Immune responses and residual SARS-CoV-2 in two critically ill COVID-19 patients before and after lung transplantation

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To the Editor:

A small number of COVID-19 patients develop critical illness resulting in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Although lung transplantation (LT) can be used to rescue patients from COVID-19-related ARDS [1,2], current information concerning the immune statues and pathogenic conditions of such transplant patients is lacking. In this study, we assessed the immune responses and the residual SARS-CoV-2 nucleic acids in patients before and after LT, including the first COVID-19 lung transplant patient in the world.

There were two critically ill COVID-19 patients in Wuxi of China, from January 25 to March 31, 2020, who eventually recovered after LT (Ethics No. 2020-014) [2 3]. For comparison purposes, we analyzed the whole blood lymphocytes, immunocyte subclasses (T, B and NK cells), blood cytokines and Ag-specific IgM and IgG of hospitalized COVID-19 patients in Wuxi (Ethics No. 2020-010-1). We focused on the analyses of the two critically ill COVID-19 patients (Patient 1: a 58-year-old male had COVID-19-associated ALI and ARDS; Patient 2: a 73-year-old male had COVID-19-associated multiple organ failure and ARDS).

In view of the limited number of critically ill patients, we merged severe and critical illness, designated as severe illness (Fig 1). Most of the severely ill patients had low levels of blood lymphocytes during hospitalization, and in particular, the blood lymphocytes in the two critically ill patients remained below the normal value before and after LT ($<1.1\times10^9$/L) (Fig. 1A). There were statistically significant lower levels of

54 blood $CD3^+$ $CD45^+$ T (<60%) (Fig. 1B and 1C), $CD4^+$ T, $CD8^+$ T and NK cells in
55 individuals with critical clinical manifestations ($P < 0.05$), and a reduction of $CD8^+$ T
56 cells was the most statistically significant in the severely ill patients ($P < 0.01$) (Fig. 1B
57 and 1D). Compared with untreated mildly ill patients, no significant increase of T and
58 NK cells was observed in blood before and after LT, and only B cells increased slightly
59 in the two critically ill patients, likely owing to mesenchymal stem cell infusion therapy
60 (Fig. 1B and 1D). The two critically ill patients developed mildly positive SARS-CoV-
61 2-specific IgM and IgG before LT, and such humoral immune responses became
62 negative post LT (data not shown), likely due to blood transfusion therapy in the
63 absence of new pathogen stimulation. It was reported that IL-6 and IL-10 play distinct
64 roles in immune tolerance [4, 5]. In our study of the two critically ill patients, long-lasting
65 IL-6 and IL-10 levels in plasma exceeded the upper limits of normal values,
66 accompanied by viral replication. The concentrations of proinflammatory cytokines
67 (IL-6, IFN-γ, and TNF-α), anti-inflammatory cytokine IL-10 and B-/T-cell stimulating
68 factor IL-4 in the severe period were significantly higher than those in the recovery
69 period ($p<0.05$), especially for the critically ill patients post LT (Fig. 1E). The above
70 findings together indicate that SARS-CoV-2 infection in critically ill patient results in
71 lower levels of cellular and humoral immune responses.

72 Pathological analyses were performed by immunostaining for $CD3^+$ T, IgA^+ and SARS-
73 CoV-2 S $protein^+$ cells in the diseased lungs. In immunohistochemistry, critically ill
74 patient' lungs (Patient 2) showed obscure mature $CD3^+$ cells in tissues, and extensively
75 fibrosis (Fig. 1F), interstitial hemorrhage (Fig. 1G) and mucous exudative necrosis in

the bronchioles (Fig. 1H), as well as alveolar epithelial atrophy, hyperplasia and shedding in the alveolar cavity (Fig. 1I). The number of IgA^+ cells from alveoli epithelial cells decreased in both the right and the left pulmonary lobes (Fig. 1J-1M). Residual SARS-CoV-2 in the lungs has been suggested to be the main reason for viral positivity of discharged COVID-19 patients [6, 7]. We observed a direct evidence of residual SARS-CoV-2 in excised lungs (Fig. 1N-1Q), suggesting that antiviral therapy may not completely eliminate the virus in the dysfunctional lungs. For the two critically ill COVID-19 patients under therapies, convalescent plasma and mesenchymal stem cell infusions appeared unable to restore a systemic immunity, including cellular and mucosal (IgA) immune responses in the diseased lungs. A previous pathologic study showed that SARS-CoV-2 was highly destructive to the immune system, resulting in reduced splenic T and B cell compositions due to necrosis and apoptosis [8]. This may account for the long-term low systemic immunity of COVID-19 transplant patients.

