The Kinetics of Humoral Response and its Relationship with the Disease Severity in COVID-19

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Research Article

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Abstract

Coronavirus Disease 2019 (COVID-19) has caused global pandemic. Here we profiled the humoral response against SARS-CoV-2 by measuring immunoglobulin (Ig) A, IgM and IgG against nucleocapsid, spike proteins and IgM, IgG antibodies against receptor-binding domain (RBD) of the spike protein along with total neutralizing antibodies. We tested 279 plasma samples collected from 176 COVID-19 patients. We demonstrate more severe cases have a late onset in the humoral response compared to mild/moderate infections. All the antibody titers continue to rise in patients with COVID-19 over the disease course. However, these levels are mostly unrelated to the disease severity. The appearance time and titers of neutralizing antibodies showed significant positive correlation to the antibodies against spike protein. Our results suggest late onset of antibody response as a risk factor for disease severity, however there is a limited role of antibody titers in predicting disease severity of COVID-19.

Main Text

Coronavirus disease 2019 (COVID–19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV–2) emerged as a major pandemic that spread across the globe with enormous healthcare and economic costs with more than 4.2 million infections and 292,000 deaths as of May 14, 2020[1,2].

Humoral responses have been the major indicators of the disease severity during other viral infections in the lung including infections with SARS-CoV and influenza virus[3–5]. SARS-CoV–2 genome encodes four structural proteins including spike (S), nucleocapsid (N), envelope and membrane proteins[1]. The S and N proteins are the two major antigens in coronavirus that induce immunoglobulin (Ig) production[6]. Antibodies against the N protein are often induced in relative higher abundance than others, which is the main target for serological diagnosis[6,7]. The receptor-binding domains (RBD) present in the S1 region of S protein are the main target of neutralizing antibodies (NAbs) and can be a potential target for vaccine development[6,8,9]. Presence of NAbs is one of the most important indicators in clinical outcome and vaccination effectiveness during other respiratory viral infections[9,10].

Previously, we described the diagnostic value of SARS-CoV–2 antibody tests in the detection of COVID–19 in asymptomatic and possibly convalescent patients[7]. However, the prognostic value of the humoral responses against SARS-CoV–2 infections has not been described in a systemic manner describing detailed immune response against various viral proteins and the relationship between humoral responses and disease severity in COVID–19. In this study, we evaluate the onset time, positive rate and titers of NAbs, IgA, IgM and IgG against N, S and IgM and IgG against RBD domains of SARS-CoV–2. The correlations between the onset time and titers to the disease severity were also investigated.

A total of 176 COVID–19 inpatients were recruited from three independent cohorts, in which 113 (64.2%) were males. The patients were aged from 18 to 82 years (mean of 49.0). Majority of the patients had

clinical symptoms included fever (151, 85.8%), cough (136, 77.3%), and dyspnea (56, 31.8%) upon admissions (Table S1). Comorbidities in form of underlying diseases were recorded in 83 (47.1%) patients, including hypertension, diabetes, chronic respiratory diseases, coronary heart disease, stroke and *etc.*

The disease severity of the patients was classified as mild/moderate (79.5%, 140/176) or severe infections (20.5%, 36/176) which included ten (5.7%) deaths. Compared to mild/moderate (termed as mild hereafter) cases, the disease symptoms including fatigue (20.7% *vs* 41.7%) (P = 0.010), headache (12.1% *vs* 38.9%) (P = 0.000), muscle pain (14.3% *vs* 47.2%) (P = 0.000), sore throat (6.4% *vs* 41.7%) (P = 0.000) and dyspnea (22.1% *vs* 69.4%) (P = 0.000) were present at a higher frequency in severe cases (^{2} test) (Table S1). The laboratory tests showed that the albumin (P = 0.001) decreased, and globulin increased (P = 0.013) significantly. The leukocytes (P = 0.000) and neutrophil ((P = 0.000)) counts were higher, while lymphocytes were lower (P = 0.009) in severe cases compared to mild/moderate cases (Table S2).

