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Inflammatory Profiles and Clinical Features of Coronavirus 2019 Survivors 3 Months After Discharge in Wuhan, China

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Background. Postdischarge immunity and its correlation with clinical features among patients recovered from coronavirus disease 2019(COVID-19) are poorly described. This prospective cross-sectional study explored the inflammatory profiles and clinical recovery of patients with COVID-19 at 3 months after hospital discharge.

Methods. Patients with COVID-19 discharged from 4 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. The72 age-, sex- and body mass index–matched participants included 19 patients with severe/critical COVID-19 (SPs), 20 patients with mild/moderate COVID-19 (MPs), 16 APs, and 17 HCs. At 3 months after discharge, levels of proinflammatory cytokines and factors related to vascular injury/repair in patients recovered from COVID-19 had not returned to those of the HCs, especially among recovered SPs compared with recovered MPs and APs. These cytokines were significantly correlated with impaired pulmonary function and chest computed tomographic 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 patients with COVID-19 and were correlated with abnormal clinical features 3 months after discharge, especially in recovered SPs.

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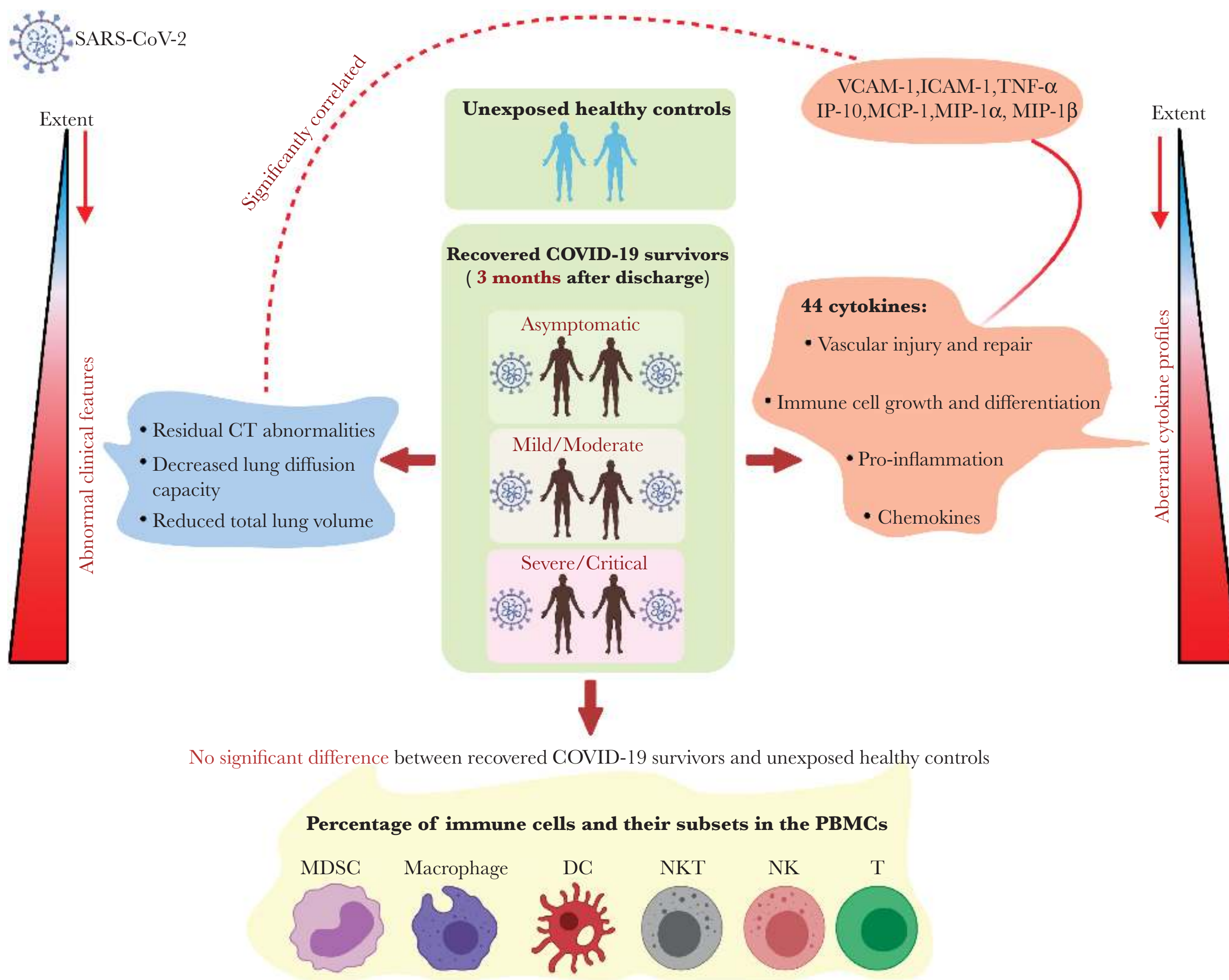
Graphical Abstract



Keywords: Recovered COVID-19 patients; 3 months after discharge; cytokine profiles; immune cells; clinical features.

The ongoing coronavirus disease 2019 (COVID-19) pandemic has, as of 12 November 2020, caused >50 million cases and >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 proinflammatory cytokines, including interleukin 6 and 1β (IL-6 and IL-1β), as well as interleukin 2, 8, and 17 (IL-2, IL-8, and IL-17), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN) 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 proinflammation markers are strongly correlated with worse outcomes and death in patients with COVID-19 [6, 7], suggesting that poor clinical outcomes might be attributed to virus-driven hyperinflammation. It remains unknown, however, how this pathological immunity will evolve and whether it is related to undesirable sequelae among discharged patients recovered from COVID-19.

