Inflammatory profiles and clinical features of COVID-19 survivors three months after discharge in Wuhan, China

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Summary Vascular injury, aberrant pro-inflammatory cytokine and chemokine levels persisted and significantly correlated with chest CT abnormalities, and impaired pulmonary function (restrictive abnormalities and reduced diffusion capacity) in recovered COVID-19 patients at 3 months post-discharge, especially in recovered severe/critical patients.

Abstract

Background Post-discharge immunity and its correlation with clinical features among patients recovered from COVID-19 are poorly described. This prospective cross-sectional study explored the inflammatory profiles and clinical recovery of COVID-19 patients at 3 months post-discharge.

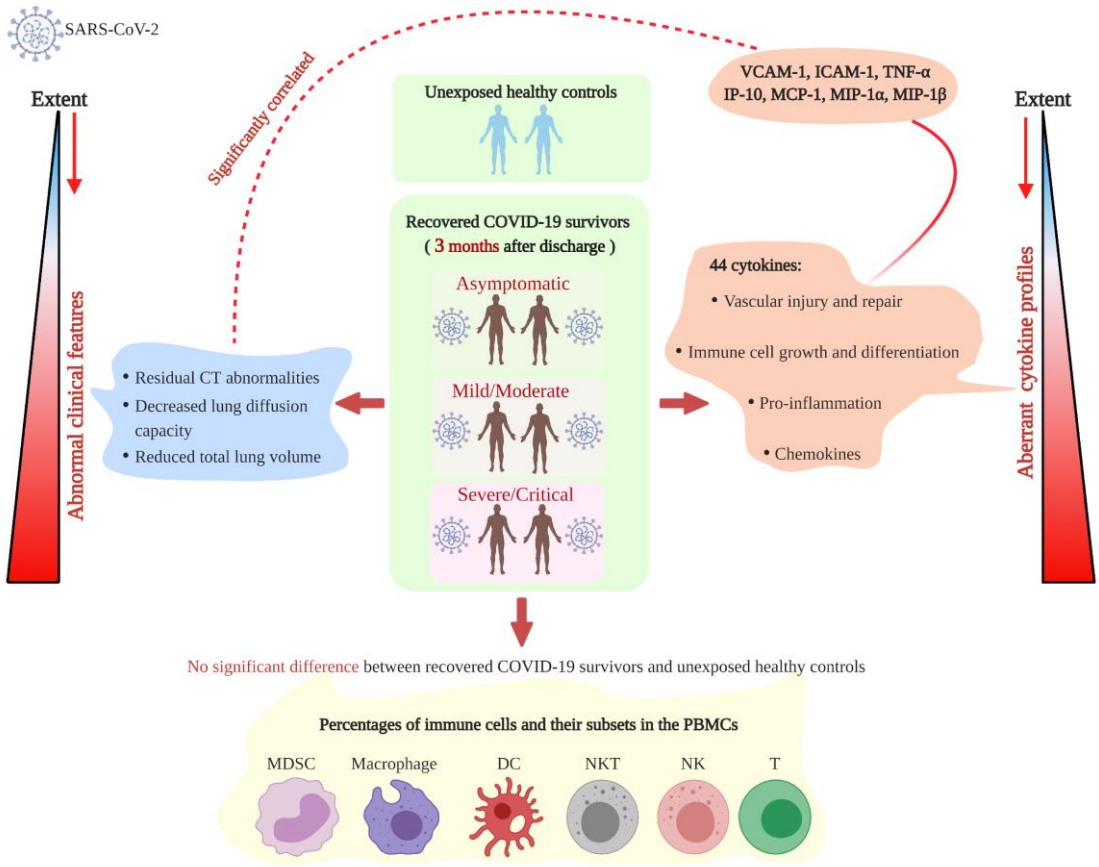
Methods COVID-19 patients discharged from four hospitals in Wuhan, recovered asymptomatic patients (APs) from an isolation hotel, and uninfected healthy controls (HCs) were recruited. Viral nucleic acid and antibody detection, laboratory examination, computed tomography, pulmonary function assessment, multiplex cytokine assay, and flow cytometry were performed.

Results The 72 age-, sex- and body mass index-matched participants included 19 severe/critical patients (SPs), 20 mild/moderate patients (MPs), 16 APs, and 17 HCs. At 3 months after discharge, levels of pro-inflammatory cytokines and factors related to vascular injury/repair in recovered COVID-19 patients had not returned to those of the HCs, especially among recovered SPs compared to recovered MPs and APs. These cytokines were significantly correlated with impaired pulmonary function and chest CT abnormalities. However, levels of immune cells had returned to nearly normal levels and were not significantly correlated with abnormal clinical features.

Conclusion Vascular injury, inflammation, and chemotaxis persisted in COVID-19 patients and were correlated with abnormal clinical features 3 months after discharge, especially in recovered SPs.

Key words: Recovered COVID-19 patients, 3 months after discharge, cytokine profiles, immune cells, clinical features.

Graphical_Abstract



Introduction

The ongoing coronavirus disease 2019 (COVID-19) pandemic has, as of November 12, 2020, caused >50 million cases and over 1,275,000 deaths[1], posing an overwhelming threat to global health. With the increasing number of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, the recovery state, regardless of asymptomatic, mild, or severe infections, has attracted attention. Determining the long-term clinical outcome and longevity of the inflammatory state after SARS-CoV-2 infection is critical for understanding the disease spectrum of COVID-19 and optimizing post-COVID-19 rehabilitation.

The immunopathology of COVID-19 is a serious issue[2]. In patients with severe COVID-19, lymphopenia is frequently observed, with reduced numbers of CD4+ T, CD8+ T, B, and natural killer (NK) cells and reduced percentages of monocytes and eosinophils[3, 4]. Most patients with severe COVID-19 exhibit elevated serum levels of pro-inflammatory cytokines, including interleukin (IL)-6 and IL-1β, as well as IL-2, IL-8, IL-17, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ-inducible protein-10 (IP-10), monocyte chemotactic protein (MCP), macrophage inflammatory protein (MIP)-1α, and tumor necrosis factor (TNF)-α, which are characterized as cytokine storms[3-5]. Moreover, specific pro-inflammation markers are strongly correlated with worse outcomes and death in COVID-19 patients [6, 7], suggesting that poor clinical outcomes might be attributed to viral-driven hyperinflammation. However, how this pathological immunity will evolve and whether it is related to undesirable sequelae among discharged COVID-19 patients remains unknown.

Two recent studies reported that the levels of immune cells, including neutrophils, monocytes, NK cells, and B and T lymphocytes, returned to nearly baseline levels in recovered untreated COVID-19 individuals[8, 9]. Convalescent-phase SARS-CoV-2-specific T cells are polyfunctional and display a stem-like memory phenotype[10]. However, the

recovery time after patient discharge was not unified in these studies, and few reports have focused on the recovery of soluble pro-inflammatory, chemotaxis, and endothelial-injury-related markers, which is important for understanding patient immune outcomes and formulating rehabilitation strategies for recovered patients. Thus, this study explored the immunological profiles and clinical characteristics of recovered COVID-19 patients with different disease severities 3 months after discharge and analyzed the correlations between aberrant levels of immune markers and abnormal clinical features to provide more rational guidance for future follow-up and rehabilitation.

Methods

Study design and participants

This prospective cross-sectional study involved COVID-19 patients discharged between March 5 and March 31, 2020, from four hospitals in Wuhan (Wuhan Union Hospital, Wuhan Pulmonary Hospital, Wuhan Central Hospital, and Fangcang Hospital), as well as recovered asymptomatic patients (APs) from an isolation hotel and uninfected healthy controls (HCs) in the community. Recruitment and testing were carried out in the outpatient clinic of Wuhan Union Hospital via telephone at 3 months after discharge by trained medical staff. All patients were contacted in the order of their discharge dates, as documented in their medical records. The exclusion criteria were chronic respiratory, hematological, autoimmune, and psychotic diseases; death before follow-up; declining to participate, or an inability to participate for reasons such as living outside Wuhan city or inability to be contacted. The recovered APs were confirmed by a previous positive SARS-CoV-2 nucleic acid test or current positive SARS-CoV-2 antibody test without symptoms throughout. Age, sex, and body mass index (BMI) were matched between the recovered COVID-19 patients and healthy

controls (recruitment details are shown in **Figure S1**). The recovered COVID-19 patients were grouped by disease severity during their infection period (severe/critical [SPs], mild/moderate [MPs], and asymptomatic [APs]) according to World Health Organization (WHO) interim guidance[11]. When interviewed, the participants were subjected to a physical checkup, pulmonary-function test, and chest computed tomography (CT) scan. Routine blood tests, biochemical tests (renal and live function markers), and coagulation tests were also completed, with peripheral venous blood samples collected for the subsequent measurement of immune cell and cytokine levels.

This project was registered on the Clinical Trials website (No. NCT04456101), and has been approved by the institutional review boards of Medical Ethics Committee of Wuhan Union Hospital (NO.0271-01). All participants or their surrogates signed informed consent.

Chest CT scanning, artificial intelligence-based quantitative analysis of CT images and pulmonary function test

The standard protocols were as previously reported[12-15], and are described in detail in the Supplementary Methods.