A total of 279 plasma samples were collected from the 176 patients, in which 103 patients provided two plasma sample, and 73 patients provided one sample. All the plasma samples were obtained between 1–46 days after disease onset, with 60 samples between days 1–7, 108 samples between days 8–14, 104 samples between days 15–28 and 7 samples between days 29 to 46. First, the appearance time of IgA, IgM, IgG antibodies against N, S proteins, and the IgM and IgG antibodies against RBD protein, as well as the presence of NAbs against SARS-CoV–2- were examined. The NAbs titers were tested by using microneutralization assay against SARS-CoV–2 in Vero cells as depicted in Fig. S1A. The presence of neutralizing antibody was further confirmed using immunofluorescence assay and viral nucleic acids quantification by infecting the plasma treated viruses on Vero cells using a qPCR method (Fig. S1). The neutralizing titers of the plasma samples were quantified based on the cytopathic effects.

All the antibodies were found to be positive as early as the first day after the symptom onset. The dynamic positive rate of serum antibodies was depicted in Figure 1A and supplementary file (Fig. S2).

The specific antibodies of IgA, IgM and IgG antibodies against N proteins were tested in 243 (87.1%), 216 (77.4%), and 207 (74.2%) samples, respectively. The N-IgA appeared at more than 90% positive rate and kept above 80% in the first three week following a decrease to 60% at >21 days. The positive rate of N-IgM increased to 80% at day 17 reaching the plateau. N-IgG had a late surge and reached at levels higher than other antibodies after day 23 of disease onset (Fig. 1A). The median seroconversion time was found to be 4 days (IQR 3.0–6.0) for N-IgM measured in the acute phase (≤7 days after symptom onset), 13 days for N-IgA (IQR 8.0–17.0) and 14 days for N-IgG (IQR 10.0–18.0).

The IgA, IgM and IgG antibodies against S proteins were tested in 119 (42.7%), 87 (31.2%) and 162 (58.1%) samples, respectively. The levels of S-IgG increased significantly and remained high and comparable to the S-IgA positive rates cross the disease course. In contrast, the positive rate of S-IgM remained lower across the disease course.

The median duration of antibody detection against N, S, RBD and appearance of NAbs is demonstrated in Fig. 1B and the median number of days since symptom onset were calculated. The titers of all the antibodies increased during the disease course including NAbs that significantly increased between week 1–2 and 2–3 and reached an apparent plateau at 3 weeks post symptoms (Fig. 1C). The tabulated data of antibody positivity along with demographic information is provided in Supplementary Table S3.

Next, we sought to evaluate whether the duration of appearance or the titers are related to the disease severity. We observed different kinetics of antibody appearance between mild/moderate and severe disease groups, with a significant delayed onset of IgA, IgM and IgG against N and S proteins, as well as IgM and IgG against RBD in severe patients (unpaired *t t*est) (Fig. 2A, Fig. S3). However, the antibody levels remained largely similar between mild/moderate and severe groups, except that S-IgA (P = 0.034) was higher in the third weeks, while RBD-IgM (P = 0.030) was lower in the fourth weeks, in severe cases, respectively (unpaired *t test)* (Fig. 2B).

NAbs are the critical indicator to evaluate clinical outcome and vaccination effects according to previous experience in that of other respiratory tract viruses[3,10]. The NAbs titers were tested by microneutralization assay. The neutralizing titers of the plasma samples determined according to cytopathic effect. Nabs increased significantly in mild/moderate cases in the third week (geometric mean titers, GMT 20.21) than that of second week (GMT 10.64) (P = 0.007). However, there are no significant increments of NAbs titers in severe cases during the disease course (Fig. 2B).

