Clinical and Antibody Characteristics Reveal Diverse Signatures of Severe and Non-severe SARS-CoV-2 Patients

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Research Article

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Abstract

Clinical and Immune response characteristics of COVID-19 between severe and non-severe patients have not been fully clarified. In this study, clinical features, antibody responses targeting SARS-CoV-2 spike protein (S) and its different domains, Ig isotypes and IgG subtypes, ACE2 competitive antibodies, binding titers with FcγIIa and FcγIIb receptors, and 14 cytokines were investigated in 119 serum samples from 37 PCR-confirmed COVID-19 patients. Severe group including 9 patients represented lower lymphocyte count, higher neutrophil count, higher level of LDH, total bile acid (TBA) (P<1×10^{-4}), r-glutaminase (P=0.011), adenosine deaminase (P<1×10^{-4}), procalcitonin (P=0.004), C-reactive protein (P<1×10^{-4}) and D-dimer (P=0.049) compared to non-severe group (28 patients). Significantly, higher-level antibody targeting S (IgA, IgM, and IgG), different S domains specificity (RBD, RBM, NTD, and CTD), FcγIIa and FcγIIb binding capability were observed in severe group than that of non-severe group, of which IgG1 and IgG3 were the main IgG subclasses. RBD-IgG were strongly correlated with S-IgG both in severe group and non-severe group. Additionally, CTD-IgG were strongly correlated with S-IgG in non-severe group. Positive RBD-ACE2 binding inhibition was strongly associated with high titers of antibody (S-IgG1, S-IgG3, NTD-IgG) especially RBD-IgG and CTD-IgG in severe group, while in non-severe group, S-IgG3, RBD-IgG and NTD-IgG titer correlated with ACE2 blocking rate. S-IgG1 was negatively associated with illness days in severe group (r=- 0.434, P=0.002), while S-IgG3 in severe group (r=0.363, P=0.011) and S-IgG1 (r=0.417, P=3×10-4) in non-severe group was positively associated with days after symptom onset. Moreover, GRO-α, IL-6, IL-8, IP-10, MCP-1, MCP-3, MIG, and BAFF were also significantly elevated in severe group. Overall, the results indicated different signatures in clinical and immune responses between the COVID-19 severe group and non-severe group, which will be markedly contributed to future therapeutic and preventive measures development.

Introduction

The coronavirus Disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been declared a threat to global health and economic. By 8th August, 2021, over 203 million COVID-2019 cases were confirmed, and accounting for over 4 million deaths.

Similar to SARS-CoV infection, the common clinical manifestations of COVID-19 include fever, cough, fatigue, sore throat, dyspnea and pneumonia, with low total lymphocyte count and percentage of T cells, increased C-reactive protein (CRP) concentration and erythrocyte sedimentation rate [1]. The COVID-19 cases can be divided into mild, moderate, or severe subtypes according to the clinical severity. Severe cases are defined by respiratory distress with pneumonia, with respiratory rate ≥30 breaths/min; or SpO_2 (oxygen saturation) ≤ 93% at rest; or PaO2/FIO2 (partial pressure of oxygen/fraction of inspired oxygen) ≤ 300mmHg. It is reported that dyspnea, myalgia or fatigue, high grade fever were the most common symptoms in severe cases. C reactive protein ‘(CRP), lactate dehydrogenase (LDH) and D-dimer level in severe cases were significantly higher than mild or moderate patients [2, 3]. Differences in clinical manifestations were primarily due to individual immune response, especially antibody titers.

Antibody plays an important role of humoral response after microbial infection. There are five antibody isotypes in serum, including IgA, IgD, IgE, IgM, and IgG. Following SARS-CoV-2 infection, virus-specific IgM, IgG, and IgA antibody have been detected [4, 5], of which IgG is the most abundant. The infection of SARS-CoV-2 relies on the interaction between the receptor binding domain (RBD) of its spike protein (S) and the angiotensin converting enzyme 2 (ACE2) on host cells [6, 7]. Multiple studies have shown that the majority of SARS-CoV-2-infected individuals produce S- and RBD-specific antibodies [8, 9]. In addition, other studies also reported isolation of N-terminal domain (NTD)-specific and S2-specific monoclonal antibodies that exhibited high neutralization potency [10, 11]. However, detailed information on antibody targeting domain of the spike protein and the frequency of the antibody was not clarified clearly.

In spite of the importance of antibody protection, concerns of antibody-dependent enhancement (ADE) arise from the possibility that existing antibody may increase the severity of disease, which may be caused by antibody-mediated endocytosis into Fc gamma receptor IIa (FcγRIIa)-expressing phagocytic cells, leading to rapid viral replication. Several studies have reported increased uptake of SARS-CoV and MERS-CoV virions into FcR-expressing monocytes or macrophages in vitro [12, 13]. However, FcγRIIb, the only inhibitory Fc receptor that cross-links with the activated receptor to intracellular transduction inhibitory signals, played a significant role in the negative regulation of immune response.

Besides the antiviral effect of antibodies, the cytokines are also important components in antiviral immune response. The proliferation of immune cells and signal factors lead to local inflammation and even cytokine storm syndrome (CSS). In COVID-19 patients, the studies reported elevated interleukins (IL) like IL-6, IL-8, IL-2R, IL-10, tumor necrosis factor (TNF-α), IL-1Ra, IP-10 (IFN-γ-induced protein 10) and macrophage inflammatory protein 1 (MCP-1) [14-18], especially in severe group.

Further comparison of differences in cytokines and immune characters between severe and non-severe patients will help to better clarify the relationship between inflammation and antibody responses. Thus in the present study, we characterized the clinical and immune features of 119 blood samples collected from 37 hospitalized patients with mild to severe symptoms, focusing on antibody isotype and IgG titers, RBD-ACE2 blocking activity, binding tiers with FcγR, B cell activation factor and cytokines. We carefully compared how these responses differentiated between the severe group and non-severe group. Finally, the interplay between antibody isotype, antibody subclasses, antibody dynamics and functional antibody characteristics were analyzed in detail to provide the full understanding of host immune response against SARS-CoV-2 infection between the severe group and non-severe group.

Methods And Materials

Study samples

Serum samples were collected from 37 COVID-19 patients at the First People's Hospital of Yueyang between January 25th and February 18th 2020. All individuals had PCR-confirmed SARS-CoV-2 infection and related symptoms. These patients were divided into severe and non-severe (mild or moderate) group based on the disease severity according to the China National Health Commission Guidelines for

Diagnosis and Treatment of SARS-CoV-2 [19]. Nine were classified severe (Severe group), while 28 were mild or moderate (Non-Severe group). The cohort included 21 females and 16 males, with a median age of 53.5（27-75）. 37 SARS-CoV-2 patients were serially sampled during the hospitalization and a total of 119 serum samples were finally collected. The serum samples were heat inactivated at 56℃ for 30 min before use.

Proteins

SARS-CoV-2 Spike Protein (S ectodomain) (cat# 40589-VO8B1) and SARS-CoV-2 Spike RBD Protein (cat# 40592-V08B) were purchased from Sino Biological (China), SARS-CoV-2 RBM were synthesized and purified by Sino Biological (China). SARS-CoV-2 NTD Protein (cat# DRA45), SARS-CoV-2 CTD Protein (cat# DRA46), human ACE2 protein (cat# C419), FcγR（a (cat# CS35), FcγR（b (cat# CS444) were purchased from Novoprotein (China).

