JCI insight

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JCI Insight. 2020. https://doi.org/10.1172/jci.insight.139834.

Research In-Press Preview COVID-19

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Methods A total of 71 patients with laboratory-confirmed COVID-19 admitted to Beijing You'an hospital in China with either mild (53 patients) or severe disease (18 patients) were enrolled with 18 healthy volunteers. We measured 34 immune mediators, cytokines and chemokines in peripheral blood every 4-7 days over one month per patient using a bio-plex multiplex immunoassay.

Results We found that the chemokine RANTES(CCL5) was significantly elevated, from an early stage of the infection, in patients with mild but not severe disease. We also found that early production of inhibitory mediators including IL-10 and IL-1RA were significantly associated with disease severity, and a combination of CCL5, IL-1Ra and IL-10 at week 1 may predict patient outcomes. The majority of cytokines that are known to be associated with the cytokine storm in virus infections such as IL-6 and IFN-gamma were only significantly elevated in the late stage of severe COVID-19 illness. TNF- alpha and GM-CSF showed no significant differences between severe and mild cases.

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Conclusion Together our data suggest early intervention to increase expression of CCL5 may prevent patients from developing severe illness. Our data also suggest that measurement of levels of CCL5, as well as IL-1Ra, IL-10 in blood individually and in combination might be useful prognostic bio-markers to guide treatment strategies.

Introduction

COVID-19 infection has been declared a global pandemic by the World Health Organisation (WHO) with 1,439,516 confirmed cases over 212 countries by 10th April 2020(1). COVID-19 is caused by a novel enveloped RNA beta coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)(2, 3). The majority of COVID-19 infections are relatively mild, with clinical features that commonly include fever and cough, with recovery within two to three weeks(4, 5). Severe infections are characterized by rapid progression to acute respiratory distress syndrome, septic shock, refractory metabolic acidosis, coagulation disorders, multi-organ failure and death(6). It is unclear why only a small proportion of patients develop severe illness, but it has been suggested that this relates to both an over reactive adaptive immune response and viral induced lung pathology(7, 8).

Recent clinical studies found patients with severe illness had lower levels of $CD4^+$ T and $CD8^+$ T cells and higher levels of plasma IL-6 and IL-10 compared to patients with mild illness(7, 9). This combination was associated with reduced patient survival, suggesting these cytokines may have an important role in viral pathogenesis(10). This has been described as a "cytokine storm" reflecting an overproduction of immune and inflammatory cells, and their cytokines(10, 11). It is thought that a cytokine storm may be an important cause of acute respiratory distress syndrome(9-11). However a more comprehensive study is required to determine both the potential protective as well as pathological role of immune mediators in disease progression, especially during the early stages of virus infection in severe cases prior to the development of lung pathology.

In this study, a cohort of 71 patients was followed up with weekly blood tests from hospital admission for up to four weeks after onset of symptoms, including mild cases who in non-pandemic circumstances do not require hospital admission. 34 immune mediators, cytokines and chemokines were measured in the blood. Longitudinal analysis was performed to demonstrate the dynamics of cytokine and chemokine production associated with disease progression to severe disease. It is hoped this will help further clarify the mechanism of immune response to COVID-19 infection, in order to guide more effective interventions for managing patients with severe illness.

Results:

Clinical and laboratory characteristics of study subjects.
Infection by SARS-Cov-2 virus in China resulted in a mild disease in the majority of the population. However, a sizeable proportion suffered a more severe and fatal disease. During this phase of the pandemic, the Beijing You'an Infectious Disease Specialist Hospital admitted many patients with mild symptoms - after the exposure to COVID-19 case and/or clinical signs and symptoms reported, nasal and pharyngeal swab specimens were tested by real time PCR for SARS-Cov-2 virus by the test center, if the result was positive and then they were sent to our hospital. Admission to hospital (first day of blood sampling) is normally 2-6 days after onset of symptoms.

In total 71 hospitalized patients with laboratory-confirmed COVID-19 and 18 healthy volunteers were recruited to the study. 53 patients were diagnosed with mild and 18 patients with severe disease. Age and pre-existing hypertension, cardiovascular and respiratory conditions were found to associate with progression of disease (Table 1), which is in agreement with other studies published recently(12, 13). In addition, blood oxygen saturation measured in week 2 in the severe patients was much lower than that in mild patients ($p<1.0E-06$). The ratio of arterial oxygen partial pressure (PaO2) to fraction of inspired oxygen (FiO2) in severe group was also lower than that in mild group ($p=1.6E-05$). Demographic and clinical data are shown in Table 1. In addition, the absolute number of white blood cell, neutrophils, and monocyte in severe group were much higher than that in mild group during the first three weeks ($p<0.05$). While, there was no significant difference of lymphocyte between mild and severe group. (Figure 1)

IFN-gamma-inducible protein 10 (IP-10) and Monocyte chemotactic protein-1 (MCP-1) are significantly associated with disease severity
IP10 level was significantly elevated in COVID patients in week 1 of onset of symptoms in both mild and severe groups when compared with healthy volunteer controls ($p=1.36E-08$ and $4.39E-08$). Then, in the mild group of patients, IP10 levels declined from week 2 and returned back to normal on week 4. In severe cases, IP10 levels remained high level at week 2 and started to decline at week 3 and further by week 4. Significantly higher levels of IP-10 in serum were observed in severe infection compared to mild infection at weeks 2, 3 and 4.($p=5.31E-03$, 0.02 and $1.52E-03$, Supplemental table 1 and figure 1).
Monocyte chemotactic protein-1 (MCP-1) was also significantly elevated in COVID patients in both mild and severe cases at the beginning of infection and remained high at all time intervals when compared with healthy controls ($P<0.05$, respectively). Significantly higher levels of MCP-1 in severe cases were observed when compared to mild cases at early time point of the infection (week 1 and 2; $p=0.047$ and $8.62E-05$) but not at later time points (week 3 and 4; $p=0.136$ and 0.030, respectively, Supplemental table 1 and figure 2).

Inhibitory cytokines IL-1Ra and IL-10 are significantly elevated in severe cases at an early stage of infection
We also found that IL-1Ra levels were elevated in both severe and mild cases and remained at a high level during the four weeks of follow up. A significant difference was only observed in the first two weeks after onset of symptoms when we compared severe cases and mild cases ($p=0.037$ and $3.78E-03$, Supplemental table 1 and figure 2).
IL-10 was only elevated in severe but not mild cases after the virus infection, and similar to IL-1Ra, the levels in severe cases were significantly higher than those in mild cases in the first two weeks ($p=0.055$ and $3.85E-03$, Supplemental table 1 and figure 3).