Two 2020 studies reported that the levels of immune cells, including neutrophils, monocytes, NK cells, and B and T lymphocytes, returned to nearly baseline levels in individuals recovered from untreated COVID-19

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[8, 9]. Convalescent-phase SARS-CoV-2–specific T cells are polyfunctional and display a stemlike memory phenotype [10]. However, the recovery time after patient discharge was notunified in these studies, and few reports have focused on the recovery of soluble proinflammatory, chemotaxis, and endothelial injury–related markers, which is important for understanding patient immune outcomes and formulating rehabilitation strategies for recovered patients. Thus, the current study explored the immunological profiles and clinical characteristics of patients recovered from COVID-19 with different disease severities 3 months after hospital 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 patients with COVID-19 discharged between 5 and 31 March 2020, from 4 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 (by telephone) and testing, 3 months after hospital discharge, were carried out by trained medical staff in the outpatient clinic of Wuhan Union Hospital. 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 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 patients recovered from COVID-19 and HCs (recruitment details are shown in Supplementary Figure 1). The patients recovered from COVID-19 were grouped by disease severity during their infection period (patients with severe/critical COVID-19 [SPs], patients with mild/moderate COVID-19 [MPs], and APs) according to World Health Organization interim guidance [11]. When interviewed, the participants underwent physical examination, pulmonary function testing, and chest computed tomography (CT). 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 Web site (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, Artificial Intelligence–Based Quantitative Analysis of CT Images, and Pulmonary Function Test

The standard protocols were as reported elsewhere [12–15] and are described in detail in the Supplementary Methods.

Multiplex 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 6 Meso Scale Discovery V-PLEX multiplex assay panels (V-PLEX; K15198D, K15190D, K15049D, K15050D, K15084D, and K15047D) on a Meso Scale Discovery SQ120 instrument, 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, VEGF receptor 1, GM-CSF, IL-2, interleukin 4, 5, 7, 9, and 15 (IL-4, IL-5, IL-7, IL-9, and IL-15), thymic stromal lymphopoietin (TSLP), serum amyloid A (SAA) protein, IFN-γ, TNF-α, TNF-β, interleukin 1α (IL-1α), IL-1β, interleukin 1RA (IL-1RA), IL-6, interleukin 10 (IL-10), interleukin 12 (IL-12)/interleukin 23 (p40), IL-12p70, interleukin 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 interleukin 16 (IL-16).

Flow Cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by means of standard density gradient centrifugation and were used immediately. The isolated PBMCs were stained with fluorochrome-conjugated human monoclonal antibodies (all from BD Biosciences; Supplementary Table 1) to determine the percentage of immune cells in PBMCs, that is, T lymphocytes (anti-CD3, anti-CD4, andanti-CD8), NK cells, NK T (NKT) cells, macrophages, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), and their subsets. Cell acquisition was performed with 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 (with interquartile range) or means (with standard deviations) for continuous variables and absolute values with percentages for categorical variables. For the comparison of clinical characteristics (laboratory

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Table 1. Clinical Characteristics 3 Months After Hospital Discharge in Patients Recovered From Coronavirus Disease 2019

	Median (IQR) Value[a]				P Value[b]			
						Comparison with HCs		
Characteristics	Recovered SPs (n = 19)	Recovered MPs (n = 20)	Recovered APs (n = 16)	HCs (n = 17)	Overall	Recovered SPs	Recovered MPs	Recovered APs
Age, y	60.00 (57.0–64.00)	56.50 (52.25–63.00)	57.00 (52.75–62.00)	57.00 (51.50–61.50)	.19[c]	…	…	…
Male sex, no. (%)	8 (42.1)	5 (25.0)	7 (43.8)	7 (41.2)	.60[d]	…	…	…
BMI, mean (SD)[e]	24.84 (2.62)	24.32 (2.31)	24.73 (2.20)	23.10 (3.32)	.20[f]	…	…	…
Comorbid conditions, no. (%)	14 (73.7)	10 (50.0)	6 (37.5)	3 (17.6)	.008[d]	.001[g]	.08	.26
Hypertension	9 (47.4)	7 (35.0)	3 (18.8)	3 (17.6)	.16[d]	…	…	…
Diabetes	8 (42.1)	3 (15.0)	2 (12.5)	0 (0.0)	.008[h]	.003[g]	.23	.23
Heart disease	2 (10.5)	1 (5.0)	1 (6.2)	0 (0.0)	.73[h]	…	…	…
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)	.54[h]	…	…	…
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)	>.99[h]	…	…	…
Time from illness onset to follow-up, mean (SD), d	139.79 (7.41)	133.75 (9.64)	…	…	.04	…	…	…
Length of hospital stay, d	47.00 (31.00–51.00)	22.00 (17.25–25.00)	…	…	<.001	…	…	…
Immune-related treatment, no. (%)								
Corticosteroids	5 (26.3)	2 (10.0)	…	…	.24[h]	…	…	…
Intravenous immunoglobulin	3 (15.8)	2 (10.0)	…	…	.66[h]	…	…	…
Serum antibody positive, no. (%)								
IgM	3 (16.7)	3 (15.0)	1 (6.2)	0 (0.0)	.34[h]	…	…	…
IgG	17 (94.4)	19 (95.0)	14 (87.5)	0 (0.0)	<.001[d]	<.001[g]	<.001[g]	<.001[g]
Laboratory findings								
Hematologic								
Blood cell count, $\times 10^9$/L								
WBCs	5.71 (4.97–6.22)	4.85 (4.26–5.55)	5.85 (4.52–6.66)	4.83 (4.34–6.40)	.27[c]	…	…	…
Neutrophils	3.59 (2.89–4.01)	3.02 (2.54–3.42)	3.70 (2.74–4.22)	3.44 (2.34–3.72)	.20[c]	…	…	…
Lymphocytes, mean (SD)	1.67 (0.45)	1.64 (0.36)	1.71 (0.48)	1.74 (0.37)	.90[f]	…	…	…
Monocytes	0.29 (0.25–0.36)	0.25 (0.21–0.27)	0.31 (0.26–0.35)	0.29 (0.27–0.36)	.09[c]	…	…	…
Eosinophils	0.09 (0.06–0.12)	0.06 (0.05–0.10)	0.08 (0.06–0.18)	0.06 (0.05–0.09)	.18[c]	…	…	…
Neutrophil–lymphocyte ratio, mean (SD)	2.18 (0.77)	1.88 (0.44)	2.18 (0.54)	1.94 (0.62)	.31[f]	…	…	…
Liver function								
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)	.41[c]	…	…	…
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)	.23[c]	…	…	…
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)	.53[c]	…	…	…
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)	.97[c]	…	…	…
ALP, mean (SD), U/L	85.42 (19.40)	80.80 (17.59)	78.81 (19.84)	72.41 (13.80)	.19[f]	…	…	…
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)	.89[c]	…	…	…
A/G ratio	1.50 (1.45–1.70)	1.60 (1.50–1.72)	1.60 (1.60–1.70)	1.60 (1.50–1.70)	.58[c]	…	…	…
Renal function								
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)	.70[c]	…	…	…
SUN, 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)	.99[c]	…	…	…
UA, mean (SD), μmol/L	369.58 (103.90)	328.93 (66.82)	341.52 (81.03)	361.76 (95.67)	.47[f]	…	…	…
CysC, 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)	.02[c]	…	…	…
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)	.08[c]	…	…	…