Measurements of SARS-CoV-2-specific antibodies

Antibody responses, mainly target the spike protein, which make it important to evaluate S-specific antibody responses. Using an in-house enzyme linked immunosorbent assay (ELISA), we measured the presence of anti-SARS-CoV-2 antibody isotypes and IgG subtypes. ELISA was used to measure the SARS-CoV-2-specific IgG, IgM, IgA, and subclasses of IgG (IgG1-G4). 1 μg/ml of the recombinant S, RBD, RBM, NTD, or CTD proteins in phosphate buffered saline (PBS) (pH 7.4) were used to coat the 96-well plates (Corning, costar) at 4°C overnight. Plates were washed with phosphate-buffered saline, 0.05% Tween-20 (PBST) five times after each binding step. Plates were blocked with blocking buffer (PBS containing 5% BSA) at 37°C for 2 hours. The serum samples diluted in PBS containing 1% BSA at 1:100 were added to plates for screening assay, and serially diluted serum samples staring from 100 fold dilution were added to plates for EC50 test. The bound antibodies were detected with horseradish peroxidase (HRP)-conjugated goat anti-human IgG, IgM, and IgA (1:10,000, Abcam) and mouse anti-human IgG1, IgG2 (1:1,000, Abcam), IgG3 (1:1,000, Thermo Fisher, USA), and IgG4 (1:4,000, Abcam). The plates were then washed five times and incubated with TMB substract (Solarbio, Beijing, China) at room temperature for 15 min and stop solution (Solarbio, Beijing, China) was then added. The absorbance at 450 nm (OD450) was measured using an ELISA microplate reader (Molecular Devices). Absorbance value at 650 nm (OD650) were also measured and subtracted to eliminate background color and absorbance value of pore plate itself. Each sample was tested in duplicate, and the results are reported as the mean values.

ACE2 blocking assay

To test the effect of serum on blocking ACE2 binding RBD, 2 μg/ml the recombinant ACE2 (Sino iological, Beijing, China) was added in 96-well plates and overnight at 4°C, followed by blocking with the blocking buffer and washing. RBD-mouse-Ig-Fc at a concentration of 0.15 μg/ml was pre-incubated with serum diluted at 1:20 at 37°C for 1 hour, followed by adding into the wells coated with ACE2 and incubated at 37°C for 1 hour. Then the proportion of RBD-Fc proteins that were blocked by serum were not able to bind with ACE2 and were washed away. Goat anti mouse IgG were added and incubated at 37°C for 1 hour,

followed by adding TMB substrates and incubated at room temperature for 15 min. Stop solution was added and measured as above. The blocking percentage were calculated 100 × (1 - (OD450 value of serum sample/OD450 value of PBS control). Each sample was tested in duplicate, and the results are reported as the mean values.

Cytokine Measurements

ELISA were used to measure the serum levels of APRIL (BioLegend, USA) and BAFF (R&D Systems, USA) according to the manufacturer's instructions. Serum cytokines (GRO-α, IFN-γ, IL-1β, IL-1-ra, IL-6, IL-8, IL-15, IP-10, MCP-1, MCP-3, MIG, and VEGFA) were measured with a multiplex assay (Human Cytokine/Chemokine Panel I, Millipore, USA) on a Luminex200 platform. Each sample was tested in duplicate, and the results are reported as the mean values.

Statistical Analyses

All the continuous variables and categorical variables in this study were expressed as median (IQR) and number/sum (%). Differences in continuous variables between severe group and non-severe groups were compared using Mann-Whitney U test. Fisher's exact test was used to analyze two-group categorical variables. The correlations were determined by the Spearman rank method. *P* values < 0.05 and r > 0.3 or < -0.3 were considered statistically significant. P values between 0.01-0.05, 0.001-0.01, 0.0001-0.001, and <0.0001 were considered statistically significant (*), very significant (**), extremely significant (***) and super significant (****), respectively, whereas "ns" represents not significant. The analyses were performed using GraphPad Prism 7 software (GraphPad, La Jolla, California, USA).

Results

Demographic and Clinical features

A total of 37 COVID-19 patients were included in the current study, including 21 females and 16 males. These patients were divided into severe and non-severe (mild or moderate) group based on the disease severity according to the China National Health Commission Guidelines for Diagnosis and Treatment of SARS-CoV-2. The median age of the patients was 53.5 years, ranging from 27 to 76 years. There were no significant differences in age and gender between two groups **(Supplementary Table 1)**. A total of 119 serum samples from the 37 patients were serially collected, ranging from 6 days after symptom onset to 45 days during hospitalization. The median sample days in severe and non-severe group were 18.5 and 19 days after symptoms onset.

For clinical manifestations, common symptoms in our cohort included fever, cough, fatigue, sore throat and chest tightness **(Supplementary Table 1)**. High grade fever ($P<1\times10^{-4}$), chest tightness ($P=0.007$), shortness of breath ($P=6\times10^{-4}$), nausea or vomiting ($P=9\times10^{-4}$) were reported significantly more in severe group compared to non-severe group. Severe group also had more comorbidities such as diabetes ($P=1.9\times10^{-4}$) **(Supplementary Table 1)**.

As shown in **Table 1**, blood examination results showed that both absolute count and percentage of leukocyte and neutrophil were significantly higher in severe group than non-severe group (P<0.05), while the percentage of lymphocyte and monocyte were significantly lower in severe group. Serum biochemical study showed that the severe cases had significantly higher levels of lactate dehydrogenase (LDH) (P=0.005), total bile acid (TBA) (P<1×10^{-4}), r-glutaminase (P=0.011), adenosine deaminase (P<1×10^{-4}), procalcitonin (P=0.004), C-reactive protein (P<1×10^{-4}) and D-dimer (P=0.049) compared to non-severe cases (Table 1). Lower percentage of $CD3^+$ T cell, $CD3^-CD16/56^+$ NK cell and higher $CD3^-CD19^+$ B cell percentage in severe groups were also observed, but due to the limited flow cytometry analysis data (severe case N=7, non-severe case N=17), the differences were not statistically significant (P>0.05). These results suggest increased systemic inflammation, dysfunction of liver and compromised T cell response are associated with the severity of COVID-19 patients.

Anti-SARS-CoV-2 antibody responses

Serum anti-SARS-CoV-2 S-specific IgG, IgA and IgM antibodies were detected in all samples **(Fig 1a)**, and antibody level in severe group were all significantly higher than non-severe group (P<0.001)**.** The four IgG subclasses targeting SARS-CoV-2 S protein were detected in all samples, with overall IgG1 and IgG3 responses higher than IgG2 and IgG4 responses. The severe group also showed higher IgG1-IgG4 levels than non-severe group (P< 1×10^{-4}) **(Fig 1b)**. Serum antibodies titers against SARS-CoV-2 S, RBD, receptor binding motif (RBM), N terminal domain (NTD), and C-terminal domain (CTD) were measured by ELISA. S-targeting antibody titer in severe group ranged from 4818 to 65392 (median 17803), followed by RBD-specific antibody titers (233 - 4871, median 1406), NTD-specific antibody titers (111 - 4795, median 579), CTD-specific antibody titers (66 - 1038, median 247), and RBM-specific antibody titers were the lowest (67 - 438, median 228) **(Fig 1c)**. The similar trend of antibody titers was observed in non-severe group. As expected, the S-targeting antibody titer is the highest, ranging from 889 to 36571 (median 8282), followed by RBD-specific antibody titers (123 - 2574, median 437), NTD-specific antibody titers (67 - 2448, median 192), CTD-specific antibody titers (50 - 1353, median 125), and RBM-specific antibody titers were the lowest (55 - 754, median 153). Similar to Ig isotypes and IgG subclasses, the IgG titers of antibody targeting different domains of S protein were also significantly higher in severe group (P<0.001).