Elevated IL-6, IL-17, IL-12, IL1beta, IFN gamma and IL-27 were only observed in late stage severe cases
Most cytokines observed in previous publications of "cytokine storms" in association with disease severity (9, 10, 14) were observed only in the late stage of severe cases, mostly at four weeks after onset of symptom, for example IL-6 , IL-12, IL1beta ; IFN gamma; IL-17; IL-27. No differences were observed in TNF alpha, GM-CSF or IL-4 between mild and severe cases (Supplemental table 1 and figure 4).

Raised levels of CCL5 (RANTES) in sera of mild but not severe COVID-19 patients in the first month of infection.

RANTES, also known as CCL5, was elevated significantly in mild but not severe cases when compared to healthy controls, and remain high in mild cases after recovery (week 3 and 4). In the first week RANTES in the mild group (638.62±174.81) was much higher than that in healthy controls (358.36±123.44, P=1.0E-06), and remained high in mild cases during their recovery phase (630.57±171.00 in week 3 and 654.14±162.86 in week 4). No elevation of RANTES was observed in the severe group during the disease progression, suggesting that RANTES may play an important role in protecting COVID-19 patients tfrom developing severe illness (Supplemental table 1 and figure 5A). We also found significant correlation between RANTES level and Lymphocyte count (Figure 5 B)

A combination of CCL5, IL-1Ra and IL-10 at week 1 may predict patient outcomes

To test whether or not the combination of CCL5, IL-1Ra and IL-10 could predict the patient outcomes (mild/severe) at an early stage of the infection, we performed K-means and agglomerative hierarchical clustering analyses using data from the patients in week 1 of onset of symptoms (n=34) and for whom, the CCL5, IL-1Ra and IL-10 levels were assayed (n=25, Supplemental table 1). We extracted two K-means clusters based on the expression levels of these 3 cytokines across the patients, and then assigned each cluster to patients with known disease severity and determined whether the cluster assignment could distinguish disease severity. We found 9 out of 15 cluster 2 patients (Figure 6A, highlighted in blue) had severe disease compared to only 1 out of 10 in cluster 1 ($p = 0.018$, Fisher's exact test; Figure 6A-B). Similarly, when we defined the patient clusters by hierarchical clustering, which is an alternative approach to k-means analysis, and does not pre-specify the number of clusters, we found two distinct clusters (Figure 6C; 10 out of 16 cluster 2 patients had severe relative to 0 out of 9 in cluster 1 ($p = 0.0028$; Figure 6D)). Together, these observations lend further support to a potentially valuable role of CCL5, IL-1Ra and IL-10 measurements in predicting disease severity at an early stage of the COVID-19 infection.

Discussion

In this study, we have analysed levels of key immune mediators in the blood of COVID-19 patients with either mild or severe disease followed up for four weeks.

We found that the early production of inhibitory mediators such as IL-10 and IL-1RA were significantly associated with severe disease.

Importantly we also found the chemokine CCL5/RANTES was significantly elevated in mild but not severe disease from an early stage of the infection and remained significantly higher compared to severe cases during the four week follow up. This suggests a protective role in disease progression although it could be secondary to other factors such as activated cytotoxic T cells which produce CCL5 upon antigen stimulation.

Significant elevation of IP-10 and MCP-1 in both mild and severe cases was also observed compared to healthy controls, with significantly higher levels in severe cases when compared to mild cases. This shows similarity to influenza virus infections, where similar associations with disease severity were also observed in both pandemic (pdm2009) H1N1 and avian H5N1 infections (15-18).

The majority of cytokines that are known to be associated with ‘cytokine storm’ such as IL-6 and IFN-gamma were only significantly elevated in the late stages of severe COVID-19 illness; while TNF alpha, GM-CSF, showed no difference between severe and mild cases. Our data imply that a classical cytokine storm may not be the major cause of severe illness in COVID-19 patients(9), highlighting the need for further work in this area.

The interleukin-1 receptor antagonist (IL-1Ra) is an early inhibitory cytokine that suppresses pro-inflammatory cytokines and T-lymphocyte responses. IL-1Ra is a cytokine that controls inflammatory responses during an early stages of immune activation(19). IL-1Ra competitively binds to the interleukin-1 receptor(20) and is produced by monocytes, macrophages or dendritic cells(21, 22). IL-1Ra can modulate the production of IL-1 and TNF-alpha(23) and type I IFN(24). Therefore, early IL-1Ra production could affect induction of pro-inflammatory and antiviral cytokines during the early phase of this coronavirus infection. The role of IL-1Ra in the immune response may vary given the different measured serum concentrations in severe and mild infections. In mild cases the inhibitory role of elevated IL-1Ra may be overridden by the robust adaptive immune responses to the virus. However in the severe cases, much higher levels of IL-1Ra were observed in comparison to mild cases, suggestive of an overactive immune response, which may contribute to the switch from controlled and protective immune environment to inflammation induced tissue damage. IL-10 was only elevated in severe cases, therefore the inhibitory role of this cytokine likely contributes to the overall suppression of the immune system, viral control and disease severity.

RANTES, also known as CCL5, is a chemokine important for T cell homing and migration during acute virus infection as well as sustaining CD8 T cell responses during a systemic chronic viral infection(25); RANTES can be expressed by a number of cell types including T cells, epithelial cells and platelets and functions as a chemoattractant for a number of different cells such as monocytes, dendritic cells, T cells, NK cells, and eosinophils. RANTES is produced by CD8 T cells upon antigen stimulation and is known for its anti-viral function in HIV by competing with the virus for the CCR5 receptor(26). Elevated serum levels of the chemokines RANTES observed in mild cases compared to severe cases in early stage of SARS-Cov-2 infection are likely to be produced by virus-specific CD8 T cells. This is in keeping with the higher percentage of total lymphocytes counts in mild cases at all time points when compared with severe cases. This could imply a protective role for such adaptive T cell responses in mild cases to clear the virus before lung inflammation take place. This hypothesis is futher supported by our data showing significant correlation between RANTES and lymphocyte counts, as well as a recent single cell study showing clonal expansion of CD8 T cells in broncho-alveolar fluid of mild but not severe COVID patients(27).