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Table 1. Continued

Characteristics	Median (IQR) Value[a]				*P* Value[b]			
						Comparison with HCs		
	Recovered SPs (n = 19)	Recovered MPs (n = 20)	Recovered APs (n = 16)	HCs (n = 17)	Overall	Recovered SPs	Recovered MPs	Recovered APs
CRP, 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)	.09[c]	…	…	…
Coagulation function								
Platelet count, mean (SD), $\times 10^9$/L	199.05 (48.37)	206.50 (46.61)	231.81 (64.25)	216.47 (57.65)	.32[f]	…	…	…
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)	.32[c]	…	…	…
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)	.16[c]	…	…	…
APTT, mean (SD), s	37.91 (2.93)	34.99 (2.88)	35.89 (3.23)	35.79 (3.47)	.03[f]	…	…	…
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)	.09[c]	…	…	…
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)	.16[c]	…	…	…
Chest CT findings								
Residual CT lesion, no. (%)	18 (94.7)	16 (80.0)	4 (25.0)	5/14 (35.7)	<.001[d]	<.001[g]	.01[g]	.69
Bilateral lung involvement, no. (%)	16 (84.2)	11 (55.0)	4 (25.0)	1/14 (7.1)	<.001[d]	<.001[g]	.009[g]	.34
Volume of total lesion in lungs, %	0.59 (0.08–2.19)	0.04 (0.01–0.18)	0.00 (0.00–0.02)	0.01 (0.00–0.02)	<.001[c]	<.001[g]	.02[g]	.47
GGO lesion, no. (%)	16 (84.2)	14 (70.0)	4 (25.0)	4/14 (28.6)	<.001[d]	.003[g]	.04	>.99
Volume of GGO in lungs, %	0.56 (0.07–2.17)	0.04 (0.01–0.14)	0.00 (0.00–0.01)	0.01 (0.00–0.01)	<.001[c]	<.001[g]	.01[g]	.28
SC, no. (%)	17 (89.5)	7 (35.0)	2 (12.5)	2/14 (14.3)	<.001[d]	<.001[g]	.25	>.99
Volume of SC in lungs, %	0.02 (0.01–0.11)	0.00 (0.00–0.01)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	<.001[c]	<.001[g]	.22	.68
Striplike fibrosis, no. (%)	17 (89.5)	6 (30.0)	2 (12.5)	2/14 (14.3)	<.001[d]	<.001[g]	.42	>.99
Reticular opacity, no. (%)	2 (10.5)	3 (15.0)	0 (0.0)	0 (0.0)	.23[h]	…	…	…
Pulmonary function								
Lung volume								
TLC (L), % predicted	83.50 (74.00–89.55)	100.60 (91.67–111.12)	95.55 (91.68–103.20)	98.90 (94.80–108.10)	<.001[c]	<.001[g]	.84	.20
TLC <80% predicted, no. (%)	8 (42.1)	0 (0.0)	2 (12.5)	0 (0.0)	<.001[h]	.003[g]	…	.23
FVC (L), % predicted	102.70 (91.50–111.40)	117.95 (107.77–134.73)	106.2 (102.40–115.42)	105.60 (99.90–122.60)	.02[c]	.38	.049	.82
FVC <80% predicted, no. (%)	1 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)	.72[h]	…	…	…
RV (L), % predicted	74.50 (61.15–90.50)	98.70 (91.62–104.98)	96.55 (84.22–111.78)	105.50 (92.20–112.20)	<.001[c]	<.001[g]	.28	.26
RV <65% predicted, no. (%)	6 (31.6)	0 (0.0)	0 (0.0)	0 (0.0)	<.001[h]	.02	…	…
Diffusion capacity								
DLCO (mmol/min/kPa), % predicted	74.90 (66.15–77.95)	85.40 (75.85–95.02)	87.95 (83.88–92.55)	93.80 (86.40–99.00)	<.001[c]	<.001[g]	.13	.21
DLCO <80% predicted, no. (%)	19 (100.0)	7 (35.0)	2 (12.5)	0 (0.0)	<.001[d]	<.001[g]	.009[g]	.23
DLCO/VA, mean (SD), % predicted	90.33 (13.08)	89.50 (12.24)	98.08 (14.34)	95.71 (12.11)	.15[f]	…	…	…
DLCO/VA <80% predicted, no. (%)	5 (26.3)	3 (15.0)	1 (6.2)	2 (11.8)	.47[h]	…	…	…

Abbreviations: A/G, albumin-globulin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; APs, asymptomatic patients; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; CT, computed tomographic; CysC, cystatin C; DLCO, diffusing capacity of the lung for carbon monoxide; FIB, fibrinogen; FVC, forced vital capacity; GGO, ground-glass opacity; HCs, healthy controls; Ig, immunoglobulin; IQR, interquartile range; LDH, lactate dehydrogenase; MPs, patients with mild/moderate coronavirus disease 2019 (COVID-19); PT, prothrombin time; RV, residual volume; SC, solid component; SD, standard deviation; SPs, patients with severe/critical COVID-19; SUN, serum urea nitrogen; TLC, total lung capacity; TT, thrombin time; UA, uric acid; VA, alveolar ventilation; WBCs, white blood cells.

