Severe Adaptive Immune Suppression May be Why Patients with Severe COVID-19 Cannot be Discharged From the ICU Even After Negative Viral Tests

Yue Zhou

Sichuan University West China Hospital

Xuelian Liao

Sichuan University West China Hospital

Xiangrong Song

Sichuan University West China Hospital

Min He

Sichuan University West China Hospital

Fei Xiao

Sichuan University West China Second University Hospital

Xiaodong Jin

Sichuan University West China Hospital

Xiaoqi Xie

Sichuan University West China Hospital

Zhongwei Zhang

Sichuan University West China Hospital

Bo Wang

Sichuan University West China Hospital

Chenliang Zhou

Renmin Hospital of Wuhan University: Wuhan University Renmin Hospital

Yan Kang

Sichuan University West China Hospital

Wei Zhang (✉ zhangwei197610@163.com)

Sichuan University West China Hospital <https://orcid.org/0000-0001-7588-7249>

Research

Keywords: COVID-19, adaptive immune suppression, secondary infections, transcriptome sequencing

Posted Date: July 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-709952/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Frontiers in Immunology on November 19th, 2021. See the published version at <https://doi.org/10.3389/fimmu.2021.755579>.

Abstract

Background: During the COVID-19 pandemic, a phenomenon emerged in which some patients with severe disease were critically ill and could not be discharged from the ICU even though they exhibited negative viral tests. In general, continuous negative viral tests are thought to indicate that the virus has been cleared from the body and that the patients can be considered "recovered". However, because these patients were still critically ill, they obviously had not truly recovered from the disease. We sought to investigate why these patients were still critically ill even though they exhibited negative viral tests by analyzing the gene expression profiles of their peripheral immune cells using transcriptome sequencing.

Methods: Fourteen severe COVID-19 patients with at least 3 negative virus tests but were still in critical ill and could not be discharged from the ICU were enrolled. Blood samples from 14 patients and 5 healthy donors were collected. Total RNA was extracted from nucleated cells for RNA-Sequencing. FeatureCounts v1.5.0-p3 was used to count the reads numbers mapped to each gene.

Results: All enrolled patients, regardless of changes in genes related to different symptoms and inflammatory responses, showed universally and severely decreased expression of adaptive immunity-related genes, especially those related to T/B cell arms and HLA molecules, and that these patients exhibited long-term secondary infections. This adaptive immune suppression is unlikely due to classic immune checkpoint molecules such as PD-1 or long-term use of glucocorticoids but may be caused by an unknown mechanism that has not yet been discovered.

Conclusions: Our findings strongly suggest that an initial recovery of these severe COVID-19 patients, as indicated by negative viral tests, may not indicate actual recovery. They still suffer from secondary infections for a long period of time because of severe adaptive immunosuppression and need to receive a variety of antibiotics, antifungal drugs, or combination therapies. Appropriate methods should be used to detect their adaptive immune function, and appropriate immunotherapy that can activate the adaptive immune response should be developed.

Trial registration: Not applicable (this study does not involve intervention on human participants).

Introduction

Coronavirus disease 2019 (COVID-19), caused by the infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 2], has spread throughout the world, causing a devastating medical and social crisis [3, 4]. As a highly heterogeneous syndrome, it shows an extensive range of clinical presentations and variable disease progression. Most infected patients are generally asymptomatic or develop mild symptoms [5–8]. However, a small proportion (~ 5%) of patients with COVID-19 progress to a severe condition [6, 9, 10]. These patients often require intensive medical treatment because of acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), or multiorgan dysfunction (MODS) with a considerable risk of mortality.

In our clinical practice, we observed that some severe COVID-19 patients with consecutive negative viral tests (SARS-CoV-2 detection by reverse transcriptase polymerase chain reaction of nasopharyngeal swab specimens) remained critically ill and could not be discharged from the ICU. In general, continuous negative viral tests are thought to indicate that the virus has been cleared from the body and that the patients can be considered "recovered". However, because these patients were still critically ill, they obviously had not truly recovered from the disease. To determine why these patients were still critically ill even though they exhibited negative viral tests, we collected blood samples and analysed the gene expression profiles of the peripheral immune cells.