We believe our study is timely and the information should be able to help with the design of planned clinical trials and novel drug development as well as the management of individual partients. However, limitations of this study include relatively sample size (n=73), with a relatively high proportion of female cases with severe disease. Larger cohorts are needed to validate these findings.

In conclusion, our data immune suppression mediated by IL10 and ILRa may be important in promoting progression of infection, which merits further investigation especially to understand their significance in terms of the pathophysiology occurring at the cellular/tissue level; in addition our data also suggest increased activity of T cells that produce the chemokines CCL5 may help to prevent patients from developing severe illness. Our data also indicate that measurement of levels of CCL5, as well as IL-1Ra, IL-10 in blood individually and/or in combination could provide useful prognostic biomarkers to guide treatment strategies.

Materials and methods

Study population

Patients were recruited from Beijing You'an Hospital, Capital Medical University, Beijing, between January 2020 and March 2020. Inclusion criteria: all available cases presenting consecutively over this period, recruited based on lab confirmation of COVID-19 and independent of severity of illness. Exclusion criteria: HBV and HIV infection, cancer, immmunosupression with high dose steroids or chemotherapy, pregnancy. Patient clinical data was collected from Electronic Medical Record System (EMRS), Laboratory Information System (LIS) and Picture Archiving and Communication System (PACS). Plasma was separated from whole blood samples and stored in a -80℃ freezer.

Clinical definitions

COVID-19 was diagnosed according to recommendations by the National Health Commission of China(6). Laboratory-confirmed patients were defined as showing a positive result on high throughput sequencing or real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay of nasal and pharyngeal swab specimens. The degree of severity was identified as mild infection or severe infection. Severe infection was defined as COVID-19 confirmed patients with one of the following conditions: respiratory distress with RR>30/min; blood oxygen saturation<93%; arterial oxygen partial pressure (PaO2) / fraction of inspired O2 (FiO2) <300mmHg; respiratory failure with mechanical ventilation; shock; or other organ failures requiring admission to ICU. Blood tests were taken every 4-7 days over the course of four weeks, and 1st day of onset of symptom was defined as the first day with clinical signs or symptoms consistent with COVID-19 infection. Patients with severe disease showed mild symptoms during the 1st and 2nd week and developed severe illness in the 3rd and 4th week after the onset of symptoms. Samples from 20 patients were collected 3-4 time points, and for the remaining patients sample were collected 1-2 time points. Healthy volunteers were selected to match the gender and age of patients and to be equal in number to the smallest comparator group (patients with severe disease). The healthy volunteers were recruited in 2017 by Youan bio-bank before COVID-19 started and did not have infection with SARS-CoV-2, or diabetes mellitus, hypertension, cardiovascular diseases, respiratory disease, kidney disease and liver disease.

Measurements of cytokines and chemokines

We determined the serum cytokine and chemokine levels in healthy volunteers (n=18) and COVID-19 infection patients in the 1st (n=34), 2nd (n=40), 3rd (n=31), 4th (n=22) week of onset of symptom. Serum cytokine and chemokine levels were measured using

ProcartaPlex Humna Cytokine&Chemokine Panel 1A 34plex, (Invitrogen, Carlsbad, Calif., United States) in Luminex 200 multiplexing instrument (EMD Millipore, Billerica, MA, USA). This system allowed us to generate quantitative measurements for 34 different chemokines, cytokines, growth factors and immune mediators, including IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, G-CSF, GM-CSF, IFN-alfa, IFN-γ, IP-10, MCP-1, MIP-1a (CCL3), MIP-1b (CCL4), RANTES (CCL5), VEGF, in 12.5ul-vol samples (Supplemental table 1, and as supplemental figure 1).

Statistical analysis

Statistical analysis of the data was performed using the Chi-square test for gender analysis. Two tailed Student's t-test was used to compare parametric continuous data between mild and severe infection groups (evaluated with Kolmogorov-Smirnov test), and nonparametric t-test (Mann-Whitney test) was used when data was not normally distributed. Statistical test differences were considered significant if the P values were less than 0.05. Analyses were performed with SPSS software v25.5 (IBM, NY, USA).

Study approval

The study was approved by the Institutional Review Board of Beijing Youan Hospital. Written informed consent was obtained from all patients.

Clustering analysis

We defined patient clusters by either K-means (Hartigan-Wong algorithm) or agglomerative hierarchical clustering (Ward's method) based on a similarity measure (Euclidean distance). The analyses were performed using R software (version 3.6.0). We used R package factoextra for visualizing the clusters.

Author Contribution: Conception and design: YHZ and TD; Data analysis: YHZ, PZ , LQ, YZ, KL; Clinical sample and data collection: RHJ, CZ, LQ, KL, BX, LCL, YCD, YMF, AL, JPS, XML, ZJH, HPX; Writing the manuscript: TD and YHZ; Data interpretation; reviewing and editing of the manuscript: TD, YHZ, JCK, AM, GO and LPH

Declaration of interests

All authors declare no competing interests.

Acknowledgments
This work was supported by the National Key R&D Program of China(2020YFE0202400),Beijing Natural Science Foundation (7191004 and 7202069), Beijing Municipal Science & Technology Commission (Z171100001017078), Beijing municipal administration of hospitals (DFL20181701 and ZYLX201711), Beijing Key Laboratory (BZ0373), and Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Sciences (CIFMS), China (grant number: 2018-I2M-2-002); TD is supported by Medical Research Council (MRC), UK (MR/L018942/1 and MRC Human Immunology Unit Core) and Nuffield Department of Medicine, Oxford University. JCK is supported by a Wellcome Trust Investigator Award (204969/Z/16/Z) , JCK, LPH and GO are in part supported by the NIHR Oxford Biomedical Research Centre. We would like acknowledge Dr Richard Newman and Dr Lucy Li for reading through the manuscript and for providing very helpful comments and suggestions.

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Table 1 Patient demographics and clinical phenotype.

