

The Lancet

Longitudinal profiling of cytokines and chemokines in COVID-19 reveals inhibitory mediators IL-1Ra and IL-10 are associated with disease severity while elevated RANTES is an early predictor of mild disease --Manuscript Draft--

Manuscript Number:	
Article Type:	Fast Track Article
Keywords:	COVID-19; cytokine; RANTES/CCL5, IL1Ra, IL-10
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Manuscript Region of Origin:	UNITED KINGDOM
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	<p>month per patient using a bio-plex multiplex immunoassay.</p> <p>Findings 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. 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.</p> <p>Interpretations Together our data strongly suggest a classical cytokine storm may not be the major cause of severe COVID illness. Early intervention to reduce IL-10 and IL-1Ra mediated inhibition or 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.</p> <p>Funding Beijing Natural Science Foundation, Beijing Municipal Science & Technology Commission, Chinese Academy of Medical Sciences (CAMS) Innovation Fund Medical Research Council, UK and Wellcome Trust</p>
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Longitudinal profiling of cytokines and chemokines in COVID-19 reveals inhibitory mediators IL-1Ra and IL-10 are associated with disease severity while elevated RANTES is an early predictor of mild disease

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Abstract:

Background Identifying immune correlates of COVID-19 disease severity is an urgent need for clinical management, vaccine evaluation and drug development. Here we present a temporal analysis of key immune mediators, cytokine and chemokines in blood of hospitalised COVID-19 patients from serial sampling and follow up over four weeks.

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.

Findings 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. 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.

Interpretation Together our data strongly suggest a classical cytokine storm may not be the major cause of severe COVID illness. Early intervention to reduce IL-10 and IL-1Ra mediated inhibition or 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.

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Research in Context

Evidence before this study

We have searched Pubmed up to 10th April 2020 using key words “cytokine” and “COVID-19” or “novel coronavirus”. We found two main studies. The first study conducted a 27 multiplex cytokine analysis in a cohort study of 41 patients with laboratory confirmed 2019-nCoV infection, all patients had serious, sometimes fatal, pneumonia, with an increased cytokine profile in association with ICU patients (n=13) when compared with non ICU patients (28), involving interleukin (IL)-2, IL-7, granulocyte-colony stimulating factor, interferon-γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumour necrosis factor-α. A further retrospective multicentre study of 68 fatal cases and 82 discharged cases with laboratory-confirmed infection of SARS-CoV-2 suggested elevated ferritin and IL-6 as predictors for fatality, however discharged cases included patients with varying degrees of disease severity from mild to severe, and the precise timing of the sampling was not mentioned. We did not find any peer reviewed published data comparing cytokines in mild and severe disease, nor longitudinal sampling and analysis from onset of symptoms with follow up to recovery or severe illness.

Added value of this study

During the COVID-19 pandemic, in addition to severe cases of the disease the Beijing You'an Infectious Disease Specialist Hospital in China admitted many patients with mild symptoms which would not occur under non-pandemic circumstances. This enabled us to collect longitudinal samples (73) from symptom onset with subsequent follow up sampling every 4-7 days over the course of the disease. This provides a unique opportunity to gain a comprehensive understanding of the temporal change and comparison of cytokines and chemokines throughout the disease course in two distinct groups of patients – those with mild disease and those with severe illness.

Implications of all the available evidence

Early intervention to reduce IL-10 and IL-1Ra mediated inhibition or to increase expression of CCL5 may prevent patients from developing severe illness; measurement of levels of CCL5, as well as IL-1Ra, IL-10 in blood could provide 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 2020¹. 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 death⁶. 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 pathogenesis¹⁰. 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 syndrome⁹⁻¹¹. 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.

Materials and methods

Study population

Patients were recruited from Beijing You'an Hospital, Capital Medical University, Beijing, between January 2020 and March 2020. All participants were hospitalized patients with laboratory-confirmed COVID-19. Their 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°C freezer. The study was approved by the Institutional Review Board of Beijing Youan Hospital. Written informed consent was obtained from all patients.

Clinical definitions

COVID-19 was diagnosed according to recommendations by the National Health Commission of China⁶. 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.

Measurements of cytokines and chemokines

Serum cytokine and chemokine levels were measured using Human Cytokine 34-plex assay kit (Bio-Rad, Hercules, CA) with Bio-Plex Manager software version 6.0 in Bio-PlexTM 200 system (Bio-Rad). This system allows quantitative measurement of 34 different chemokines, cytokines, growth factors and immune mediators, including IL-1 β , 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- α , IFN- γ , IP-10, MCP-1, MIP-1a (CCL3), MIP-1b (CCL4), RANTES (CCL5), VEGF, in 12.5 μ l-vol samples. Amongst these, 13 cytokines were under the detection level, and a total of 21 detectable cytokines were included in the analysis (table 2, and supplemental figure 1).

Statistical analysis

Statistical analysis of the data was performed using the Chi-square test for gender analysis. 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). 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). We used R package factoextra for visualizing the clusters.

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 ($p < 1.0 \times 10^{-6}$). 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 ($p = 1.6 \times 10^{-5}$). Demographic and clinical data are shown in Table 1 and Figure 1.

IFN- γ -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 ($p = 1.36 \times 10^{-8}$ and 4.39×10^{-8}). 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 ($p = 5.31 \times 10^{-3}$, 0.02 and 1.52×10^{-3} , table 2 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 8.62×10^{-5}) but not at later time points (week 3 and 4; $p = 0.136$ and 0.030 , respectively, table 2 and figure 1).

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 3.78×10^{-3} , table 2 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 3.85×10^{-3} , table 2 and figure 3).

Elevated IL-6, IL-17, IL-12, IL1 β , IFN γ and IL-27 were only observed in late stage severe cases

Most cytokines observed in previous publications of "cytokine storms" were observed only in the late stage of severe cases, mostly at four weeks after onset of symptom, for example IL-6, IL-12, IL1 β ; IFN γ ; IL-17; IL-27. No differences were observed in TNF α , GM-CSF or IL-4 between mild and severe cases (table 2 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 , $P = 1.0 \times 10^{-6}$), 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 from developing severe illness (table 2 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, first we used K-means clustering analysis. We extracted two

clusters by scaling the values of these 3 cytokines across all 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 in the 1st week of onset of symptom, 9 out of 15 cluster 2 patients 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 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 infections¹⁴⁻¹⁷.