Mesoscale-discovery (MSD) Multiplexed Immunoassay

Peripheral venous blood was collected into ethylenediaminetetraacetic acid (EDTA)-coated vacutainer tubes. Supernatant was obtained subsequently for cytokine profiling assays. Plasma levels of 44 soluble markers were measured using six MSD V-PLEX multiplex assay panels (V-PLEX, K15198D, K15190D, K15049D, K15050D, K15084D, and K15047D) on an MSD SQ120 instrument (Rockville, MD, USA) according to the manufacturer's instructions. The 44 cytokines include: vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), basic fibroblast growth factor (bFGF), placental

growth factor (PlGF), tyrosine receptor kinase in the endothelium-2 (Tie-2), vascular endothelial growth factor (VEGF)-A, VEGF-C, VEGF-D, vascular endothelial growth factor receptor-1 (Flt-1), GM-CSF, IL-2, IL-4, IL-5, IL-7, IL-9, IL-15, thymic stromal lymphopoietin (TSLP), serum amyloid A protein (SAA), interferon-γ (IFN-γ), TNF-α, TNF-β, IL-1α, IL-1β, IL-1RA, IL-6, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-17A, IL-17B, IL-17C, IL-17D, Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-4, MIP-1α, MIP-1β, macrophage-derived chemokine (MDC), thymus activation-regulated chemokine (TARC), IL-8, and IL-16.

Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood via standard density gradient centrifugation and were used immediately. The isolated PBMCs were stained with fluorochrome-conjugated human monoclonal antibodies (all from BD Biosciences, USA, **Table S1**) to determine the percentage of immune cells in PBMCs; that is, T lymphocytes (anti-CD3, CD4, CD8), natural killer (NK) cells, NKT cells, macrophages, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), and their subsets. Cell acquisition was performed on a BD LSRFortessa X-20 flow cytometer (BD Biosciences). Data were analyzed using FlowJo V10 software.

Statistical analysis

The clinical characteristics and inflammatory consequences of all participants are presented as medians (IQR) or means (± SD) for continuous variables and absolute values with percentages for categorical variables. For the comparison of clinical characteristics (laboratory findings, pulmonary function, and CT scans), percentage of immune cells, and 44 cytokines between the four groups (recovered SPs, MPs, APs, and HCs), we used Kruskal–Wallis tests (data with non-normal distribution), one-way analysis of variance (ANOVA)

(data with normal distribution), $\chi 2$ tests, or Fisher's exact tests, as appropriate, to obtain an overall p-value for each variable. To adjust for multiple hypothesis testing, false discovery rate (FDR) correction was first performed for the overall p-value using the Benjamini–Hochberg procedure at the FDR <0.05 significance threshold. Then, for variables with an overall p <0.05 and FDR <0.05, which were deemed statistically significant, we performed pairwise subgroup comparisons (recovered SPs vs. HCs, MPs vs. HCs, APs vs. HCs, SPs vs. MPs, SPs vs. APs, and MPs vs. APs). We conducted Mann–Whitney U test (data with non-normal distribution), independent t-test (data with normal distribution), $\chi 2$, and Fisher's exact tests as appropriate, with Bonferroni correction and took the two-sided p <0.05/n (n = the number of comparisons) as the threshold to determine whether the comparison between the two subgroups was statistically significant. The associations between cytokine profiles and abnormal clinical features in recovered COVID-19 patients were examined by Spearman correlation analysis and visualized with correlation matrix plots.

All tests were two-sided and performed using R software (version 4.0.2, R Foundation) or SPSS (version 26).

Results

1. Clinical characteristics of the study populations

This study enrolled three groups of COVID-19 patients at 3 months after discharge (recovered SPs: n= 19; recovered MPs: n=20; and recovered APs: n=16). Seventeen HCs were recruited at the same time and were matched for age, sex, and BMI. As shown in **Table 1**, the mean duration from illness onset to follow-up was 4.5 months (recovered SPs =139.79 vs. recovered MPs = 133.75 days), and the median length of hospital stay was significantly longer in recovered SPs than that in recovered MPs (47.0 vs. 22.0 days, p <0.0001). Most

recovered patients tested positive for serum SARS-COV-2 IgG, with a few still positive for IgM. The laboratory findings of the four groups revealed that all indicators had returned to normal levels and were comparable to those in the HCs. Comparison of levels of C-reactive protein (CRP) and some hematologic markers at discharge and 3 months later revealed that only the monocyte counts were significantly decreased after 3 months (**Table S2**). Artificial intelligence (AI)-assisted CT findings showed persisting residual lesions on chest CT images and were more frequently observed in recovered SPs (94.7%), followed by recovered MPs (80%). Consistently, the volume percentages of total lesion, ground-glass opacity (GGO), and solid component in the lungs increased with the severity of previous COVID-19. In general, the volume of residual lesions in the whole lungs was not large, indicating that the pneumonia lesions on CT images were well absorbed in recovered COVID-19 patients 3 months after discharge. However, strip-like fibrosis, a solid component newly formed during the recovery period, was more common in recovered SPs than in MPs (89.5% vs. 30%). Correspondingly, anomalies of pulmonary function were mainly noted in diffusion capacity and lung volume (**Table 1**), as revealed by the significantly reduced diffusing capacity for carbon monoxide (DLCO)%, total lung capacity (TLC)%, and residual volume (RV)% values in the recovered SPs, but not in MPs and APs. The ventilatory capacity of pulmonary function showed no significant differences (**Table S3)**.

2. Cytokine profiles of recovered COVID-19 patients 3 months after discharge

The abnormal clinical manifestations above indicated that the COVID-19 survivors had not yet fully recovered at 3 months after hospital discharge and had suffered post-COVID-19 organ damage (fibrosis on CT images and decreased lung volume and DLCO pred%). To assess the state of inflammation in these survivors, we measured the levels of 44 plasma cytokines in SPs, MPs, Aps, and HCs using an MSD multiplexed immunoassay **(Figure 1-4)**. The plasma cytokines were categorized into four classes.

Class-1 cytokines are associated with vascular injury and repair/angiogenesis **(Figure 1)**. We discovered that VCAM-1, ICAM-1, PlGF, and Tie-2 were significantly elevated in recovered SPs compared to HCs, whereas bFGF exhibited the opposite change, but MPs and APs bore no significant difference with HCs. Meanwhile, VEGF family and its receptor Flt-1 showed no significant differences between these four groups **(Figure 1C)**.

Class-2 cytokines promote immune cell growth and differentiation (**Figure 2**). Comparison of these cytokines revealed that IL-7 levels significantly decreased in recovered SPs compared to those in APs and HCs, while TSLP levels were relatively higher in recovered SPs. However, no significant differences were found in GM-CSF, IL2, IL-4, IL-5, IL-9, and IL-15. IL-7 exerts anti-apoptotic properties and induces potent proliferation of naive and memory T cells, causing replenishment of the circulating pool ($CD4^+$ and $CD8^+$)[16, 17]. TSLP is reportedly involved in the development of acute Th2-dependent allergic airway inflammation[18]. Accordingly, recovered SPs tended to have a certain degree of T cell immune perturbation.

Class-3 cytokines are pro-inflammatory immune factors **(Figure 3).** We found significant upregulation of SAA and TNF-α in recovered SPs, but not in MPs and APs. IL-1α and IL-β levels did not differ significantly between the four groups. However, IL-1RA was significantly elevated in SPs. The levels of IL-6 and IL-10 in recovered COVID-19 patients, which have been widely reported for the stratification of disease severity during acute COVID-19, almost back to the level of HCs, while IL-17A and IL-17D levels remained significantly higher in recovered SPs.

Class-4 cytokines were characterized as chemokines **(Figure 4)**. Levels of chemokines that stimulate the migration of eosinophils (Eotaxin and Eotaxin-3) and chemotaxis for monocytes or lymphocytes (IP-10, MCP-1, MIP-1α, MIP-1β, MDC) were also significantly higher in

recovered SPs than those in recovered MPs, APs, and HCs. However, levels of TARC (chemotactic factor for T-lymphocytes), IL-8 (involved in neutrophil trafficking), and IL-16 (stimulates a migratory response in $CD4^+$ lymphocytes) did not differ significantly among the four groups.

Moreover, among these 44 cytokines, only VCAM-1, ICAM-1, TNF-α, MIP-1α, and MIP-1β were significantly higher in recovered SPs than in recovered MPs.

3. Proportions of immune cells in PBMCs among recovered COVID-19 patients

Several plasma cytokines remained at abnormal levels in recovered COVID-19 patients 3 months after discharge, especially in recovered SPs, which prompted consideration of immune cell recovery. We explored the proportions of six types of immune cells (T, NK, NKT, DC, macrophage, and MDSC) by isolating PBMCs from whole blood and phenotypically analyzed them by flow cytometry **(Figure S2)**. As shown in **Table 2**, a total of 51 age-, sex-, and BMI-matched subjects were analyzed (recovered SPs: n=20; MPs n=14; APs: n=9; HCs: n=8). The proportions of total $CD3^+$ T, $CD4^+$ T, $CD8^+$ T cells, NK, and NKT cells were slightly higher in the recovered SPs at 3 months after discharge, with the ratio of $CD4^+/CD8^+$ T cells relatively lower than that in HCs, although the differences were not statistically significant. Similarly, no significant difference was observed in the percentage of DCs ($CD11C^+HLA\text{-}DR^+$) between recovered COVID-19 individuals and HCs. The mean fluorescence intensity (MFI) of CD80 and CD86 in DCs was comparable across the four groups. We also did not observe any significant differences in the percentages of macrophages ($CD11b^+CD14^+$) and the CD80 and CD86 MFIs of macrophages between recovered COVID-19 individuals and HCs. Within the MDSC lineage, no significant differences were found in the frequencies of total MDSCs, PMN-MDSCs, and MO-MDSCs between recovered patients and HCs (all $P > 0.05$).