Serum antibody blocking RBD binding to ACE2

To examine whether the serum could result in antiviral activity, we next detected whether the serum antibody could block SARS-CoV-2 RBD to bind the ACE2 receptor, which will exert potential neutralizing activity of SARS-CoV-2 in infected patient. In severe group, the blocking percentages ranged from -20.4% to 94.7% (median 7.3%), which was significantly higher than non-severe group (-20.8%-65.9%, median -2.7%, P=5×10^{-4}) **(Fig 2a)**. While only some samples exhibited good inhibitory effect, other samples did not block RBD-ACE2 engagement and seemed the ACE-2 binding-enhanced signal. Obviously, the severe group showed higher positive blocking rate (75.0%) than non-severe group (42.3%) **(Fig 2b)**. Positive correlations were found between antibody titers and blocking percentage. In severe group, the blocking percentage were positively correlated with S-IgG1 (r=0.372, P=0.009), S-IgG3 (r=0.594, P<1×10^{-4}), S-IgG

(r=0.454, *P*=0.001), NTD-IgG titer (0.414, *P*=0.004), especially strongly correlated with RBD-IgG (r=0.803, $P<1\times10^{-4}$) and CTD-IgG titer (r=0.802, $P<1\times10^{-4}$) **(Fig 3a-3d, 3f and 3g)**. In non-severe group, IgG3 (r=0.364, *P*=0.002), RBD-IgG (r=0.331, *P*=0.005), and NTD-IgG titer (r=0.480, $P<1\times10^{-4}$) were positively associated with blocking percentage **(Fig 3b, 3d and 3f)**.

Serum antibody binding titers with Fc receptors

To detect whether the difference of serum samples in inhibition or enhancement RBD binding with ACE2 was non-specifically induced by Fc function of serum antibodies, we examined the binding activity of serum sample to Fc receptors, which included an activating receptor FcγR□a and an inhibitory receptor FcγR□b. The binding titer of serum antibody to FcγR□a ranged from 635 to 345005 (median 12953) in severe group and 437-94649 (median 2653) in non-severe group, while binding titers to FcγR□b ranged from 111 to 8375 (median 276) in severe group and 111 to 3287 (median 204) in non-severe group. Notably, both FcγR□a and FcγR□b binding titer were significantly higher in severe group than non-severe group ($P<1\times10^{-4}$ and P=0.030, respectively)(**Fig 4a**). However, no correlation was found between the blocking rate and FcγR□titer in both severe group (r=0.053, *P*=0.723) and non-severe group (r=-0.082, *P*=0.498) (**Fig 4c)**, nor was the correlation between blocking rate and FcγR□b titer in severe group (r=0.113, *P*=0.444) and non-severe group (r=-0.161, *P*=0.180) (**Fig 4d**). In addition, we performed an analysis using the ratio of FcγR□a and FcγR□b binding titers in severe group and non-severe group. Consistent with the binding titers in the separate groups, this ratio in severe group is significantly higher than non-severe group ($P<1\times10^{-4}$), and no correlation with ACE2-blocking was found (r=0.059, *P*=0.690 and r=-0.049, *P*=0.685, respectively) (**Fig 4b-4e**), indicating severe group's Igs FcγR-binding activity is much stronger.

Differential expression profile of cytokines in severe and non-severe case

To assess other immune factors in blood samples, we continued to analyze the profile of cytokines in COVID-19 patients. Elevated level of nine pro- and anti-inflammatory cytokines were observed in the severe cases as compared with that of the non-severe cases. For severe group, IL-6, IL-8, IP-10, MCP-3, and MIG showed the most significant elevation ($P<1\times10^{-4}$), followed by MCP-1 ($P=3\times10^{-4}$), GRO-α (*P*=0.006) and BAFF (*P*=0.003). Differences of IFN-γ, IL-1β, IL-1Ra, IL-15, VEGF-A, and APRIL between two groups were not statistically significant (*P*>0.05) **(Fig 5)**. These results suggest that significantly higher inflammation responses in severe group than non-severe group infected by SARS-CoV-2.

Specificity and Correlation of antibody responses in severe and non-severe group

The results above indicated that the severe group' antibody level were much higher than non-severe group's. To investigate the feature of Ig, we analyzed the correlations of Ig isotypes and IgG titers of different domain targeting antibody. As shown in **Fig 6a**, significant correlation between S-IgG and S-IgM was observed both in severe group (r=0.499, $P=3\times10^{-4}$) and non-severe group (r=0.584, $P<1\times10^{-4}$), S-IgA was positively correlated with S-IgM (r=0.786, *P*=0.040) and S-IgG (r=0.528, $P<1\times10^{-4}$) only in non-severe

group. Notably, RBD-IgG were strongly correlated with S-IgG both in severe group (r=0.676, $P<1\times10^{-4}$) and non-severe group (r=0.665, $P<1\times10^{-4}$) (**Fig 6b**). Besides, RBM-IgG were positively correlated with S-IgG in severe group (r=0.365, $P=0.011$), while CTD-IgG were positively correlated with S-IgG in non-severe group (r=0.648, $P<1\times10^{-4}$). In addition, strong correlation between RBD-IgG and CTD-IgG were found in both severe group (r=0.657, $P<1\times10^{-4}$) and non-severe group (r=0.586, $P<1\times10^{-4}$), RBD-IgG were positively correlated with RBM-IgG in severe group (r=0.248, $P=0.037$), while NTD-IgG with RBD-IgG (r=0.315, $P=0.008$), NTD-IgG with CTD-IgG (r=0.306, $P=0.030$) showed significant correlation only in non-severe group (**Fig 6c**). Together, these results indicated that RBD domain was the main target on S for SARS-CoV-2 specific antibody in severe group, whereas RBD and CTD were both frequently targeted in non-severe group.

Correlations between antibody responses and days after symptoms onsets in two groups

In analyzing the specificity of antibody responses, RBD was the main domain for SARS-CoV-2' S spike specific antibody in severe group. We continued to investigate the correlations between antibody responses and days since symptom onset against two groups. S-IgG titer increased significantly with longer days after symptom onsets in non-severe group (r=0.451, $P<1\times10^{-4}$) (**Fig 7a**). Similarly, accompanied with more time after symptom onsets, CTD-IgG titer were higher when symptom lasts (r=0.385, $P=0.007$), while that correlation with RBD-IgG titer or NTD-IgG titer were not significant (r<0.3) (**Fig 7b-d**). In addition, S-IgM were negatively correlated with days after symptom onset only in severe group (r=- 0.511, $P=2\times10^{-4}$) (**Fig 7e**), while correlations between S-IgA and days after symptom onset were not statistically significant (**Fig 7f**). Notably, S-IgG1 was negatively associated with illness days in severe group (r=- 0.434, P=0.002), while S-IgG3 in severe group (r=0.363, P=0.011) and S-IgG1 (r=0.417, P=3×10-4) in non-severe group was positively associated with days after symptom onset (**Fig 7g and 7h**). These results suggest that different antibody dynamics between the severe group and non-severe group induced by SARS-CoV-2 infection.

Discussion

In this study, we investigated clinical features and antibody response, including antibody level, specificity, Ig isotypes and IgG subtypes, ACE2 competitive antibodies function, FcγR-binding activity, and a panel of 14 cytokine levels of COVID-19 patients. We also sought to understand the clinical and Immune response characteristics of severe SARS-CoV-2 and non-severe patients. Finally, we determined the different signatures in clinical and antibody responses in these two groups.

Consistent with what was previously reported [22], we observed that LDH, D-dimer, CRP, concentration of prothrombin, TBA, r-glutaminase, adenosine deaminase in severe group were significantly higher than non-severe group. We also found significantly lower proportion of lymphocytes and higher neutrophil count and percentage in severe group than non-severe group. These results indicated these markers found in laboratory could be used for predicting severe cases, and should be paid more attention during treatment.