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 patients⁹.

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 activation¹⁸. IL-1Ra competitively binds to the interleukin-1 receptor¹⁹ and is produced by monocytes, macrophages or dendritic cells^{20,21}. IL-1Ra can modulate the production of IL-1 and TNF-alpha²² and type I IFN²³. 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 infection²⁴; RANTES is mainly 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 receptor²⁵. 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 further 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 patients²⁶.

In conclusion, our data suggest that a "cytokine storm" is not be the cause of progression of COVID patients to severe illness, although this may contribute to late stage lung injury. Immune suppression mediated by IL10 and

ILRa may be important in promoting progression of infection, and early intervention to ameliorate this or increase activity of T cell that produce the chemokines CCL5 may help to 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/or in combination could provide useful prognostic biomarkers to guide treatment strategies.

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.

Data sharing

The data that support the findings of this study are available from the corresponding author on reasonable request. Participant data without names and identifiers will be made available after approval from the corresponding author and National Health Commission. After publication of study findings, the data will be available for others to request. The research team will provide an email address for communication once the data are approved to be shared with others.

Acknowledgments

This work was supported by the 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.Definition of abbreviations. N.D.= Not detected; P/F = PaO₂/FiO₂; SaO₂ = oxygen saturation; RR = respiratory rate.

	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,n/total (%)					
Diabetes	N.D.	5/71 (7.04%)	3/53 (5.66%)	2/18(11.11%)	0.595
Hypertension	N.D.	13/71(18.31%)	6/53 (11.32%)	7/18 (38.89%)	0.015
Cardiovascular disease	N.D.	8/71(11.27%)	3/53(5.66%)	5/18(27.68%)	0.021
Respiratory disease	N.D.	3/71(4.23%)	0/53 (0)	3/18(16.67%)	0.014
Kidney disease	N.D.	1/71 (1.41%)	0/53 (0%)	1/18 (5.56%)	0.254
liver disease	N.D.	4/71 (5.63%)	2/53(3.77%)	2/18 (11.11%)	0.265
presenting symptoms, n/total (%)					
Fever	N.D.	57/71 (80.28%)	41/53 (77.36%)	16/18 (88.89%)	0.494
Cough	N.D.	43/71(60.56%)	31/53 (58.49%)	12/18 (66.67%)	0.540
Expectoration	N.D.	19/71 (26.76%)	13/53 (24.53%)	6/18 (33.33%)	0.542
Vomit	N.D.	1/71 (1.41%)	0/53 (0%)	1/18 (5.56%)	0.254
Diarrhea	N.D.	1/71 (1.41%)	1/53(1.89%)	0/18 (0%)	1.000
Physiological variables, median (IQR)					
RR	N.D.	20 (20.0-21.0)	20.0 (20.0-20.0)	21.0 (20.0-24.25)	3.3E-05
SaO ₂ (n=53)	N.D.	96.50 (92.0-98.1)	96.95(95.25-97.80)	88.0 (80.75-91.15)	<1.0E-06
R/F	N.D.	383.0 (264.0-461.75)	431.50 (366.75-472.75)	217.00 (19.25-260.00)	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

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.

Table 2: Dynamic changes of cytokines/chemokines in different period of infection

time of infection					P values ¹		
	healthy control	time of infection	mild	severe	P values ¹		
					mild v.s. health	severe v.s. health	mild v.s. severe
IP-10	44.71 (32.34-58.14)	1st	257.26 (112.14-409.06)	263.94 (180.68-1013.44)	1.36E-08	4.39E-08	0.067
		2nd	163.50 (87.04-261.26)	565.80 (175.28-1577.65)	6.13E-08	7.32E-07	5.31E-03
		3rd	81.30 (59.56-105.08)	145.42 (93.70-492.62)	4.69E-03	6.26E-05	0.02
		4th	68.94 (52.88-84.94)	151.68 (118.34-542.39)	0.082	1.13E-04	1.52E-03
MCP-1	70.96 (45.65-114.38)	1st	119.30 (99.74-199.86)	199.86 (111.5-307.34)	4.37E-04	1.92E-04	0.047
		2nd	98.36 (84.12-169.00)	274.66 (146.04-672.40)	5.61E-03	3.87E-05	8.62E-05
		3rd	119.94 (96.96-145.84)	167.28 (106.50-553.05)	3.37E-03	9.39E-04	0.136
		4th	95.05 (90.09-123.12)	142.59 (119.70-195.96)	0.021	8.57E-03	0.030
RANTES	358.36±123.43	1st	638.62±174.80	418.16±121.43	5.44E-07	0.157	1.43E-04
		2nd	640.36±177.73	464.82±136.40	3.09E-08	0.062	3.30E-03
		3rd	630.57±171.00	480.85±166.81	3.97E-07	0.076	0.017
		4th	654.14±162.86	502.52±132.39	1.39E-06	0.032	0.026
IL-1 RA	730.80 (325.11-1332.00)	1st	974.28 (543.13-1258.26)	1628.32 (993.72-2439.77)	0.353	0.022	0.037
		2nd	693.72 (402.30-1431.95)	1938.58 (1261.06-3447.48)	0.712	9.23E-03	3.78E-03
		3rd	1024.06 (896.34-1415.36)	1604.92 (1026.63-1981.27)	0.082	0.091	0.264
		4th	1022.59 (973.14-1072.04)	1274.60 (1119.52-2873.74)	0.550	0.044	0.254
IL-10	3.92 (2.21-6.02)	1st	3.68 (2.58-6.18)	6.82 (3.36-7.68)	0.477	0.046	0.055
		2nd	3.32 (2.14-5.22)	7.85 (4.34-14.23)	0.799	0.014	3.85E-03
		3rd	4.16 (3.52-6.48)	6.30 (3.52-9.96)	0.351	0.202	0.456
		4th	3.60 (2.80-4.84)	8.05 (3.88-13.94)	0.937	0.041	0.012
IL-12	6.68 (4.02-8.81)	1st	8.34 (6.94-9.60)	9.48 (6.78-13.18)	0.030	0.032	0.203