[a]Data represent median (IQR) values unless otherwise specified.

[b]Kruskal-Wallis test or 1-way analysis of variance (ANOVA) were used for analysis of continuous variables, and χ^2 test or Fisher exact test for analysis of all categorical variables between 4 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 pulmonary function among the 4 groups. For variables with an overall *P* value <.05 and an FDR <0.05, we performed subgroup comparisons, otherwise left as blank cells. Bonferroni correction was conducted for subgroup comparison, and the corrected significance threshold for subgroup *P* values is .017 (.05/3).

[c]*P* value determined with Kruskal-Wallis test.

[d]*P* value determined with χ^2 test.

[e]BMI calculated as weight in kilograms divided by height in meters squared.[f]*P* value determined with 1-way ANOVA.

[g]$P < .017$, statistically significant between the subgroup comparison.

[h]*P* value determined with Fisher exact test.

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findings, pulmonary function, and CT scans), percentage of immune cells, and 44 cytokines between the 4 groups (recovered SPs, MPs, APs, and HCs), we used Kruskal-Wallis tests (data with nonnormal distribution), 1-way analysis of variance (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 a significance threshold of FDR <0.05. Then, for variables with an overall *P* value <.05 and an 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* (data with nonnormal distribution), independent *t* (data with normal distribution), $\chi 2$, and Fisher exact tests as appropriate, with Bonferroni correction, and we used 2-sided $P < .05/n$ (where n is the number of comparisons) as the threshold to determine whether the difference between 2 subgroups was statistically significant. The associations between cytokine profiles and abnormal clinical features in patients recovered from COVID-19 were examined using Spearman correlation analysis and visualized with correlation matrix plots. All tests were 2 sided and performed using R (version 4.0.2; R Foundation) or SPSS (version 26) software.

RESULTS

Clinical Characteristics of the Study Populations

This study enrolled 3 groups of patients recovered from COVID-19 at 3 months after hospital discharge (19 recovered SPs, 20 recovered MPs, and 16 recovered APs). 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 (139.79 vs 133.75 days, respectively, for recovered SPs vs recovered MPs), and the median length of hospital stay was significantly longer in recovered SPs than that in recovered MPs (47.0 vs 22.0 days, respectively; $P < .001$). Most recovered patients tested positive for serum SARS-COV-2 immunoglobulin G, with a few still positive for immunoglobulin M.

The laboratory findings of the 4 groups revealed that levels of all indicators had returned to normal and were comparable to those in the HCs. Comparison of levels of C-reactive protein and some hematologic markers at discharge and 3 months later revealed that only the monocyte counts were significantly decreased after 3 months (Supplementary Table 2). Artificial intelligence–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, 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 patients recovered from COVID-19 3 months after discharge. However, striplike fibrosis, a solid component newly formed during the recovery period, was more common in recovered SPs than in recovered 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 percentage values for diffusing capacity of the lung for carbon monoxide (DLCO) , total lung capacity , and residual volume in the recovered SPs, but not in MPs and APs. The ventilatory capacity of pulmonary function showed no significant differences (Supplementary Table 3).

Cytokine Profiles of Patients Recovered From COVID-19 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 experienced post–COVID-19 organ damage (fibrosis on CT images and decreased lung volume and DLCO predicted percentage). To assess the state of inflammation in these survivors, we measured the levels of 44 plasma cytokines in SPs, MPs, APs, and HCs using a Meso Scale Discovery multiplex immunoassay (Figures 1–4). The plasma cytokines were categorized into 4 classes.

Class 1 cytokines are associated with vascular injury and repair/angiogenesis (Figure 1). We discovered that levels of VCAM-1, ICAM-1, PlGF, and Tie-2 were significantly elevated in recovered SPs compared with HCs, whereas bFGF exhibited the opposite change, but MPs and APs showed no significant difference compared with HCs. Meanwhile, VEGF family and its VEGF receptor 1 showed no significant differences between these 4 groups (Figure 1C).

Class 2 cytokines promote immune cell growth and differentiation (Figure 2). Comparison of these cytokines revealed that IL-7 levels were significantly decreased in recovered SPs compared with APs and HCs, while TSLP levels were relatively higher in recovered SPs. However, no significant differences were found in GM-CSF, IL-2, 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 T-helper 2–dependent allergic airway inflammation [18]. Accordingly, recovered SPs tended to have a certain degree of T-cell immune perturbation.

Class 3 cytokines are proinflammatory immune factors (Figure 3**).** We found significant up-regulation of SAA protein and TNF-α in recovered SPs, but not in MPs and APs. IL-1α and IL-1β levels did not differ significantly between the 4 groups. However, IL-1RA was significantly elevated in SPs. The levels of IL-6 and IL-10 in patients recovered from COVID-19, which