In antibody response, similar to previous study [23], we observed a significantly higher titer of S-specific IgA, IgG and IgM in severe group than that of non-severe patients. We also observed significant positive correlations of S-IgM and S-IgG in two groups. However, the correlations of S-IgA and S-IgG, S-IgA and S-IgM, were just shown in non-severe group. Indeed, the S specific antibody isotype switch might be different between these two groups. Meanwhile, IgA and IgG, showed no association with illness days during the hospitalization in severe group, which was different from that in non-severe group. Similar to the previous reports [5, 24], it is likely that the production of antibody is faster and stronger in severe group, and IgA and IgG antibody maintained better than non-severe group.

It is reported that IgG subclasses were negatively correlated to viral load [23]. In our study, we found that S-IgG1 and S-IgG3 were majority subclass IgG induced by SARS-CoV-2 infection. Furthermore, negative correlation between IgG1 and days after symptom onset, positive correlation between IgG3 and days after symptom onset in severe group were found in our analysis. While in non-severe group, we only found positive correlation between IgG1 and illness days, and no association between IgG3 and illness days. One possibility might be that in the early illness stage, the higher IgG1 response accompanied with symptoms, and in the later stage in severe group, level change of IgG1 and IgG3 seemed to produce unidentified antibody response's effect against illness severity. As we know, the IgG1 and IgG3 were the main antibody that could induce antibody-dependent cell-mediated cytotoxicity (ADCC) due to their high affinity with FcγRs and were helpful for elimination of viruses. We also found significantly higher IgG1 and IgG3 responses in severe group. Moreover, IgG1 in severe group, IgG1 and IgG3 in non-severe group showed significant correlation with RBD-ACE2 blocking rate, which was similar to the Luo et al. study that S-specific IgG1 and IgG3 were associated with disease severity and were correlated with reduced virus load in nasopharyngeal swab [23]. Furthermore, we found significant higher binding titer of FcγRIIa and FcγRIIb in severe group, as well as the ratio of FcγRIIa/FcγRIIb. Thus, it is worth investigating whether IgG subclasses especially IgG1 and IgG3 and binding with RcγRs exerts different antiviral activity in the progress of SARS-CoV-2 infection and leads to different severity of the disease.

Plasma anti–SARS-CoV-2 spike protein and receptor-binding domain IgG were helpful for virus neutralization by blocking the interaction between RBD and the virus receptor AEC2 [26, 27]. Our study showed that the SARS-CoV-2 specific antibody consisted of not only RBD- targeting antibody but also high titers of NTD- and CTD- targeting antibody, resulted in correlation with blocking rate, which indicated that the important function of NTD- and CTD-reactive antibody in serum. Positive correlations between RBD-targeting antibody titers and serum blocking rate of RBD-ACE2 were found in both groups. In addition, NTD-IgG was also associated with blocking rate in both severe group and non-severe group, and CTD-IgG in severe group significantly correlated with blocking rate. The receptor bind motif (RBM), however, did not show significant correlation with the blocking rate. Based on the RBM-IgG titers were much lower than that of RBD (123-4871), NTD (68-4795), CTD (50-1353), our results indicated that the linear epitope of RBM was less frequently targeted and was not a good choice of immunogen. Several studies have reported that the combined immunogens of different domain of S protein exhibited more robust and stable immunogenicity and higher neutralization potency [10, 11, 28–30]. Therefore, in the future, more attention should be paid to detect and isolate NTD-directed or CTD-directed neutralizing

antibody, and immunogens may not only just be based on RBD but also based on other domains of S such as NTD.

Previous studies have shown that elevated levels of proinflammatory cytokines, such as IL-1β, IL-1Ra, IL-6, IL-8, IL-9, IL-10, IFN-γ, IP-10, MCP-1 and MCP-3 are associated with severe lung injury and adverse outcomes in SARS-CoV or MERS CoV infection, and IP-10, IL-10 and IL-6 could anticipate subsequent clinical progression [17, 18, 32, 33, 34]. Our results also showed that the IL-6, IL-8, IP-10, MCP-1, MCP-3, and MIG were significantly different between severe cases and non-severe cases, suggesting that the magnitude of these cytokines is associated with the disease severity, which reflect dysregulated immune response. The combinatorial analysis of clinical classification with serum cytokines can contribute to better evaluate the severity of COVID-19 and optimize the therapeutic strategies. Besides, we found significantly higher BAFF level in severe COVID-19 group than non-severe group, indicating robust activation of B cell response associated with BAFF in severe COVID-19 patients when corresponding to overall higher antibody responses in severe group. Since BAFF and APRIL, the agents associated with B cell activation and maturation have been reported to play roles in the pathogenesis of HIV-1 and HCV [39, 40], next, we should explore the functional characteristics of BAFF during SARS-CoV-2 infection.

Clinical and demographic features of COVID-19 patients have recently been reported [1, 20, 21], and some immunological features were subsequently reported [3]. Characterization of the clinical and immune response of COVID-19 patients, such as our now study about clinical features and antibody responses signatures of severe SARS-CoV-2 and non-severe patients is still valuable to understand SARS-CoV-2 infection. Although the relatively small sample size was one of limitations in the current study, we still detected dysregulated antibody responses, hyperinflammation and lymphopenia due to the severity infected by SARS-CoV-2. In the future, the roles of specific Igs, Fc effector function, influences of uncertain cytokines in COVID-19 patients should be further investigated in larger cohorts.

Declarations

Ethics approval and consent to participate

The current study obtained the approval of the Institutional Review Board of the First People's Hospital of YueYang city (No.2021-016). The protocol used by this investigation was in accordance with the principles expressed in the Helsinki Declaration of 1975, which was revised in 2008.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article or the supplementary information.

Competing interests

The authors declare that there are no competing interests.

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Author contributions

HW, MS, YY and YZ conceived and designed the studies. YG, LX, HJ and MZ collected reagents and study materials. HW, YD, YL, BL, XZ, and XW, performed laboratory experiments. HW, YD, YL, GY, YM, MS, YY and YZ analyzed data. HW, YM, MS, YY and YZ wrote and revised the manuscript. All authors approved the final manuscript.