		2nd	7.87 (6.66-10.97)	9.18 (5.49-15.50)	0.084	0.172	0.600
		3rd	9.85 (7.34-11.43)	9.58 (6.44-16.94)	0.020	0.088	0.830
		4th	8.77 (6.09-10.88)	14.92 (9.85-21.32)	0.144	3.55E-03	6.29E-03
IL-6	77.78 (25.85-114.08)	1st	25.18 (21.26-41.82)	55.73 (31.62-103.22)	0.038	0.798	0.044
		2nd	57.42 (19.26-86.84)	34.53 (16.06-95.86)	0.238	0.391	0.907
		3rd	50.58 (32.75-65.81)	54.04 (8.62-132.55)	0.323	0.943	0.441
		4th	17.24 (9.69-56.89)	137.00 (20.34-319.84)	7.66E-03	0.137	4.38E-03
IL-17	42.50 (18.90-57.93)	1st	50.33 (22.88-75.82)	53.22 (32.86-107.42)	0.279	0.148	0.502
		2nd	48.88 (9.00-87.58)	56.04 (16.51-155.52)	0.908	0.570	0.566
		3rd	56.10 (32.09-96.09)	94.54 (20.93-165.14)	0.158	0.415	0.992
		4th	61.86 (41.62-81.88)	129.12 (84.64-255.37)	0.279	8.39E-04	0.032
Eotaxin	9.68 (7.43-13.05)	1st	10.50 (6.64-11.68)	13.56 (10.08-25.54)	0.792	0.049	0.033
		2nd	9.25 (6.22-12.15)	12.04 (5.84-19.50)	0.479	0.433	0.221
		3rd	14.85 (9.77-17.89)	21.11(13.38-33.67)	0.061	2.28E-04	9.16E-03
		4th	14.75 (9.84-17.45)	27.65 (14.78-40.52)	0.058	1.67E-04	0.016
GMCSF	110.99 (48.69-129.66)	1st	65.05 (34.92-123.11)	77.09 (34.02-171.22)	0.459	0.619	0.960
		2nd	79.84 (50.46-128.66)	78.47 (51.26-231.37)	0.532	0.872	0.602
		3rd	101.80 (73.27-135.58)	143.33 (89.13-185.04)	0.611	0.278	0.291
		4th	74.34 (52.06-113.14)	189.22 (55.75-337.86)	0.709	0.044	0.035
IFNgamma	29.87 (11.08-44.79)	1st	23.48 (16.08-36.12)	30.44 (18.08-55.20)	0.743	0.465	0.150
		2nd	23.48 (15.42-45.56)	28.03 (20.42-62.28)	0.908	0.593	0.522
		3rd	29.02 (15.42-44.82)	18.75 (9.70-51.47)	0.842	0.924	0.959
		4th	29.72 (18.74-31.84)	74.07 (25.62-131.19)	0.888	0.067	9.55E-03
IL-13	23.77 (8.68-45.18)	1st	13.34 (7.68-20.18)	14.90 (11.87-30.66)	0.074	0.904	0.086
		2nd	13.40 (7.82-20.16)	15.38 (11.60-43.34)	0.137	0.916	0.297
		3rd	18.76 (11.20-27.08)	26.36 (9.58-32.41)	0.573	0.816	0.909
		4th	18.08 (9.46-20.63)	36.08 (13.01-61.08)	0.184	0.420	0.086

IL-15	29.35 (14.69-46.07)	1st	30.24 (17.98-41.90)	60.52 (33.98-98.31)	0.900	0.010	7.46E-03
		2nd	33.63 (13.46-55.04)	52.76 (23.94-91.60)	0.683	0.332	0.284
		3rd	41.92 (25.56-55.38)	60.52 (41.76-104.94)	0.085	0.020	0.077
		4th	40.51 (27.91-48.39)	55.80 (41.84-140.01)	0.095	0.011	0.016
IL-18	65.80 (37.36-171.66)	1st	68.96 (46.34-118.76)	85.78 (72.52-157.88)	0.980	0.298	0.117
		2nd	80.50 (50.02-118.76)	141.80 (86.22-194.77)	0.734	0.091	0.018
		3rd	85.78 (57.28-140.96)	95.82 (86.44-198.82)	0.462	0.138	0.141
		4th	84.00 (57.73-123.90)	123.73 (81.31-209.23)	0.691	0.192	0.104
IL-1beta	16.42 (6.46-26.96)	1st	22.58 (11.54-35.68)	29.08 (17.22-59.46)	0.245	0.013	0.096
		2nd	17.29 (9.13-41.78)	35.68 (24.34-115.56)	0.339	0.040	0.146
		3rd	32.68 (17.30-57.18)	25.89 (4.58-75.70)	0.028	0.562	0.391
		4th	34.22 (22.92-47.57)	47.18 (39.74-120.68)	0.017	8.77E-04	0.046
IL-2	42.84 (17.69-91.64)	1st	36.76 (28.47-54.40)	45.96 (24.98-70.42)	0.609	0.904	0.474
		2nd	29.64 (17.18-50.86)	38.96 (21.72-85.51)	0.229	0.956	0.346
		3rd	45.33 (28.12-59.58)	56.78 (23.98-75.99)	0.937	0.862	0.749
		4th	45.96 (34.02-57.88)	64.48 (42.43-109.42)	0.899	0.272	0.061
IL-27	227.85 (100.28-395.16)	1st	207.68 (118.10-292.58)	167.54 (123.22-529.68)	0.829	0.954	0.929
		2nd	227.98 (141.62-354.54)	227.98 (101.20-789.40)	0.905	0.409	0.497
		3rd	324.28 (159.56-422.12)	517.88 (128.60-650.61)	0.452	0.757	0.918
		4th	235.31 (167.28-373.48)	692.37 (212.74-1490.42)	0.759	0.026	0.034
IL-7	11.90 (7.19-20.13)	1st	16.32 (11.43-19.85)	14.22 (8.38-40.82)	0.631	0.409	0.598
		2nd	11.66 (6.73-21.60)	18.72 (2.83-28.95)	0.853	0.456	0.459
		3rd	19.76 (12.02-24.36)	10.94 (6.18-38.40)	0.044	0.589	0.156
		4th	15.56 (9.84-18.38)	27.76 (14.78-40.52)	0.921	7.00E-03	0.031
MIP-1beta	70.11±51.08	1st	68.61±31.90	35.56±24.99	0.441	0.074	6.94E-03
		2nd	64.50±21.25	79.07±52.70	0.490	0.568	0.301
		3rd	72.80±44.19	85.35±23.41	0.534	0.346	0.423