	Healthy Volunteers (18)	All hospitalized patients (n=71)	Mild disease (n=53)	Severe (n=18)	P value[1]
Age, median (IQR), yr	48.00 (40.75-52.25)	48.00 (37.00-63.00)	44.0 (34.50-56.00)	66.0 (51.50-74.25)	2.2E-05
Gender, Men, n/total (%)	7/18 (38.9%)	30/71 (42.25%)	23/53 (43.40%)	7/18 (38.89%)	0.738
Pre-existing conditions					
Diabetes,n/total (%)	N.D.	5/71 (7.04%)	3/53 (5.66%)	2/18(11.11%)	0.595
Hypertension,n/total (%)	N.D.	13/71(18.31%)	6/53 (11.32%)	7/18 (38.89%)	0.015
Cardiovascular disease,n/total (%)	N.D.	8/71(11.27%)	3/53(5.66%)	5/18(27.68%)	0.021
Respiratory disease,n/total (%)	N.D.	3/71(4.23%)	0/53 (0)	3/18(16.67%)	0.014
Kidney disease,n/total (%)	N.D.	1/71 (1.41%)	0/53 (0%)	1/18 (5.56%)	0.254
liver disease,n/total (%)	N.D.	4/71 (5.63%)	2/53(3.77%)	2/18 (11.11%)	0.265
presenting symptoms					
Fever, n/total (%)	N.D.	57/71 (80.28%)	41/53 (77.36%)	16/18 (88.89%)	0.494
Cough, n/total (%)	N.D.	43/71(60.56%)	31/53 (58.49%)	12/18 (66.67%)	0.540
Expectoration, n/total (%)	N.D.	19/71 (26.76%)	13/53 (24.53%)	6/18 (33.33%)	0.542
Vomit, n/total (%)	N.D.	1/71 (1.41%)	0/53 (0%)	1/18 (5.56%)	0.254
Diarrhea, n/total (%)	N.D.	1/71 (1.41%)	1/53(1.89%)	0/18 (0%)	1.000
Physiological variables					
RR, mean (95% CI)	N.D.	20.9 (19.9-21.9)	20.1 (19.7-20.5)	23.2 (19.5-26.9)	3.3E-05
SaO_2, mean (95% CI)	N.D.	92.4 (89.6-95.2)	95.7(93.6-97.9)	82.9 (76.9-88.9)	<1.0E-06
R/F, mean (95% CI)	N.D.	372.6 (331.3-413.8)	425.0 (386.1-463.9)	225.7 (194.5-256.9)	1.6E-05
ICU admission, n/total (%)	N.D.	7/71 (9.86%)	0/53 (0%)	7/18 (38.89%)	2.6E-04
Mechanical ventilation, n/total (%)	N.D.	6/71 (8.45%)	0/53 (0%)	6/18(33.3%)	0.001

Definition of abbrevations. N.D.= Not detected; P/F = PaO2/FiO2; SaO2 = oxygen saturation; RR = respiratory rate.

[1]Values comparing severe and mild infection patients were calculated by Chi-square test and Fisher's exact test. Student's t test was used where data were normally distributed (evaluated with Kolmogorov-Smirnov test), and non-parametric t test (Mann-Whitney test) was used when data were not normally distributed.

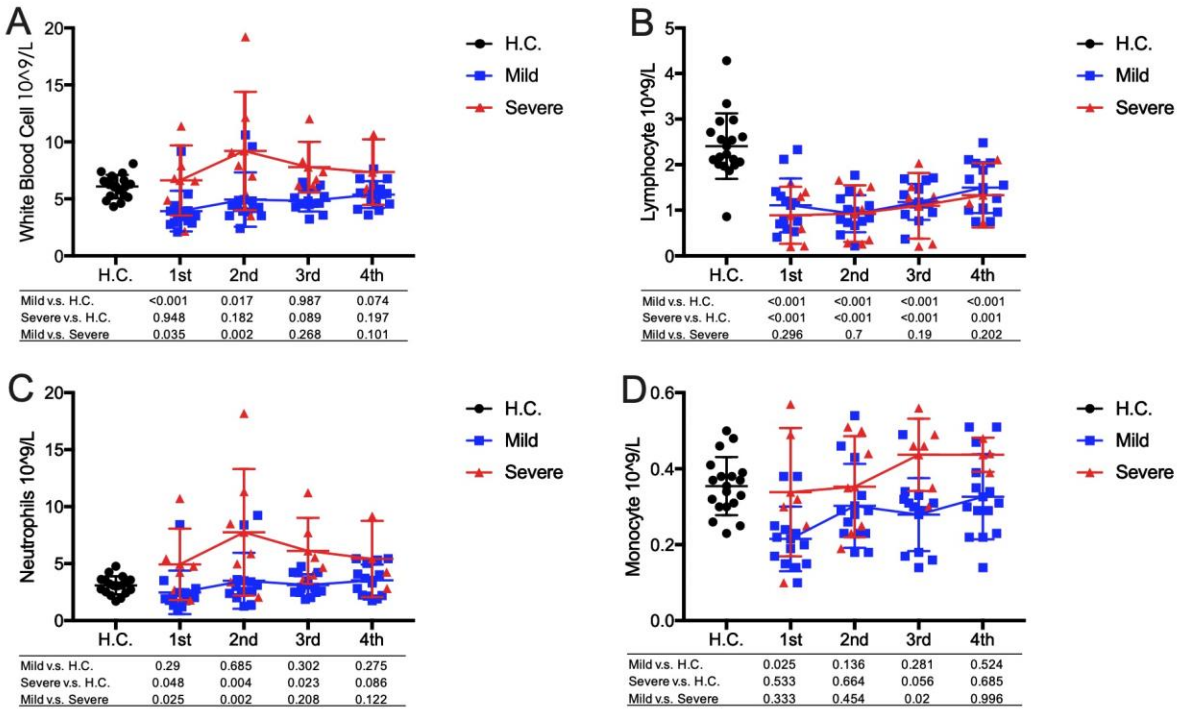
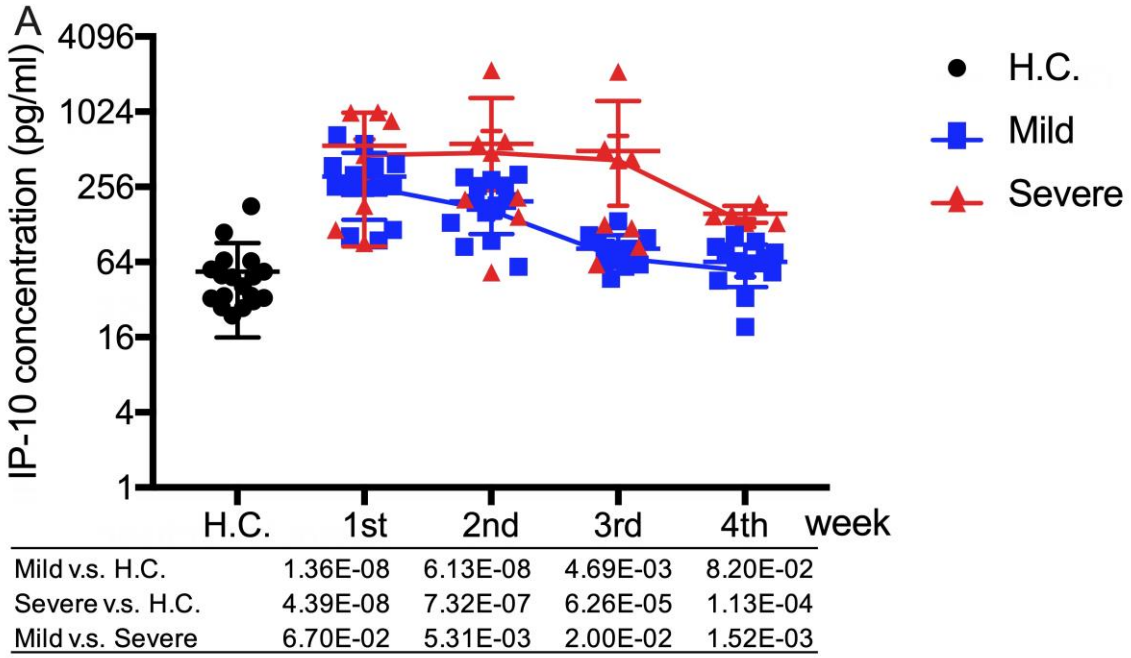
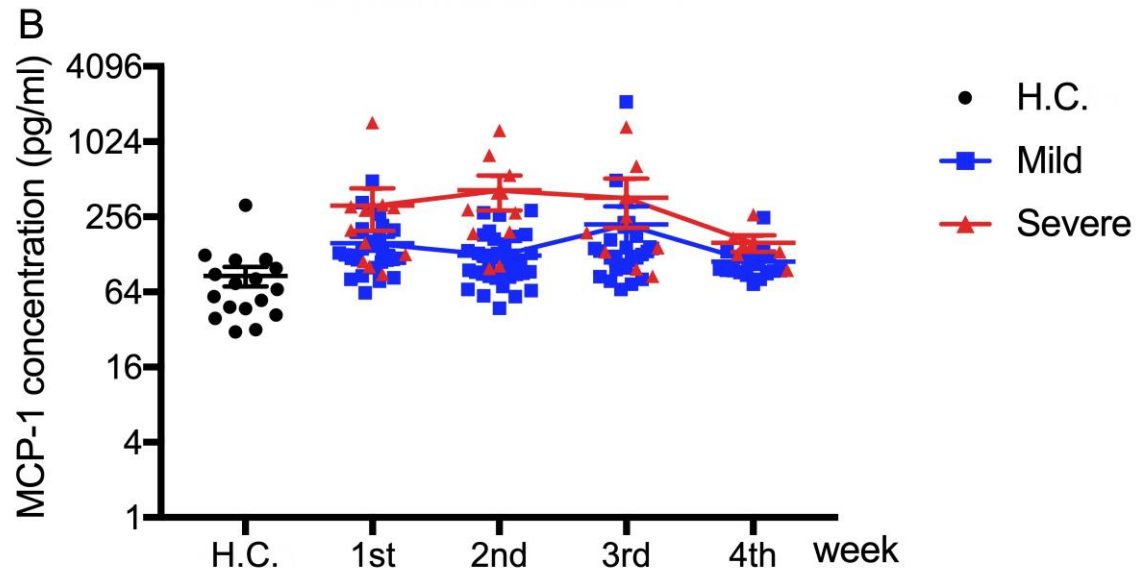


