom onset, respectively. The seropositive median day of Ag was two days after symptom onset (Supplementary Figure 2a).

When virus-specific antibodies and antigen were both taken into account, another three types of serological changes were observed: 1) four-fold elevation without seroconversion in the titers of antibodies (two patients); 2) antigen seropositivity preceded antibody seroconversion (10 patients), 3) antigen maintained negative while antibody underwent seroconversion (one patent) (Supplementary Figure 2b). The longitudinal antibodies and antigen alterations of the 13 individuals are shown in Supplementary Figure 3.

The dynamic analysis of antibodies and antigen levels revealed that TAb levels of the patients who experienced TAb seroconversion during the follow-up period reached plateau 3–6 days after the first positive TAb measurement (Supplementary Figure 4a). Plateaued TAb levels varied broadly (≥24-fold) among patients. IgG and IgM also exhibited similar changes (Supplementary Figure 4b–c). IgA levels reached plateau about three days after seroconversion, after which some of them underwent obvious decrease (Supplementary Figure 4d). The varying Ag levels in patients who underwent Ag seropositivity became negative at the third week after the disease onset (Supplementary Figure 4e–f). During the first five weeks after onset, there was obvious growth in virus-specific TAb, IgG, IgM, and IgA antibody titers. However, IgM demonstrated a subtle reduction in the third week after the onset (Supplementary Figure 4f).

Correlation analysis between serological detection results and clinical laboratory tests showed that IgA positively correlated with alpha1-antitrypsin (rho=0.714, *P*=0.008); IgG positively correlated with lymphocyte percentage (rho=0.832, *P*=0.001); IgM (rho=−0.718, *P*=0.006) and TAb (rho=−0.709, *P*=0.007) negatively correlated with eosinophil rate (eosinophil count/total number of white blood cells). Notably, there was a significant negative correlation between eosinophil count and the average level of Ag during the first week after the symptom onset (rho=−0.859, *P*<0.001).

Discussion

The outbreak of the SARS-CoV-2 has created a big challenge for public health laboratory sciences. Although serological tests have been frequently applied for viral infection diagnosis and exclusion [1], there were few reports on virus-specific antigen assays in the detection of SARS-CoV-2. In this study, we evaluated the application of the CMIA for the detection of SARS-CoV-2 antibodies and antigen in admitted hospital patients with confirmed SARS-CoV-2 infections. The results showed that during the first week after the symptom onset, the positive rate of antigen was obviously higher than that of antibodies, indicating that antigen test was more sensitive and accurate than antibody detection to diagnose SARS-CoV-2 infection in the acute phase of virus infection. More importantly, the combination of "Ag and/or TAb" maintained the positive rate at 81.82% during the first three days after the symptom onset and quickly enhanced to 92.31% during the 4th–6th days after the symptom onset. Our small-scale survey strongly demonstrated that along with virus-specific antibodies, virus-specific antigen could be helpful because it showed high sensitivity to RT-qPCR-based viral RNA detection, especially in the first week after the symptom onset.

The specific antibodies generated by SARS-CoV-2 infection mainly include IgA, IgM, and IgG, which are biologically active immunoglobulins that can specifically bind to the viruses. The detection of antibodies can help in COVID-19 diagnosis and infection status evaluation. IgA mainly occurs early in the blood and mucosa, and it plays a crucial role in mucosal immunity, such as in the respiratory tract. Compared with IgG, IgM is also produced at a relatively early stage by the body's initial immune response, which is usually applied as an important serological indicator of an early infection. Considering its short maintenance period, low concentration, and relatively low affinity, IgM can temporarily protect the host. In contrast, IgG is the major immunoglobulin distributed in tissue fluid and blood, which possesses a high affinity and can helpfully resist secondary infections. Usually, the creation of IgG lags after IgM. IgG is maintained for a longer time than IgM and has a protective effect on the host. A positive IgG can be recognized as a sign of infection or past infections [2]. The TAb test kit of Innodx Biotech for COVID-19 is actually a combination of IgG, IgM and IgA. According to Supplementary Figure 2a, after excluding patients without seroconversion, the seropositive median day of Ag was 2 days, while the seroconversion median days of IgA, TAb, IgM, and IgG were 9.5 days, 10 days, 11 days, and 11.5 days, respectively. Basically, the order of seroconversion median days of different antibodies was in line with the previous literature [2]. However, when all the patients were included, the seroconversion median days of IgM and IgG were simultaneous (10 days after the symptom onset). Our findings closely align with those of a recently published paper in which the seroconversion median days of IgM and IgG also