		4th	41.33±11.00	48.20±46.05	0.456	0.426	0.775
TNF-alpha	31.70 (13.83-40.53)	1st	14.18 (10.58-22.56)	17.76 (13.88-35.20)	0.144	0.776	0.353
		2nd	16.56 (7.02-36.40)	18.96 (12.68-50.95)	0.171	0.835	0.450
		3rd	20.16 (15.36-30.38)	18.37 (6.42-28.12)	0.745	0.192	0.152
		4th	19.56 (12.98-34.00)	39.44 (10.58-61.85)	0.551	0.625	0.330
IL-4	34.86±24.42	1st	32.51±22.71	69.70±65.16	0.84	0.081	0.22
		2nd	41.11±24.69	36.46±10.49	0.521	0.914	0.761
		3rd	50.71±53.49	68.9	0.396	0.197	0.753
		4th	55.50±17.61	103.79±37.91	0.185	2.53E-04	0.100

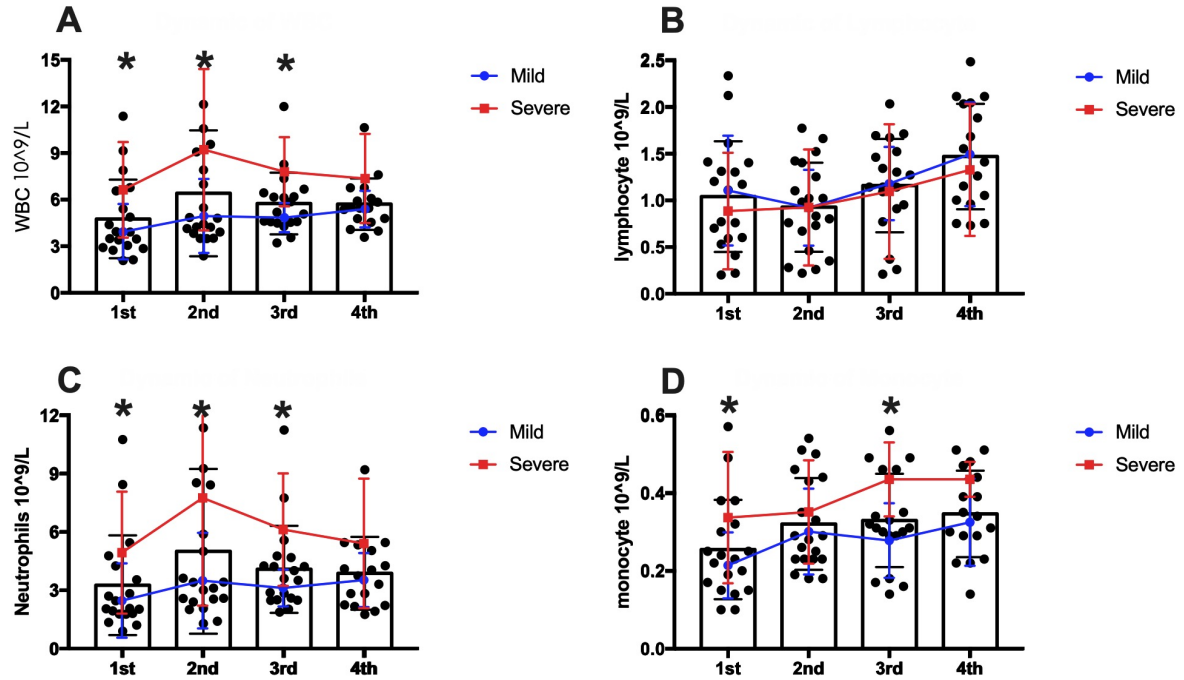


Figure 1 Dynamic changes of blood cells during COVID-19 infection. The values of white blood cell (A), lymphocyte count (B), neutrophils count (C) and monocyte count (D) in COVID-19 infection patients in 1st, 2nd, 3rd, 4th week of onset of symptom was presented with black dot in scatter diagram. The dynamic changes of WBC, lymphocyte, neutrophils and monocyte are presented with red line in severe patients and blue line in mild patients. * indicates that the difference between mild and severe group was significant (p < 0.05).

