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Kinetics of viral load and antibody response in relation to COVID-19 severity

Running Title: Antibody Response in COVID-19 patients

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Keywords: SARS-CoV-2, COVID-19, Antibody response, Cross-reactivity, Neutralizing antibodies
Abstract

The SARS-CoV-2 is the causative agent for COVID-19 pneumonia. Little is known about the kinetics, tissue distribution, cross-reactivity and neutralization antibody response in COVID-19 patients. Two groups of RT-PCR confirmed COVID-19 patients were enrolled in this study, including 12 severe patients in ICUs who needed mechanical ventilation and 11 mild patients in isolation wards. Serial clinical samples were collected for laboratory detection. Results showed that most of the severe patients had viral shedding in a variety of tissues for 20~40 days post onset of disease (8/12, 66.7%); while the majority of mild patients had viral shedding restricted to the respiratory tract and had no detectable virus RNA after 10 days post-onset (9/11, 81.8%). Mild patients showed significantly lower IgM response compared with that of the severe group. IgG responses were detected in most patients in both severe and mild groups at 9 days post onset and remained high level throughout the study. Antibodies cross-reactive to SARS-CoV and SARS-CoV-2 were detected in COVID-19 patients but not in MERS patients. High-levels of neutralizing antibodies were induced after about 10 days post onset in both severe and mild patients which were higher in the severe group. SARS-CoV-2 pseudotype neutralization test and focus reduction neutralization test with authentic virus showed consistent results. Sera from COVID-19 patients, but not convalescent SARS and MERS patients inhibited SARS-CoV-2 entry. Anti-SARS-CoV-2 S and N IgG level exhibited moderate correlation with neutralization titers in patients’ plasma. This study improves our understanding of immune response in human after SARS-CoV-2 infection.
Introduction

Before November 2019, six coronaviruses (CoVs) were known to infect humans and cause respiratory disease, including four community-acquired CoVs OC43, 229E, HKU1, NL63 that are endemic in humans (1-4), and two highly pathogenic CoVs, severe acute respiratory syndrome CoV (SARS-CoV) (5) and Middle East respiratory syndrome CoVs (MERS-CoV) (6) which have zoonotic transmission followed by variable transmission between humans. Recently, a novel coronavirus first identified in Wuhan, Hubei province, China in late 2019 has spread worldwide to cause a pandemic of pneumonia (7). The novel CoV was named “SARS-CoV-2” and the disease was called “coronavirus disease 2019” (COVID-19) (8). Robust human to human transmission of SARS-CoV-2 has led to a pandemic (9) involving every province of China and then to more than 203 countries and territories. As of May 12, 2020, 4,088,848 cases of COVID-19 have been reported World Health Organization (WHO), including 283,153 deaths (10).

SARS-CoV-2 belongs to lineage B betacoronavirus and has high nucleotide homology with bat SARS-like CoV and SARS-CoV (11). SARS-CoV-2 causes severe respiratory illness similar to SARS-CoV. Older adults and individuals with comorbidities are at higher risk for severe disease (12-14). Little is known about the immune response and their relationship with the clinical outcomes in SARS-CoV-2 infected patients. Information about kinetics of virus replication, neutralizing antibody responses and cross-reactivity with other human respiratory CoV are also required for diagnosis, prognosis and epidemiology investigations.

Here, by monitoring viral shedding and antibody responses in patients with severe and mild disease in different tissues, we found that COVID-19 patients with different severity of disease showed different patterns of viral shedding and antibody responses. Severe patients had more prolonged viral shedding in a variety of tissues than mildly ill patients. IgM responses in mild patients were much lower than those observed in severe patients indicating IgM detection in mild patients was not sensitive and efficient. SARS-CoV-2-specific antibodies were found in tissues outside respiratory tract in severe patients.
Detection of antibody responses in urine and other body fluids could be used to as a marker to determine disease severity. By using plasma from SARS, MERS and COVID-19 patients, strong cross-reactivities were detected between SARS-CoV-2 and SARS-CoV, but not MERS-CoV which is an important information for differential diagnosis in Middle East countries. Antibody against N or S protein were correlated with neutralizing antibody titers which may be useful when screening of convalescent plasma for passive transfusion therapy.
Results

Patients and clinical information.