SARS-CoV-2 RNA can be detected in the gastrointestinal tract using swabs and stool sampling [9], and in particular, SARS-CoV-2 particles can be found in the gut endothelium [10], suggesting the potential significance of the gut in viral transmission and pathogenesis. Prior to LT, the two critically ill patients in the current study were under treatments with convalescent plasma infusion, mesenchymal stem cell infusion and antiviral agents until SARS-CoV-2 nucleic acid turned into negative in blood and nasopharyngeal and anal swabs. Post LT, residual SARS-CoV-2 in nasopharyngeal and anal swabs was also examined. In anal swabs, SARS-CoV-2 was mildly positive at day 26, 30, and 43 post LT in Patient 1 (Fig. 2A), and mildly positive at day 28 post

hospitalization and day 17 post LT in Patient 2 (Fig. 2B). Of note, in the absence of antiviral treatment and under immune suppression therapy (doses of drugs were only 1/6 of those for ordinary transplant patients) post LT, there was no indication of SARS-CoV-2 infection in the new (donor) lungs according to the chest radiographs and axial pulmonary CT graphs in the negative-pressure ward up to 45 days (Fig. 2A and 2B), while viral nucleic acid remained negative after the patients were transferred to the general ward. There was no medical staff infected by SARS-CoV-2 during medical care in general ward. The above results indicated that the detection of viral positive nucleic acids by anal swapping does not necessarily reflect a contagious SARS-CoV-2 in the gut.

In conclusion, following LT, the two critically ill COVID-19 patients in the absence of antiviral treatment have not had a second SARS-CoV-2 infection in the new lungs. For the first time, our study provides information relating to the immune status and SARS-CoV-2 positivity of lung transplant patients.

References

1. Lang C, Jaksch P, Hoda MA, et al. Lung transplantation for COVID-19-associated acute respiratory distress syndrome in a PCR-positive patient. *The Lancet Respiratory medicine* 2020;**8**(10):1057-60.
2. Chen JY, Qiao K, Liu F, et al. Lung transplantation as therapeutic option in acute respiratory distress syndrome for coronavirus disease 2019-related pulmonary fibrosis. *Chinese medical journal* 2020;**133**(12):1390-96.
3. Yan Y, Liu B, Ding H, et al. Characterizing COVID-19 severity, epidemiology and SARS-CoV-2 genotypes in a regional business hub of China. *The Journal of infection* 2020; **S0163-4453**(20):30566-1.
4. Dembek A, Laggai S, Kessler SM, et al. Hepatic interleukin-6 production is maintained during endotoxin tolerance and facilitates lipid accumulation. *Immunobiology* 2017;**222**(6):786-96.
5. Schmetterer KG, Pickl WF. The IL-10/STAT3 axis: contributions to immune tolerance by thymus and peripherally derived regulatory T-cells. *European journal of immunology* 2017;**47**(8):1256-65.
6. Li J, Long X, Fang X, et al. SARS-CoV-2 positivity in a discharged COVID-19 patient: a case report. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2020;**26**(8):1115-17.
7. Ye G, Pan Z, Pan Y, et al. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. *The Journal of infection* 2020;**80**(5):e14-e17.
8. Tabary M, Khanmohammadi S, Araghi F, Dadkhahfar S, Tavangar SM. Pathologic features of COVID-19: a concise review. *Pathology, research and practice* 2020;**216**(9):153097.
9. Wong SH, Lui RN, Sung JJ. Covid-19 and the digestive system. *Journal of clinical gastroenterology and hepatology* 2020;**35**(5):744-48.
10. Stahl K, Bräsen JH, Hoeper MM, David S. Direct evidence of SARS-CoV-2 in gut endothelium. *Intensive care medicine* 2020;**46**(11):2081-82.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure legends

Figure 1. Immune responses of critically ill COVID-19 patients and immunohistochemistry of the diseased lungs.