The S and RBD region are the key targets for vaccine design (9). We then defined the associations of anti-S and -RBD antibodies with NAbs in reference to the clinical disease severity. The appearance time of the anti-S IgA, IgM and IgG (r = 0.996, 0.993, 0.993, respectively, P = 0.000) and anti-RBD IgM, IgG (r = 0.996, r = 0.994, P = 0.000) showed strong positive correlation with the NAbs in all the patients (Fig. 3A). The titers of IgA, IgM, IgG antibodies against S indicated as OD value were positively correlated with NAbs titers both in mild/moderate and severe cases (Fig. 3B). However, the titers of IgM, IgG antibodies against RBD protein showed positive correlations with NAbs titers only in mild/moderate cases, but not in severe cases (Fig. 3B). To Define the time kinetics of the correlation, we found that the NAbs titers showed positive correlations with the IgA (*P* = 0.010) and IgM (*P* = 0.002) antibodies against S protein in the second week, with IgA (*P* = 0.000), and IgG (*P* = 0.001) antibodies against S protein and IgM (*P* = 0.028) antibodies against RBD protein in the third week (Fig. 3C).

In summary, our data show that the host mounts a robust humoral response against SARS-CoV–2, which is mediated by antibodies against a wide range of antigens present in the viral structure along with the presence of neutralizing antibodies. Levels of antibodies, especially IgG increase over the course of the disease while a limited increase is observed in IgA and IgM over the course of the disease. Further, significantly delayed onset of antibodies was observed in more severe cases, but at the same time, similar titers were observed in severe cases compared to those of the mild/moderate cases. These data indicate that delayed onset of antibodies may contribute to the disease severity, however, more detailed studies are warranted to describe the clear relationship between antibody response and disease outcome.

Materials And Methods

Patients and plasma specimens

In this study, a total of 279 plasma sample were collected from 176 COVID–19 patients from three cohorts, including 218 samples from 140 mild/moderate patients and 61 samples from 36 severe patients. In the first cohort, we recruited a total of 123 inpatients (31 severe and 92 mild/moderate cases) from Wuhan hospitals during the early phase of the pandemic in January 2020. A total of 215 blood sample were taken from the patients (two serial samples from 92 patients with a 4-day interval and one sample from remaining 31 patients). The second cohort included a total of 28 hospitalized patients recruited from Beijing hospitals (5 severe and 23 mild/moderate cases) which provided one blood sample from 17 patients and two samples from 11 patients. Samples of most of these two cohorts have been used in a previous study[7]. Additionally, we recruited a third cohort which included a cluster of 25 patients from Harbin in April. One plasma sample was taken from each patient. All the blood samples were collected between 1–46 days of the disease onset. The diagnosis was based on the examinations of nucleic acids and lung computed tomography (CT) according to the Diagnostic Guidelines[11]. The plasma specimens were collected from the patients. The demographic data and clinical diagnosis were recorded.

Enzyme-linked immunosorbent assay (ELISA)

The presence of antibodies against SARS-CoV–2 N, S and RBD proteins were examined by indirect enzyme-linked immunosorbent assay (ELISA) established as previously reported[3]. The N protein was expressed by our group as reported previously. The full-length ectodomain of S protein and RBD protein were produced by 293T cells with a purity of ≥90% (Sino Biological, Beijing, China). The optimized coating concentrations of N, S and RBD proteins were determined to be 5, 10 and 5 ng per well. The optimal plasma dilution was 1:400. The ELISAs cut-off values were decided by the mean values and standard deviation (S. D.) of healthy plasma samples by calculating the mean absorbance at 450 nm (A450). The cut-off values of IgA, IgM and IgG were 0.1, 0.13, and 0.3 for N, and 0.14, 0.2, and 0.21 for S protein, respectively. The cut-off values of IgM and IgG for RBD was 0.2 and 0.3, respectively.

Microneutralization assay

The plasma samples were diluted in serial two-fold dilutions from 1:10 to 1:320, then mixed with equal volumes of SARS-CoV–2 at a dose of 100 TCID50 (50% tissue culture infective dose) determined by Vero cells. The mixtures were incubated at 37°C for 1 h, then 100μl of the mix was added in quadruplicate to a monolayer of Vero cells (ATCC, CCL–81) cultured in 96-well microtiter plates. The virus-plasma mixture was removed after 1 h and 200ul fresh growth medium was added to each well. The virus back-titration was included in each test. The plates were incubated for 5 days and the cytopathic effect was observed. The NAbs titers were calculated by using Reed-Muench method and showed as geometric mean titers (GMTs)[12]. The schematic procedure and representative results showed in supplementary file (Fig. S1).