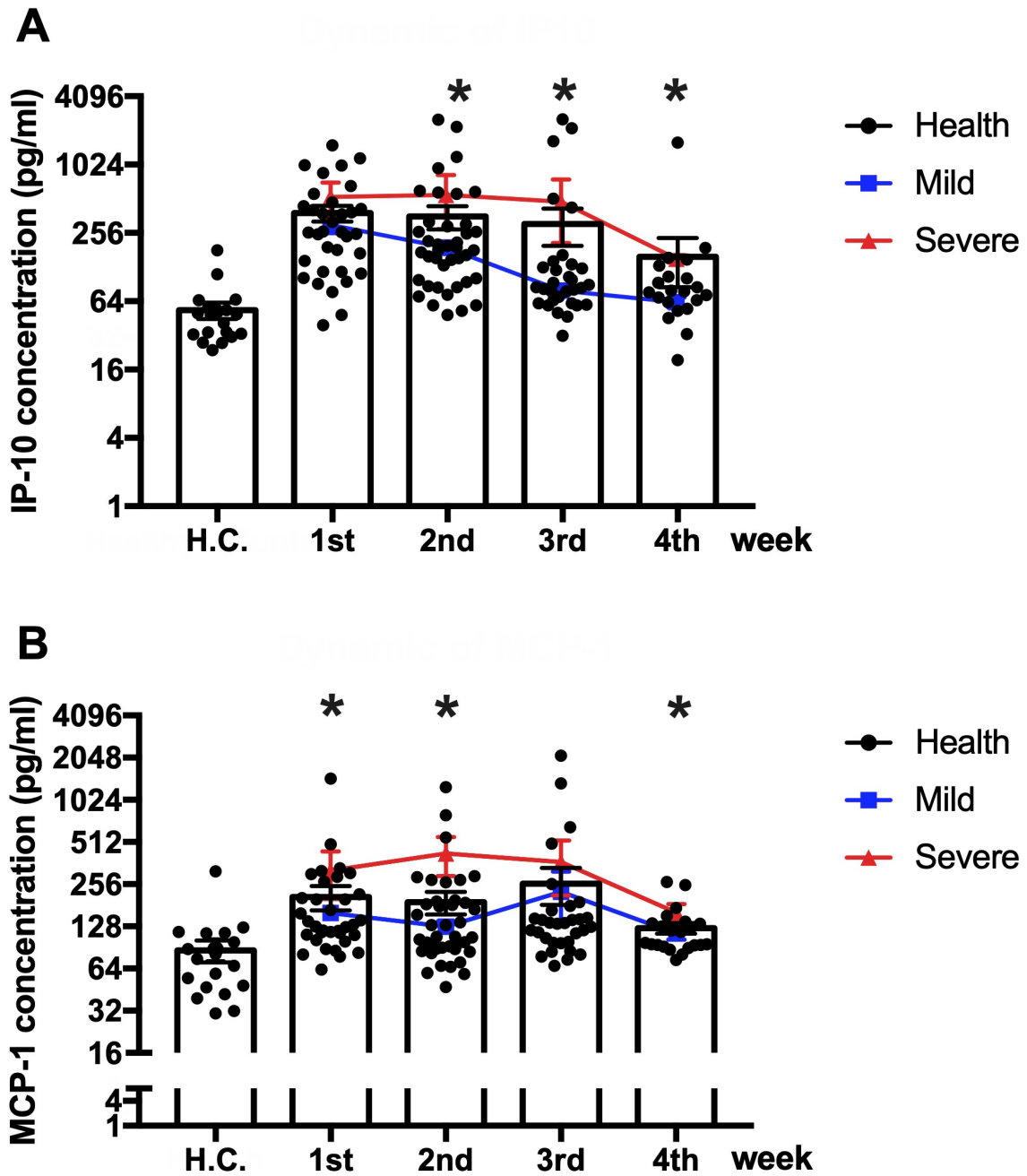


Figure 2. The dynamic changes of IP-10 and MCP-1 plasma levels between mild and severe COVID-19 infection. The values of IP-10 (A) and MCP-1 (B) in healthy controls and COVID-19 infection patients in 1st, 2nd, 3rd and 4th week of onset of symptom are presented with black dot in scatter diagram, the dynamic changes of IP-10 and MCP-1 are presented with red line in severe patients and blue line in mild patients. * indicates that the difference between mild and severe group was significant ($P < 0.05$).