We further divided the recovered COVID-19 patients into two groups according to the results of DLCO% and CT scans. However, none of the immune cells exhibited significant differences between the group with normal DLCO% (NDLCO) and abnormal DLCO% group (ADLCO) (**Figure S3**), while the proportion of $CD4^+$ T cells and PMN-MDSC was significantly lower in the abnormal CT group (ACT) than in the normal CT group (NCT) (**Figure S4**).

4. Correlations between cytokine profiles and abnormal clinical features in recovered COVID-19 patients

Based on the above findings, we examined the potential associations between cytokine profiles and abnormal clinical features by Spearman correlation analysis in recovered COVID-19 patients. Variables with significant correlations are shown in **Figure 5 and Table S4-6**. Mainly class-1, -3, and -4 cytokines were significantly correlated with the indicated laboratory findings, residual CT abnormalities, and pulmonary function test (PFT). VCAM-1, ICAM-1 (two vascular injury factors); TSLP, SAA, TNF-α, IL-1RA, IL-17C (five inflammatory cytokines); and IP-10, MCP-1, MIP-1α, MIP-1β, and IL-16 (five chemokines) were positively correlated with Cys-C and lactate dehydrogenase (LDH) (two organ damage indicators) **(Figure 5A)**. Meanwhile, levels of cytokines including ICAM-1, PlGF (two vascular injury/repair factors), TSLP, TNF-α, IL-17C (three inflammatory cytokines), MCP-1, MIP-1α, and MIP-1β (three chemokines) were positively correlated with GGO% and solid component% on CT images, whereas bFGF (tissue repair) and TARC (may play a role in mature T-cell activation) were negatively correlated **(Figure 5B)**, consistent with the lower median levels of bFGF and TARC in recovered SPs than in HCs **(Figure 1B, 4C)**. Furthermore, VCAM-1, ICAM-1, PlGF (three vascular injury/repair factors); TNF-α, IL-12(p40), IL-17A (three inflammatory cytokines); and IP-10, MIP-1α, and MIP-1β (three chemokines) showed significant negative relationships with DLCO pred% and TLC pred% of

pulmonary function, except for IL-7, which showed a positive correlation **(Figure 5C)**, indicating that IL-7 may play a protective role in lung recovery. Overall, cytokines including VCAM-1, ICAM-1, TNF-α, IP-10, MCP-1, MIP-1α, and MIP-1β were positively correlated with abnormal clinical features among recovered COVID-19 patients at 3 months after discharge.

Discussion

The results of this study showed persisting respiratory sequelae (reduced lung volume, diffusion capacity disorder, and chest CT abnormalities) in recovered COVID-19 patients at 3 months post-discharge, more frequently and conspicuous in recovered SPs compared to their MP and AP counterparts. Several factors associated with vascular injury and repair/angiogenesis (class-1 cytokines), inflammation (class-3 cytokines), and chemotaxis (class-4 cytokines) were upregulated in recovered COVID-19 patients, particularly in SPs. Furthermore, the percentage of immune cells in PBMCs, including T, NK, and NKT cells; DCs; macrophages; and MDSCs, did not differ significantly between recovered COVID-19 patients and HCs, whereas the proportion of $CD4^+$ T cells was significantly lower in the ACT than in the NCT groups of recovered COVID-19 patients. Additionally, cytokines, such as VCAM-1 and ICAM-1 (two class-1 cytokines); TNF-α (class-3 cytokine); and IP-10, MCP-1, MIP-1α, and MIP-1β (four class-4 cytokines) were positively correlated with all the above abnormal clinical features observed in recovered COVID-19 patients.

Immunopathology, especially cytokine release syndrome, is thought to be a major cause of disease severity and death in patients infected with SARS-CoV-2, SARS-CoV, and MERS-CoV [19, 20]. COVID-19 usually involves a cytokine storm, a phlogistic phenomenon caused by positive feedback loops that regulate cytokine production and overwhelm counter-

regulatory mechanisms[21]. Several inflammatory cytokines (such as SAA, TNF-α, IL-6, IL-17), chemokines (IP-10, MIP-1α, MIP-1β), and vascular injury factors (ICAM-1, VCAM-1) have been widely reported to be significantly elevated in acute phase of COVID-19[2, 22-24]. In our study, at 3 months after discharge, the levels of cytokines and chemokines related to hyper-inflammatory response, including SAA, TNF-α, IL-17A, IL-17D, eotaxin, eotaxin-3, IP-10, MCP-1, MIP-1α, MIP-1β, and MDC, remained elevated in recovered SPs, except for that of IL-7 (a T cell growth-promoting factor), which was decreased. Unexpectedly, factors related to vascular injury and angiogenesis, such as VCAM-1, ICAM-1, Tie-2, and PlGF, were significantly elevated in SPs; as pro-inflammatory proteins are key danger signals that cause endothelial function to shift from the homeostatic to the defensive mode[25], inflammation and vascular damage might coexist and aggravate each other in SARS-COV-2 infection, a vicious cycle that persisted in SPs 3 months after discharge and may lead to long-term undesirable consequences in recovered SPs, as this cycle is associated with heart disease and stroke in normal populations.

Furthermore, VCAM-1 and ICAM-1 (two vascular injury factors) were significantly negatively correlated with DLCO pred% of pulmonary function, suggesting that the reduction in DLCO pred% in recovered COVID-19 patients may be caused by endothelial cell activation, leading to disturbance of alveolar-capillary gas exchange. However, compared to HCs, IL-7 levels were significantly decreased in recovered COVID-19 SPs rather than in MPs and APs and were positively correlated with DLCO pred%, indicating the protective role of IL-7 in improving clinical outcomes. The ex vivo administration of IL-7 reportedly restored T cell IFN-ɣ production in COVID-19 patients[26], and current evidence has favored the effective role and safety of IL-7 in improving T cell immunity among critical COVID-19 patients[27, 28]. IL-7 therapy may help improve ongoing immune disorders in recovered

COVID-19 patients, thereby improving the corresponding clinical outcomes, especially in those who recovered from severe/critical illness.

Levels of cytokines including VCAM-1, ICAM-1, TNF-α, IP-10, MCP-1, MIP-1α, and MIP-1β were not only significantly elevated in recovered COVID-19 SPs but were also positively correlated with all abnormal clinical features (residual CT abnormalities and impaired pulmonary function) experienced by recovered COVID-19 patients at 3 months after discharge. This implies that these aberrant vascular-injury related cytokines, inflammatory factors, and chemokines may explain the residual clinical abnormalities and may also lead to undesirable future clinical sequelae, which still needs further studies to confirm and follow up.

Furthermore, dysfunction of myeloid, NK, T, and B cells and their subsets occur in acute-phase COVID-19[29-31]. In this study, we found that immune cells, including DCs, macrophages and their CD80 and CD86 MFI, as well as NK, NKT cells, T cells (total, $CD4^+$, $CD8^+$), and MDSCs (total, PMN-, MO-), returned to normal levels in recovered COVID-19 patients 3 months after discharge. These data were consistent with those of a recent study[8] that reported comparable numbers of $CD4^+$ T, $CD8^+$ T cells, B cells, and NK cells in recovered COVID-19 patients to those in unexposed HCs. Moreover, a study[10] on individuals with asymptomatic or mild COVID-19 reported that convalescent-phase SARS-CoV-2-specific T cells were polyfunctional and displayed a stem-like memory phenotype, even in the absence of detectable humoral responses. Thus, the numbers of most immune cells returned to normal in recovered COVID-19 patients 3 months after hospital discharge and their function began to shift towards protective immunity against reinfection.

This study has several limitations. First, the sample size was limited. Second, this cross-sectional study focused only on intermediate-term (3 months after discharge) follow-up

findings. Third, we did not assess the functional capabilities of SARS-CoV-2-specific immune cells or monitor antibody titers in convalescent individuals. Fourth, we did not evaluate related damage to the cardiovascular system, although it is an important target organ in vascular injury.

In conclusion, we found that vascular injury, aberrant pro-inflammatory cytokine and chemokine levels, and abnormal clinical features persisted in COVID-19 patients at 3 months after discharge, especially in recovered SPs compared to MPs and APs. These findings raise concerns regarding ongoing aberrant-cytokine-mediated underlying organ damage in some recovered COVID-19 patients, especially severe/critical survivors. Whether they return to normal or continue to progress in later stages requires further research. Most importantly, attention should be paid to vascular injury, inflammation, and chemotaxis in recovered COVID-19 SPs. These three classes of cytokines persist and aggravate each other, forming a vicious cycle that may cause long-term irreversible, life-threatening sequelae such as cardiovascular and cerebrovascular diseases and lung fibrosis (abnormal blood gas exchange). Our study focused on the cytokine profiles and their correlation with clinical sequelae in recovered COVID-19 patients with different disease severities 3 months after discharge, which may improve understanding of the full spectrum of COVID-19 and provide guidance for long-term rehabilitation in recovered patients.

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Author contributions

YJ and MZ designed the study. MZ, JX, ZY, SW, TL, YL YF, ZW, and GY collected the clinical data and information based on the follow-up protocols. MZ, ZY, SW and TL collected the peripheral blood samples. MZ, ZY, and TL did the experiment of multiplex-cytokine assay and flow cytometry. MZ, ZY, and JX summarized and checked all data. MZ, KW and ZY conducted the statistical analysis and draw all article figures. The manuscript was drafted by MZ and ZY. YJ, MZ and JZ critically revised the manuscript and all the authors approved the final submission.