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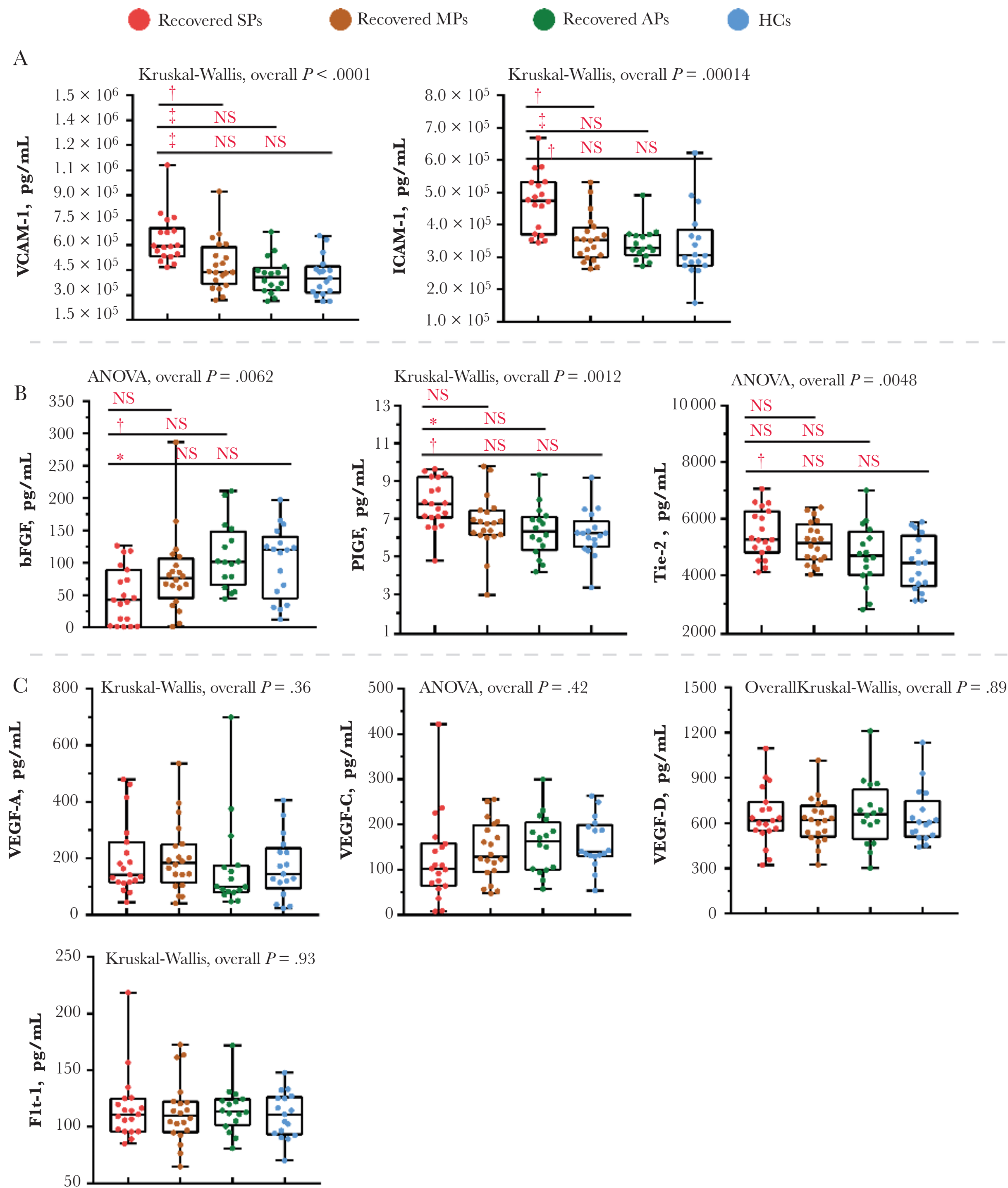

Figure 1. Plasma level of cytokines involving vascular injury and repair in patients recovered from coronavirus disease 2019 (COVID-19) and healthy controls. Class 1 cytokines involving vascular injury and repair/angiogenesis were measured in recovered patients with severe/critical COVID-19 (SPs; n = 19), patients with mild/moderate COVID-19 (MPs; n = 20), and asymptomatic patients (APs; n = 16) and healthy controls (HCs; n = 17), using a multiplex immunoassay (Meso Scale Discovery). *A,* Statistical analysis of cytokines related to vascular injury, including vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1). *B,* Statistical analyses of cytokines related to vascular repair, including basic fibroblast growth factor (bFGF), placental growth factor (PIGF), and tyrosine receptor kinase in the endothelium 2 (Tie-2). *C,* Statistical analysis of cytokines related to angiogenesis, including the vascular endothelial growth factor (VEGF) family (VEGF-A, VEGF-C, and VEGF-D) and its receptor (Flt-1). Data are expressed as box plots showing median and interquartile range. Dots represent individual subjects, including recovered SPs (*red*), recovered MPs (*brown*), recovered APs (*green*), and HCs (*blue*). The significance of differences for comparisons of the 4 groups was determined using the Kruskal-Wallis test (for data with nonnormal distribution) or 1-way analysis of variance (ANOVA; for data with normal distribution) and presented as an absolute overall *P* value. False discovery rate (FDR) correction was first applied for all 44 cytokines. For variables with an overall *P* value <.05 and an FDR <0.05, pairwise subgroup comparisons (recovered SPs vs HCs, MPs vs HCs, APs vs HCs, SPs vs MPs, SPs vs APs, and MPs vs APs) were performed with Bonferroni correction. *adjusted $P < .05$; †adjusted $P < .01$; ‡adusted $P < .001$; NS, not significant.