Figure 1 Dynamic changes of blood cells during COVID-19 infection.
The values of white blood cell (A), lymphocyte count (B), neutrophils count (C) and monocyte count (D) in severe and mild infection patients and healthy control were compared each other. Two tailed Student's t-test, was used to compare parametric continuous data (evaluated with Kolmogorov-Smirnov test), and nonparametric t-test (Mann-Whitney test) was used when data was not normally distributed. The differences between each group were presented with P value in the table under the diagram. The absolute numbers of white blood cell (A), lymphocyte count (B), neutrophils count (C) and monocyte count (D) in COVID-19 infection patients in 1^{st}, 2^{nd}, 3^{rd}, 4^{th} week of onset of symptom and health control were presented in scatter diagram, in which, health control was with black circle, mild patients with blue square, and severe with red triangle. The dynamic changes of WBC, lymphocyte, neutrophils and monocyte are presented with red line in severe patients and blue line in mild patients.



Mild v.s. H.C.	1.36E-08	6.13E-08	4.69E-03	8.20E-02
Severe v.s. H.C.	4.39E-08	7.32E-07	6.26E-05	1.13E-04
Mild v.s. Severe	6.70E-02	5.31E-03	2.00E-02	1.52E-03

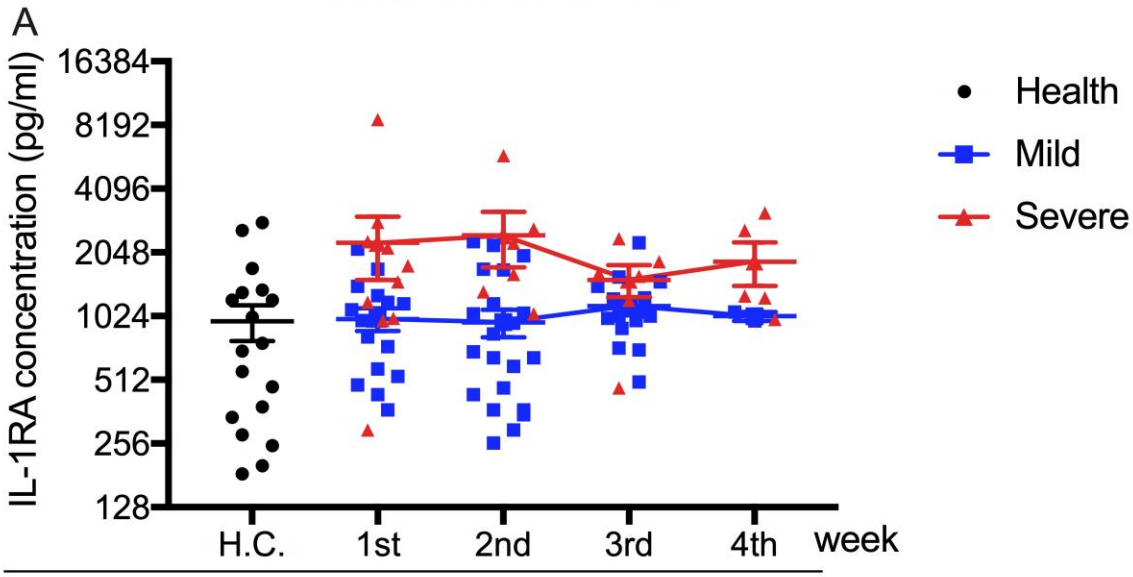


Mild v.s. H.C.	4.37E-04	5.61E-03	3.37E-03	2.10E-02
Severe v.s. H.C.	1.92E-04	3.87E-05	9.39E-04	8.57E-03
Mild v.s. Severe	4.70E-02	8.62E-05	1.36E-01	3.00E-02