Twenty-three laboratory-confirmed SARS-CoV-2 patients were enrolled in this study. Serial clinical samples were collected every 3~4 days for profiling the kinetics of antibody responses and viral loads during SARS-CoV-2 infection. As showed in Table 1, a total of 12 severely ill patients and 11 mildly ill patients from three hospitals were enrolled in this study. Most patients were older than 50 years old with a median age of 56 years (24~82 years old). At the time of writing, most mild patients (8/11, 72.7%) were discharged from the hospital while the majority of severe patients (10/12, 83.3%) were still in the ICU. Most of the patients had visited Wuhan or had direct contact with other confirmed cases.

Prolonged viral shedding from multiple sites in severely patients.

A total of 461 clinical samples were obtained from 23 severe and mild patients, including 84 nasal swabs, 59 throat swabs, 36 sputum, 90 fecal samples, 79 urine samples, 113 samples of plasma, and one biopsy of gastric juice. As showed in Fig. 1, most of the patients with severe disease had viral shedding for up to 30~40 days post-onset; a majority of mild patients had no detectable viral loads after 15 days post-onset. There was significant difference in the peak viral load (P<0.001, T test) between severe and mild cases. The viral loads in respiratory samples were higher in the severe group and gradually declined over time. SARS-CoV-2 virus was mainly detected in respiratory samples (nasal swabs, throat swabs and sputum) (Fig. 1A, B, C). However, in most of the severely ill patients, feces remained positive for viral RNA over a prolonged time (Fig. 1D). Periodically, urine and plasma were also tested positive (Fig 1E, F).

Mildly ill patients have lower IgM responses against SARS-CoV-2.

To understand the kinetics of the antibody responses against SARS-CoV-2 in patients, IgM and IgG antibody responses against the N protein of SARS-CoV-2 in plasma were assayed (Fig. 2). One hundred and twenty plasma specimens were obtained from 23 patients at
different time points. IgM responses in patients with severe disease increased within one to
two weeks post onset and gradually decreased after four weeks (Fig. 2A), while IgM
responses were much lower in mildly ill patients. Most of the mild patients (8/11) did not
produce significant IgM antibody throughout the whole disease course indicating IgM
diagnosis for mild patients was not sensitive and efficient (Fig. 2A). IgG responses emerged
at 10~15 days post onset (Fig. 2B). Most patients showed high levels of IgG antibodies,
which were maintained for at least for 6 weeks (Fig. 2B). Forty-eight plasma samples
collected from healthy voluntary donors (HD) in 2017-2018 were used as controls to assess
the specificity of the tests. As compared with positive and negative controls provided in the
kit, healthy donors did not have any SARS-CoV-2 specific antibodies.

Detection of IgM and IgG antibodies in respiratory specimens and other body fluids.
To investigate the presence of SARS-CoV-2-specific antibodies in other tissues with viral
shedding, 93 urine, 60 feces, 67 sputum, one BALF and one pleural effusion samples were
collected from severe and mild patients. IgM and IgG antibody response against SARS-CoV-
2 nucleocapsid protein were detected. As shown in Fig. 3A and B, viral specific IgM were
detected in urine (3/10) and sputum (4/10) in severe patients. While, viral specific IgG were
present in urine (7/10) and sputum (7/10) in 10 severe patients. In contrast, no antibody was
detected in the mild group indicating severe infection might result in tissue damage including
airways in the lung and kidneys (Fig. 3A, B). Detection rates of IgM were lower in the urine
and sputum of severely ill patients which was consistent with the larger size of IgM
pentamers than monomer IgG. Appearance of SARS-CoV-2 specific IgG in urine and
sputum could be a potential marker to determine disease severity. No antibody was detected
in fecal samples (Fig. 3C). SARS-CoV-2 specific IgM and IgG antibodies were also detected
in bronchoalveolar lavage fluid (BALF) and pleural effusion from the same severe patient
PT7 indicating various tissue damage and antibody distribution in severe patients (Fig. 3D).
S2 fragment of spike protein was preferentially recognized by SARS-CoV-2-specific antibodies in patients.