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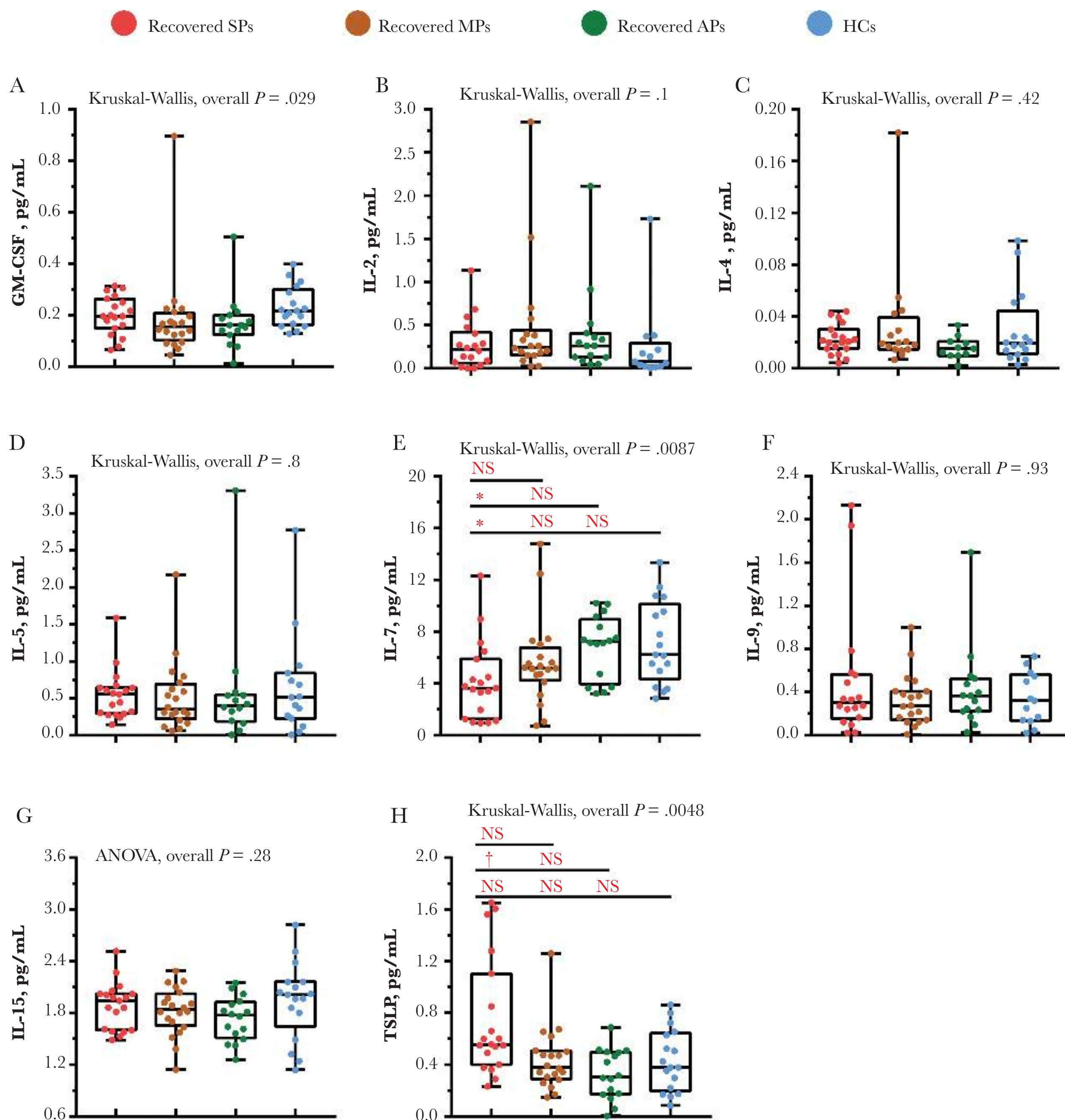

Figure 2. Plasma level of cytokines involving immune cell growth and differentiation in patients recovered from coronavirus disease (COVID-19) and healthy controls. Class 2 cytokines involving immune cell growth and differentiation were measured in recovered patients with severe/critical COVID-19 (SPs; n = 19), patients with mild/moderate COVID-19 (MPs; n = 20), and symptomatic patients (APs; n = 16) and healthy controls (HCs; n = 17), using a multiplex immunoassay (Meso Scale Discovery). Statistical analyses are displayed as scatterplots for granulocyte-macrophage colony-stimulating factor (GM-CSF) (*A*), interleukin 2 (IL-2) (*B*), interleukin 4 (IL-4) (*C*), interleukin 5 (IL-5) (*D*), interleukin 7 (IL-7) (*E*), interleukin 9 (IL-9) (*F*), interleukin 15 (IL-15) (*G*), and thymic stromal lymphopoietin (TSLP) (*H*). Data are expressed as box plots showing median and interquartile range. Dots represent individual subjects, including recovered SPs (*red*), recovered MPs (*brown*), recovered APs (*green*), and HCs (*blue*). The significance of differences for comparisons of the 4 groups was determined using the Kruskal-Wallis test (for data with nonnormal distribution) or 1-way analysis of variance (ANOVA; discovery rate (FDR) correction was first applied for all 44 cytokines. For variables with an overall *P* value <.05 and an FDR <0.05, pairwise subgroup comparisons (recovered SPs vs HCs, MPs vs HCs, APs vs HCs, SPs vs MPs, SPs vs APs, and MPs vs APs) were performed with Bonferroni correction. *adjusted *P* < .05; †adjusted *P* < .01; NS, not significant.

have been widely reported for the stratification of disease severity during acute COVID-19, were almostback to the levels in 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, and MDC) were

