Dear Editor,
This study found that the concentrated antibody therapy was able to clear residual virus reservoirs, increase neutralizing antibody levels and the overall immune response by enhancing peripheral lymphocyte counts and neutrophil–lymphocyte ratio when given during the early phase of SARS-CoV-2 infection.

To the Editor:
Due to the absence of specific and effective treatment for coronavirus disease-2019 (COVID-19), antibody therapy is of great interest. With the strong specificity, the antibody therapy is suitable for emergency use. While therapeutic antibodies and convalescent plasma were promising and facilitating patient recovery, the clinical benefit and immunological effects of therapies using plasma and concentrated antibody from convalescent donors remain uncertain. Here, we conducted a non-randomized clinical trial using concentrated antibodies to treat critically ill COVID-19 patients.

Thirteen COVID-19 patients were enrolled in this study at the Shenzhen Third People's Hospital from late January to August, 2020. The demographic data of the patients were shown in Table 1. Seven patients (S1–S7) received concentrated antibody and (or) convalescent plasma treatment as the intervention group, and the other six patients (S8–S13) without plasma or antibody therapy were followed as the control group. Health history of the both groups showed a very slight and nonsignificant difference (Table S1). Concentrated antibodies were derived from the plasma of fourteen convalescent COVID-19 donors (Table 2, Supplemental Methods and Materials).

We first investigated the effects of concentrated antibody on viral clearance. The virus Ct values began to increase after two days of concentrated antibody treatment and were then remained undetectable within 10 days posttransfusion for 6 treated patients (Figure 1A). In contrast, SARS-COV-2 in the control group was cleared at 14–48 days post-symptom onset (Figure S1A). The virus titer decreased significantly after concentrated antibody treatment ($P$ = 0.00204) (Figure S1B).

We and others have detected residual virus in lung tissues even though continuous SARS-CoV-2 negativity in the nasal, throat swabs and sputum. It was proposed that the ongoing viral activity may contribute to COVID-19 severity. Thus, we questioned whether the concentrated antibody therapy could clear the virus reservoir. We applied Viral-Track mining the scRNA-seq data of Bronchoalveolar lavage fluid (BALF) samples from two patients before and after the antibody treatment. Total numbers of viral reads mapped to the SARS-CoV-2 viral genome were 7,460 for S1, and 178 for S2 before the antibody treatment, respectively (Figure 1C); in contrast, no viral read was detected in samples after the treatment. We further confirmed the SARS-CoV-2 as the only virus in the analyzed data with Viral-Track (Figure 1B). The viral reads were found to be enriched in the epithelial cells, plasma cells, macrophages and T cells (Figure S1C). The pathway analysis indicated an enhanced antiviral function of T cells after treatment (Figure S2).