To compare antigenicity of different SARS-CoV-2 structural proteins, S (aa 1-1213 of spike protein), S1 (aa 1-685 of spike protein), S2 (aa 686-1213 of spike protein), RBD (receptor binding domain, aa 319-514 of spike protein) and N proteins (aa 1-419 of nucleocapsid protein) were used as coating antigens for ELISA assays (Fig. 4). All of the S, S1, S2, RBD and N proteins were recognized by patient plasma and peaked at 3-4 weeks post-onset. The seroconversion rates against S and S2 proteins reached 100% between 7~14 days after illness onset. They were lower against S1 (10/20, 50%), RBD (13/20, 65%) and N (18/20, 90%) proteins at 7~14 days, and reached 100% after 3 weeks after illness onset (Fig. 4B, C, E). S2 and S2 containing full-length S protein based ELISAs performed better than the others and could detect SARS-CoV-2-specific antibody in all patients even at the first two weeks post-onset (Fig. 4A, D), while healthy donors did not have any SARS-CoV-2 specific antibodies. No obvious differences in IgG response against viral proteins (S, S2, RBD and N) were observed between severe and mild patients, except anti-S1 IgG response due to limited samples. The correlations between IgG levels against different viral proteins (S, S1, S2, N and RBD) were performed and compared as showed in Fig 4F. Most of IgG responses against different viral proteins (RBD, N, S, and S1) showed moderate to strong correlations with each other, except for the correlation between anti-S2 IgG and anti-S1/N IgG. In addition, the anti-S and anti-S2 IgG (r²=0.6902) was better correlated than that between anti-S and anti-N IgG (r²=0.4255).

Strong cross-reactive antibody responses between SARS-CoV-2 and SARS-CoV but not MERS-CoV.

Spike (S) and nucleocapsid (N) proteins of six hCoVs were used to establish in-house ELISA assays for IgG antibody detection. Ninety-six healthy donors were included as controls. As shown in Fig 5A-D, plasma from both severe and mild groups recognized HCoV-229E, NL63, HKU1 and OC43, which shared similar trend as healthy donors since these viruses were
prevalent worldwide, and most of adults were serologically positive for these viruses (15, 16). To further investigate the cross reactivity with SARS-CoV and MERS-CoV, plasma was obtained from 18 SARS convalescents in 2018, 15 years after SARS-CoV infection and 12 MERS convalescents in 2015, 6-18 months after MERS-CoV infection were also included in this study. SARS convalescent plasma showed high-levels of cross-reactivity against SARS-CoV-2 S and N proteins as predicted since SARS-CoV shared 88.6% with N and 69.2% with S homologies, respectively (Fig 5E) (17). No obvious cross-reactivity was observed between MERS-CoV and SARS-CoV-2-specific antibodies. MERS-CoV is still circulating in Middle East countries. Patients infected with MERS-CoV represented similar clinical symptoms as observed in COVID-19 patients. However, our result indicated that it was unlikely to misdiagnose MERS and COVID-19 patients using serological tests. (Fig 5F).

Neutralizing activities were correlated with the magnitude of SARS-CoV-2 S and N antibody responses.