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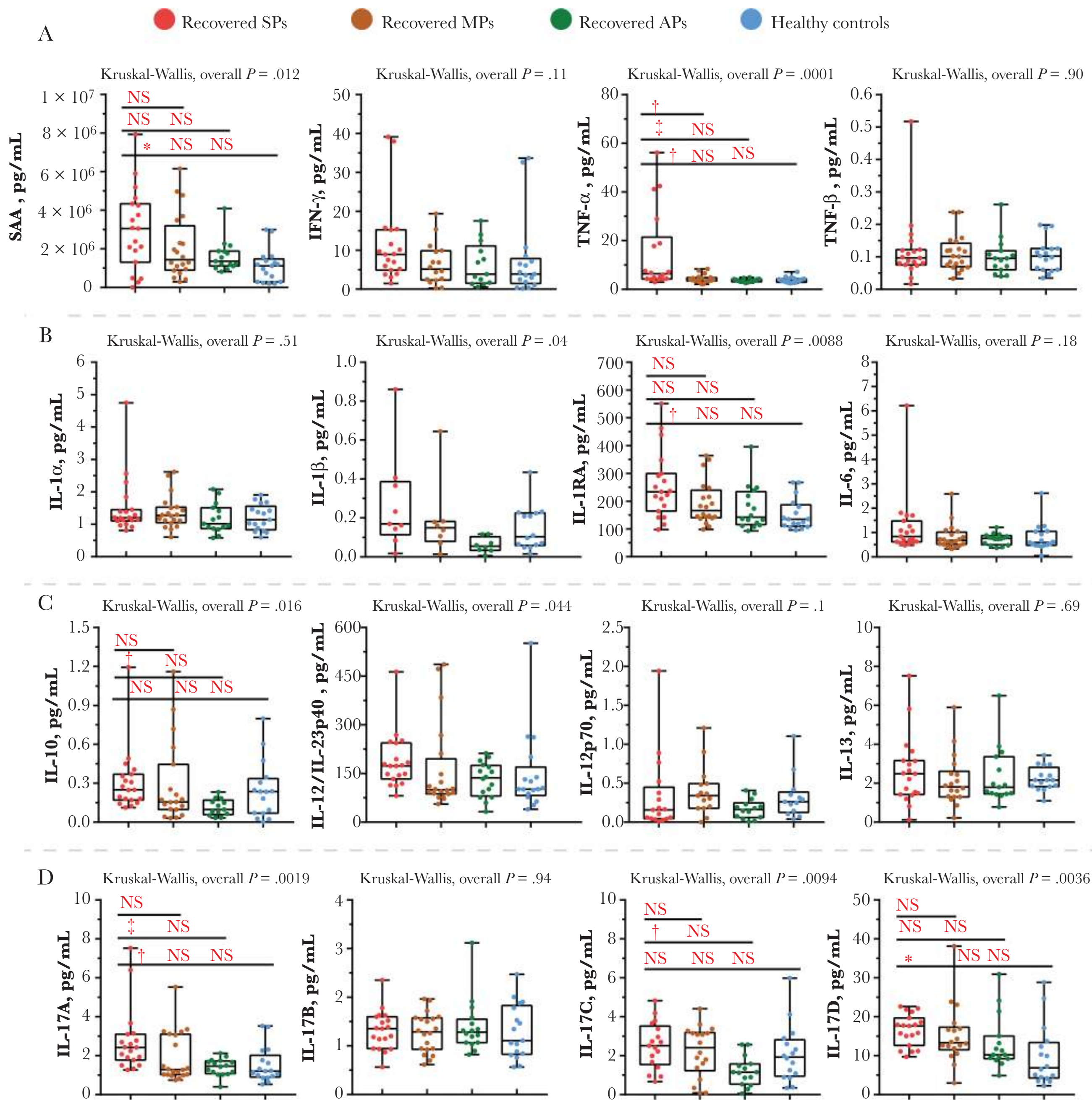

Figure 3. Plasma level of proinflammatory and anti-inflammatory cytokines in patients recovered from coronavirus disease 2019 (COVID-19) and healthy controls (HCs). Class 3 cytokines that were to pro- or anti-inflammatory were measured in recovered patients with severe/critical COVID-19 (SPs; n = 19), patients with mild/moderate COVID-19 (MPs; n = 20), and asymptomatic patients (APs; n = 16) and healthy controls (HCs; n = 17), using a multiplex immunoassay (Meso Scale Discovery). Statistical analyses are displayed as scatterplots for serum amyloid A (SAA) protein, interferon (IFN) γ, tumor necrosis factor (TNF) α, and TNF-β (*A*); interleukin 1α, 1β, 1RA, and 6 (IL-1α, IL-1β, IL-1RA, and IL-6) (*B*); interleukin 10, 12/23 (p40), 12 (p70), and 13 (IL-10, IL-12/IL-23p40, IL-12p70, and IL-13) (*C*); and the interleukin 17 family (IL-17A, IL-17B, IL-17C, and IL-17D) (*D*). Data are expressed as box plots showing median and range. Dots represent individual subjects, including recovered SPs (*red*), recovered MPs (*brown*), recovered APs (*green*), and HCs (*blue*). The significance of differences for comparisons of the 4 groups was determined using the Kruskal-Wallis test (for data with nonnormal distribution) or 1-way analysis of variance (for data with normal distribution) and presented as an absolute overall *P* value. False discovery rate (FDR) correction was first applied for all 44 cytokines. For variables with an overall *P* value <.05 and an FDR <0.05, pairwise subgroup comparisons (recovered SPs vs HCs, MPs vs HCs, APs vs HCs, SPs vs MPs, SPs vs APs, and MPs vs APs) were performed with Bonferroni correction. *adjusted *P* < .05; †adjusted *P* < .01; ‡adusted *P* < .001; NS, not significant.

also significantly higher in recovered SPs than in recovered MPs or 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 4 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.