Data availability

Anonymized clinical and laboratory test data are available on request, subject to an internal review by YJ and MZ to ensure that the participants' anonymity and confidentiality are protected, with completion of a data-sharing agreement, and in accordance with the Wuhan Union hospital's institutional review boards and institutional guidelines. Please submit requests for participant-related clinical and other data to YJ (whuhjy@126.com).

Conflicts of interest

The authors have no conflict of interest or financial relationships to disclose.

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Table 1. Clinical characteristics for patients who recovered from COVID-19 three months after discharge

Characteristics	Group (N=72)				*P* value			
	Recovered SPs RS (n=19)	Recovered MPs RM (n=20)	Recovered Aps RA (n=16)	Healthy controls HC (n=17)	Overall	RS *vs.* HC	RM *vs.* HC	RA *vs.* HC
Age, median (*IQR*), years	60.00 (57.0-64.00)	56.50 (52.25-63.00)	57.00 (52.75-62.00)	57.00 (51.50-61.50)	0.19[*]	-	-	-
Sex								
Male, n (%)	8 (42.1%)	5 (25.0%)	7 (43.8%)	7 (41.2%)	0.60[†]	-	-	-
BMI, median (*IQR*), kg/m^2	24.84 (±2.62)	24.32 (±2.31)	24.73 (±2.20)	23.10 (±3.32)	0.20[&]	-	-	-
Comorbidities	14 (73.7%)	10 (50.0%)	6 (37.5%)	3 (17.6%)	0.008[†]	0.001[¶]	0.082	0.26
Hypertension	9 (47.4%)	7 (35.0%)	3 (18.8%)	3 (17.6%)	0.16[†]	-	-	-
Diabetes	8 (42.1%)[c]	3 (15.0%)	2 (12.5%)	0 (0.0%)	0.008[#]	0.003[¶]	0.23	0.23
Heart disease	2 (10.5%)	1 (5.0%)	1 (6.2%)	0 (0.0%)	0.73[#]	-	-	-
Cerebrovascular disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	-	-	-
Liver disease	2 (10.5%)	1 (5.0%)	2 (12.5%)	0 (0.0%)	0.54[#]	-	-	-
Kidney disease	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-	-	-	-
Solid tumor	1 (5.3%)	1 (5.0%)	0 (0.0%)	0 (0.0%)	1.00[#]	-	-	-
Duration from illness onset to follow-up, days	139.79 (±7.41)	133.75 (±9.64)	-	-	0.035	-	-	-
Length of hospital stay, days	47.00 (31.00-51.00)	22.00 (17.25-25.00)	-	-	<0.0001	-	-	-
Immune related treatment								
Corticosteroids	5 (26.3%)	2 (10.0%)	-	-	0.24[#]	-	-	-
Intravenous immunoglobulin	3 (15.8%)	2 (10.0%)	-	-	0.66[#]	-	-	-
Serum antibody								

Characteristics	Group (N=72)				*P* value			
	Recovered SPs RS (n=19)	Recovered MPs RM (n=20)	Recovered Aps RA (n=16)	Healthy controls HC (n=17)	Overall	RS *vs.* HC	RM *vs.* HC	RA *vs.* HC
IgM Positive, n (%)	3 (16.7%)	3 (15.0%)	1 (6.2%)	0 (0.0%)	0.34[#]	-	-	-
IgG Positive, n (%)	17 (94.4%)	19 (95.0%)	14 (87.5%)	0 (0.0%)	<0.0001[†]	<0.0001[¶]	<0.0001[¶]	<0.0001[¶]
Laboratory findings								
Hematologic indicators								
WBCs, x10^9/L	5.71 (4.97-6.22)	4.85 (4.26-5.55)	5.85 (4.52-6.66)	4.83 (4.34-6.40)	0.27[*]	-	-	-
Neutrophil count, x10^9/L	3.59 (2.89-4.01)	3.02 (2.54-3.42)	3.70 (2.74-4.22)	3.44 (2.34-3.72)	0.20[*]	-	-	-
Lymphocyte count, x10^9/L	1.67 (±0.45)	1.64 (±0.36)	1.71 (±0.48)	1.74 (±0.37)	0.90[&]	-	-	-
Neutrophil-to-lymphocyte ratio	2.18 (±0.77)	1.88 (±0.44)	2.18 (±0.54)	1.94 (±0.62)	0.31[&]	-	-	-
Monocyte count, x10^9/L	0.29 (0.25-0.36)	0.25 (0.21-0.27)	0.31 (0.26-0.35)	0.29 (0.27-0.36)	0.086[*]	-	-	-
Eosinophil count, x10^9/L	0.09 (0.06-0.12)	0.06 (0.05-0.10)	0.08 (0.06-0.18)	0.06 (0.05-0.09)	0.18[*]	-	-	-
Liver function indicators								
Total bilirubin, μmol/L	15.40 (13.00-17.70)	14.40 (11.00-19.52)	16.80 (14.20-18.85)	16.90 (14.50-17.90)	0.41[*]	-	-	-
Direct bilirubin, μmol/L	5.10 (4.35-5.75)	5.20 (3.72-6.23)	5.90 (5.03-6.63)	5.80 (5.10-6.40)	0.23[*]	-	-	-
ALT, U/L	21.00 (12.50-29.00)	21.00 (15.50-28.25)	22.00 (16.75-26.25)	15.00 (13.00-31.00)	0.53[*]	-	-	-
AST, U/L	21.00 (17.50-26.50)	21.50 (18.00-25.00)	21.50 (18.00-24.75)	20.00 (17.00-24.00)	0.97[*]	-	-	-
ALP, U/L	85.42 (±19.40)	80.80 (±17.59)	78.81 (±19.84)	72.41 (±13.80)	0.19[&]	-	-	-
Total protein, g/L	77.10 (73.70-78.70)	75.50 (73.67-79.92)	75.85 (74.15-79.20)	76.60 (74.20-82.10)	0.89[*]	-	-	-
A/G	1.50 (1.45-1.70)	1.60 (1.50-1.72)	1.60 (1.60-1.70)	1.60 (1.50-1.70)	0.58[*]	-	-	-
Renal function indicators								
Creatinine, μmol/L	68.40 (64.10-75.95)	67.35 (62.17-73.22)	65.75 (63.25-69.98)	68.50 (63.50-75.90)	0.70[*]	-	-	-

Characteristics	Group (N=72)				*P* value			
	Recovered SPs RS (n=19)	Recovered MPs RM (n=20)	Recovered Aps RA (n=16)	Healthy controls HC (n=17)	Overall	RS *vs.* HC	RM *vs.* HC	RA *vs.* HC
BUN, mmol/L	5.00 (4.55-5.50)	4.95 (4.68-5.53)	4.95 (4.52-5.62)	5.20 (4.60-5.60)	0.99*	-	-	-
UA, μmol/L	369.58 (±103.90)	328.93 (±66.82)	341.52 (±81.03)	361.76 (±95.67)	0.47&	-	-	-
Cys-C, mg/L	1.11 (1.04-1.36)	0.98 (0.89-1.12)	0.93 (0.83-1.06)	0.96 (0.87-1.10)	0.018*	-	-	-
LDH, U/L	224.0 (208.75-258.25)	207.00 (189.75-232.50)	205.5 (186.25-234.00)	190.0 (183.00-213.00)	0.076*	-	-	-
CRP, median (IQR), mg/L	1.30 (0.46-3.75)	0.63 (0.29-1.50)	1.06 (0.56-1.50)	0.42 (0.11-1.23)	0.086*	-	-	-
Coagulation function indicators								
PLT, $x10^9$/L	199.05 (±48.37)	206.50 (±46.61)	231.81 (±64.25)	216.47 (±57.65)	0.32&	-	-	-
D-Dimer, μg/ml	0.34 (0.28-0.51)	0.38 (0.29-0.46)	0.32 (0.25-0.46)	0.29 (0.25-0.38)	0.32*	-	-	-
PT, s	12.80 (12.35-13.00)	12.80 (12.50-13.22)	13.10 (12.85-13.80)	12.90 (12.70-13.10)	0.16*	-	-	-
APTT, s	37.91 (±2.93)	34.99 (±2.88)	35.89 (±3.23)	35.79 (±3.47)	0.034&	-	-	-
FIB, g/l	3.21 (2.97-3.69)	3.10 (2.94-3.38)	3.08 (2.79-3.37)	2.90 (2.55-3.22)	0.090*	-	-	-
TT, s	16.50 (16.40-17.20)	16.40 (16.08-17.33)	16.25 (15.55-16.83)	16.20 (15.70-16.60)	0.16*	-	-	-
Chest CT findings								
Residual CT lesion, n (%)	18 (94.7%)	16 (80.0%)	4 (25.0%)	5/14 (35.7%)	<0.0001†	0.0004¶	0.014¶	0.69
Bilateral lungs involved, n (%)	16 (84.2%)	11 (55.0%)	4 (25.0%)	1/14 (7.1%)	<0.0001†	<0.0001¶	0.009¶	0.34
Volume percentage of total lesion in lungs, median (*IQR*), %	0.59 (0.08-2.19)	0.04 (0.01-0.18)	0.00 (0.00-0.02)	0.01 (0.00-0.02)	<0.0001*	0.0002¶	0.015¶	0.47
GGO lesion, n (%)	16 (84.2%)	14 (70.0%)	4 (25.0%)	4/14 (28.6%)	<0.0001†	0.003¶	0.035	1.00
Volume percentage of GGO in lungs, median (*IQR*), %	0.56 (0.07-2.17)	0.04 (0.01-0.14)	0.00 (0.00-0.01)	0.01 (0.00-0.01)	<0.0001*	0.0005¶	0.014¶	0.28
Solid components (SC), n (%)	17 (89.5%)	7 (35.0%)	2 (12.5%)	2/14 (14.3%)	<0.0001†	<0.0001¶	0.25	1.00