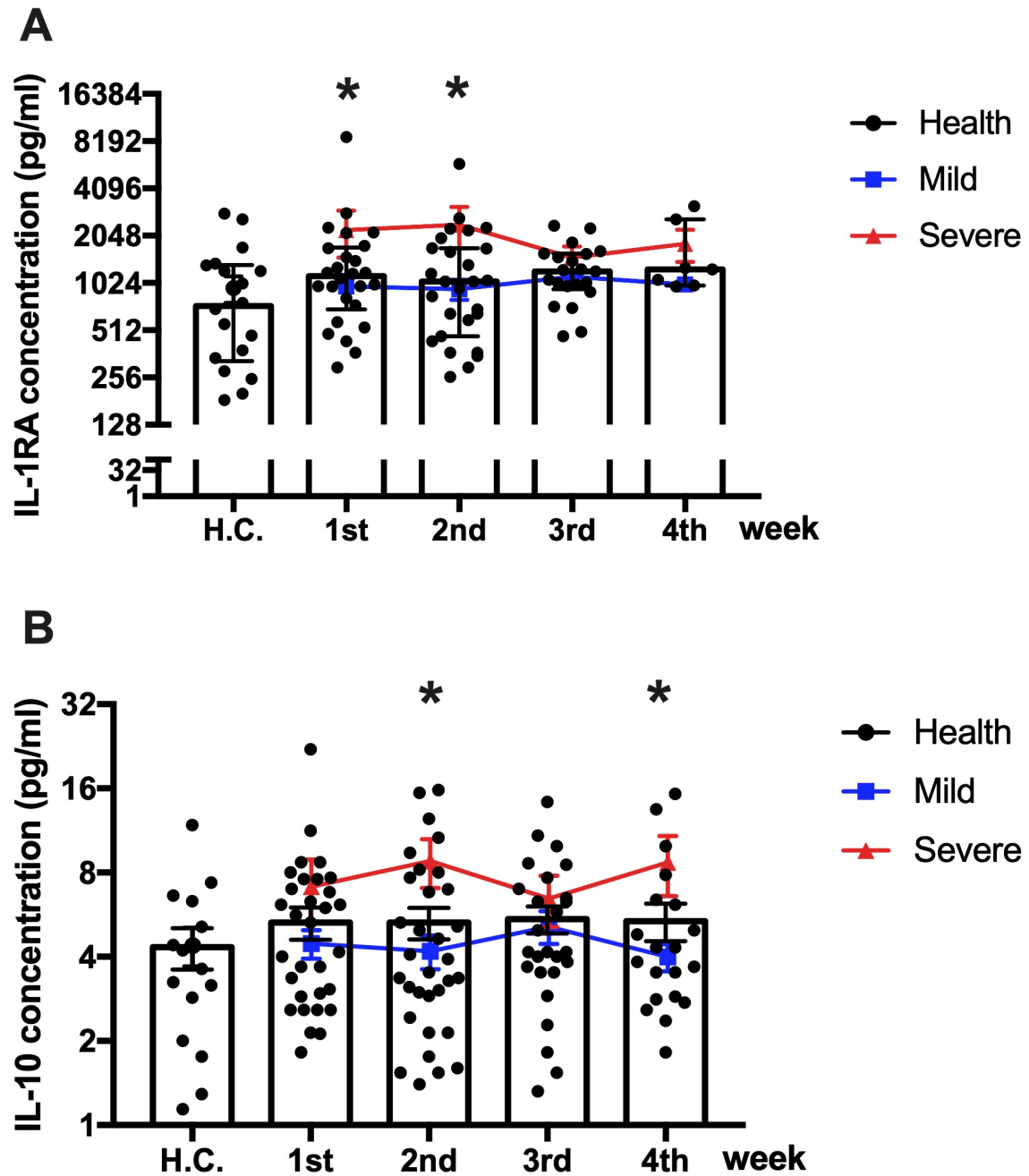


Figure 3. Inhibitory cytokines IL-1Ra and IL-10 are significantly elevated in severe cases at early stage of infection

The values of IL-1RA (A) and IL-10 (B) in healthy controls and COVID-19 infection patients in 1st, 2nd, 3rd and 4th week of onset of symptoms are presented with black dot in scatter diagram, the dynamics of IL-1RA (A) and IL-10 (B) were presented with red line in severe patients and blue line in mild patients. * indicates that the difference between mild and severe group was significant ($p < 0.05$).

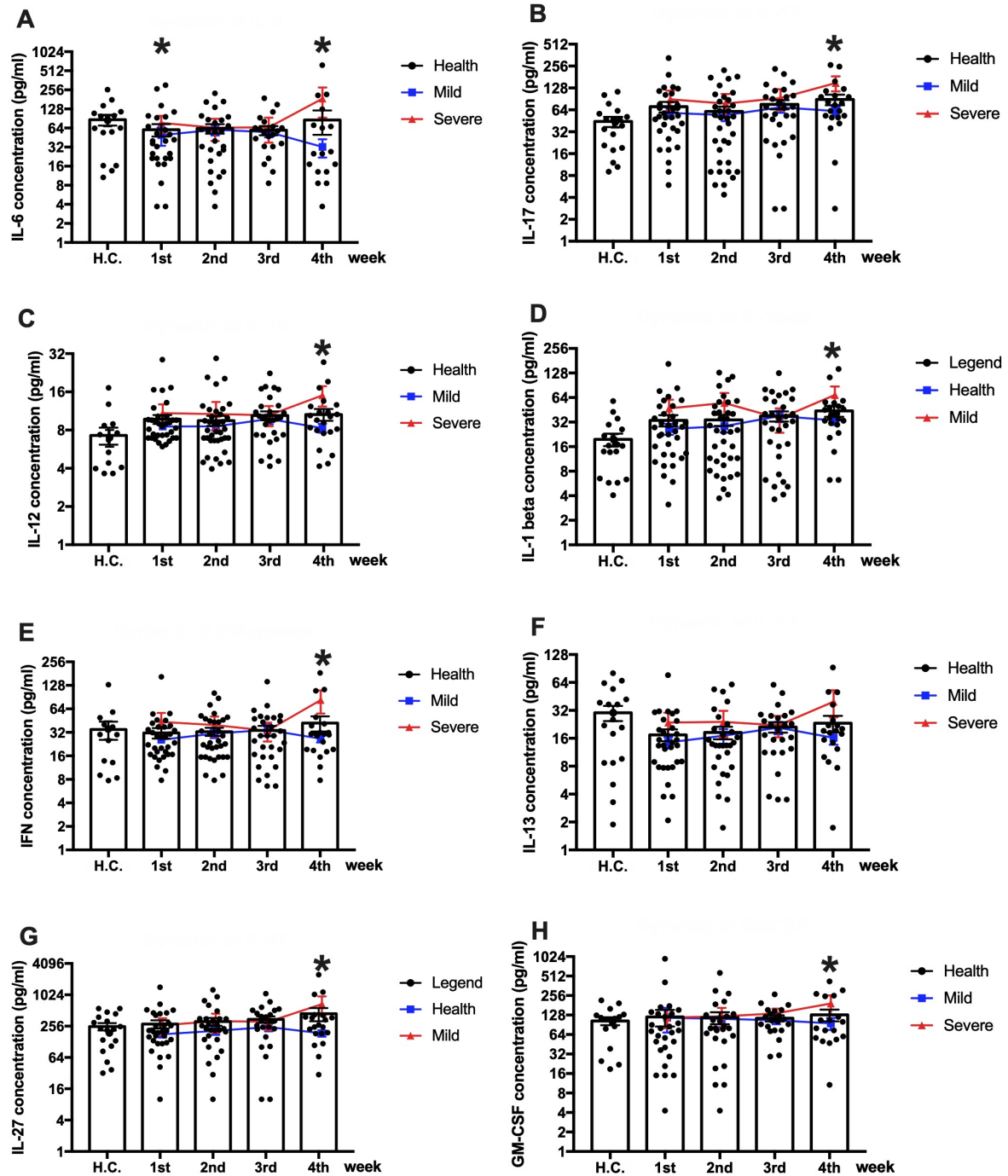


Figure 4. Elevated IL-6, IL-17, IL-12, IL-1beta, IFN-gamma, IL-13, IL-27 and IL-7 in late stages of severe cases. The values of IL-6 (A), IL-17 (B), IL-12 (C), IL-1beta (D), IFN-gamma (E), IL-13 (F), IL-27 (G) and IL-7 (H) in healthy controls (H.C.) and COVID-19 infection patients in 1st, 2nd, 3rd and 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. * indicates that the difference between mild and severe groups was significant (p<0.05).