We found that all patients, regardless of the changes in genes related to different symptoms and inflammatory responses, showed universally and severely decreased expression of adaptive immunity-related genes, especially those related to T/B cell arms and HLA molecules. This severe adaptive immune suppression makes these patients susceptible to secondary infections. In fact, although not all the patients had direct laboratory evidence of secondary infections, they all had clinical symptoms of infections and were treated with broad-spectrum antibiotics or antifungal drugs. Corresponding to this is the recent outbreak of COVID-19-related mucormycosis in India [11]. A prior history of COVID-19 was present in 37% of the patients who developed mucormycosis after an initial recovery [12], indicating that this "initial recovery" may not indicate a real cure. These patients who appeared to be cured were likely to have severe adaptive immunosuppression, which made them vulnerable to severe secondary infections such as mucormycosis. For these patients, appropriate methods should be used to detect their adaptive immune function, and appropriate immunotherapy that can activate the adaptive immune response should be considered. Our data also suggest that this adaptive immune suppression is unlikely to be due to classic immune checkpoint molecules such as PD-1 or long-term use of glucocorticoids but may be caused by an unknown mechanism that has not yet been discovered, and it is worthy of further exploration.

Methods

Study design:

This cross sectional, single center observational study consisted of 14 patients with severe COVID-19 and 5 healthy donors. The patients were enrolled in 2 batches at two different time points (April 2020 and May 2020 separately) in the intensive care unit (ICU) of *East Campus of Renmin Hospital of Wuhan University*, including 5 patients in batch 1 and 9 patients in batch 2. Nasopharyngeal swab specimen which was positive for SARS-CoV-2 by reverse transcriptase polymerase chain reaction (RT-PCR) was used as the diagnosis criteria of COVID-19. In this study, the patients with severe COVID-19 with at least 3 negative virus tests were still in critical ill and could not be discharged from the ICU were enrolled.

Sample Collection:

For each enrolled subject, peripheral venous blood (3mL) was obtained in sodium heparin-coated vacutainers. 3mL Trizol was added to per sample to prevent RNA from degradation. All samples were kept in -80°C until use.

Rna Sequencing And Data Analysis:

Total RNA was extracted from nucleated cells in whole blood. RNA purity was checked using NanoPhotometer spectrophotometer (IMPLEN, CA, USA), and RNA integrity was assessed using the RNA Nano 6000 Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). A total amount of 1 µg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext Ultra™ RNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's instructions. The library quality was determined on the Agilent Bioanalyzer 2100 system. Sequencing was performed on an Illumina Novaseq platform. FeatureCounts v1.5.0-p3 was used to count the reads numbers mapped to each gene. Differential expression analysis of two conditions (COVID-19 versus healthy) was performed using the DESeq2 R package (1.16.1).

Statistics:

Sequencing data are presented in the form of volcano plots (integrating log2 fold values and multiple-test adjusted probabilities) and heat map plots, generated in R studio and Graphpad prism 8 (GraphPad Software Inc., La Jolla, USA). The resulting P-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. The significance threshold was set to an adjusted P-value < 0.05 found by DESeq2. Categorical variables were represented directly as numbers and continuous variables were represented with medians and IQRs. A two-sided P value of < 0.05 was used to indicate statistical significance.

Results

Patients infected with SARS-CoV-2 at different periods have significant differences in genes controlling smell and taste functions

To obtain a comprehensive understanding of the impact of SARS-CoV-2 infection on patients with severe COVID-19 with at least 3 negative virus tests, we analysed the transcriptional profiles of whole blood cells via RNA-Seq analysis. Five healthy volunteers and 14 patients with severe COVID-19 were enrolled in this study (the characteristics of the patients are listed in Table 1). The patients were enrolled in 2 batches at two different time points (April 2020 and May 2020 separately). The first batch (including 5 patients) and the second batch (including 9 patients) both came from the *East Campus of Renmin Hospital of Wuhan University*. The common feature of these two batches of patients was at least three consecutive negative SARS-CoV-2 virus tests.