Immunofluorescence assay

Vero cells were fixed in 4% formaldehyde, permeabilized with 0.5% Triton X–100, and incubated with 5% BSA. The anti-SARS-CoV–2 nucleocapsid antibody, prepared by our group was used as primary antibody and IRDye Fluor800-labeled anti-mouse IgG (Li-Cor, USA) was used as secondary antibodies. The nuclei were stained with DAPI (Sigma, St. Louis, MO, USA). The fluorescence intensity in the cells were scanned by using an Operetta high-content imaging system (PerkinElmerr, Waltham, MA, USA).

Quantitative real-time PCR

The nucleic acids were extracted by TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Reverse transcription real-time PCR was performed using the AgPath-ID One-step RT-PCR Kit (Thermo Fisher Scientific). The expression levels of SARS-CoV–2 N RNA in cultured cells were determined according to a standard curve line. Primers targeted to SARS-CoV–2 N gene were as described in previous report (1).

Ethics Approval

This study was approved by the Ethical Review Board of Wuhan Jinyintan Hospital, Infectious Disease Hospital of Heilongjiang Province, and Institute of Pathogen Biology, Chinese Academy of Medical Sciences & Peking Union Medical College. Written informed consent was obtained from each healthy volunteer and patient suffering from common respiratory infections before enrollment. For the COVID–19 patients, the written informed consent was waived in the light of this emerging infectious disease of high public health relevance.

Statistical analysis

The categorical variables were described as frequency rates and percentages, and continuous variables were described using median with interquartile range (IQR). Two-sided unpaired t test was used for group comparison of continuous variables, and ^{2} test was used for group comparison of proportional categorical variables. Correlation analysis was evaluated by the Spearman's rank correlation test. Two-sided $P<0.05$ was considered to be statistically significant. All statistical analysis were conducted using SPSS version 19.0 and R version 3.6.1.

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Declarations

Author contributions: JWW, DYZ, LS and LLR conceived and designed experiments. LG, CW, YYW, YW, HCW and GW performed the experiments. DYZ, SYY, LHW, YFH, HC, JunWW, DYZ contributed clinical samples and clinical data collection. LLZ, LLR, DC, CDC, DYZ and JWW analyzed the data. LLR, DC, LLZ, LS, CDC, DYZ and JWW wrote the manuscript. All authors reviewed the manuscript.

Conflict of Interest Disclosures: All authors declare no competing interests.