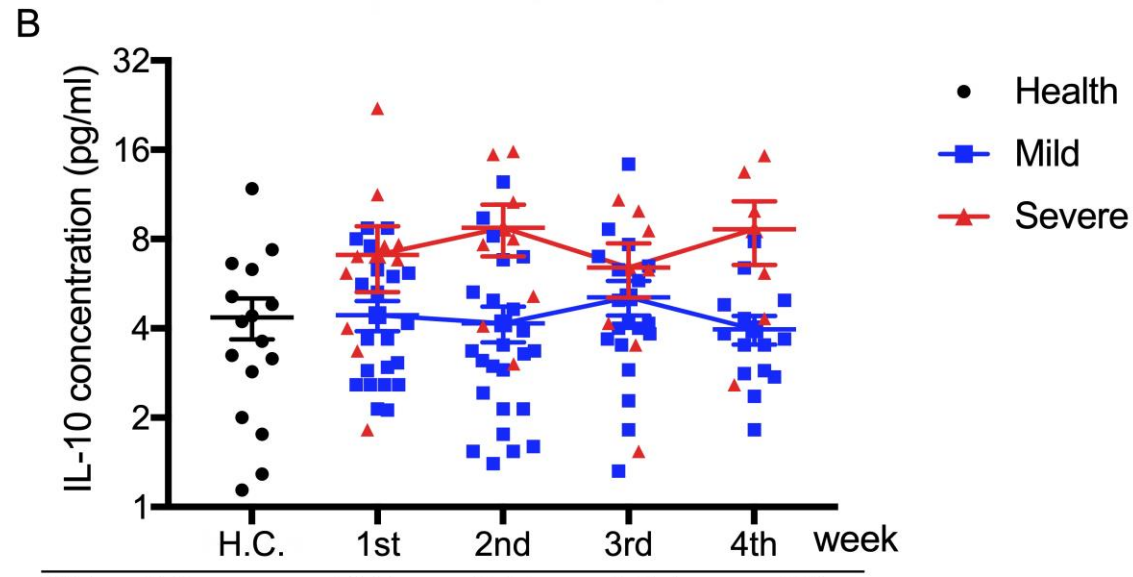
Figure 2. The dynamic changes of IP-10 and MCP-1 plasma levels between mild and severe COVID-19 infection.

The values of IP-10 (A) and MCP-1 (B) in severe and mild infection patients and healthy control were compared each other. Two tailed Student's t-test, was used to compare parametric continuous data (evaluated with Kolmogorov-Smirnov test), and nonparametric t-test (Mann-Whitney test) was used when data was not normally distributed. The differences between each group were presented with P value in the table under the diagram. The values of IP-10 (A) and MCP-1 (B) in healthy controls and COVID-19 infection patients in 1st, 2nd, 3rd and 4th week of onset of symptom are presented in scatter diagram, in which, health control was with black circle, mild patients with blue square, and severe

with red triangle. The dynamic changes of IP-10 and MCP-1 are presented with red line in severe patients and blue line in mild patients.



Mild v.s. H.C.	0.353	0.712	0.082	0.55
Severe v.s. H.C.	0.022	0.00923	0.091	0.044
Mild v.s. Severe	0.037	0.00378	0.264	0.254



Mild v.s. H.C.	0.477	0.799	0.351	0.937
Severe v.s. H.C.	0.046	0.014	0.202	0.041
Mild v.s. Severe	0.055	0.00385	0.456	0.012

Figure 3. Inhibitory cytokines IL-1Ra and IL-10 are significantly elevated in severe cases at early stage of infection

The values of IL-1RA (A) and IL-10 (B) in severe and mild infection patients and healthy control were compared each other. Two tailed Student's t-test, was used to compare parametric continuous data (evaluated with Kolmogorov-Smirnov test), and nonparametric t-test (Mann-Whitney test) was used when data was not normally distributed. The differences between each group were presented with P value in the table under the diagram. The values of IL-1RA (A) and IL-10 (B) in healthy

controls and COVID-19 infection patients in 1^{st}, 2^{nd}, 3^{rd} and 4^{th} week of onset of symptoms are presented in scatter diagram, in which, health control was with black circle, mild patients with blue square, and severe with red triangle. The dynamics of IL-1RA (A) and IL-10 (B) were presented with red line in severe patients and blue line in mild patients.