Characteristics	Group (N=72)				*P* value			
	Recovered SPs RS (n=19)	Recovered MPs RM (n=20)	Recovered Aps RA (n=16)	Healthy controls HC (n=17)	Overall	RS *vs.* HC	RM *vs.* HC	RA *vs.* HC
Volume percentage of SC in lungs, median (*IQR*), %	0.02 (0.01-0.11)	0.00 (0.00-0.01)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	<0.0001*	0.0003¶	0.22	0.68
Strip-like fibrosis, n (%)	17 (89.5%)	6 (30.0%)	2 (12.5%)	2/14 (14.3%)	<0.0001†	<0.0001¶	0.42	1.00
Reticular opacity, n (%)	2 (10.5%)	3 (15.0%)	0 (0.0%)	0 (0.0%)	0.23#	-	-	-
Pulmonary function								
Lung volume								
TLC (L) % pred	83.50 (74.00-89.55)	100.60 (91.67-111.12)	95.55 (91.68-103.20)	98.90 (94.80-108.10)	<0.0001*	<0.0001¶	0.84	0.20
<80% pred, n (%)	8 (42.1%)	0 (0.0%)	2 (12.5%)	0 (0.0%)	0.0001#	0.003¶	-	0.23
FVC (L), % pred	102.70 (91.50-111.40)	117.95 (107.77-134.73)	106.2 (102.40-115.42)	105.60 (99.90-122.60)	0.020*	0.38	0.049	0.82
<80% pred, n (%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.72#	-	-	-
RV (L) % pred	74.50 (61.15-90.50)	98.70 (91.62-104.98)	96.55 (84.22-111.78)	105.50 (92.20-112.20)	<0.0001*	<0.0001¶	0.28	0.26
<65% pred, n (%)	6 (31.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.0001#	0.02	-	-
Diffusion capacity								
DLCO (mmol/min/kPa) % pred	74.90 (66.15-77.95)	85.40 (75.85-95.02)	87.95 (83.88-92.55)	93.80 (86.40-99.00)	<0.0001*	<0.0001¶	0.13	0.21
<80% pred, n (%)	19 (100.0%)	7 (35.0%)	2 (12.5%)	0 (0.0%)	<0.0001†	<0.0001¶	0.009¶	0.23
DLCO/VA% pred	90.33 (±13.08)	89.50 (±12.24)	98.08 (±14.34)	95.71 (±12.11)	0.15&	-	-	-
<80% pred, n (%)	5 (26.3%)	3 (15.0%)	1 (6.2%)	2 (11.8%)	0.47#	-	-	-

Note: Data were expressed as median (*IQR*), mean (±SD) or n (%). Kruskal-Wallis (K-W) test or One-way ANOVA were used for analysis of continuous variables and χ^2 test or fisher's exact test for analysis of all category variables between four groups. False discovery rate (FDR) correction was performed at the FDR < 0.05 significance threshold for the comparison of laboratory findings, Chest CT findings, and the pulmonary

function among the four groups. For variables with overall $p < 0.05$ and FDR <0.05, we performed subgroup comparisons. Bonferroni correction was conducted for subgroup comparison, and the corrected significance threshold of subgroup p value is 0.017 (0.05/3).

¶: $p < 0.017$; *: Kruskal-Wallis (K-W) test; †: χ^2 test; &: One-way ANOVA; #: Fisher's exact test;

Abbreviations: RS: recovered severe/critical patients; RM: recovered mild/moderate patients; RA: recovered asymptomatic patients; IQR: interquartile range; BMI: body mass index; WBC: white blood cell; PLT: platelet; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; A/G: albumin/globin; BUN: blood urea nitrogen; UA: uric acid; Cys-C: cystain C; LDH: lactate dehydrogenase; CRP: C-reactive protein; PT: prothrombin time; APTT: activated partial thromboplastin time; FIB: fibrinogen; TT: thrombin time; GGO: ground-glass opacity; SC: solid components; TLC: total lung capacity; FVC: forced vital capacity; RV: residual volume; DLCO: diffusing capacity of the lung for carbon monoxide; VA: alveolar ventilation.

Table 2. Percentage of immune cells in PBMCs of recovered COVID-19 survivors 3 months after discharge

Characteristics	Group (N=51)				*Overall*
	Recovered SPs (n=20)	**Recovered MPs (n=14)**	**Recovered APs (n=9)**	**Healthy controls (n=8)**	***p* value**
Age, median (IQR), years	59.50 (56.25-64.00)	56.00 (52.75-63.00)	56.00 (53.50-61.00)	56.50 (51.25-60.25)	0.13*
Sex					
Male, n (%)	11 (55.0%)	6 (42.9%)	4 (44.4%)	4 (50.0%)	0.90†
BMI, median (IQR), kg/m^2	23.96 (±3.48)	23.69 (±2.72)	24.11 (±1.91)	22.33 (±1.99)	0.54&
Lymphocytes% of PBMSs, median (IQR)					
CD3$^+$ T cells, %	58.00 (49.60-67.85)	53.90 (48.62-58.62)	48.05 (37.77-53.67)	48.40 (35.88-57.35)	0.13*
CD3$^+$ CD4$^+$ T cells, %	30.21 (26.02-37.16)	30.01 (26.43-38.34)	29.04 (25.46-29.75)	26.67 (23.05-33.05)	0.77*
CD3$^+$ CD8$^+$ T cells, %	19.34 (13.84-23.05)	13.48 (10.16-19.37)	11.92 (10.77-18.39)	12.08 (7.76-16.54)	0.33*
CD4$^+$ / CD8$^+$ T cells	1.44 (1.01-2.52)	2.04 (1.79-3.72)	2.04 (1.64-2.61)	1.92 (1.48-3.08)	0.51*
NK (CD3$^-$ CD16$^+$ CD56$^+$) cells% of PBMCs	14.82 (13.89-18.59)	17.31 (11.80-19.79)	18.43 (12.77-32.07)	11.61 (9.42-14.24)	0.24*
NKT (CD3$^+$ CD56$^+$) cells % of PBMCs	9.22 (6.19-13.00)	7.24 (5.27-11.15)	6.88 (5.13-7.68)	6.20 (4.35-7.87)	0.087*
Dendritic cells% of PBMCs, median (IQR)					
DC (CD11C$^+$ HLA-DR$^+$), %	13.70 (9.84-15.70)	13.35 (10.62-15.40)	15.20 (13.50-17.80)	14.75 (11.62-17.10)	0.72*
MFI of HLA-DR in DCs	1835.0 (1504.0-2252.0)	2075.0 (1602.8-2537.5)	1679.0 (1560.5-2044.0)	1851.5 (1605.5-2472.3)	0.71*
MFI of CD80$^+$ in DCs	57.30 (49.20-63.80)	63.15 (58.03-65.97)	76.60 (58.10-81.20)	58.30 (50.33-68.40)	0.24*
MFI of CD86$^+$ in DCs	2277.0 (2084.0-2424.0)	2100.5 (1940.5-2259.25)	2329.0 (2196.0-2536.0)	2324.5 (2240.25-2603.5)	0.039*
Macrophages% of PBMCs, median (IQR)					
Macrophages (CD11b$^+$ CD14$^+$), %	12.80 (10.10-14.80)	10.20 (8.20-12.10)	12.60 (10.40-15.10)	14.60 (10.86-18.27)	0.30*
MFI of CD80$^+$ in Macrophages	256.0 (186.0-363.0)	247.0 (183.5-323.0)	287.0 (193.5-341.5)	212.0 (173.8-321.5)	0.92*
MFI of CD86$^+$ in Macrophages	1200.0 (1046.0-1356.5)	1113.0 (1010.5-1275.0)	1190.0 (919.5-1502.0)	1236.5 (1140.3-1400.8)	0.48*
MDSCs% of PBMCs, median (IQR)					

Characteristics	Group (N=51)				*Overall* *p* value
	Recovered SPs (n=20)	Recovered MPs (n=14)	Recovered APs (n=9)	Healthy controls (n=8)	
Total MDSCs ($CD11b^+$ $CD33^+$ I	1.61 (0.78-2.21)	1.36 (0.78-1.50)	2.33 (1.22-2.50)	1.85 (1.49-2.10)	0.30*
PMN-MDSCs ($CD11b^+$ $CD33^+$ HLA-DR^- $CD14^-$ $CD15^+$), %	0.16 (0.05-0.23)	0.17 (0.12-0.58)	0.51 (0.24-0.81)	0.15 (0.07-0.33)	0.13*
MO-MDSCs ($CD11b^+$ $CD33^+$ HLA-DR^- $CD14^+$ $CD15^-$), %	0.05 (0.03-0.08)	0.05 (0.03-0.07)	0.05 (0.03-0.15)	0.07 (0.05-0.08)	0.59*
PMN-MDSCs/MO-MDSCs	3.27 (1.54-6.59)	5.94 (1.87-9.29)	7.25 (4.78-10.55)	2.33 (1.64-4.70)	0.34*

Note: Data were presented as median (interquartile range, IQR) for continuous variables and n (%) for category variables. Kruskal-Wallis (K-W) test was used for continuous variables and chi-square test or fisher's exact test for all category variables. False discovery rate (FDR) correction was performed at the FDR < 0.05 significance threshold for the comparison of these variables, but all > 0.05. *: Kruskal-Wallis (K-W) test. †: χ^2 test. &: One-way ANOVA;

Abbreviations: SPs: severe/critical patients; MPs: mild/moderate patients; Aps: asymptomatic patients; IQR: interquartile range; BMI: body mass index; PBMC: peripheral blood mononuclear cells; DC: dendritic cells; MFI: mean fluorescence intensity; NK cells: natural killer cells; NKT cells: natural killer T cells; MDSC: myeloid-derived suppressor cell; PMN: polymorphonuclear; MO: mononuclear.