Table 1. Demographic and clinical characteristics of the 14 enrolled patients. Values are expressed as medians (interquartile ranges) except sex and outcome. Abbreviations: APACHE II score, Acute Physiology and Chronic Health Evaluation II score; FiO₂, fraction of inspired oxygen; PaO₂, oxygen partial pressure; PaCO₂, carbon dioxide partial pressure; SpO₂, pulse oxygen saturation.

Parameter	Patient Batch 1 (N=5)	Patient Batch 2 (N=9)
gender (male/female)	3/2	7/2
Age (year)	65 (63, 73)	66 (65, 73)
APACHE II score	-	21 (17, 22)
Mean arterial pressure (mmHg)	91 (79, 105)	75 (65, 99)
Leukocytes (10 ⁹ /L)	7.78 (6.04, 8.94)	10.09 (8.78, 14.59)
Neutrophils%	66.5 (65.1, 67.1)	73.6 (66.7, 79.5)
Lymphocytes%	-	12.5(4.8,15.2)
PLT (10 ⁹ /L)	179 (95.75, 265.25)	148 (135, 213)
pH	7.45 (7.41, 7.49)	7.36 (7.31, 7.5)
Lactate (mmol/L)	1.11 (0.65, 1.61)	1.8 (1.2, 2.4)
FiO ₂ (%)	40 (40, 50)	60 (45, 70)
PaO ₂ (mmHg)	123 (110.75, 145.25)	123 (74, 168)
PaCO ₂ (mmHg)	61.2 (56, 62.5)	48 (38, 54)
SpO ₂	98.5 (98, 99.25)	95 (93, 99)
outcome (alive/dead)	4/1	4/5

Compared with the healthy donors, the severe COVID-19 patients exhibited 33788 upregulated genes, 1007 downregulated genes, and 20347 genes that remained unchanged. A volcano map (Supplementary Fig. 1) was used to visually display the differentially expressed gene (DEG) distribution between the patients and the healthy donors. We used Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses to investigate gene enrichment. The most significantly enriched GO terms were "detection of stimulus involved in sensory perception" and "sensory perception of smell" (Fig. 1A). The most significantly enriched KEGG terms were "olfactory transduction", "neuroactive ligand-receptor interaction", and "taste transduction" (Fig. 1B). Most of the genes described in those terms overlapped and were classified as genes controlling sensory functions, mainly smell and taste. The expression levels of these genes varied among the different batches of patients. In the first batch of patients, only one patient showed abnormally increased expression of the smell- (Fig. 2A) and taste-related (Fig. 2B) genes; however, in the second batch, more than half of the patients showed obviously elevated expression of these genes. The results indicate that patients infected with SARS-CoV-2 at different periods have significantly different symptoms regarding the genes controlling smell and taste functions.

Changes in the expression of genes related to “cytokine storm” and inflammatory responses

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, including bacterial, fungal, parasitic, or viral infections (The Third International Consensus Definitions for Sepsis and Septic Shock, Sepsis-3) [13]. Since SARS-CoV-2 is an infectious viral pathogen, it is reasonable to consider that severe COVID-19 is a subtype of sepsis [14, 15]. The “cytokine storm” and inflammatory-related responses are the main focusses of sepsis studies. However, our RNA-Seq results showed that in the patients with severe COVID-19, there were large individual differences in the expression of inflammatory factors (Fig. 3A), chemokines (Fig. 3B), and adhesive molecules (Fig. 3C). Patient A5 from batch 1 and patients B1, B2, B3, B4, and B8 from batch 2 showed significant upregulation of these genes, whereas no obvious changes were found in patients A1, A2, A3, and A4 from batch 1 and patients B5, B6, B7, and B9 from batch 2 (Fig. 3A-C). In addition, the expression levels of cytokines, chemokines, and adhesive molecules showed no significant difference between the survival and death groups, which is in line with recent COVID-19 studies [16, 17] (Supplementary Fig. 2–4). According to the results, in those patients with severe COVID-19 but negative viral tests, there were large individual differences in inflammatory responses, and there was no obvious relationship between the inflammatory responses and severity and mortality.