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Tables

Table 1. Laboratory findings in COVID-19 patients

Laborotory items	Normal range	All patients (n=37)	Severe (n=9	Non-severe (n=28)	P value
White blood cell ($\times10^9$/L)	3.5-9.5	5.61 (4.21-10.21)	11.40 (7.20-15.59)	5.45(4.130-7.113)	0.0010
Neutrophil ($\times10^9$/L)	1.8-6.3	3.58 (2.39-8.07)	10.81(5.17-13.29)	3.30 (2.350-5.198)	0.0005
Lymphocyte ($\times10^9$/L)	1.1-3.2	1.02 (0.675-1.695)	0.78 (0.46-0.96)	1.22 (0.8875-1.833)	0.0530
Monocyte ($\times10^9$/L)	0.1-0.6	0.54 (0.335-0.73)	0.78 (0.10-1.33)	0.54 (0.35-0.70)	0.0430
NEUT(%)	40-75	65.40 (56.40-84.20)	84.20 (74.20-87.95)	60.80(54.98-76.43)	0.0019
LYMPH(%)	20-50	23.00 (11.05-31.20)	11.20 (5.75-18.95)	25.10(14.23-32.03)	0.0047
MONO(%)	3-10	8.50 (6.05-11.00)	6.00 (2.40-8.45)	9.60 (7.325-11.78)	0.0047
Lactate dehydrogenase (U/L)	120-250	178.0 (155.0-208.7)	334.7 (191.0-476.5)	163.6 (149.4-185.5)	0.0047
ALkaline Phosphatase (U/L)	45-125	61.70 (46.85-72.45)	59.13 (42.90-61.95)	68.15 (48.50-73.28)	0.0911
Total bile acid(μmol/L)	0-12	4.15 (2.15-5.92)	5.48 (2.325-6.99)	0.80 (2.07-5.65)	<0.0001
r-glutaminase (U/L)	10-60	28.28 (14.90-85.74)	100.10 (23.25-187.60)	27.25 (11.98-43.60	0.0107
Adenosine deaminase (U/L)	4-24	9.22 (8.04-11.47)	12.04 (10.11-15.70)	8.87 (7.87-10.11)	<0.0001
Procalcitonin (ng/ml)	<0.046	0.10 (0.0425-0.70)	0.80 (0.415-3.12)	0.06 (0.04-0.10)	0.0335
C-reactive protein (mg/L)	0-10	25.37 (1.963-58.70)	70.13 (58.70-168.50)	8.39 (1.70-33.47)	<0.0001
D-dimer (ng/mL)	<0.5	340.3 (175.0-485.0	416.0 (350.0-910.0)	290.0 (150.0-445.0)	0.0495
CD3+T (%)	50-84	73.67 (60.61-79.61)	65.23 (56.16-75.80)	75.05 (62.44-80.84)	0.3117
CD3+T cell count	955-2860	887.0 (586.3-1509)	991.0 (618.0-1216)	880.0 (571.5-1553)	0.9518
CD3+CD4+T (%)	27-51	41.31 (31.04-49.54)	47.71 (28.48-50.06)	41.03 (32.04-49.06)	0.7164

CD3+CD4+T cell count	550-1440	501.0 (335.8-673.8)	612.0 (313.0-682.0)	499.0 (353.5-647.5)	0.4794
CD3+CD8+T (%)	15-44	24.41 (17.10-30.37)	24.41 (17.10-30.37)	21.56 (17.59-31.49)	0.8991
CD3+CD8+T cell count	320-1250	319.5 (192.5-523.0)	338.0 (301.0-540.0)	282.0 (170.0-512.0)	0.8843
CD3-CD16/56+NK (%)	7-40	12.48 (8.78-17.87)	9.37 (5.55-30.43)	13.06 (9.67-17.29)	0.8086
CD3-CD16/56+ cell count	150-1100	174.5 (121.3-226.0)	164.0 (76.00-335.0)	185.0 (133.5-219.5)	0.5544
CD3-CD19+B (%)	5-18	11.63 (8.02-17.50)	18.36 (11.57-22.38)	11.40 (7.51-13.70)	0.0892
CD3-CD19+B cell count	90-560	141.0 (97.5-300.8)	250.0 (127.0-354.0)	107.0 (96.00-259.5)	0.1228

Figures

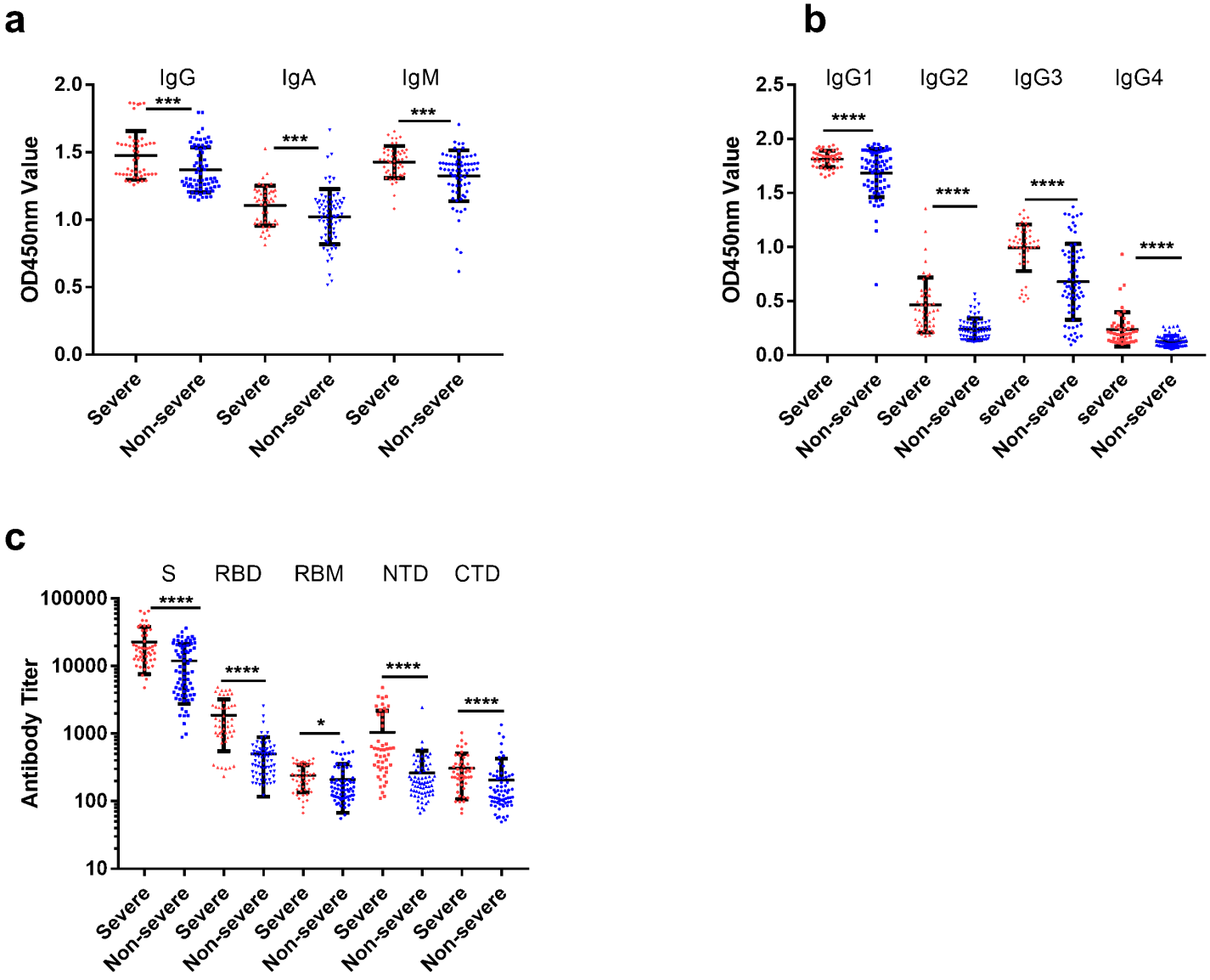

Figure 1

SARS-CoV-2 specific antibodies in COVID-19 severe cases and non-severe cases. Serum samples from severe cases (N=48) and non-severe cases (N=71) were compared for SARS-CoV-2 S specific antibody isotypes: IgG, IgA, and IgM (a), different anti-S IgG subtypes (IgG1, IgG2, IgG3, and IgG4) (b), IgG titers targeting S, RBD, RBM, NTD, and CTD (c). The OD450nm values were normalized by subtracting OD650nm values. The antibody titers were the dilution fold that reached half-maximal binding with corresponding antigens, and the values were calculated by Graphpad Prism 7. Mann-Whitney U test was used to compare differences between the two groups. Significances were marked as follows: $p < 0.05$ (*), $p < 0.01$(**), $p < 0.001$ (***), and $p < 0.0001$ (****), respectively.