Figure legends

Figure 1. Plasma level of cytokines involving vascular injury and repair in recovered COVID19 patients and healthy controls.

Class-1 cytokines involving vascular injury and repair/angiogenesis were measured in recovered severe/critical patients (SPs, n=19), mild/moderate patients (MPs, n=20), asymptomatic patients (APs, n=16) and healthy controls (HCs, n=17) by Meso Scale Discovery (MSD) Multiplexed Immunoassay. **(A)** Statistical analysis of cytokines related to vascular injury, including VCAM-1, ICAM-1. **(B)** Statistical analysis of cytokines related to vascular repair, including bFGF, PlGF, Tie-2. **(B)** Statistical analysis of cytokines related to angiogenesis, including VEGF family (VEGF-A, VEGF-C, VEGF-D) and its receptor (Flt-1). Data were expressed as boxplots with median and range. Each dot represents an individual subject: recovered SPs (red), recovered MPs (brown), recovered APs (green) or HCs (blue). Significance of comparisons of the four groups was determined by the Kruskal-Wallis test (data with non-normal distribution) or one-way ANOVA (data with normal distribution) and indicated as an absolute overall *p* value. False discovery rate (FDR) correction was first applied for all the 44 cytokines. For variables with overall p value < 0.05 and FDR < 0.05, pairwise subgroup comparison (recovered SPs vs. HCs, MPs vs. HCs, APs vs. HCs, SPs vs. MPs, SPs vs. APs, and MPs vs. APs) was performed with Bonferroni correction method, and the significance was indicated as: ns, not significant; *$p < 0.0083$ (0.05/6), **$p < 0.0017$ (0.01/6), ***$p < 0.00017$ (0.001/6), ****$p < 0.000017$ (0.0001/6).

Figure 2. Plasma level of cytokines involving immune cell growth and differentiation in recovered COVID19 patients and healthy controls.

Class-2 cytokines involving immune cell growth and differentiation were measured in recovered severe/critical patients (SPs, n=19), mild/moderate patients (MPs, n=20), asymptomatic patients (APs, n=16) and healthy controls (HCs, n=17) by Meso Scale Discovery (MSD) Multiplexed Immunoassay. Statistical analysis of **(A)** GM-CSF, **(B)** IL-2, **(C)** IL-4, **(D)** IL-5, **(E)** IL-7, **(F)** IL-9, **(G)** IL-15, and **(H)** TSLP were displayed in the form of scatter plots. Data were expressed as boxplots with median and range. Each dot represents an individual subject: recovered SPs (red), recovered MPs (brown), recovered APs (green) or HCs (blue). Significance of comparisons of the four groups was determined by the Kruskal-Wallis test (data with non-normal distribution) or one-way ANOVA (data with normal distribution) and

presented as an absolute overall *p* value. False discovery rate (FDR) correction was first applied for all the 44 cytokines. For variables with overall p value < 0.05 and FDR < 0.05, pairwise subgroup comparison (recovered SPs vs. HCs, MPs vs. HCs, APs vs. HCs, SPs vs. MPs, SPs vs. APs, and MPs vs. APs) was performed with Bonferroni correction method, and the significance was indicated as: ns, not significant; *p < 0.0083 (0.05/6), **p < 0.0017 (0.01/6), ***p < 0.00017 (0.001/6), ****p < 0.000017 (0.0001/6).

Figure 3. Plasma level of pro/anti-inflammatory cytokines in recovered COVID19 patients and healthy controls.

Class-3 cytokines related to pro/anti-inflammation were measured in recovered severe/critical patients (SPs, n=19), mild/moderate patients (MPs, n=20), asymptomatic patients (APs, n=16) and healthy controls (HCs, n=17) by Meso Scale Discovery (MSD) Multiplexed Immunoassay. Statistical analysis of **(A)** SAA, IFN-γ, TNF-α, TNF-β, **(B)** IL-1α, IL-1β, IL-1RA, IL-6, **(C)** IL-10, IL-12 (p40), IL-12 (p70), IL-13, and **(D)** IL-17 family (17A, 17B, 17C, 17D) were displayed in the form of scatter plots. Data were expressed as boxplots with median and range. Each dot represents an individual subject: recovered SPs (red), recovered MPs (brown), recovered APs (green) or HCs (blue). Significance of comparisons of the four groups was determined by the Kruskal-Wallis test (data with non-normal distribution) or one-way ANOVA (data with normal distribution) and presented as an absolute overall *p* value. False discovery rate (FDR) correction was first applied for all the 44 cytokines. For variables with overall p value < 0.05 and FDR < 0.05, pairwise subgroup comparison (recovered SPs vs. HCs, MPs vs. HCs, APs vs. HCs, SPs vs. MPs, SPs vs. APs, and MPs vs. APs) was performed with Bonferroni correction method, and the significance was indicated as: ns, not significant; *p < 0.0083 (0.05/6), **p < 0.0017 (0.01/6), ***p < 0.00017 (0.001/6), ****p < 0.000017 (0.0001/6).

Figure 4. Plasma level of chemokines in recovered COVID19 patients and healthy controls.

Class-4 cytokines involving chemotaxis were measured in recovered severe/critical patients (SPs, n=19), mild/moderate patients (MPs, n=20), asymptomatic patients (APs, n=16) and healthy controls (HCs, n=17) by Meso Scale Discovery (MSD) Multiplexed Immunoassay. Statistical analysis of chemotactic factor **(A)** Eotaxin, Eotaxin-3, **(B)** IP-10, MCP-1, MCP-4, MIP-1α, MIP-1β, MDC, and **(C)** TARC, IL-8,

IL-16 were displayed in the form of scatter plots. Data were expressed as boxplots with median and range. Each dot represents an individual subject: recovered SPs (red), recovered MPs (brown), recovered APs (green) or HCs (blue). Significance of comparisons of the four groups was determined by the Kruskal-Wallis test (data with non-normal distribution) or one-way ANOVA (data with normal distribution) and presented as an absolute overall *p* value. False discovery rate (FDR) correction was first applied for all the 44 cytokines. For variables with overall p value < 0.05 and FDR < 0.05, pairwise subgroup comparison (recovered SPs vs. HCs, MPs vs. HCs, APs vs. HCs, SPs vs. MPs, SPs vs. APs, and MPs vs. APs) was performed with Bonferroni correction method, and the significance was indicated as: ns, not significant; *p < 0.0083 (0.05/6), **p < 0.0017 (0.01/6), ***p < 0.00017 (0.001/6), ****p < 0.000017 (0.0001/6).

Figure 5. Correlation between cytokines and abnormal clinical features in recovered COVID-19 patients.

Correlation matrices of cytokines and abnormal clinical features in 55 recovered COVID-19 patients (including recovered APs: n=19, MPs: n=20, and APs: n=16).

(A) Spearman correlation of cytokines with laboratory markers (Cys-C, LDH, CRP, and APTT). **(B)** Spearman correlation of cytokines with residual CT abnormities (Total lesion%, GGO%, and solid component%). **(C)** Spearman correlation of cytokines with pulmonary function tests (PFT, including DLCO pred%, TLC pred%, and RV pred%). These correlations were calculated by 55 recovered COVID-19 patients pooled as one group, using the value of each variable for each patient. Only cytokines of significant correlations were displayed. Significance was determined by two-tailed, Spearman correlation analysis: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. And the correlation coefficients were visualized by colour intensity and dot size.