overlapped (13 days after the symptom onset) [7]. These data suggested that the sensitivities of chemiluminescence-based serological detection kits for IgM and IgG might have been improved (10 days vs. 13 days), while the resolution for IgM and IgG seroconversion median days needs to be further optimized.

Recent studies have shown that COVID-19 severity, clinical outcomes, and serum inflammatory factors significantly correlated with the levels of antibodies[10] . Limited by the small sample size, in this study, we only analyzed the correlation between blood test results (Supplementary Table 2) and serological test results. A few significant correlations between plateaued antibody levels and the clinical laboratory tests were observed. Moreover, significant negative correlation was observed between eosinophil count and the average level of Ag during the first week after the symptom onset. The above analysis might provide data for further studies.

Conclusion

Despite being a preliminary CMIA for SARS-CoV-2, our study reported the virus-specific antibodies and antigen kinetics and their relationship. We highlighted that the combination of antibodies and antigen assays might be a useful technique for the surveillance and control of COVID-19, especially at the very beginning of the regional outbreaks, where there are many suspected patients and asymptomatic infections needing confirmation of diagnosis.

Supplementary Files

Supplementary Table 1 Baseline characteristics of patients with COVID-19

Supplementary Table 2 Laboratory results of patients with COVID-19

Supplementary Figure 1 Detection flowchart and main results of serological tests. (a), detection flowchart; (b), antibodies and antigen detection results in patients with COVID-19. TAb: SARS-CoV-2 total antibody.

Supplementary Figure 2 Serological changes in the SARS-CoV-2-specific antibodies and antigen. (a), serological patterns of patients who underwent IgM and IgG seroconversion (n=10); (b), serological patterns of all patients (n=13). Type 1: IgG seroconversion earlier than that of IgM; Type 2: synchronous seroconversion of IgG and IgM; Type 3: IgG seroconversion later than that of IgM; Type A: four-fold elevation without seroconversion in the titers of antibodies; Type B: antigen seropositivity earlier than antibody seroconversion; Type C: antigen maintained negative. TAb: SARS-CoV-2 total antibody.

Supplementary Figure 3 Antibodies and antigen kinetics of each patient with COVID-19. (a), Patients with IgG seroconversion earlier than IgM; (b), Patients with synchronous seroconversion of IgG and IgM; (c), Patients with IgM seroconversion earlier than IgG; (d), Patients without IgG and IgM seroconversion. TAb: SARS-CoV-2 total antibody.

Supplementary Figure 4 The antibodies and antigen kinetics in patients with COVID-19 at different time points. Dynamic changes in the (a) TAb, (b) IgG, (c) IgM, (d) IgA, and (e) Ag. (f), Levels of SARS-CoV-2-specific antibodies and antigen in patients from the first week to the fifth week after the symptom onset. The box plots in (f) show medians (middle line) with the third and first quartiles (boxes), while the whiskers show 1.5× the interquartile range (IQR) above and below the box. The numbers of patients were 13, 13, 12, 10, and four for the first week, second week, third week, fourth week, and fifth week, respectively. TAb: SARS-CoV-2 total antibody.

Abbreviations

COVID-19: Coronavirus disease 2019; TAb: total antibodies; Ag: antigen; CMIA: chemiluminescent microparticle immunoassays; RT-qPCR: reverse transcription polymerase chain reaction; RLU: relative luminescence unit; NP: nucleocapsid protein; S/CO: signal-to-cutoff; IQR: interquartile range.

Declarations

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Conflict of interest

The authors report that they have no conflicts of interest.

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Authors' contributions

HJZ, MZ, and DDL conceived the study and designed experimental procedures; MZ, QFL, YLZ, DDL, and YFW performed the experiment; HJZ, MZ, GQL, KPW, fulfilled data acquisition, data analysis, and data interpretation; HJZ, GQL, MZ, XC, KD, RL, and XZ wrote the paper; All authors have reviewed and approved the final version.