Little is known about the kinetics of anti-SARS-CoV-2 neutralizing antibodies in patients with severe or mild disease. Neutralizing antibodies against authentic SARS-CoV-2 in severe and mild patients were evaluated. We found that 73.9% (17/23) of patients generated robust neutralizing antibodies (FRNT<sub>50</sub>&gt;500) 3 weeks post disease onset (Fig. 6A, 6B). Higher neutralizing antibody titers were induced in the severely ill group as compared to mildly ill patients. Plasma collected from patients 3 weeks post-onset were also tested to compare their neutralizing activities against SARS-CoV-2 pseudotype (Fig 6C) and authentic virus (Fig 6D) at a fixed dilution (1:40). Similar results were obtained with both neutralizing tests indicating there was a good correlation between these two detection methods (21/23 pseudotype, 19/23 live virus-FRNT). SARS and MERS convalescents plasma could not neutralize SARS-CoV-2 pseudotype and authentic virus indicating that SARS and MERS convalescent patients could still be vulnerable to SARS-CoV-2 infection. (Fig. 6C, D). Anti-SARS-CoV-2 S and N IgG level exhibited moderate correlation with neutralization titers of patients' plasma ((Pearson r=0.5393 P value&lt;0.0001 S, r= 0.6709 P value&lt;0.0001 N),
suggesting that monitoring S and N antibody levels could be useful to determine neutralizing titer before convalescent plasma transfusion (Fig. 6E-F). No obvious correlation between viral load and neutralizing titer was observed (Fig. 6G).
DISCUSSION

Temporal profile of serial viral loads from different tissue samples of patients indicated that viral shedding was more common in respiratory and fecal material, especially in severe patients, and to a much less extent in urine and blood. A recently study reported that detectable SARS-CoV-2 viral RNA in blood strongly correlated with clinical severity (18).

A lower level of IgM response was observed in mildly ill patients compared with that of the severe group. The lower level of IgM response associated with mild disease probably reflects lower viral loads and viral antigens. Similar IgG responses were detected in both severe and mild patients. Remarkably, virus-specific IgM and IgG were detectable in serial urine and sputum samples of most of severe patients, but not in mild patients indicating severe tissue damage in these patients which could be used as a marker to determine disease severity.

Several SARS-CoV-2 proteins induced IgG responses in severe and mild patients. Although all proteins including S, S1, S2, RBD and N could be used to detect antibody response, S2 and S2 containing full length S proteins performed better in these ELISA assays and antibodies could be detected in most infected patients in the first two weeks post onset. The sensitivity of detection method was associated with abundance, conservation and antigenicity of viral proteins indicating S2 region possessed more epitopes recognized by viral-specific antibodies.

Six human coronaviruses have been identified. Although SARS-CoV disappeared, HCoV-229E, NL63, HKU1 and OC43 are circulating worldwide and MERS-CoV primarily is in Middle East countries (19, 20). Antigenic cross-reactivity was observed previously between SARS-CoV and HCoV-229E, OC43 (21). Cross-reactivity analysis between SARS-CoV-2 and other six CoVs provided essential information for diagnosis, epidemiological studies and help to dissect the roles of pre-existing antibodies against other CoVs in SARS-CoV-2 and MERS-CoV infected patients. Our ELISA results showed that although our healthy controls were often infected with 229E, NL63, HKU1 of OC43 viruses, there was no cross reaction in the SARS-CoV-2 ELISA assays, indicating that the SARS-CoV-2 S, S1,
RBD, S2 and N protein assays are specific for the virus. Our results showed that most of the SARS-CoV-2 infected patients were infected with the 4 low pathogenic CoVs previously. Plasma from COVID-19 patients also showed high-level of antibody binding to SARS-CoV N and S proteins which was consistent with high homology between the genomes of these two viruses. Of note, stronger cross-reactivities against SARS-CoV proteins were observed in severe patients. Although SARS-CoV-2 and HCoV-OC43 full-length spike proteins share relative low homology (27.2%), some regions in S2 fragments show high homology (70%~80%), which might account for the cross-reactivity between HCoV-OC43 and SARS-CoV-2. Lack of obvious cross-reactivity between MERS-CoV and SARS-CoV-2 plasma ruled out the possibility of misdiagnosis using serologic tests in Middle East region where MERS-CoV has been prevalent for over 7 years.