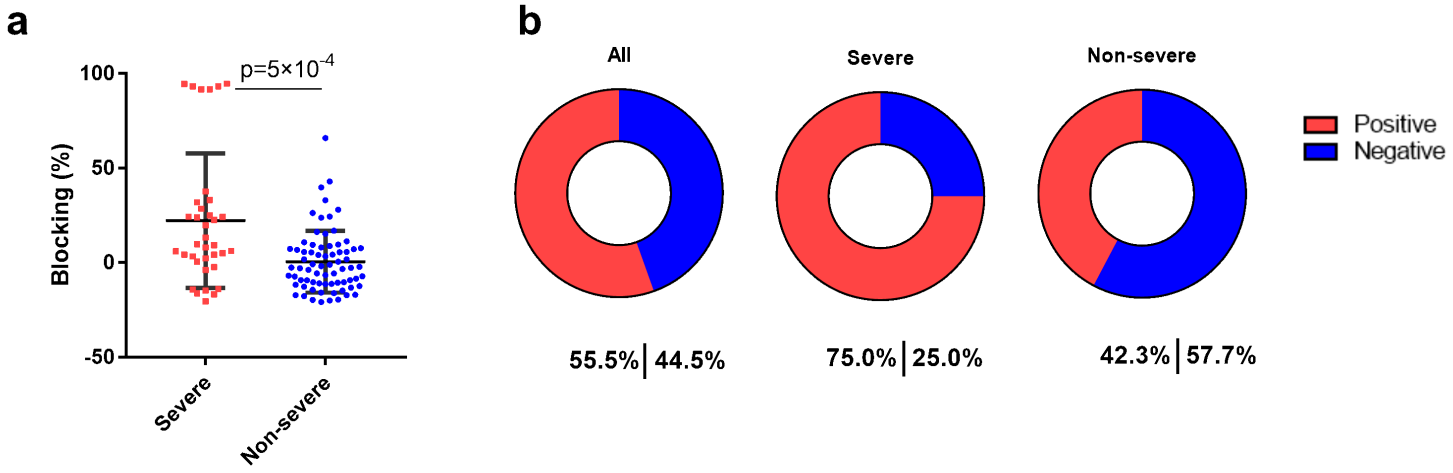

Figure 2

Comparison of RBD–ACE2 binding inhibition of serum samples between severe group and non-severe group. (a) The blocking percentage of serum to inhibit RBD-ACE2 interaction were showed. Serum were diluted at a final dilution of 1:40. The blocking percentages were calculated as 100 × (1 − (OD450 value of serum sample/OD450 value of PBS control)). (b) Pie charts showing the proportions of samples with positive (Red) or negative (Blue) RBD-ACE2-binding inhibition. Mann-Whitney U test was used to compare differences between the two groups.

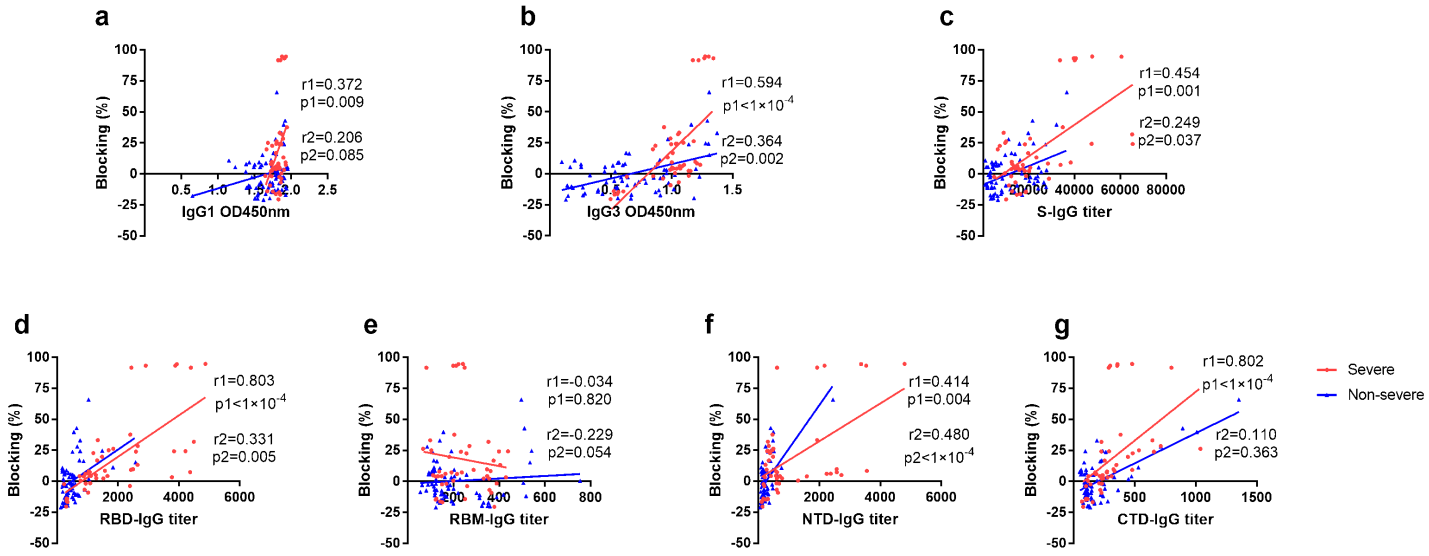

Figure 3

Correlations between blocking percentage and antibody response in severe group and non-severe group. Correlation of blocking percentage with IgG1 (a), IgG3 (b), S-IgG (c), RBD-IgG (d), RBM-IgG (e), NTD-IgG (f), CTD-IgG (g). The correlations were determined by the Spearman rank method, P values < 0.05 and r > 0.3 or < -0.3 were considered statistically significant. Red dots, r1 and p1 represent sample from severe cases; blue dots, r2 and p2 represent samples from non-severe cases.