The adaptive immune function of all the patients was severely impaired

Compared with the number of upregulated genes (33,788), only 1,007 genes were downregulated. Among them, many genes were related to immune functions. According to the KEGG enrichment analysis results, there were 10 significantly changed KEGG pathways ($p\text{-adj} < 0.05$), and 4 of them were related to immune responses, namely, “primary immunodeficiency”, “T cell receptor signalling pathway”, “haematopoietic cell lineage”, and “Th1 and Th2 cell differentiation”. Seven genes were shared by these 4 categories: CD3D, CD3E, CD3G, CD4, CD8A, CD8B, and CD40LG. Compared with those in the healthy volunteers, the expression levels of these genes in the patients were dramatically decreased (Fig. 4A). In addition, genes related to B cell function, including CD5, CD19, CD20, CD21, CD22, CD23, CD79a, and CD79b, were also significantly downregulated (Fig. 4B).

The major histocompatibility complex (MHC) is a collection of genes that code for MHC molecules found on the surface of all nucleated cells [18, 19]. In humans, MHC genes are referred to as human leukocyte antigen (HLA) genes. MHC molecules play an important role in the antigen presentation process, which is a key step in the activation of the adaptive immune response. As shown in Fig. 4C, the expression of multiple HLA genes, in addition to the most reported HLA-DR gene, was decreased to a very low level.

Antigen-induced signals transferred from antigen-presenting cells by MHC molecules to the T cell receptor (TCR) alone are insufficient to activate T cells. Costimulatory and coinhibitory receptors play a pivotal role in T cell activation or inhibition, as they determine the functional outcome of TCR signalling [20, 21]. The expression of four classic costimulatory molecules, namely, CD27, CD28, CD40LG, and TNFRSF25,

was reduced to a very low level (Fig. 4D). However, the expression of other costimulatory molecules (Supplementary Fig. 5A) as well as coinhibitory molecules [22], such as CTLA-4, PD-1, and PD-L1 (Supplementary Fig. 5B), did not show a significant increasing or decreasing trend.

Together, these results strongly suggest that the adaptive immune response is still severely and universally impaired in patients with severe COVID-19 who have negative viral tests and that this immunosuppression is not due to the upregulation of classic immune checkpoint molecules such as PD-1.

Discussion

To investigate why some severe COVID-19 patients with negative virus tests were still critically ill and could not be discharged from the ICU, we analysed the changes in the transcription level of all genes using whole blood cells from 14 patients and 5 healthy volunteers. The RNA-Seq analysis showed that those patients had different expression patterns in genes controlling some disease-related symptoms (such as smell and taste disorders) and that the changes in inflammation-related genes, including cytokines, chemokines, and adhesive molecules, were varied and exhibited obvious individual differences. However, all patients, regardless of the changes in their symptoms and inflammatory responses, showed severely and universally decreased expression of adaptive immunity-related genes, especially those related to T/B cell arms and HLA molecules. The results suggest that those patients were in a state of severe adaptive immunosuppression, which may contribute to their critical illness even though they exhibited negative virus tests.

Long-term severe immunosuppression makes these patients susceptible to secondary infections [23]. In fact, among the 14 enrolled patients, 5 were confirmed to have secondary infections, as evidenced by laboratory tests, including bloodstream, urinary tract, and multisite infections. Although other patients had no direct laboratory evidence of secondary infections, they all had clinical symptoms of infections and were treated with broad-spectrum antibiotics or antifungal drugs. Among all the patients, patients A4 and A5 showed the lowest expression of adaptive immunity-related genes. Patient A4 was a 73-year-old male patient who had complex infections, including respiratory carbapenem-resistant *Acinetobacter baumannii* infection, bloodstream gram-negative bacterial infection, and possible urinary tract infection. The patient finally died despite the use of broad-spectrum antibiotics and other supportive treatments. Patient A5 was a 65-year-old male patient with severe *Candida parapsilosis* infection. After 6 months of intensive medical treatment, including extracorporeal membrane oxygenation (ECMO) treatment and lung transplantation, the patient finally recovered. Overall, the severe adaptive immunosuppression that occurs after negative virus tests does put the patients at high risk of secondary infection and may lead to eventual death. Indeed, even after receiving critical care, including systemic administration of broad-spectrum antibiotics/antifungal drugs, the use of ventilators or ECMO, 6 patients (A4, B3, B4, B5, B6, B9) eventually died during hospitalization.