A. Blood T lymphocyte counts. Blood T lymphocyte counts were detected by clinical blood cell analyzer. The gray area is the location of the dangerous values ($<1.1\times10^9$/L). **B.** Gating strategies for blood immunocytes. $CD3^+$ and $CD45^+$ T lymphocytes were gated for $CD4^+$ and $CD8^+$ T cells. $CD3^-$ and $CD45^+$ immunocytes were gated for B ($CD19^+$) and NK ($CD16/56^+$) cells. **C.** Percentage comparison of blood $CD3^+$ and $CD45^+$ lymphocyte levels. **D.** Absolute percentage comparison of blood immunocytes. **E.** Plasma cytokine levels. Plasma samples from COVID-19 patients (n=6) were collected during the early and recovery periods (mildly and severely ill patients were at around day 30; transplant patients were before being transferred to the general ward). IL-6, IL-10, IFN-γ, IL-17A, TNF-α, and IL-4 beads were used for staining the cytokines in plasma samples. Quantitative detection and comparison of inflammatory cytokine expression levels in different periods were conducted by flow cytometry. Mildly ill patients (Mild): individuals who had mild manifestation and no apparent or long-term decrease in lymphocyte levels. Severely ill patients (Severe): individuals who had lasting lower levels of lymphocytes and needed supplemental oxygen and intensive care. Critically ill patients: individuals who had failure of respiratory organs leading to dependency on ventilators. The red solid symbol (▲ or ●) stands for critically ill patients. The results are expressed as the mean ± SEM; NS, not statistically significant,

$P < 0.05$ was considered statistically significant. **F-I.** Hominine CD3 immunostaining of diseased lung tissues. **J-M.** Hominine IgA immunostaining. **N-Q.** SARS-CoV-2 S protein immunostaining. Post LT, approximately 2-cm segments from excised human lungs were collected and fixed in formalin for 24 h. After fixation, tissue was embedded and sectioned. For the detection of CD3-, IgA- and SARS-CoV-2 S protein-positive cells in lungs, the slides were stained with CD3ε rabbit monoclonal antibody (Cell Signaling Technology, U.S.A, 1: 1000, E4T1B), human IgA heavy chain rabbit polyclonal Ab (Proteintech, U.S.A., 1: 400), and SARS-CoV-2 S protein rabbit polyclonal Ab (Sino Biological Inc., China, 1: 50), respectively, overnight at 4°C. After labeling with a goat anti-rabbit secondary IgG Ab, a color reaction was developed with the addition of 3, 3'-diaminobenzidine free base (DBA), followed by counterstaining with hematoxylin. Data shown are representative immunohistochemistry results. S protein: spike protein; R, right upper lobe; L, left lower lobe; The red arrow shows the positive cells.

Figure 2. Chest imaging and viral nucleic acid detection before and after LT.

A. Chest radiographic images and SARS-CoV-2 nucleic acid results of patient 1. Chest radiographic images were obtained before and post LT. Axial CT images on day 45 post LT, and SARS-CoV-2 nucleic acid results. **B.** Axial chest CT images before and after LT and SARS-CoV-2 nucleic acid results of patient 2. Nasopharyngeal and anal swabs were placed in viral transport medium at a low temperature. Total RNA extraction and SARS-CoV-2 detection were performed using the commodity reagents of Da An Gene

(Da An Gene, China) according to the manufacturer's instructions. Briefly, SARS-CoV-2 RNA was detected by real-time RT-PCR. Target genes (ORF1ab + N) were set as described in the reagent instructions. A cycle threshold value (Ct value) less than 36 was defined as a positive (+), and Ct value between 36 and 37 was defined as mildly positive (±). 1, 2 and 3 on CT images mean the different axial images within the lungs. ph: post-hospitalization; plt: post LT.