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Figures

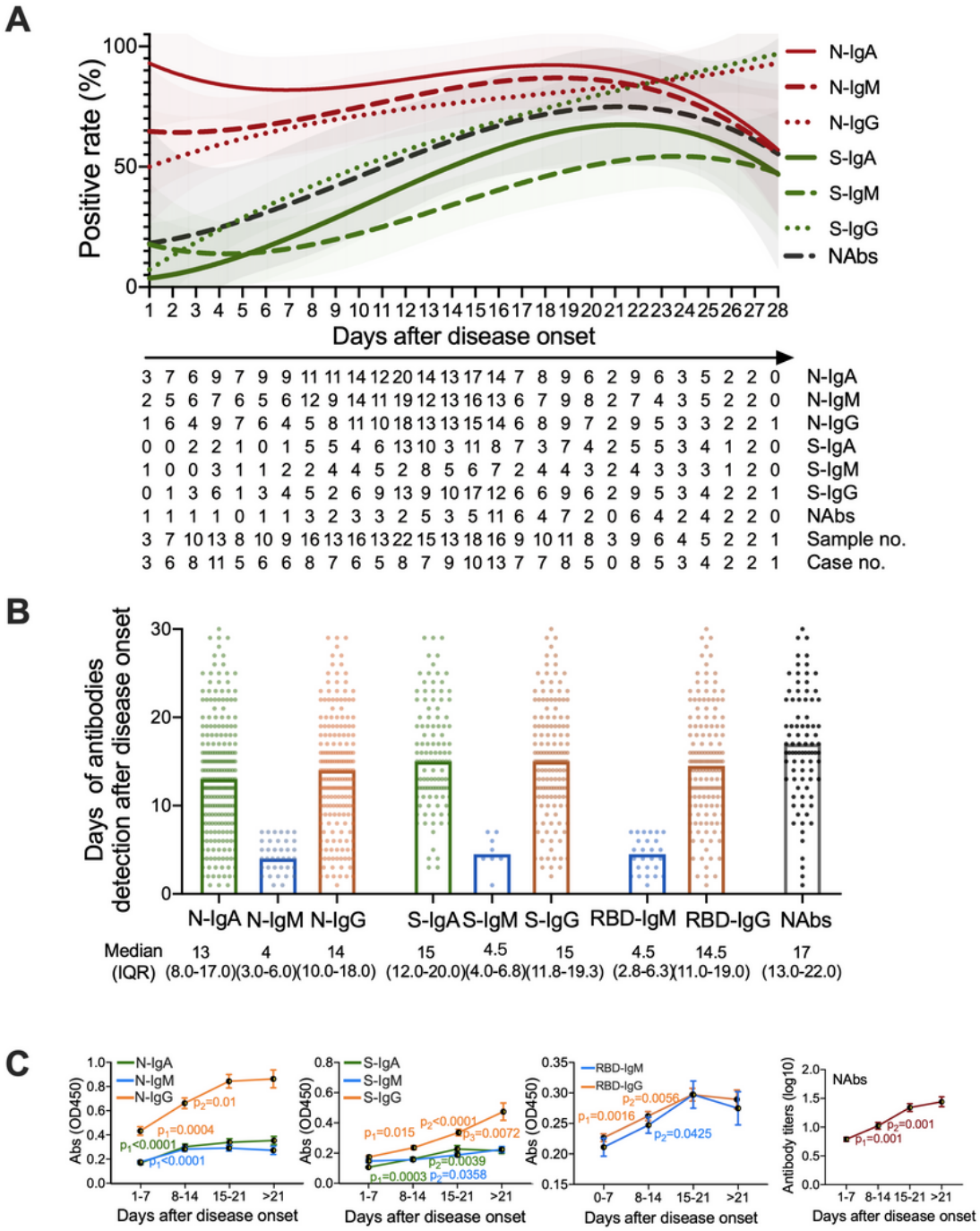

Figure 1

The characteristics of humoral responses in COVID-19. (A). Positive rate of IgA, IgM, and IgG antibodies against SARS-CoV-2 N, S proteins and neutralizing antibodies (NAbs) in all the cases. The fitted cure lines are created by Fit Spline program of Graphpad software where shadows indicating 95% confidence intervals. The lower table denotes the number of samples tested positive at each time point. (B) The seroconversion median time of the antibodies calculated using the samples collected within 7 days after

disease onset to show the acute phase (IgM). The median time of IgA and IgG antibodies were calculated by using all the patients with IgA or IgG antibodies. (C) Levels of IgM, IgA, and IgG antibodies and titers of NAbs against SARS-CoV-2 in plasma samples over course of the disease. Antibody titers were expressed as optical density (OD) value and NAbs titers are shown as geometric mean titers (GMTs).