The plaque/focus reduction neutralization assay is considered to be “gold standard” to quantity antibody neutralization titer. In this study, SARS-CoV-2 pseudotype neutralization system and FRNT assay were compared. SARS-CoV-2 pseudotype expressing SARS-CoV-2 spike protein was successfully used to detect neutralizing antibody with similar sensitivity which could be useful for hospitals without BSL-3 labs where they could do neutralizing test before convalescent plasma transfusion. In addition, anti-SARS-CoV-2 S and N IgG level exhibited moderate correlation with neutralization titers of patients’ plasma, which also provided an alternative method to determine neutralizing titers. Convalescent plasma from SARS and MERS survivors could not inhibit SARS-CoV-2 pseudotype and authentic virus entry. Although SARS-CoV shared 88.6% homology with SARS-CoV-2, a recent study showed their RBD which is the major domain for neutralizing antibody induction was different in structure observed under CryoEM (22). In summary, this study provides comprehensive information on kinetics, tissue distribution, cross-reactivities and neutralization of antibody responses in COVID-19 patients, and will improve our understanding of humoral immune response in human after SARS-CoV-2 infection as well as will shed light on diagnosis, prognosis, convalescent plasma transfusion therapy and epidemiology studies of SARS-CoV-2 infection in human.
Methods

Patient enrollment and sample collection.

Between January 28th to February 24th, 2020, 23 patients with confirmed novel coronavirus (SARS-CoV-2) infection by real-time PCR were hospitalized in the First Affiliated Hospital of Guangzhou Medical University (n=13 patients), the Sixth Affiliated hospital of Guangzhou Medical University, Qingyuan People’s Hospital (n=3 patients) and Yangjiang People’s Hospital (n=7 patients). Respiratory swabs, sputum, body fluids and plasma specimens were collected every three to four days following admission. Clinical data including patient demographic information and clinical outcome was retrieved from the medical records. A total of 12 severely and 11 mildly ill patients were enrolled for serological analysis. Patients with severe pneumonia who were admitted to the ICU and required mechanical ventilation were enrolled in severe group; the patients with a mild clinical presentation (mainly with fever, cough, malaise and headache, including non-pneumonia or mild pneumonia) were enrolled into mild group. Eighteen SARS convalescent plasma were collected in 2018 from healthcare workers who were infected with SARS-CoV 17 years ago. Twelve MERS convalescent plasma were acquired as previously described(23), Ninety-six healthy donor plasma samples which were collected in 2017-2018 were used as controls in this study.

Real-time PCR detection of SARS-CoV-2.

Nucleic acid was extracted from respiratory samples and urine using a Viral RNA extraction kit from Zybio Inc (Chongqing). RNA extraction from feces and blood were performed by total RNA extraction kit from Sangon Biotech (Shanghai). A real-time PCR assay kit targeting SARS-CoV-2 RdRp and N gene regions was provided by Zybio Inc. Serial sampling of nasal swabs, throat swabs, sputum, anal swabs, urine and blood were used to monitor viral shedding.

IgM and IgG ELISA assays.
Clinical samples, including plasma, urine, sputum, feces, bronchoalveolar lavage fluid (BALF) and pleural fluid were collected and analyzed for the presence of SARS-CoV-2-specific IgM and IgG antibodies to the SARS-CoV-2 nucleoprotein using a commercial antibody detection kit provided by Lizhu Medicine Group Holding CO., LTD (Zhuhai). Plasma and urine were used directly in ELISA assays while feces and sputum were diluted with the same volume of PBS and centrifuged at 3000 rpm for 10 minutes before supernatant was harvested for antibody detection. According to the manufacturer’s instructions, threshold IgM value was negative control + 0.1; while IgG threshold for the presence of SARS-CoV-2 infection was negative control+0.13.

Comparison of antibody response to different SARS-CoV-2 proteins.

To assess the antibody response to different SARS-CoV-2 proteins or different fragments of the spike protein, SARS-CoV-2 S (spike protein, 1203 aa), S1 (675 aa), S2 (533 aa), RBD (228 aa) and N (424 aa) proteins were obtained from Sino Biological, Inc (Beijing) and in-house ELISAs for detection of SARS-CoV-2 specific IgG antibody were established. Briefly, 96-well plates (Jet, Biofil Co., Ltd, Guangzhou) were coated with 100 μl/well (0.5 μg/ml) of SARS-CoV-2 S, S1, S2, RBD or N protein in DPBS buffer (Thermo Fisher Scientific (China), Shanghai) overnight at 4°C. After blocking (DPBS, 10%FBS), 100 μl diluted plasma (1:100) were added and plates were incubated at 37 °C for one hour. After washing, plates were incubated with 100 μl of HRP-conjugated mouse anti-human IgG (H+L) antibody (Code: 109-035-088, Jackson ImmunoResearch, West Grove, PA) at 37 °C for one hour. Reactions were visualized by adding 50 μl TMB substrate solution (Biohao Biotechnology Co., Ltd., Beijing). Optical densities at 450 nm were then read. The mean value of healthy donor anonymous plasma (named HD group) collected in 2017-2018 plus 3 standard deviations was used as the detection threshold.