An outbreak of COVID-19-associated mucormycosis (CAM) has recently been reported. The “syndemic” of rhino-orbito-cerebral mucormycosis infections has arisen, with nearly 9000 cases reported thus far from several states in India [11]. Poor control of diabetes mellitus is considered an important predisposing factor for CAM [24, 25]. However, not all CAM patients have diabetes (approximately 20% of patients have no history of diabetes), and not all CAMs occur in India [26]. A prior history of COVID-19 was present in 37% of patients with mucormycosis developing after an initial recovery [12], which strongly suggests that this “initial recovery” may not be a real cure. These patients who appeared to be cured were likely to have severe adaptive immunosuppression, which makes them vulnerable to severe secondary infections such as mucormycosis. Therefore, we should not classify critically ill patients with negative SARS-CoV-2 tests as truly recovered because these patients are likely to still be in a state of severe immunosuppression and at high risk of secondary infection. For these patients, appropriate methods should be used to detect their adaptive immune function, and appropriate immunotherapy that can activate the adaptive immune response should be considered.

Notably, adaptive immune suppression after negative virus tests, at least in the patients included in this study, was not caused by the elevated expression of classic immune checkpoint molecules such as PD-1 [22]. In this study, we found that PD-1, PD-L1, and CTLA-4 did not show a significant increasing or decreasing trend, and the expression of these genes was too low to cause such a full range of adaptive immune suppression. Therefore, the use of PD-1 or PD-L1 antibodies may not benefit these patients.

It has been reported that some patients are immunosuppressed before infection [27]. However, in this study, the enrolled patients did not have a history of haematological tumours or the long-term use of immunosuppressants. Therefore, this adaptive immunosuppression should be regarded as a consequence of SARS-CoV-2 infection rather than a pre-existing immunodeficiency. Additionally, patients A1, B1, B5, B7, and B9 had no history of glucocorticoid therapy during hospitalization, indicating that the immunosuppression identified in these patients could not be attributed to glucocorticoid use. In this study, all enrolled patients showed severely decreased expression of adaptive immunity-related genes, which cannot be simply explained by coincidence. COVID-19 can be regarded as a subtype of sepsis caused by a specific pathogen (SARS-CoV-2) [28]. Adaptive immunosuppression is also a feature of long-term hospitalized patients with sepsis [29–31]. For these patients, the pathogen type of the initial infection is usually unverifiable, but they still suffer from secondary infections for a long period of time and need to receive a variety of broad-spectrum antibiotics, antifungal drugs, antiviral drugs, or combination therapies [32]. This indicates that the adaptive immunity suppression observed in patients with COVID-19 and sepsis is likely to be caused by an unknown mechanism that has not yet been discovered, and it is worthy of further exploration.

Conclusion

This study enrolled 14 patients with severe COVID-19. The common feature of these patients was that they were still critically ill even though they exhibited negative virus tests. We found that all enrolled patients, regardless of changes in genes related to different symptoms and inflammatory responses,

showed severely decreased expression of adaptive immunity-related genes, which put these patients at high risk of long-term secondary infections. For these patients who appear to have "recovered" due to a negative virus test, appropriate methods should be used to detect their adaptive immune function, and appropriate immunotherapy that can activate the adaptive immune response should be developed. Notably, this adaptive immunosuppression is unlikely due to classic immune checkpoint molecules such as PD-1 or long-term use of glucocorticoids but may be caused by an unknown mechanism that has not yet been discovered, and it is worthy of further exploration.

Declarations

Ethics approval and consent to participate

The local Ethics Committee of *East Campus of Renmin Hospital of Wuhan* provided ethical approval for this study. From all included participant, written informed consent was obtained before sampling.