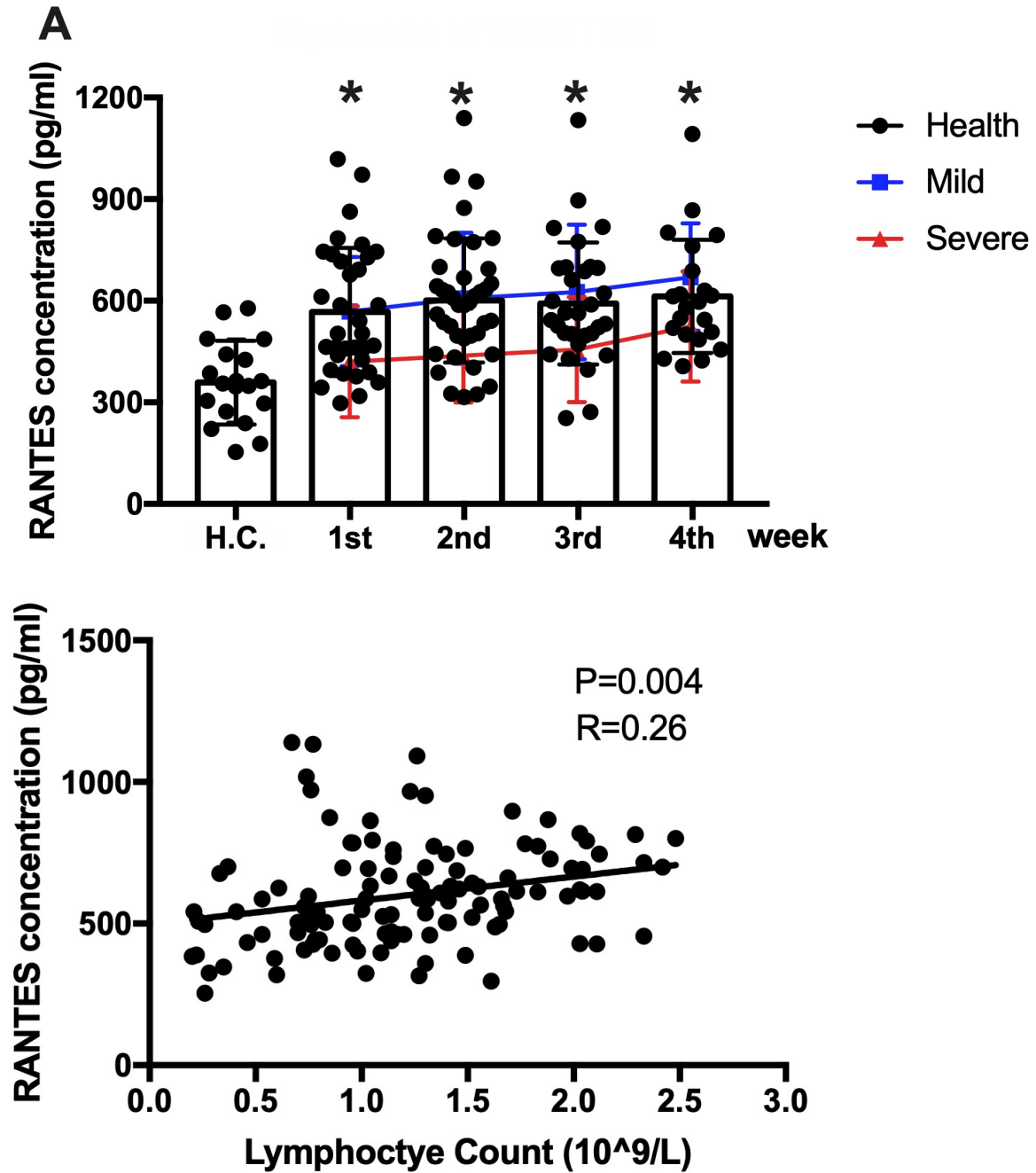


Figure 5. High level of RANTES in mild but not severe COVID-19 patients. A. The values of RANTES in health control and COVID-19 infection patients in 1st, 2nd, 3rd and 4th week of onset of symptom was presented with black dot in scatter diagram, the dynamics of RANTES was presented with red line in severe patients and blue line in mild patients. * indicates that the difference between mild and severe group was significant ($p<0.05$). B. Correlation analysis between RANTES and lymphocyte counts. Each black circle indicates individual patients, the linear correlation between RANTES and lymphocyte count was presented with black line.