Figure 1

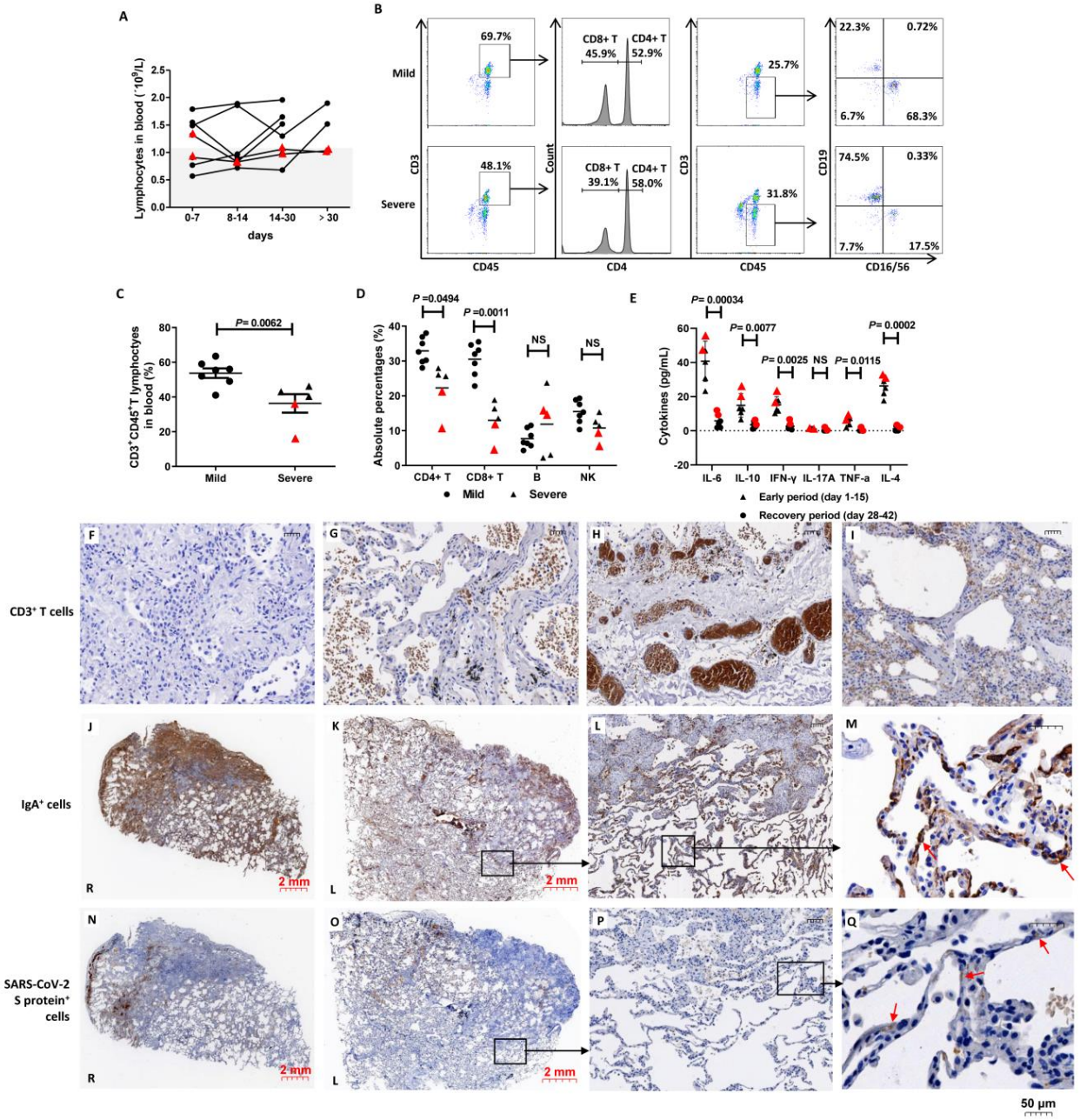
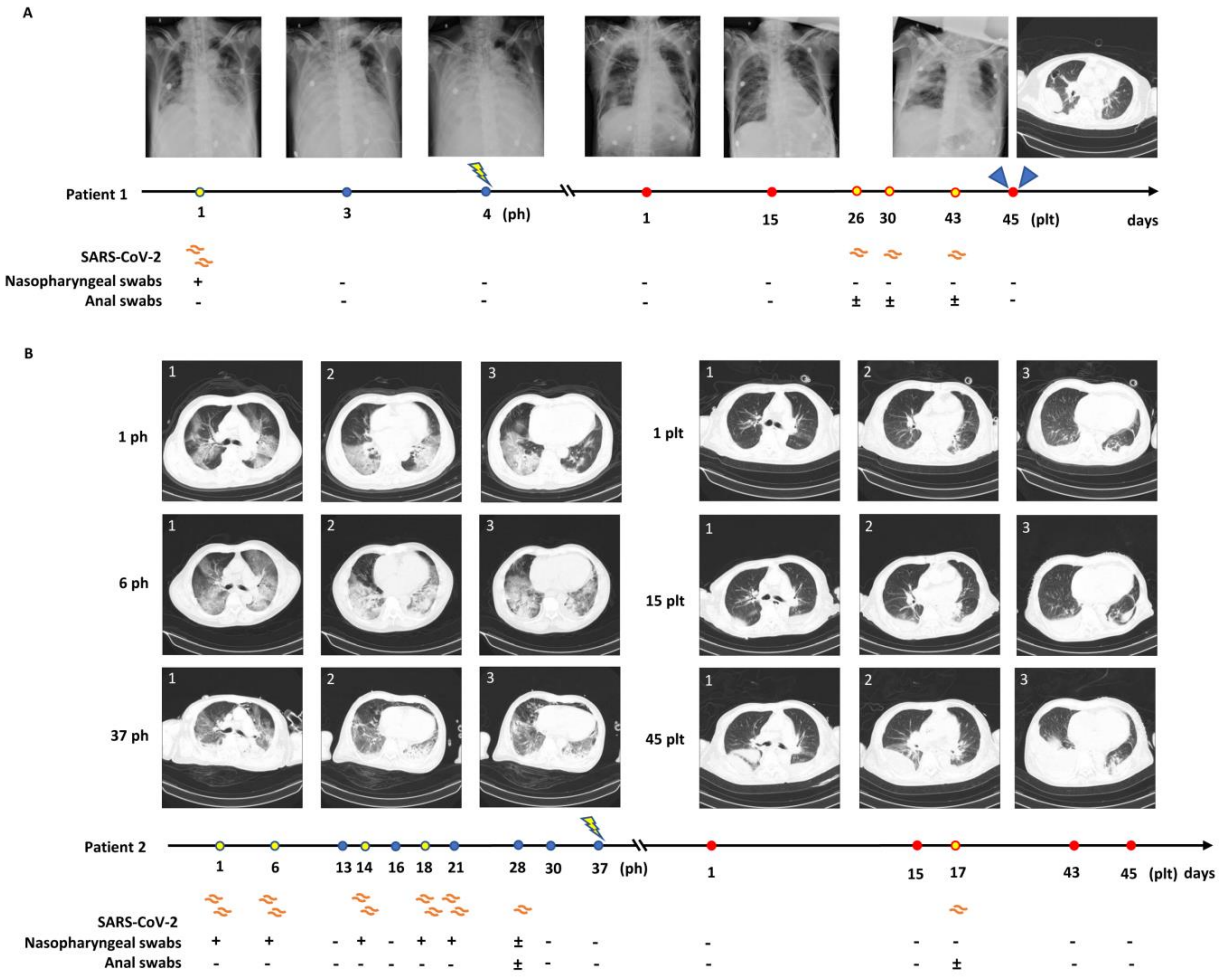