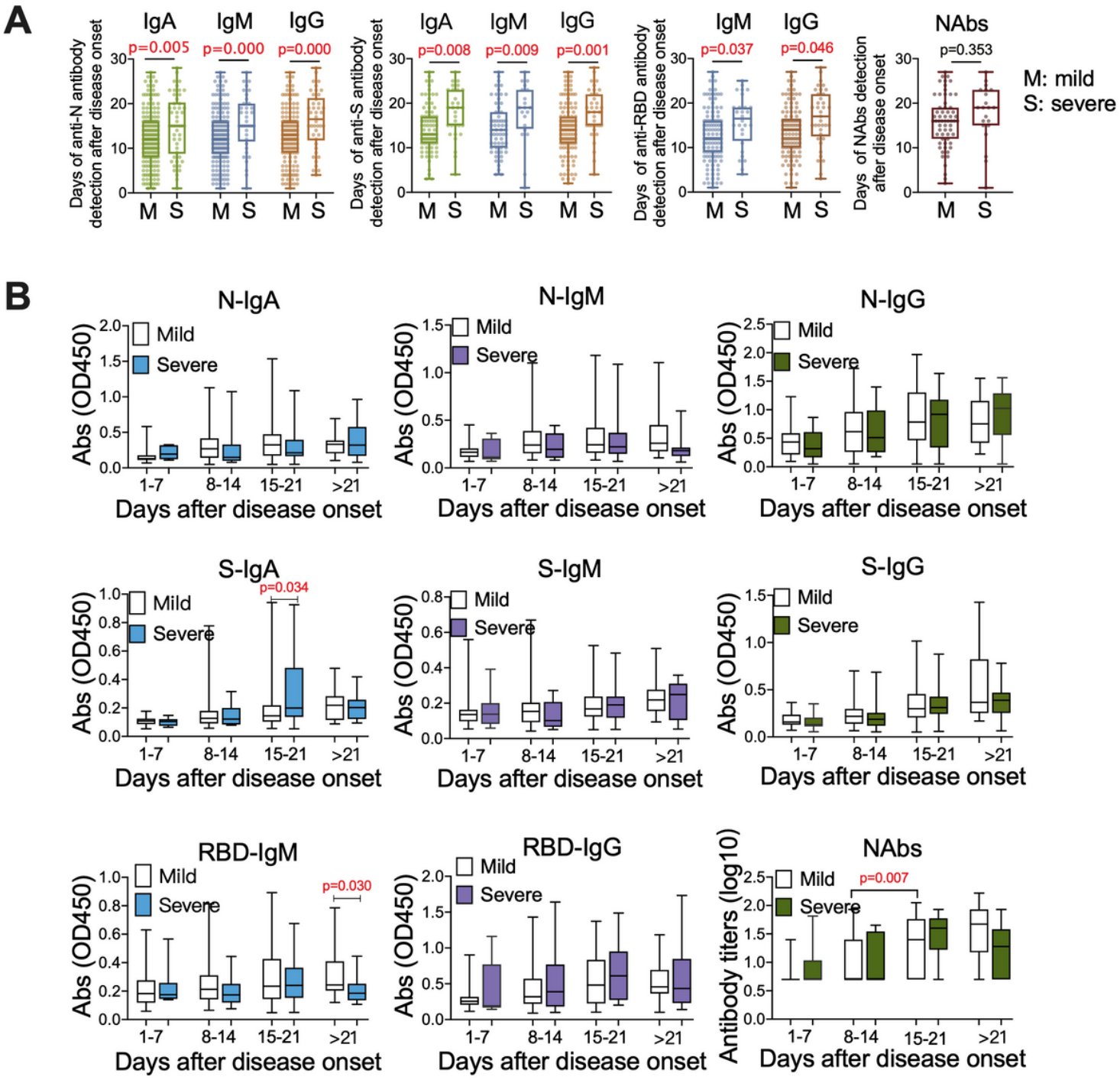

Figure 2

The profiles of antibodies in mild/moderate and severe COVID-19 patients. (A) The appearance time of IgA, IgM, IgG and total neutralizing antibodies between mild/moderate and severe cases. The onset time of anti-SARS-CoV-2 antibodies against nucleocapsid (N), and spike (S) proteins, anti-receptor binding

domain (RBD) IgM and IgG, and neutralizing antibodies (NAbs) against SARS-CoV-2 in mild/moderate (m) and severe patients is indicated. (B) The optical density (OD) value of IgA, IgM and IgG antibodies against SARS-CoV-2-N, S and RBD are shown while the NAbs titers showed by geometric mean titers over the time course of the disease.