Data Availability Statement

Raw data in this study were available from the corresponding authors on request.

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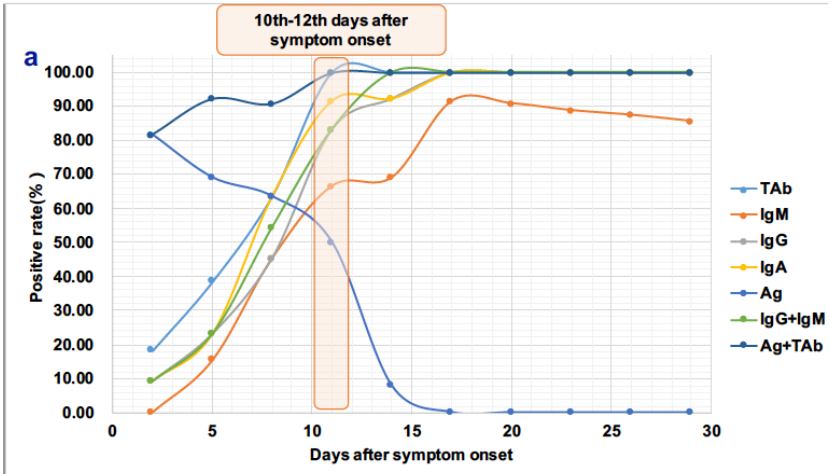
Figures



Days after symptoms onset	Samples(n)	Positive samples[n(%)]						
		TAb	IgG	IgM	IgA	Ag	IgG+IgM	Ag+TAb
1-3	11	2(18.18)	1(9.09)	0(0.00)	1(9.09)	9(81.82)	1(9.09)	9(81.82)
4-6	13	5(38.46)	3(23.08)	2(15.38)	3(23.08)	9(69.23)	3(23.08)	12(92.31)
7-9	11	7(63.64)	5(45.45)	5(45.45)	7(63.64)	7(63.64)	6(54.55)	10(90.91)
10-12	12	12(100.00)	10(83.33)	8(66.67)	11(91.67)	6(50.00)	10(83.33)	12(100.00)
13-15	13	13(100.00)	12(92.31)	9(69.23)	12(92.31)	1(7.69)	13(100.00)	13(100.00)
16-18	12	12(100.00)	12(100.00)	11(91.67)	12(100.00)	0(0.00)	12(100.00)	12(100.00)
19-21	11	11(100.00)	11(100.00)	10(90.91)	11(100.00)	0(0.00)	11(100.00)	11(100.00)
22-24	9	9(100.00)	9(100.00)	8(88.89)	9(100.00)	0(0.00)	9(100.00)	9(100.00)
25-27	8	8(100.00)	8(100.00)	7(87.50)	8(100.00)	0(0.00)	8(100.00)	8(100.00)
28-30	7	7(100.00)	7(100.00)	6(85.71)	7(100.00)	0(0.00)	7(100.00)	7(100.00)

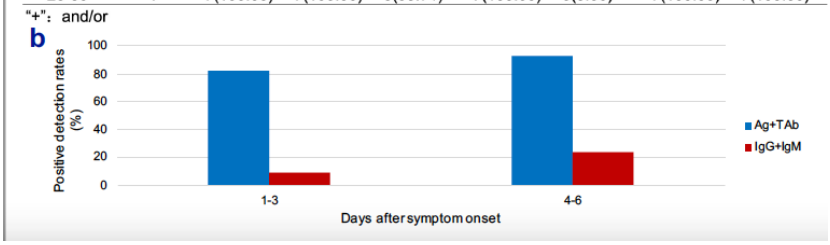


Figure 1

Antibodies and antigen responses against SARS-CoV-2. (a), Diagram of positive rates of virus-specific TAb, IgG, IgM, IgA, and Ag versus days after the symptom onset in 107 serum samples from 13 patients. (b), The positive detection rate of cases using the “detection in combination of Ag and/or TAb” versus “detection in combination of IgG and/or IgM”. TAb: SARS-CoV-2 total antibody.

Supplementary Files

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