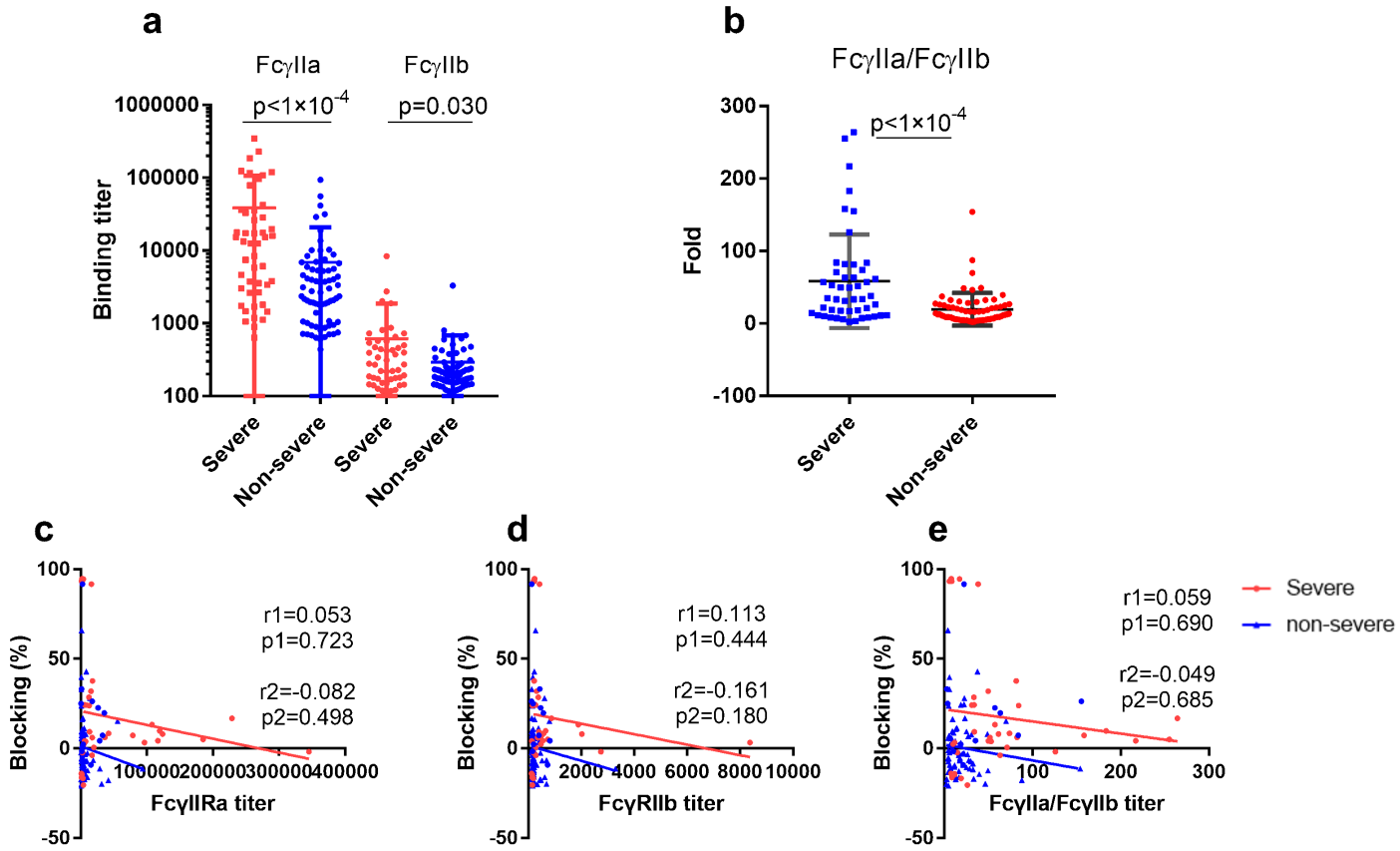

Figure 4

Comparison of binding titers with FcγRIIa and FcγIIb in serum samples and the correlations with blocking rate. (a) Binding titers of serum samples to FcγIIa and FcγIIb in severe cases and non-severe cases. (b) Comparison of specific ratio of FcγIIa/FcγIIb in severe group and non-severe group. Mann-Whitney U test was used to compare differences between the two groups. Correlations between blocking percentage and FcγIIa titer (c), FcγIIb titer (c), FcγIIa/ FcγIIb (d). Red dots, r1 and p1 represent sample from severe cases; blue dots, r2 and p2 represent samples from non-severe cases. P values < 0.05 and r > 0.3 or < -0.3 were considered statistically significant.

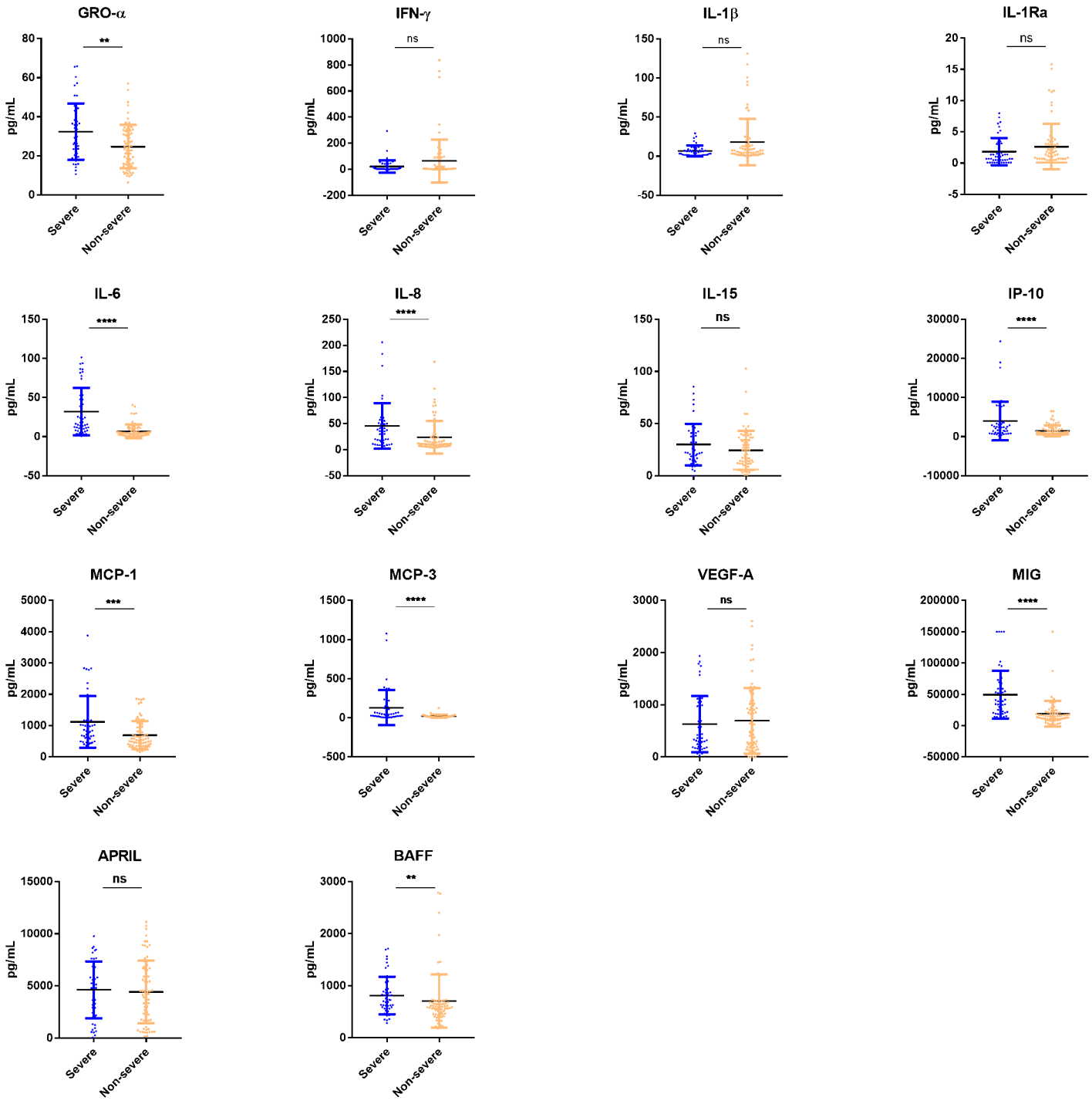

Figure 5

Comparison of serum cytokine/chemokine concentrations between severe and non-severe COVID-19 cases. Samples from severe (N=45) and non-severe COVID-19 cases (N=74) collected during hospitalization were used for measuring the concentrations of 12 cytokines and chemokine. Values were presented in units of pg/mL. Mann-Whitney U test was used to compare cytokine levels between two groups. Significances were marked as follows: $p < 0.05$ (*), $p < 0.01$(**), $p < 0.001$ (***), and $p < 0.0001$ (****), respectively.