Cross-reactivity between SARS-CoV-2 and other six human CoVs.
To determine the cross-reactivity between SARS-CoV-2 and other six human respiratory CoVs, including SARS-CoV, MERS-CoV, HCoV-NL63, HCoV-OC43, HCoV-229E and HCoV-HKU1, we purchased S and N proteins of all six human CoVs from Sino Biological (Beijing), Inc and established an in-house viral protein ELISA assays for cross-reactive IgG antibody detection as described above.

**Pseudotype-based neutralization assay.**

To safely and rapidly assess neutralization activity of patients' plasma against SARS-CoV-2, we developed a luciferase reporter-based pseudotype neutralization assay which has a non-replicative human immunodeficiency virus backbone coated with the SARS-CoV-2 spike protein. SARS-CoV-2 pseudotype neutralization assays were performed on ACE2-overexpressing HEK293 cells (ATCC, Manassas, VA) (HEK293-ACE2 cells) in 96-well microplates. Fifty microliters of 20-fold diluted patients' plasma were combined with equal volume of the SARS-CoV-2 pseudotype and incubated for 60 min at 37°C. The mixtures were then added to 96-well plates seeded with HEK293-ACE2 cells at 3.8×10^4 cell/well. Cells were further cultured for 40 hours at 37°C. Luciferase activities in cell lysates were measured using steady-Glo luciferase assay kit (Promega, Madison, WI). Neutralizing activity was defined as the ratio of inhibition of SARS-CoV-2 pseudotype (SARS-CoV-2-pp) luciferase activity comparing patients' plasma (1:40 dilution) to control.

**Focus reduction neutralization test.**

SARS-CoV-2 focus reduction neutralization test (FRNT) was performed in a certified Biosafety level 3 lab. Fifty microliters plasma samples were serially diluted, mixed with 50 μl of SARS-CoV-2 (100 focus forming unit, FFU) in 96-well microwell plates and incubated for 1 hour at 37°C. Mixtures were then transferred to 96-well plates seeded with Vero E6 cells (ATCC, Manassas, VA) for 1 hour at 37°C to allow virus entry. Inoculums were then removed before adding the overlay media (100 μl MEM containing 1.2%...
Carboxymethylcellulose, CMC). The plates were then incubated at 37°C for 24 hours.

Overlays were removed and cells were fixed with 4% paraformaldehyde solution for 30 min.

Cells were permeabilized with 0.2% Triton X-100 and incubated with cross-reactive rabbit anti-SARS-CoV-N IgG (Cat: 40143-R001, Sino Biological, Inc, Beijing) for 1 hour at room temperature before adding HRP-conjugated goat anti-rabbit IgG(H+L) antibody (1:4000 dilution) (Code: 111-035-144, Jackson ImmunoResearch, West Grove, PA). Cells were further incubated at room temperature. The reactions were developed with KPL TrueBlue Peroxidase substrates (Seracare Life Sciences Inc, Milford, MA). The numbers of SARS-CoV-2 foci were calculated using an EliSpot reader (Cellular Technology Ltd, Shaker Heights, OH).

**Statistical analysis**

Statistical analysis was performed using Graphpad Prism software, version 7.00. Pearson’s correlation coefficient was used to assess the relationship between anti-viral IgG levels and neutralizing titers. A Student’s t test was used to analyze differences in mean values between groups. A P value <0.05 was considered to be statistically significant. (*, P values of ≤0.05. **, P values of ≤0.01. ***, P values of ≤0.001.) All values are depicted as mean ± SEM. Multiple comparisons following one-way ANOVA and Kruskal-Wallis tests were performed for statistical analysis in cross-reactivity and neutralizing experiments since experiment for each virus was independently carried out. Bonferroni correction was used to avoid inflation of experiment-wise Type I error.