Previous studies have reported the decreased T cell counts in peripheral blood of COVID-19 patients; particularly, the decreased CD8 T cells were significantly correlated with disease severity. Here, we explore whether the antibody therapy affects cellular immunity. We found the concentrated antibody treatment significantly increased T cell counts in peripheral blood (Figure S3A–C). Importantly, the CD8/CD4 ratio in cured patients receiving concentrated antibody (S1, S4, S6, S7) was remained above 0.5 from 8–17 days after symptom onset, except for patient S6 whose CD8/CD4 ratio was always below 0.5 (Figure S3D; this value was the lower limitation of CD8/CD4 ratio in healthy Chinese adults). By contrast, among those patients with fatal outcomes (S2, S3, S5) even receiving concentrated antibody, the CD8/CD4 ratio decreased and reached...
| S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10 | S11 | S12 | S13 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Severity | Critical | Critical | Critical | Critical | Critical | Critical | Critical | Critical | Critical | Critical | Critical | Critical |
| Age (years) | 46 | 73 | 69 | 64 | 69 | 70 | 29 | 72 | 36 | 69 | 57 | 66 | 63 |
| Gender | Male | Male | Male | Female | Male | Female | Male | Male | Male | Female | Male | Female |
| Wuhan traveling | Yes | Yes | Yes | No | No | No | Yes | No | Yes | No | No | No |
| Symptom onset date/first symptom | 2020-1-21 | 2020-1-20 | 2020-1-29 | 2020-3-13 | 2020-4-24 | 2020-1-19 | 2020-1-11 | 2020-1-21 | 2020-1-20 | 2020-1-20 | 2020-1-12 | 2020-1-12 |
| Hospitalization date | 2020-1-22 | 2020-1-22 | 2020-1-24 | 2020-2-7 | 2020-2-3 | 2020-3-6 | 2020-4-27 | 2020-1-20 | 2020-1-28 | 2020-1-20 | 2020-1-23 | 2020-1-24 |
| Convalescent Plasma Transfusion (date) | ND | Yes 2020-2-13; 2020-2-23 | ND | Yes 2020-2-19 | ND | Yes 2020-3-7 | ND | ND | ND | ND | ND | ND |
| Neutralizing antibody Transfusion (date) | Yes 2020-2-2 | Yes 2020-2-2; 2020-3-7 | Yes 2020-2-19 | Yes 2020-2-19 | Yes 2020-3-7 | Yes 2020-5-1 | ND | ND | ND | ND | ND | ND |
| Outcome/date | Cured 2020-3-8 | Death 2020-7-23 | Death 2020-2-26 | Cured 2020-3-11 | Death 2020-10-10 | Cured 2020-4-1 | Cured 2020-5-21 | Cured 2020-2-23 | Cured 2020-2-26 | Cured 2020-2-8 | Cured 2020-3-7 | Cured 2020-3-6 |
| Chronic basic disease | None | HTN | Diabetes | SP | HTN | Coronary heart disease | Diabetes | HTN | None | HTN | None | None |
| Medication history | None | None | None | None | None | None | None | None | None | None | None | None |
| SARS-CoV-2 RT-qPCR (Ct value, Before or Transfusion Day/sample) | +30.97 | +27.67 | +36.2 | +37.8 | +27.7 | +26.3 | +40 | +21.6 | +24.8 | +23 | +22.5 | +26.5 | +23.8 |
| Flu A/B | –/- | –/- | –/- | –/- | –/- | –/- | –/- | –/- | –/- | –/- | –/- | –/- |
| RSV virus | – | – | – | – | – | – | – | – | – | – | – | – |
| Adenovirus | – | – | – | – | – | – | – | – | – | – | – | – |
| Interferon atomization | 2020-1-22 | 2020-1-24 | 2020-2-7 | 2020-2-4 | 2020-3-6 | 2020-4-27 | 2020-1-20 | 2020-1-28 | 2020-1-20 | 2020-1-20 | 2020-1-23 | 2020-1-23 |
| Ribavirin | 2020-1-23 | 2020-1-22 | NO | 2020-2-12 | 2020-2-11 | No | 2020-1-20 | No | 2020-1-20 | No | 2020-1-20 | No |
| Methylprednisolone | Yes | Yes | Yes | Yes | Yes | No | Yes | Yes | Yes | No | Yes | Yes | Yes |

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-qPCR, reverse transcription polymerase chain reaction; Ct, cycle threshold; BALF, bronchoalveolar lavage fluid; HTN, hypertension; SP, schizophrenic; Flu, influenza.
### Table 2: Characteristics and antibody titer of convalescent plasma donors

| Number | Gender | Age | Disease severity | Interval between symptom onset and discharge | Interval between discharge and plasma donation | Donated plasma volume (ml) | Blood type | For antibody therapy | For convalescent plasma | RBD-specific IgG ELISA titer | RBD-specific IgM ELISA titer | Neutralizing antibody titer | Infectious pathogen |
|--------|--------|-----|------------------|---------------------------------------------|---------------------------------------------|--------------------------|-----------|-------------------|---------------------------|--------------------------|--------------------------|--------------------------|------------------------|
| 1      | Male   | 36  | Moderate         | 17                                          | 4                                          | 500                      | B+        | Yes               | –                         | 200                      | 1800                     | NT                       | –                      |
| 2      | Male   | 35  | Moderate         | 15                                          | 8                                          | 500                      | O+        | Yes               | –                         | 600                      | 600                      | NT                       | –                      |
| 3      | Male   | 51  | Moderate         | 18                                          | 11                                         | 300                      | O+        | Yes               | –                         | 278 612                  | 670                      | NT                       | –                      |
| 4      | Female | 52  | Moderate         | 18                                          | 9                                          | 500                      | O+        | Yes               | –                         | NT                       | NT                       | NT                       | –                      |
| 5      | Male   | 42  | Moderate         | 16                                          | 14                                         | 500                      | O+        | Yes               | –                         | 259 418                  | 171 002                  | NT                       | –                      |
| 6      | Male   | 51  | Moderate         | 24                                          | 5                                          | 500                      | A+        | Yes               | –                         | 16 200                   | 48 600                   | NT                       | –                      |
| 7      | Male   | 19  | Moderate         | 22                                          | 5                                          | 500                      | A+        | Yes               | –                         | 16 200                   | 48 600                   | NT                       | –                      |
| 8      | Female | 49  | Moderate         | 20                                          | 17                                         | 480                      | A+        | Yes               | –                         | 112 720                  | 6053                     | NT                       | –                      |
| 9      | Female | 50  | Moderate         | 19                                          | 16                                         | 400                      | B+        | Yes               | –                         | 671 429                  | 102 565                  | NT                       | –                      |
| 10     | Male   | 54  | Moderate         | 16                                          | 16                                         | 400                      | B+        | Yes               | –                         | 16 200                   | 16 200                   | NT                       | –                      |
| 11     | Male   | 47  | Severe           | 34                                          | 23                                         | 400                      | B+        | Yes               | –                         | 437 400                  | 437 400                  | NT                       | –                      |
| 12     | Female | 36  | Moderate         | 22                                          | 23                                         | 400                      | O+        | Yes               | –                         | 48 600                   | 16 200                   | NT                       | –                      |
| 13     | Female | 47  | Moderate         | 27                                          | 21                                         | 400                      | O+        | Yes               | –                         | 16 200                   | 16 200                   | NT                       | –                      |
| 14     | Male   | 28  | Moderate         | 15                                          | 22                                         | 400                      | AB+       | Yes               | –                         | 16 200                   | 48 600                   | NT                       | –                      |