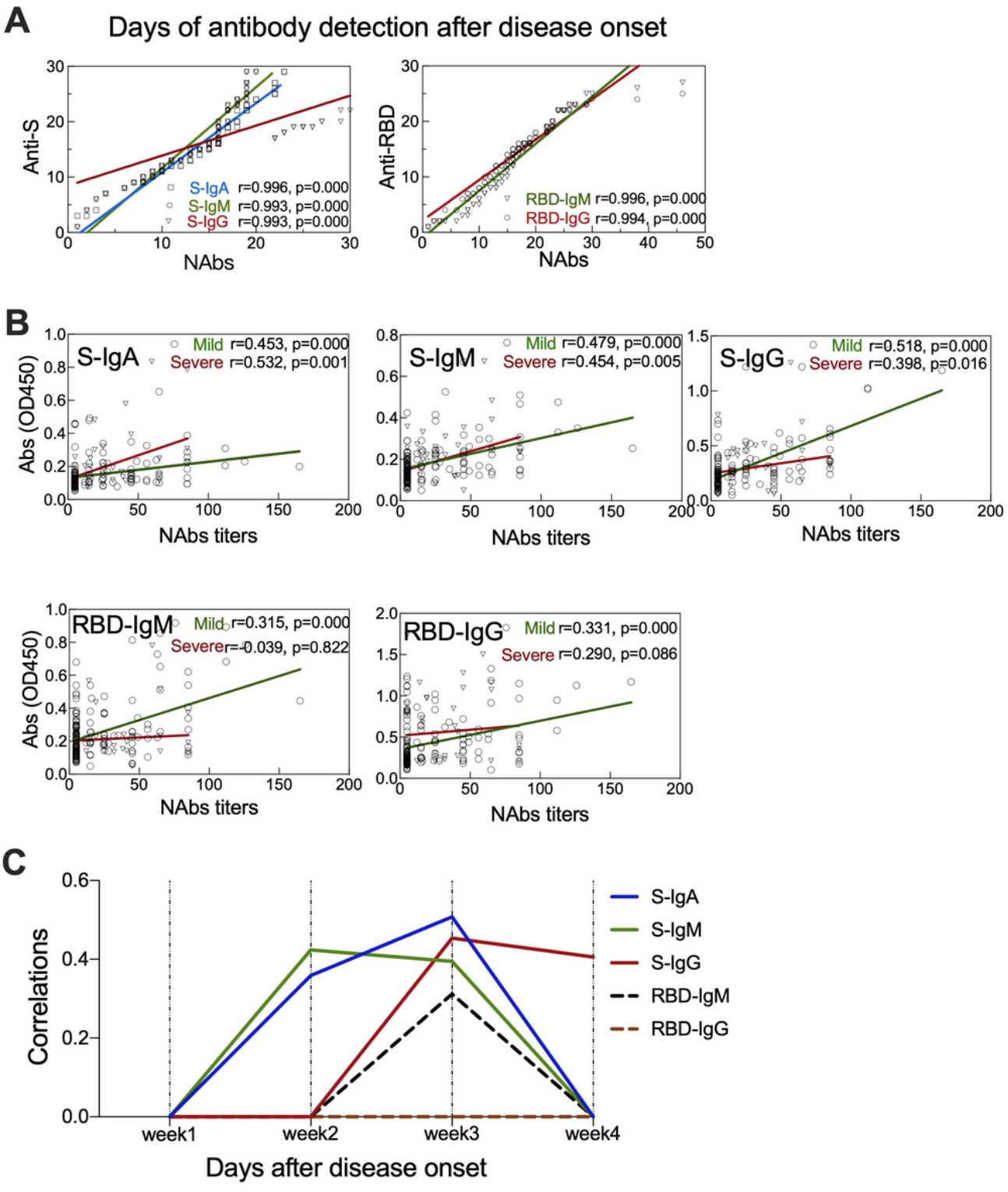

Figure 3

Correlations of binding antibodies with neutralizing antibodies (NAbs) in mild/moderate and severe patients. (A) The correlations of appearance time between antibodies against spike (S) and receptor-binding domain (RBD) proteins with NAbs. The x-and y-axis are number of days when the antibody was detected after disease onset. (B) Associations of anti-S and anti-RBD antibodies levels with NAbs titers in the mild/moderate and severe patients. (C) The correlations of NAbs titers with the antibodies against S and RBD proteins during the disease course.

Supplementary Files

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