Consent for publication

All authors reviewed and provided consent to publish the work described in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request. The authors declare that the data supporting the findings of this study are available within the paper and its supplementary information files.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (81972729 to W.Z., 81971811 to F.X.), and Sichuan Science and Technology Program (2020YFS0203 to W.Z., 2021YFS0003 to Y.K., 2020ZYD008 to X.L.), and Project of Novel Coronavirus Pneumonia in West China Hospital (HX2019nCoV027 to Y.K.).

Authors' contributions

W.Z. conceived the initial concept and designed the study. Y.Z., X.L., X.S., M.H., F.X., X.J., X.X., Z.Z., B.W. contributed the clinical data and blood samples. Y.K. helped to analyze the clinical data. C.Z., and Y.K. offered opinion to improve the study. Y.Z. and W. Z. wrote the paper.

Acknowledgements

The authors thank all patients and volunteers who participated in this study.

References

1. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020, 5(4):536–544.
2. Samudrala PK, Kumar P, Choudhary K, Thakur N, Wadekar GS, Dayaramani R, Agrawal M, Alexander A. Virology, pathogenesis, diagnosis and in-line treatment of COVID-19. *Eur J Pharmacol.* 2020;883:173375.
3. Keni R, Alexander A, Nayak PG, Mudgal J, Nandakumar K. COVID-19: Emergence, Spread, Possible Treatments, and Global Burden. *Front Public Health.* 2020;8:216.
4. Mirchandani P. Health Care Supply Chains: COVID-19 Challenges and Pressing Actions. *Ann Intern Med.* 2020;173(4):300–1.
5. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet.* 2020;395(10229):1054–62.
6. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497–506.
7. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, Barnaby DP, Becker LB, Chelico JD, Cohen SL, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *Jama.* 2020;323(20):2052–9.
8. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. *Jama.* 2020;324(8):782–93.
9. Lavezzo E, Franchin E, Ciavarella C, Cuomo-Dannenburg G, Barzon L, Del Vecchio C, Rossi L, Manganelli R, Loregian A, Navarin N, et al. Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'. *Nature.* 2020;584(7821):425–9.
10. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *Jama.* 2020;323(13):1239–42.
11. Gandra S, Ram S, Levitz SM. **The "Black Fungus" in India: The Emerging Syndemic of COVID-19-Associated Mucormycosis.** *Ann Intern Med* 2021.
12. Pal RSB, Bhadada SK, Banerjee M, Bhogal RS, Hage N, Kumar A: **COVID-19-associated mucormycosis: An updated systematic review of literature.** *Mycoses* 2021.
13. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, et al: **The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).** *Jama* 2016, **315**(8):801–810.
14. Zafer MM, El-Mahallawy HA, Ashour HM. **Severe COVID-19 and Sepsis: Immune Pathogenesis and Laboratory Markers.** *Microorganisms* 2021, 9(1).
15. Vincent JL. COVID-19: it's all about sepsis. *Future Microbiol.* 2021;16:131–3.