RBD, receptor-binding domain; IgG, immunoglobulin G; ELISA, enzyme-linked immunosorbent assay.
FIGURE 1 Monitoring SARS-CoV-2 viral loads among the enrolled COVID-19 patients. (A) The serial viral Ct values in concentrated antibody therapy group; (B) Representative Viral-Track analysis data. For each potential viral genome, represented by a dot, the entropy of the sequence (how repetitive are the mapped sequences) and the percentage of the segment that is mapped are plotted. Green dots correspond to viral segments that have passed quality control. Viral genomes with more than 50 mapped reads are plotted. (C) Viral-Track analysis of SARS-CoV-2 reads in BALF scRNA-seq data collected before and after the antibody treatment. D0 is the symptom onset date in (A). The square cross indicates the reception of the convalescent plasma transfusion and the diamond plus indicates the reception of concentrated neutralizing antibody treatment as indicated. Tombstone indicates fatal patients (S2, S3, S5). Pedestrian indicates cured patients (S1, S4, S6, S7).

the lowest point at 13–16 days postsymptoms onset (Figure S3E). In the control group, the CD8/CD4 ratio gradually decreased after disease onset, reaching the lowest point 14–24 days after symptom onset (Figure S3F, Extended Data 1). Neutrophil–lymphocyte ratio (NLR) was also correlated with COVID-19 severity and prognosis, and the NLR more than 11.75 is strongly associated with the higher mortality. In the current study, the NLR was remained below 11.75 in most of the cured patients (Figure S3G) although it fluctuated greatly at higher levels in the fatal patients (Figure S3H). In the control group, the NLR fluctuated until 14–28 days postdisease onset (Figure S3I).

The right timing for antibody therapy is still unclear. We hypothesized that early treatment would favor the recipient to initialize robust antibody response by themselves. Patient S1, S5, S6, and S7 started therapy at 12, 8, 7, and 7 days after the symptoms onset, respectively. They showed increased neutralizing antibody titers along with the increased IgG, IgM, IgA, and total immunoglobulin concentrations after the concentrated antibody treatment (Figure 2A, B). In contrast, patient S3 and S4 received the concentrated antibody therapy at 29 and 21 days after the symptoms onset, respectively. Little improvement in either neutralizing or total antibodies was observed by the treatment in the two patients (Figure 2A, B). Patient S2 received the treatment at 13 days after the symptom onset. The total immunoglobulin and neutralizing antibody levels reach stable high level after the antibody transfusion (Figure 2B). These preliminary data indicated that the earlier Ab treatment possibly facilitated disease recovery from severe COVID-19.

We suspect that the antibody therapy may also affect proinflammatory responses because antibody-dependent cytokine release (ADCR). Plasma IL-6, procalcitonin, and C-reacting protein (CRP) were dynamically monitored. After antibody treatment, the IL-6 levels were increased
FIGURE 2 Monitoring binding antibody and neutralizing antibody levels among the treated COVID-19 patients. The levels of neutralizing antibody and RBD-binding antibody (IgG, IgM, IgA, and total Ig) before and after concentrated antibody therapy in treated patients S1–S7. (A) Cured patients (S1, S4, S6, S7); (B) fatal patients (S2, S3, S5).

rapidly in six of the seven patients (Figure S4A). Notably, IL-6 in patient S2 remained at a lower level for 30 days after the first antibody treatment, but showed a transient increase after the second antibody treatment then followed by decrease to low levels (Figure S4B). We also observed the similar changes of CRP, procalcitonin mirrored IL-6 patterns in patient S1 to S7 (Figure S4C–F).

In summary, we found that concentrated antibody therapy may help clear viral reservoirs in infected lung tissues of critical COVID-19 patients, likely through antibody mediated effector functions. Although the transfused antibodies may cause a transient increase of the inflammatory cytokines, they contribute to the improvement of the overall immune homeostasis and could be used during the
early phase of COVID-19. These findings provide novel evidences for ongoing monoclonal antibody therapy for COVID-19.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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