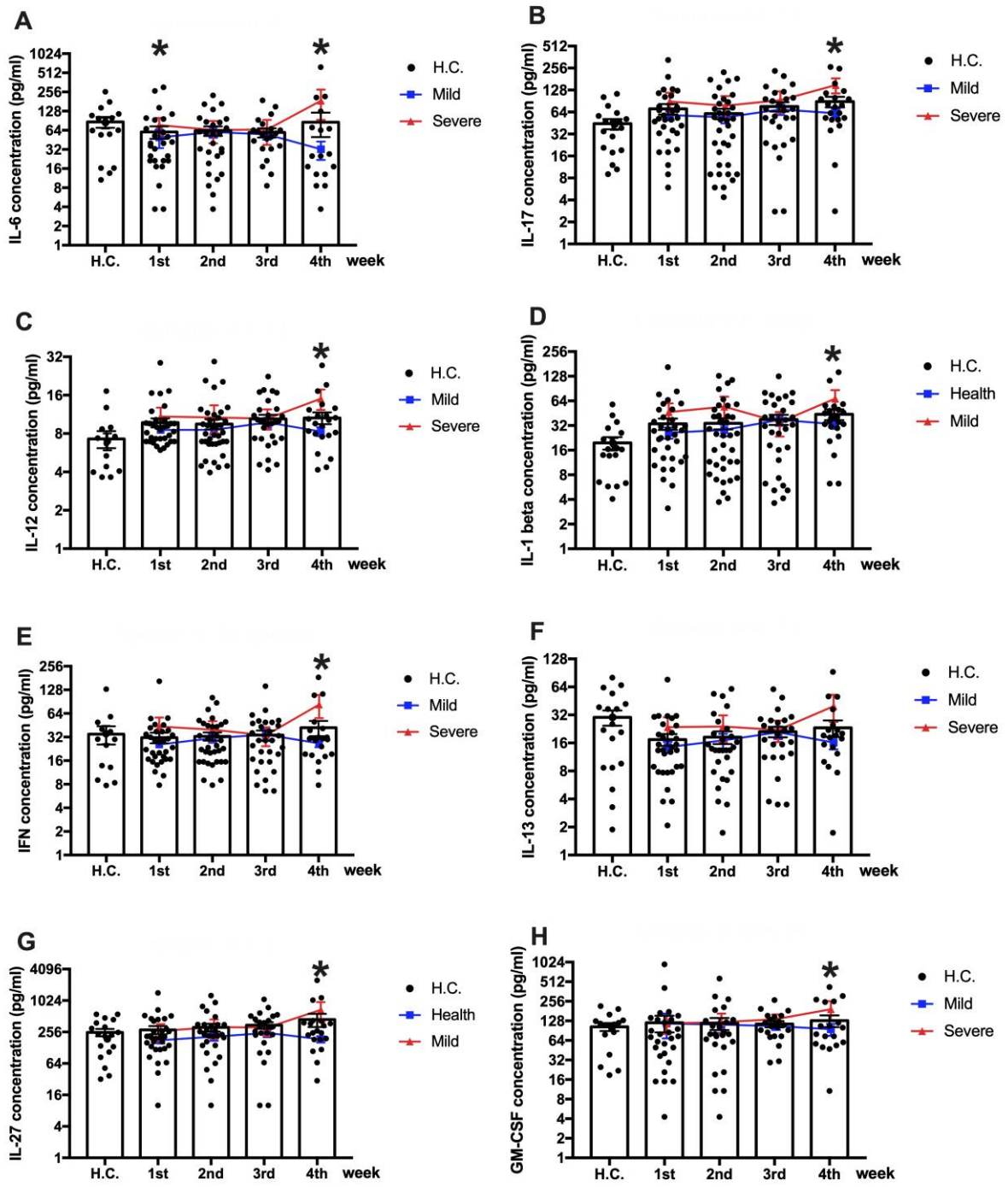
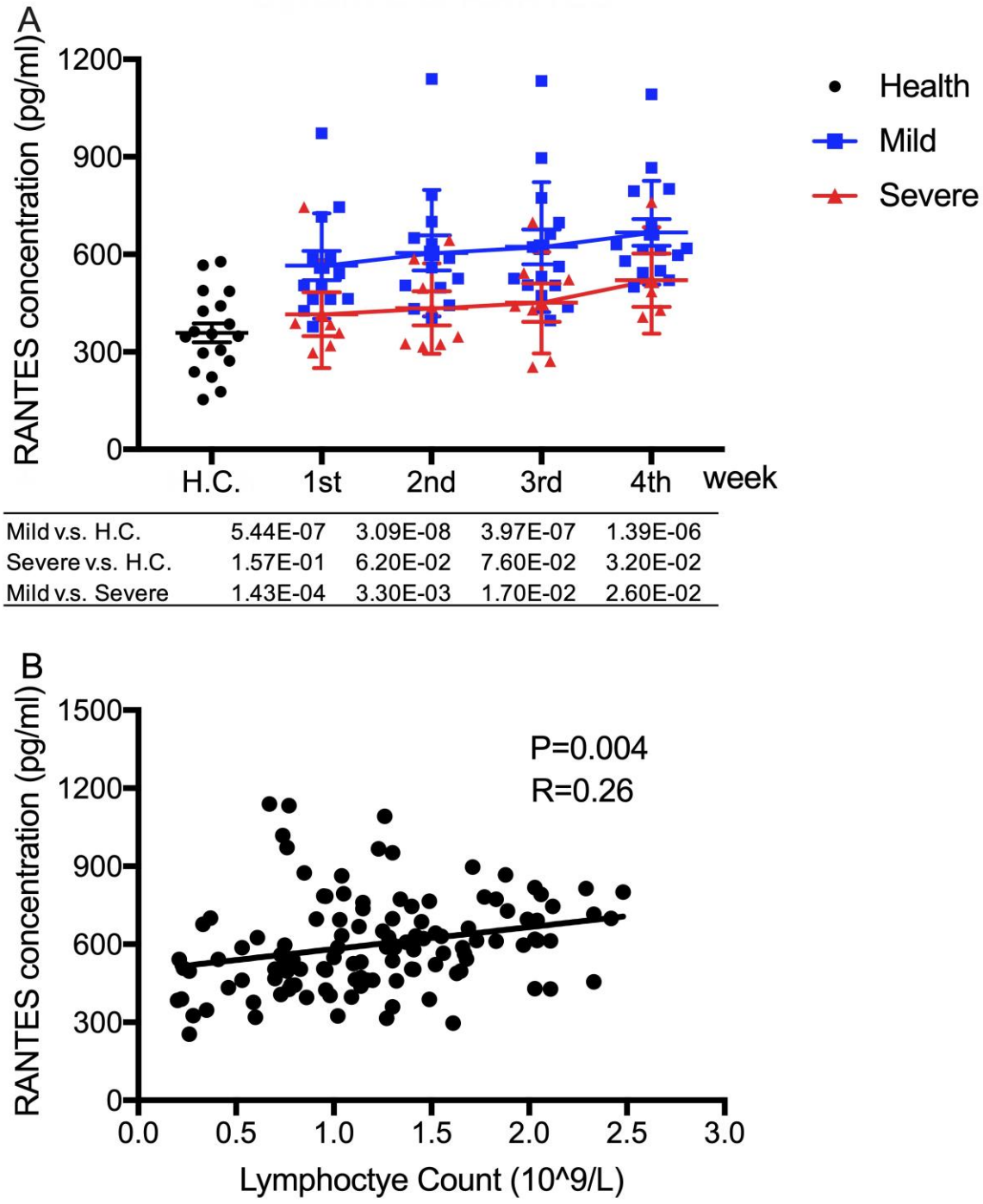


Figure 4. Elevated IL-6, IL-17, IL-12, IL-1beta, IFN-gamma, IL-13, IL-27 and IL-7 in late stages of severe cases.