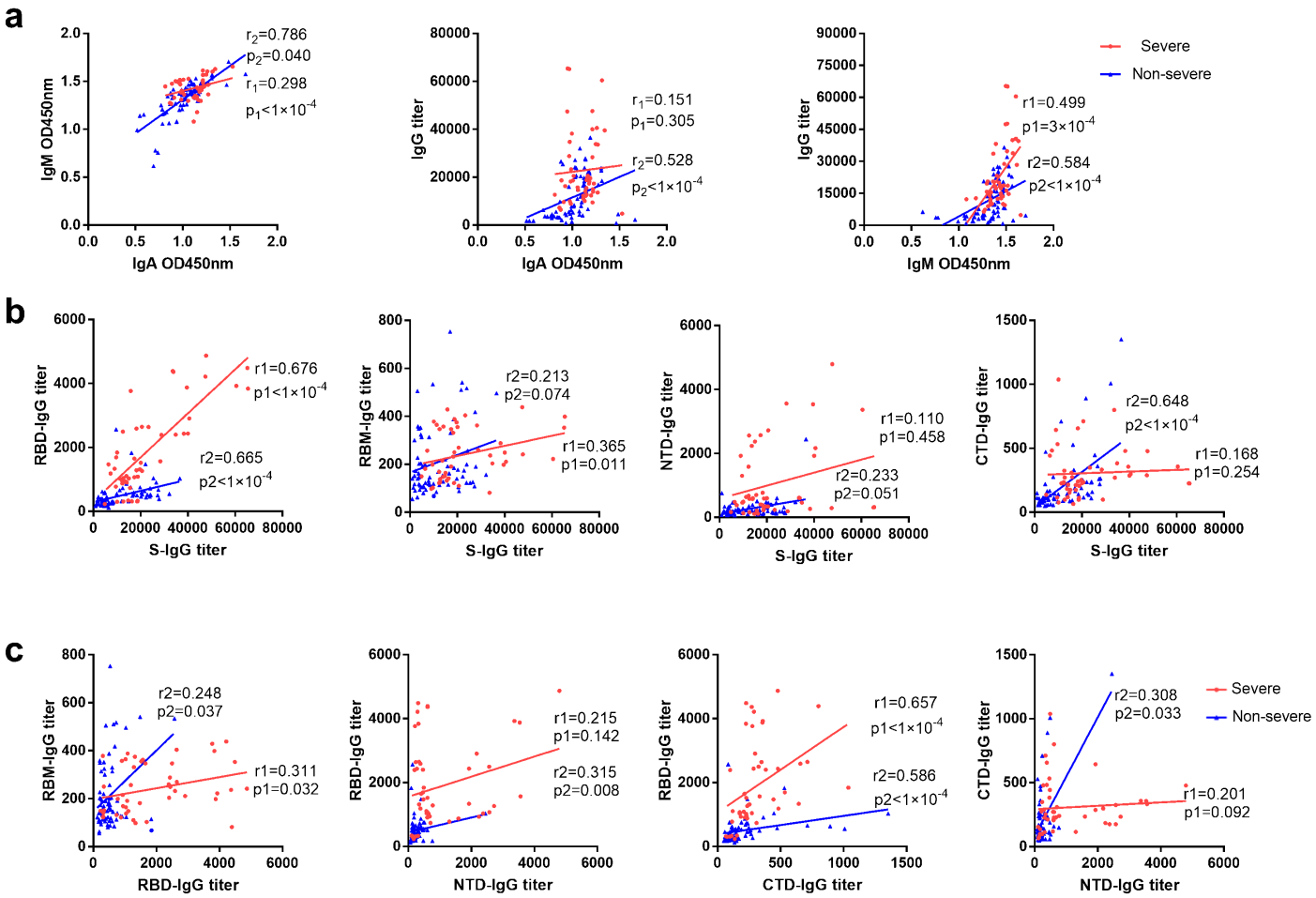

Figure 6

Correlations of antibody isotypes and specific antibodies targeting different antigens. The correlations between antibody level of IgM and IgA, IgG and IgA, IgG and IgM (a), and correlations of antibody level targeting S and different S domain (b), and correlations of antibody targeting different S domain. The correlations were determined by the Spearman rank method, P values < 0.05 and r > 0.3 or < -0.3 were considered statistically significant. Red dots, r1 and p1 represent sample from severe cases; blue dots, r2 and p2 represent samples from non-severe cases.

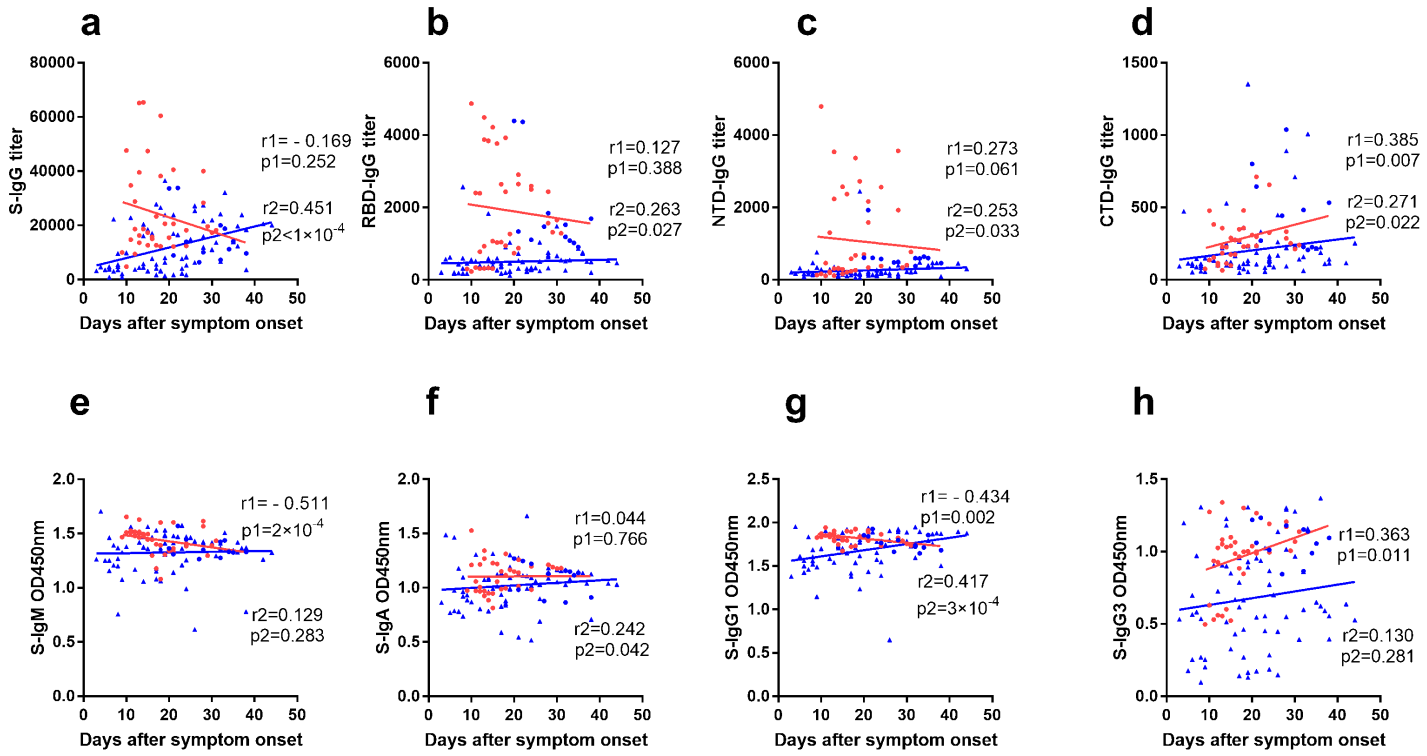

Figure 7

Correlations of specific antibody responses and illness days. The correlations between days after symptom onset and S-IgG (a), RBD-IgG (b), NTD-IgG (c), CTD-IgG (d), S-IgM (e), S-IgA (f), S-IgG1 (g), and S-IgG3 (h) were determined by the spearman rank method, P values < 0.05 and r > 0.3 or < -0.3 were considered statistically significant. Red dots, r1 and p1 represent sample from severe cases; blue dots, r2 and p2 represent samples from non-severe cases.

Supplementary Files

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- SupplementaryTable.docx