**Study approval**

Institutional Review Board approval from the Health Commission of Guangdong Province as well as the Ethics Committees of each of the hospitals to use patients and healthy donor samples in this study was obtained. Written informed consent forms were obtained from all participants.
Author contributions

JZ, YL, MP, and JZ conceived the study; YW, LZ, LS, FY, SR, BZ, TS, AA, RC, ZZ, MG, AZ, YH and LL collected clinical specimen and executed the experiments; CM, MG, HT, ZL, XH, FL, JS, YZ, LW, YL, ZC, ZZ, JZ, CC, LK, JW, HL, YJ, ML, YL, YD, LT and JL analyzed the data; NZ, PS and JH contributed to critical revision of the manuscript; JZ, MP, YW and LZ wrote the manuscript. All authors revised and approved the final version.

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DISCLOSURE STATEMENT

No conflict of interest was reported by the authors


Table 1. Demographic and clinical outcomes of SARS-CoV-2 infected patients

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Gender</th>
<th>Age</th>
<th>City, Country</th>
<th>Visited Wuhan Hubei, China</th>
<th>Direct contact with confirmed cases</th>
<th>Receiving mechanical ventilation</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A: Severe patients (n=12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT1</td>
<td>M</td>
<td>67</td>
<td>Guangzhou, China</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Still in ICU</td>
</tr>
<tr>
<td>PT2</td>
<td>M</td>
<td>49</td>
<td>Guangzhou, China</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Still in ICU</td>
</tr>
<tr>
<td>PT3</td>
<td>M</td>
<td>50</td>
<td>Guangzhou, China</td>
<td>Y</td>
<td>NA</td>
<td>Y</td>
<td>Still in ICU</td>
</tr>
<tr>
<td>PT4</td>
<td>M</td>
<td>53</td>
<td>Guangzhou, China</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Still in ICU</td>
</tr>
<tr>
<td>PT5</td>
<td>M</td>
<td>61</td>
<td>Guangzhou, China</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Still in ICU</td>
</tr>
<tr>
<td>PT6</td>
<td>M</td>
<td>42</td>
<td>Guangzhou, China</td>
<td>Y</td>
<td>NA</td>
<td>Y</td>
<td>Transferred out of ICU</td>
</tr>
<tr>
<td>PT7</td>
<td>M</td>
<td>72</td>
<td>Guangzhou, China</td>
<td>N</td>
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</tr>
<tr>
<td>PT8</td>
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<td>58</td>
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</tr>
<tr>
<td>PT9</td>
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<td>Y</td>
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</tr>
<tr>
<td>PT10</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Group B: mild patients (n=11)</td>
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<tr>
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<td>PT16</td>
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<tr>
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<td>NA</td>
<td>N</td>
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</tr>
</tbody>
</table>

1Gender: M male, F female
2Visited Wuhan Hubei, China. Y yes, N no, NA not available.
3Direct contact with confirmed cases. Y yes, N no, NA not available
4Receiving mechanical ventilation
Figure 1. Temporal profile of serial viral load from different tissue samples. Viral loads in patients in the ICU (PT1~PT12) and mild patients with mild disease (PT13~PT23) in nasal swabs (A), pharyngeal swabs (B), sputum (C), feces (D), urine (E) and blood (F) were measured. X-axis indicates the days post-onset, y-axis indicates patient numbers. Heat map of Ct values of viral loads were shown. Ct value < 37 indicates the presence of SARS-CoV-2 nucleic acid in the samples. Each square represents one sample detected and gray squares indicate viral nucleotide acid negative.
Figure 2. Kinetics of IgM and IgG responses against SARS-CoV-2 in severe and mild patients. IgM (A) and IgG (B) antibody response against the N protein of SARS-CoV-2 in plasma were detected. Serial plasma samples were collected from 12 severe and 11 mild SARS-CoV-2 infected patients. Forty-eight plasma previously collected from healthy voluntary donors in 2017-2018 were used as healthy donor group (HD). Positive (PC) and negative (NC) controls provided by detection kit were included to ensure test validity.
Figure 3. Kinetics of IgM and IgG responses against SARS-CoV-2 in different tissues.