A
Lymphocytes in blood (*10⁹/L)
days
0-7
8-14
14-30
> 30
B
Mild
Severe
69.7%
CD8+ T 45.9%
CD4+ T 52.9%
25.7%
22.3%
0.72%
6.7%
68.3%
48.1%
CD8+ T 39.1%
CD4+ T 58.0%
31.8%
74.5%
0.33%
7.7%
17.5%
CD3
Count
CD19
CD45
CD4
CD16/56
C
P= 0.0062
CD3+CD45+T lymphoctyes in blood (%)
Mild
Severe
D
P =0.0494
P =0.0011
NS
Absolute percentages (%)
CD4+ T
CD8+ T
B
NK
E
P= 0.00034
P= 0.0077
P= 0.0025
P= 0.0115
P= 0.0002
Cytokines (pg/mL)
IL-6
IL-10
IFN-γ
IL-17A
TNF-a
IL-4
Early period (day 1-15)
Recovery period (day 28-42)
CD3+ T cells
IgA+ cells
SARS-CoV-2 S protein+ cells
2 mm
50 μm

Figure 2


A
Patient 1
1
3
4 (ph)
1
15
26
30
43
45 (plt)
days
SARS-CoV-2
Nasopharyngeal swabs
Anal swabs
B
1 ph
6 ph
37 ph
1 plt
15 plt
45 plt
Patient 2
1
6
13
14
16
18
21
28
30
37 (ph)
1
15
17
43
45 (plt) days
SARS-CoV-2
Nasopharyngeal swabs
Anal swabs