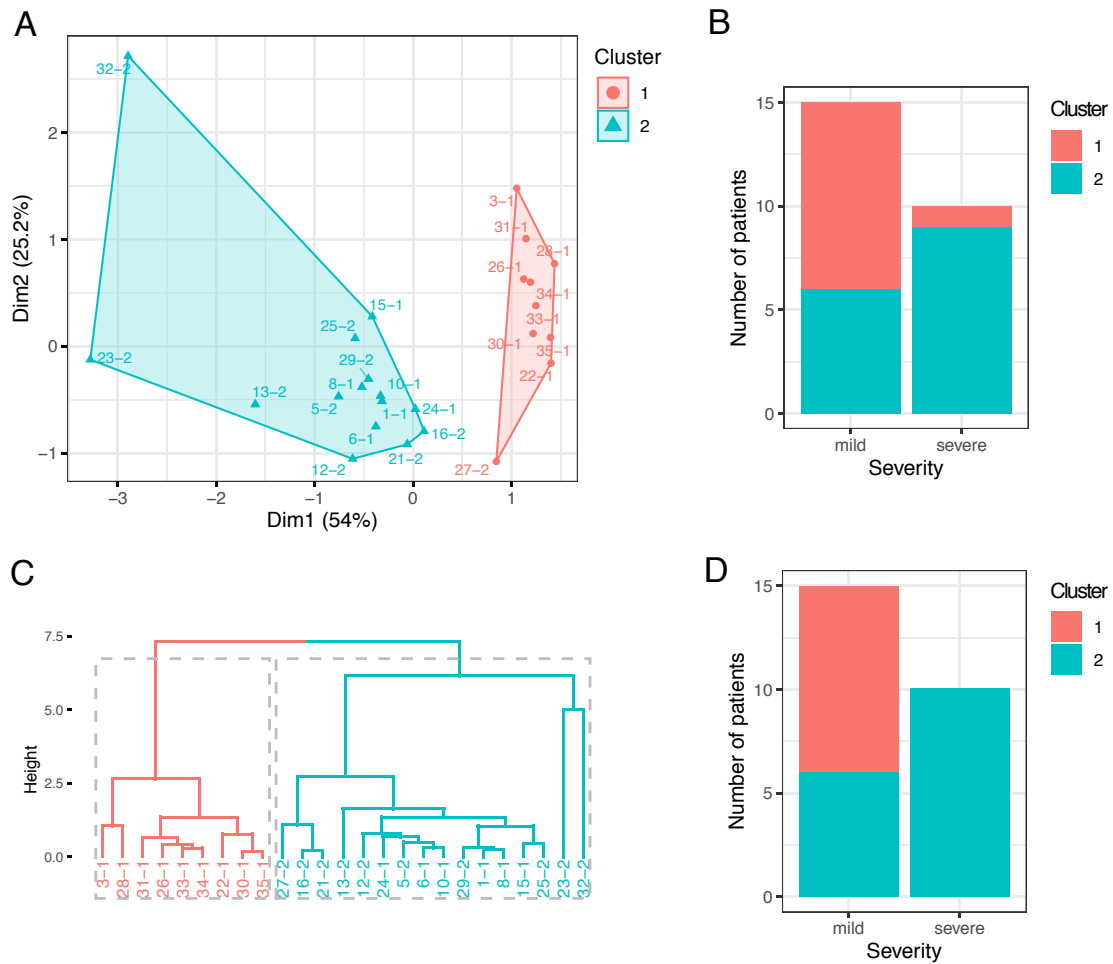


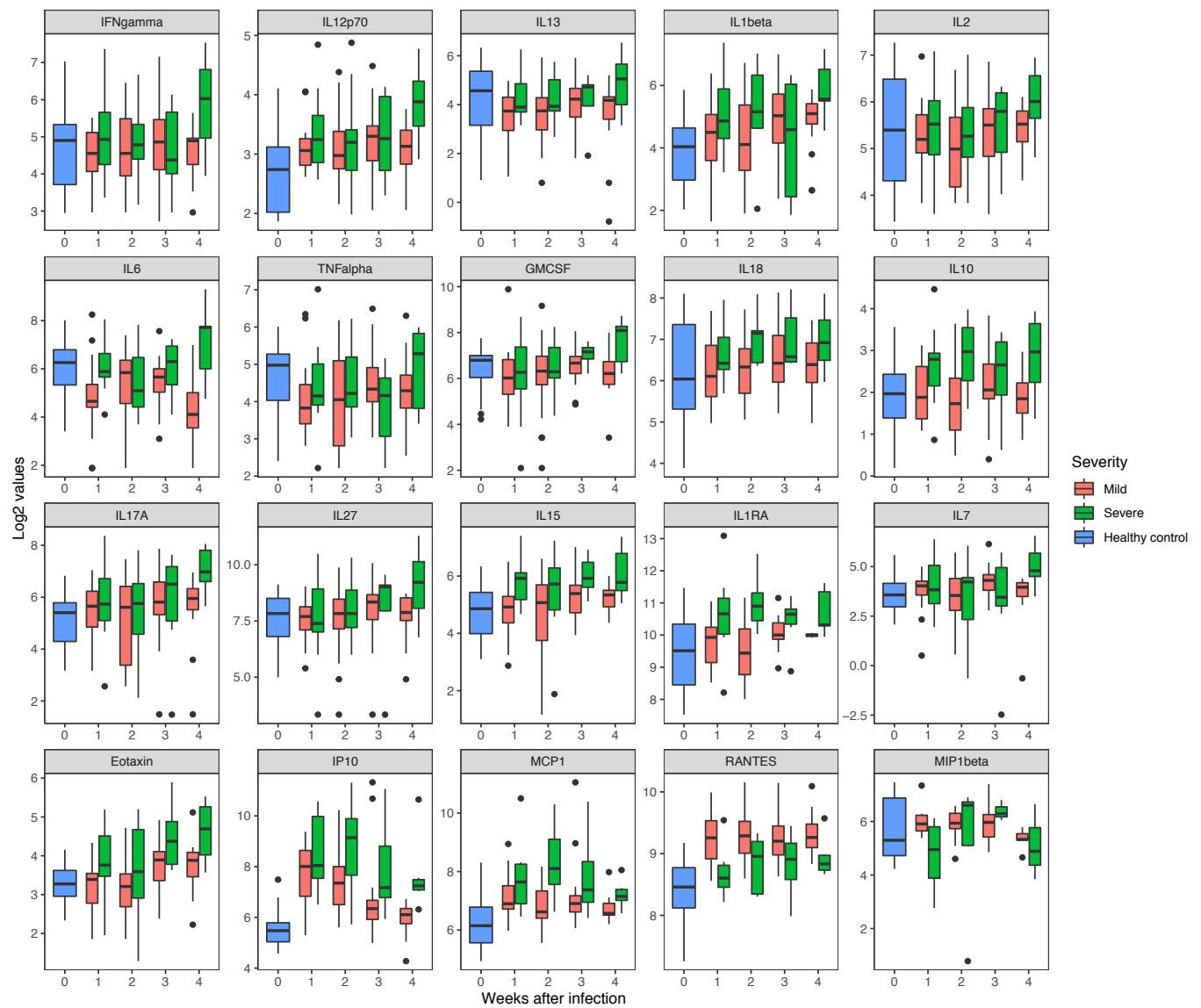
Figure 6: Combination of CCL5, IL-1Ra and IL-10 predict the disease severity.

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.



Supplemental figure 1

Box plots of cytokine levels on the y-axis (Log2 scale) in blood samples from health control and COVID-19 infection patients in 1st, 2nd, 3rd and 4th week of onset of symptom. The central horizontal line indicates the median of each distribution, upper and lower boundaries of the boxes indicate the 3rd and 1st quartiles.