Specimens from urine (A), sputum (B), feces (C), BALF and pleural effusion (D) from COVID-19 patients were detected for the presence of IgM and IgG antibody against the N protein of SARS-CoV-2. Positive (PC) and negative (NC) control provided by detection kit were included to ensure test validity. Plasma from 48 health donors (HD) were also included.
Figure 4. IgG antibody response against different SARS-CoV-2 proteins or fragments.

Plasma collected at different time points after admission were used for IgG detection in different protein-coated ELISA assays, including S (1209 aa) (A), S1 (681 aa) (B), RBD (457 aa) (C), S2 (539 aa) (D), N (430 aa) (E). Eleven plasma from healthy donors (HD) were used as controls. The correlations between IgG levels against different viral proteins were analyzed and summarized. Pearson’s correlation coefficient was used to assess the relationship among anti-viral IgG levels of different proteins (F). A Student’s t test was used to analyze differences in mean values between groups (A-E), a P value <0.05 was considered to be statistically significant.
Figure 5. IgG cross-reactivity analysis between the other six human CoVs and SARS-CoV-2. Spike (S) and nucleoprotein (N) of the other six human CoVs were used as coated target antigen to establish in-house ELISA to detect IgG antibody for HCoV-229E (A), HCoV-NL63 (B), HCoV-HKU1 (C), HCoV-OC43 (D), SARS-CoV (E), MERS-CoV (F). Plasma from 96 healthy donors and 23 SARS-CoV-2 infected patients were used (A-F). Severe: severe COVID-19 patient, mild: mild COVID-19 patients; HD: healthy donors. Plasma from 18 SARS (E) and 12 MERS (F) convalescents were used as control, respectively. A Student’s t test was used to analyze differences in mean values between groups (A-F). Experiment for each virus was independently carried out. Multiple comparisons following one-way ANOVA and Kruskal-Wallis test were performed for statistical analysis. Bonferroni correction was used to avoid inflation of experiment-wise Type I error. In Fig 5A-5D, a difference was considered statistically significant when the p-value is lower than 0.0167 (=0.05/3) (*, P values of ≤0.0167, **, P values of ≤0.0033, *** P values of ≤0.00033). In Fig 5E-5F, a difference was considered statistically significant when the p-value is lower than 0.0083 (=0.05/6) (*, P values of ≤0.0083, **, P values of ≤0.0017, *** P values of ≤0.00017).
Figure 6. Neutralizing and cross protection of antibody response against SARS-CoV-2 in severe and mild patients. Serial plasma were collected from severe (A) and mild (B) SARS-CoV-2 infected patients, and used for authentic SARS-CoV2 neutralizing test FRNT50 to evaluate kinetics of neutralizing antibodies in SARS-CoV-2 infected patients. Plasma collected three weeks post-onset were used to compare cross neutralizing antibodies between severe, mild SARS-CoV-2 patients and SARS-CoV convalescents using SARS-CoV-2 pseudotype (C) and authentic virus (D) at a fixed dilution (1:40). A Student’s t test was used to analyze differences in mean values between groups. Experiment for each virus was independently carried out. Multiple comparisons following one-way ANOVA and Kruskal-Wallis tests were performed for statistical analysis. Bonferroni correction was used to avoid inflation of experiment-wise Type I error. There were a total of 10 pairwise comparisons among 5 groups. Hence, a difference was considered statistically significant when the p-value is lower than 0.005 (=0.05/10) (’, P values of ≤0.005. **, P values of ≤0.001. ***, P values of ≤0.0001) (C-D). Pearson’s correlation coefficient was used to assess the relationship between neutralizing titer and S and N-specific IgG level (E, F) and viral loads of respiratory specimens (G) were analyzed.