The values of IL-6 (A), IL-17 (B), IL-12 (C), IL-1beta (D), IFN-gamma (E), IL-13 (F), IL-27 (G) and IL-7 (H) were compared between severe and mild infection patients by two tailed Student's t-test with parametric continuous data (evaluated with Kolmogorov-Smirnov test), and nonparametric t-test (Mann-Whitney test) when data was not normally distributed. * indicates that the difference between mild and severe groups was significant ($p<0.05$). The values of IL-6 (A), IL-17 (B), IL-12 (C), IL-1beta (D), IFN-gamma (E), IL-13 (F), IL-27 (G) and IL-7 (H) in healthy controls (H.C.) and COVID-19 infection patients in 1st, 2nd, 3rd and 4th week of onset of symptom are presented with black dots in scatter diagram, the dynamics of cytokines and chemokines are presented with red line in severe patients and blue line in mild patients.



Mild v.s. H.C.	5.44E-07	3.09E-08	3.97E-07	1.39E-06
Severe v.s. H.C.	1.57E-01	6.20E-02	7.60E-02	3.20E-02
Mild v.s. Severe	1.43E-04	3.30E-03	1.70E-02	2.60E-02

Figure 5. High level of RANTES in mild but not severe COVID-19 patients.
A. The value of RANTES in severe and mild infection patients and healthy control were compared each other. Two tailed Student's t-test, was used to compare parametric continuous data (evaluated with Kolmogorov-Smirnov test), and nonparametric t-test (Mann-Whitney test) was used when data was not normally distributed. The differences between each group were presented with P value in the table under the diagram. The values of RANTES in health control and COVID-19 infection patients in 1st, 2nd, 3rd and 4th week of onset of symptom was presented in scatter diagram, in which, health control was with black circle, mild patients with blue square, and severe with red triangle. The dynamics of RANTES was presented with red line in severe patients and blue line in mild patients.
B. Correlation analysis between RANTES and lymphocyte counts.

Correlation analysis between RANTES and lymphocyte counts was performed by linear regression analysis. Each black circle indicates individual patients, the linear correlation between RANTES and lymphocyte count was presented with black line.

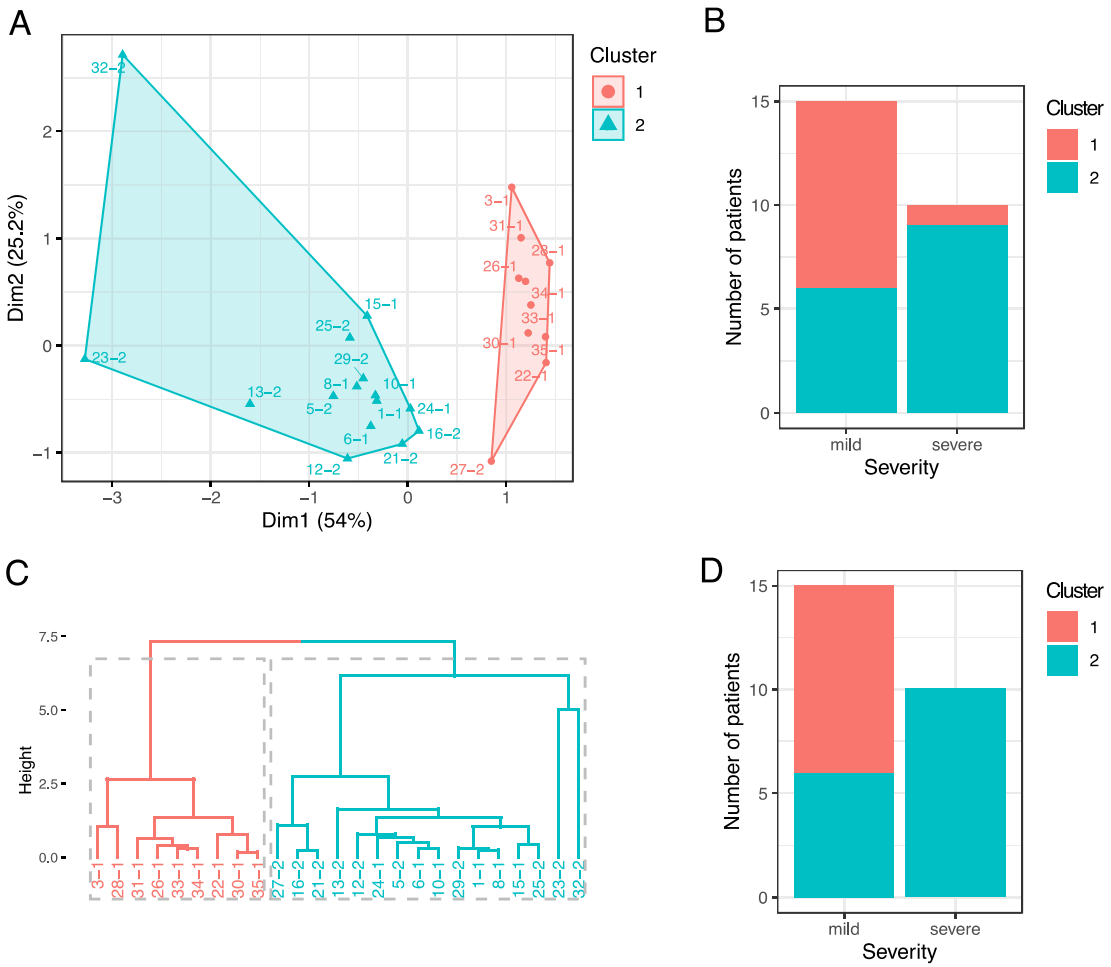


Figure 6: Combination of CCL5, IL-1Ra and IL-10 predict the disease severity.
A. a cluster plot to visualize k-means clusters with the proportion of variance explained by each component. The two distinct clusters were highlighted in red and blue. The patient IDs were shown next to each dots.
B. a bar plot of the number of K-mean cluster1 and cluster2 patients with either mild or severe COVID-19 disease.
C. a dendrogram showing agglomerative hierarchical clusters. The height on the y-axis represents the distance between two clusters. Two major clusters were highlighted in red and blue. The patient IDs were shown at the bottom of the dendrogram.
D. a bar plot of the number of hierarchical cluster1 and cluster2 patients with either mild or severe COVID-19 disease.