Figure 1 Recovered SPs Recovered MPs Recovered APs Healthy controls

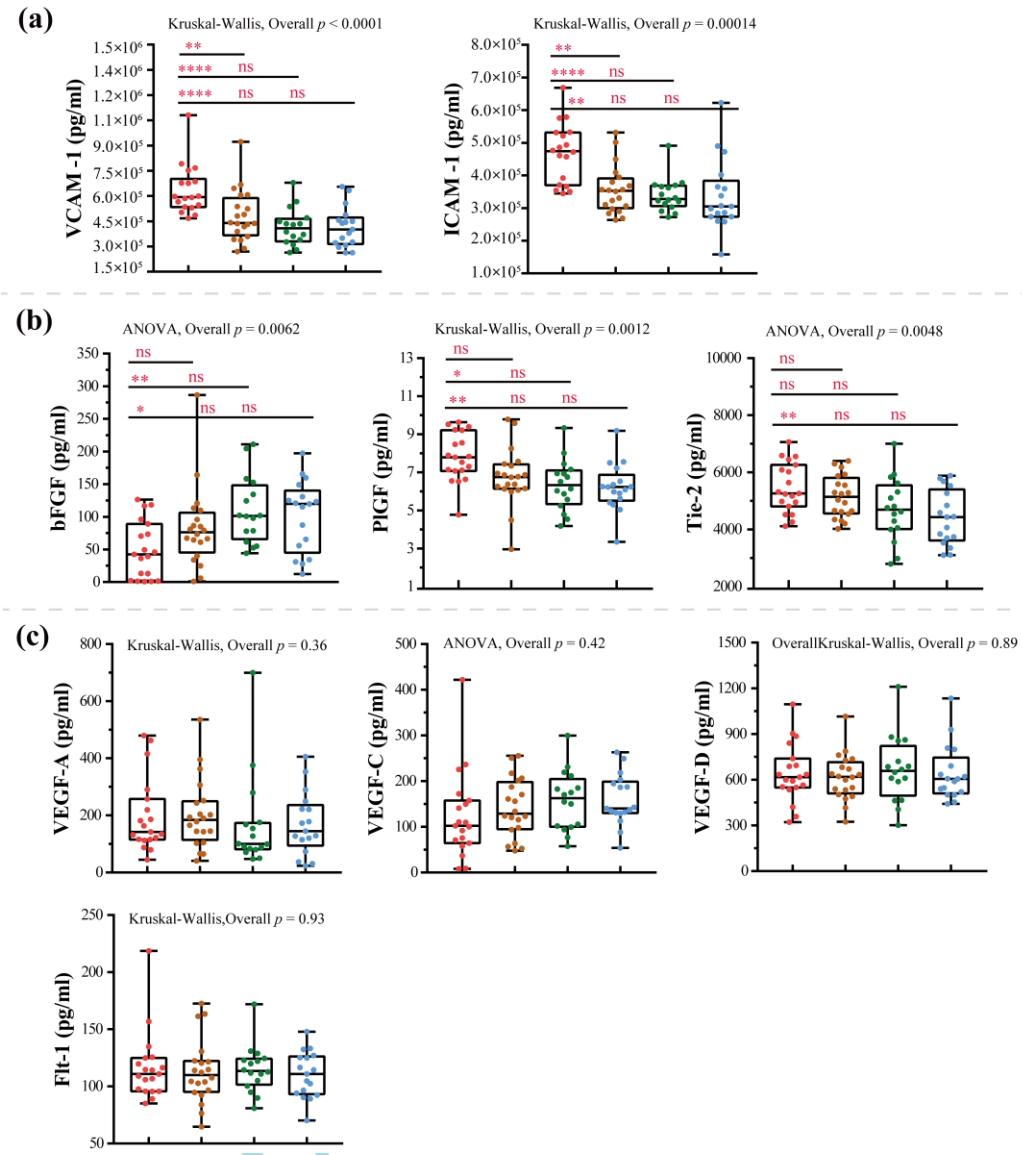


Figure 2

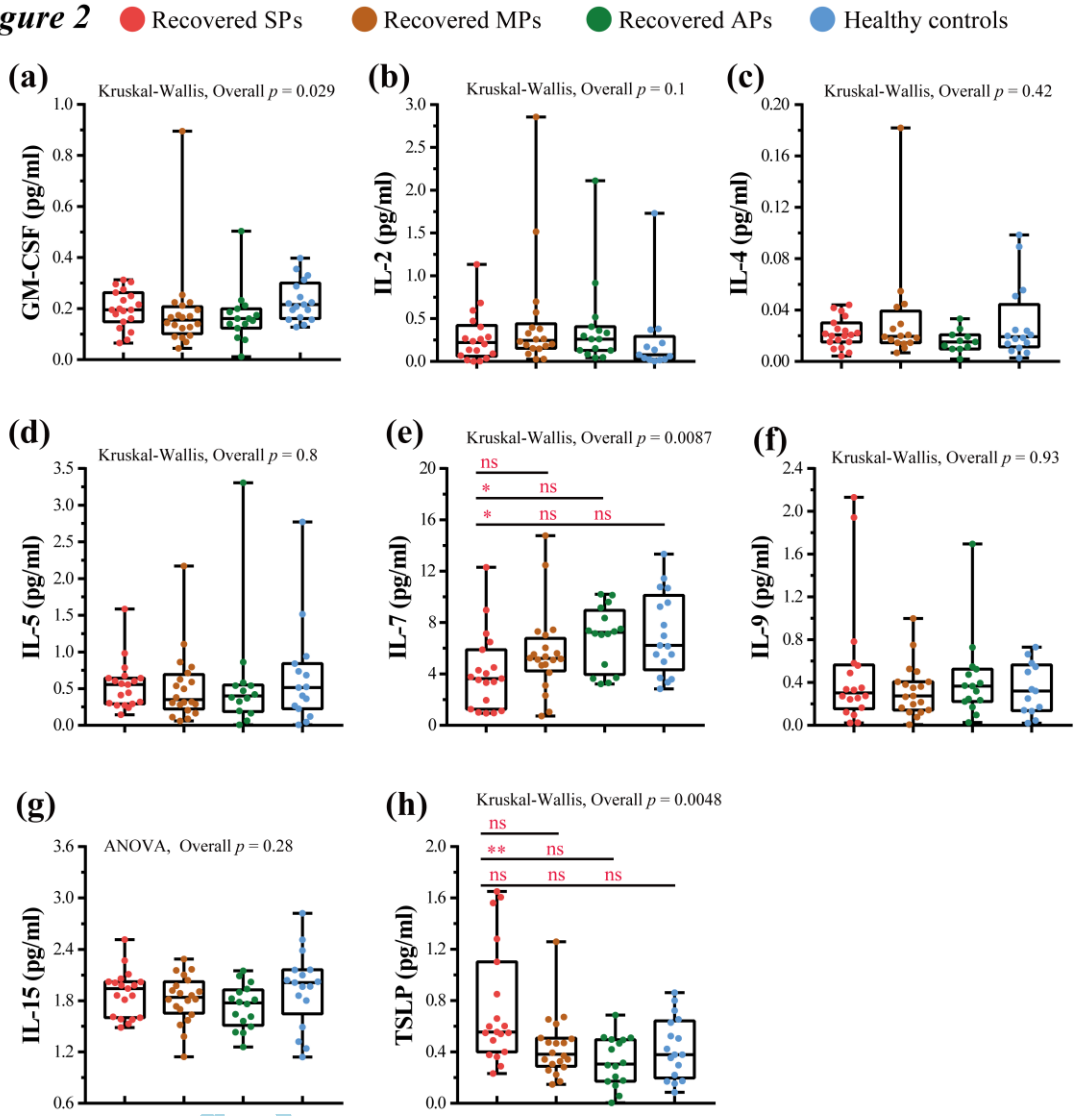


Figure 3 Recovered SPs Recovered MPs Recovered APs Healthy controls

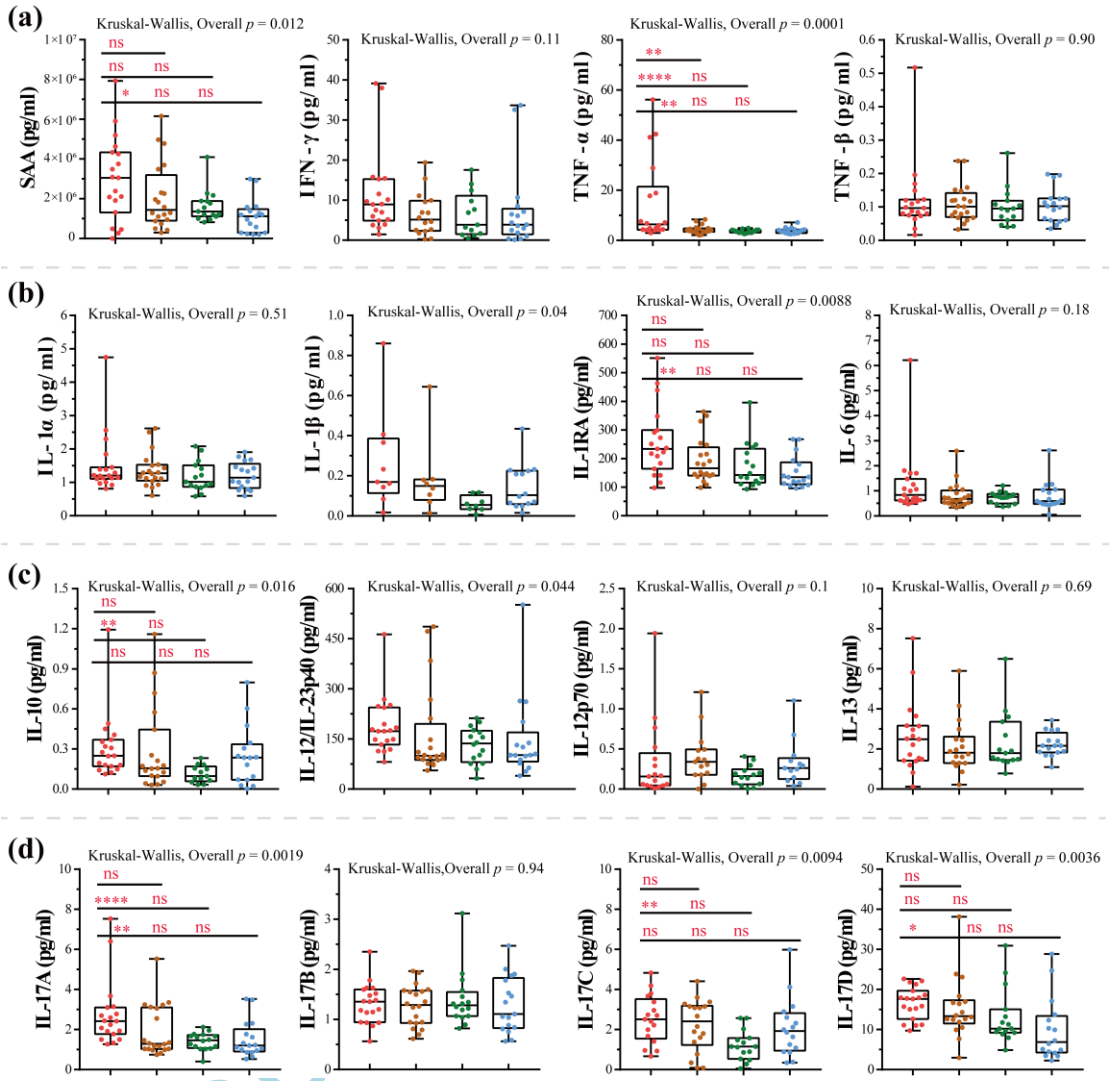


Figure 4 Recovered SPs Recovered MPs Recovered APs Healthy controls

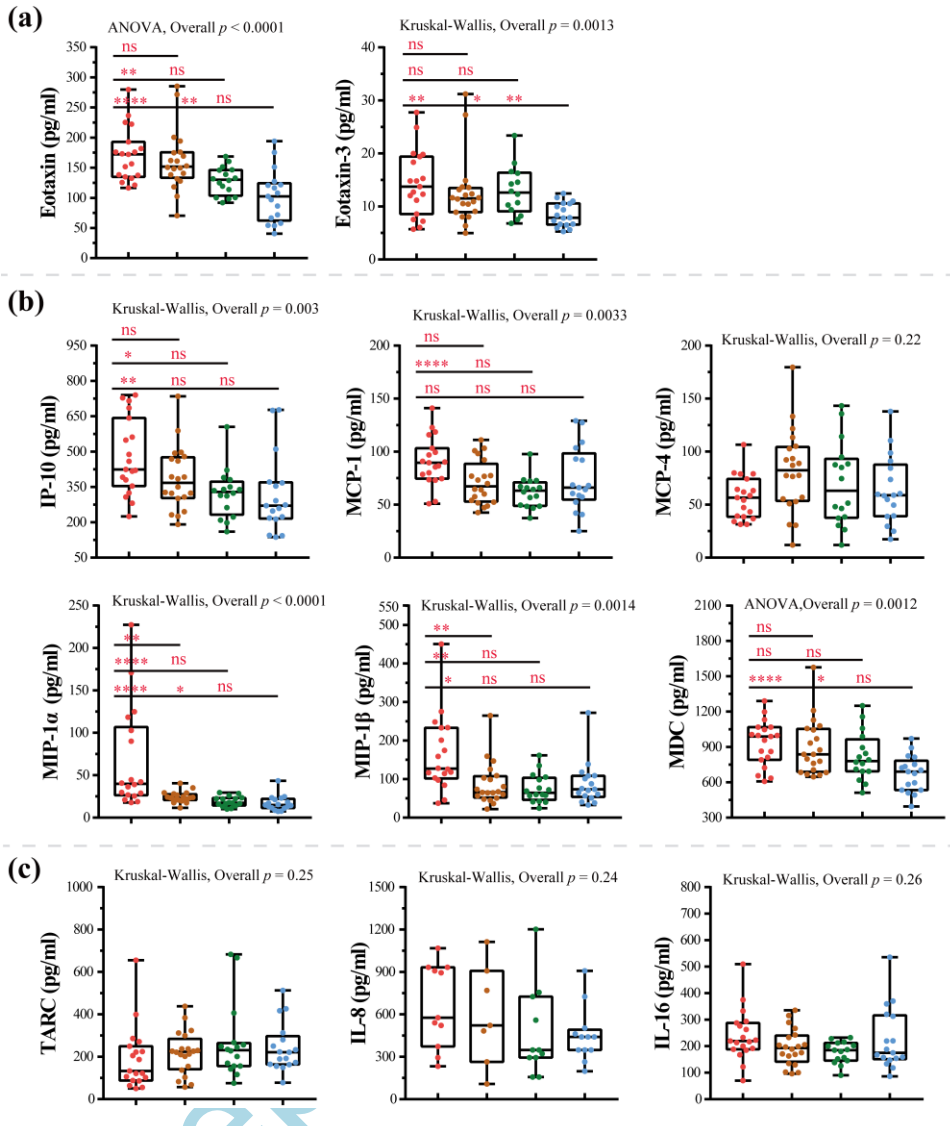
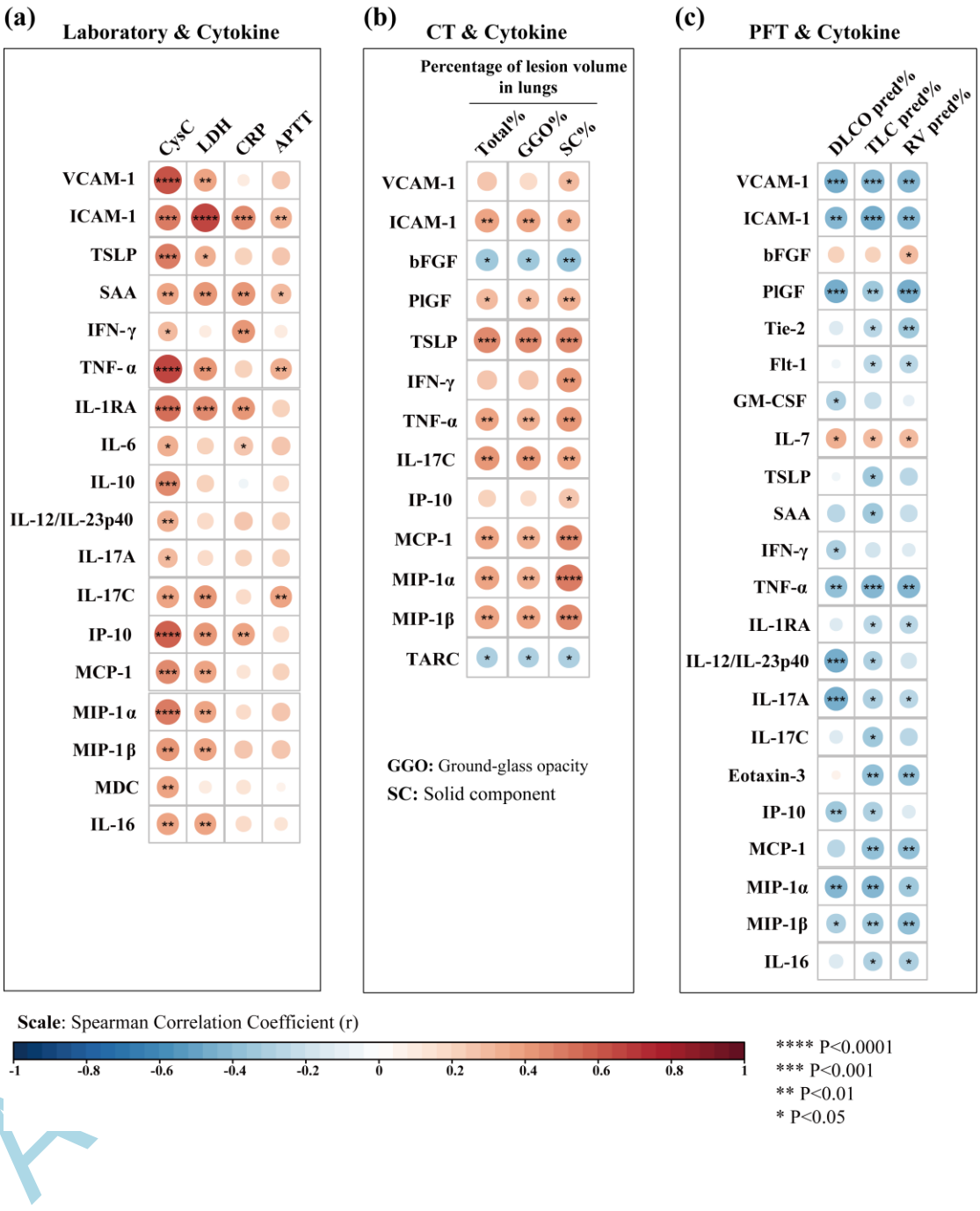


Figure 5


(a)
Laboratory & Cytokine
CysC
LDH
CRP
APTT
VCAM-1
ICAM-1
TSLP
SAA
IFN-γ
TNF-α
IL-1RA
IL-6
IL-10
IL-12/IL-23p40
IL-17A
IL-17C
IP-10
MCP-1
MIP-1α
MIP-1β
MDC
IL-16
(b)
CT & Cytokine
Percentage of lesion volume in lungs
Total%
GGO%
SC%
VCAM-1
ICAM-1
bFGF
PlGF
TSLP
IFN-γ
TNF-α
IL-17C
IP-10
MCP-1
MIP-1α
MIP-1β
TARC
GGO: Ground-glass opacity
SC: Solid component
(c)
PFT & Cytokine
DLCO pred%
TLC pred%
RV pred%
VCAM-1
ICAM-1
bFGF
PlGF
Tie-2
Flt-1
GM-CSF
IL-7
TSLP
SAA
IFN-γ
TNF-α
IL-1RA
IL-12/IL-23p40
IL-17A
IL-17C
Eotaxin-3
IP-10
MCP-1
MIP-1α
MIP-1β
IL-16
Scale: Spearman Correlation Coefficient (r)
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
**** P<0.0001
*** P<0.001
** P<0.01
* P<0.05