16. Liu X, Wang H, Shi S, Xiao J. **Association between IL-6 and severe disease and mortality in COVID-19 disease: a systematic review and meta-analysis.** *Postgrad Med J* 2021.
17. Wilk AJ, Lee MJ, Wei B, Parks B, Pi R, Martínez-Colón GJ, Ranganath T, Zhao NQ, Taylor S, Becker W, et al: **Multi-omic profiling reveals widespread dysregulation of innate immunity and hematopoiesis in COVID-19.** *J Exp Med* 2021, 218(8).
18. Neefjes J, Jongsma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol.* 2011;11(12):823–36.
19. Roche PA, Furuta K. The ins and outs of MHC class II-mediated antigen processing and presentation. *Nat Rev Immunol.* 2015;15(4):203–16.
20. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol.* 2013;13(4):227–42.
21. Attanasio J, Wherry EJ. Costimulatory and Coinhibitory Receptor Pathways in Infectious Disease. *Immunity.* 2016;44(5):1052–68.
22. Schildberg FA, Klein SR, Freeman GJ, Sharpe AH. Coinhibitory Pathways in the B7-CD28 Ligand-Receptor Family. *Immunity.* 2016;44(5):955–72.
23. Le Balc'h P, Pinceaux K, Pronier C, Seguin P, Tadié JM, Reizine F. Herpes simplex virus and cytomegalovirus reactivations among severe COVID-19 patients. *Crit Care.* 2020;24(1):530.
24. Pakdel F, Ahmadikia K, Salehi M, Tabari A, Jafari R, Mehrparvar G, Rezaie Y, Rajaeih S, Alijani N, Barac A, et al: **Mucormycosis in patients with COVID-19: A cross-sectional descriptive multicenter study from Iran.** *Mycoses* 2021.
25. Steinbrink JM, Miceli MH. Mucormycosis. *Infect Dis Clin North Am.* 2021;35(2):435–52.
26. Buil JB, van Zanten ARH, Bentvelsen RG, Rijpstra TA, Goorhuis B, van der Voort S, Wammes LJ, Janson JA, Melchers M, Heusinkveld M, et al: **Case series of four secondary mucormycosis infections in COVID-19 patients, the Netherlands, December 2020 to May 2021.** *Euro Surveill* 2021, 26(23).
27. Abbasi J. Researchers Tie Severe Immunosuppression to Chronic COVID-19 and Virus Variants. *Jama.* 2021;325(20):2033–5.
28. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, Su X, Cao B. SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet.* 2020;395(10235):1517–20.
29. Rubio I, Osuchowski MF, Shankar-Hari M, Skirecki T, Winkler MS, Lachmann G, La Rosée P, Monneret G, Venet F, Bauer M, et al. Current gaps in sepsis immunology: new opportunities for translational research. *Lancet Infect Dis.* 2019;19(12):e422–36.
30. Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman SD 2nd, Kreisel D, Krupnick AS, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. *Jama.* 2011;306(23):2594–605.
31. Delano MJ, Ward PA. Sepsis-induced immune dysfunction: can immune therapies reduce mortality? *J Clin Invest.* 2016;126(1):23–31.

32. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43(3):304–77.

Figures

Figure 1

The outline of the RNA-Seq results. (A) The GO enrichment items with upregulated genes (33788 genes). The abscissa shows the GO terms, the ordinate shows the significance value (p-adj) of each GO term, and the number at the top of each histogram indicates how many changed genes in each GO term. Different colours represent the three GO subcategories biological process (BP), cellular component (CC), and molecular function (MF). (B) The KEGG enrichment items with upregulated genes (33788 genes). The abscissa shows the KEGG terms, the ordinate shows the significance value (p-adj) of each KEGG term, and the number at the top of each histogram indicates how many changed genes in each KEGG term.

Figure 2

Patients infected with SARS-CoV-2 at different periods have significant differences in genes controlling smell and taste functions. The expression of genes related to smell (A) and taste (B) function. The abscissa shows the sample source, including healthy controls (HCs) and patients from two batches, and the ordinate shows the gene transcription level by RNA-sequencing read counts.

Figure 3

Changes in the expression of genes related to “cytokine storm” and inflammation-related responses. These heatmaps show the relative expression levels of inflammation-related genes: (A) cytokines, (B) chemokines, and (C) adhesive molecules. In the heatmap, each row depicts a different gene, and each column depicts an individual subject, including healthy volunteers and enrolled patients. The relative expression levels of genes were calculated as $\log_{10}\text{FPKM}$, standardized with the z-score method, and then presented with a pseudocolour scale from -3 to 3. Blue represents downregulation, and red represents upregulation.

Figure 4

The adaptive immune function of all patients was severely impaired. The transcription level of genes related to T cells (A), B cells (B), HLA molecules (C) and costimulatory molecules (D). The abscissa shows the sample source, including healthy controls (HCs) and patients from two batches, and the ordinate shows the gene transcription level by RNA-sequencing read counts. Significance was assessed

by p.adj comparison in the RNA-Seq DEG analysis. All p-values < 0.01 (between the healthy control group and patient batch 1 or patient batch 2).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigures.docx](#)