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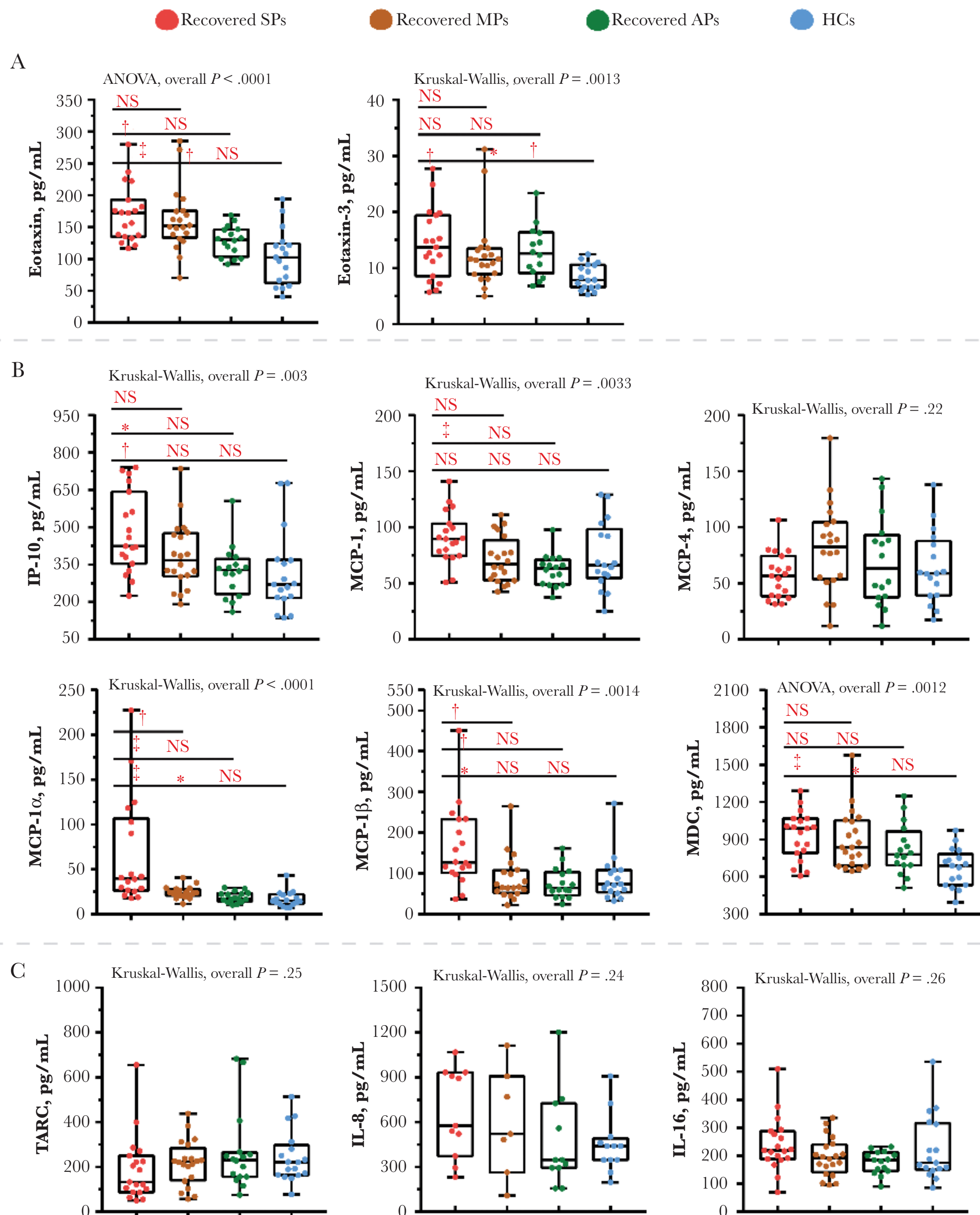

Figure 4. Plasma level of chemokines in patients recovered from coronavirus disease 2019 (COVID-19) and healthy controls. Class 4 cytokines involving chemotaxis were measured in recovered patients with severe/critical COVID-19 (SPs; n = 19), patients with mild/moderate COVID-19 (MPs; n = 20), and asymptomatic patients (APs; n = 16) and healthy controls (HCs; n = 17), using a multiplex immunoassay (Meso Scale Discovery). Statistical analyses are displayed as scatterplots for chemotactic factor eotaxin (*A*); interferon γ–inducible protein 10 (IP-10), monocyte chemotactic protein (MCP) 1, MCP-4, macrophage inflammatory protein (MIP) 1α, MIP-1β, and macrophage-derived chemokine (MDC) (*B*); and thymus activation–regulated chemokine (TARC), interleukin 8 (IL-8), and interleukin 16 (IL-16) (*C*). Data are expressed as box plots showing median and range. Dots represent individual subjects, including recovered SPs (*red*), recovered MPs (*brown*), recovered APs (*green*), and HCs (*blue*). The significance of differences for comparisons of the 4 groups was determined using the Kruskal-Wallis test (for data with nonnormal distribution) or 1-way analysis of variance (ANOVA; for data with normal distribution) and presented as an absolute overall *P* value. False discovery rate (FDR) correction was first applied for all 44 cytokines. For variables with an overall *P* value <.05 and an FDR <0.05, pairwise subgroup comparisons (recovered SPs vs HCs, MPs vs HCs, APs vs HCs, SPs vs MPs, SPs vs APs, and MPs vs APs) were performed with Bonferroni correction. *adjusted *P* < .05; †adjusted *P* < .01; ‡adusted *P* < .001; NS, not significant.

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Table 2. Immune Cells in Peripheral Blood Mononuclear Cells 3 Months After Hospital Discharge in Patients Recovered From Coronavirus Disease 2019

	Median (IQR) Value[a]				
Characteristics	Recovered SPs (n = 20)	Recovered MPs (n = 14)	Recovered APs (n = 9)	HCs (n = 8)	Overall *P* Value[b]
Age, y	59.50 (56.25–64.00)	56.00 (52.75–63.00)	56.00 (53.50–61.00)	56.50 (51.25–6.25)	.13
Male sex, no. (%)	11 (55.0)	6 (42.9)	4 (44.4)	4 (5.0)	.90[c]
BMI, mean (SD)[d]	23.96 (3.48)	23.69 (2.72)	24.11 (1.91)	22.33 (1.99)	.54[e]
T cells, % of PBMCs					
$CD3^+$	58.00 (49.60–67.85)	53.90 (48.62–58.62)	48.05 (37.77–53.67)	48.40 (35.88–57.35)	.13
$CD3^+CD4^+$	3.21 (26.02–37.16)	3.01 (26.43–38.34)	29.04 (25.46–29.75)	26.67 (23.05–33.05)	.77
$CD3^+CD8^+$	19.34 (13.84–23.05)	13.48 (1.16–19.37)	11.92 (1.77–18.39)	12.08 (7.76–16.54)	.33
$CD4^+/CD8^+$ T-cell ratio	1.44 (1.01–2.52)	2.04 (1.79–3.72)	2.04 (1.64–2.61)	1.92 (1.48–3.08)	.51
NK cells ($CD3^-CD16^+CD56^+$), % 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)	.24
NKT cells ($CD3^+CD56^+$) , % 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)	.09
DCs					
DCs ($D11C^+HLA\text{-}DR^+$), % of PBMCs	13.70 (9.84–15.70)	13.35 (1.62–15.40)	15.20 (13.50–17.80)	14.75 (11.62–17.10)	.72
MFI in DCs					
HLA-DR	1835.0 (1504.0–2252.0)	2075.0 (1602.8–2537.5)	1679.0 (156.5–2044.0)	1851.5 (1605.5–2472.3)	.71
$CD80^+$	57.30 (49.20–63.80)	63.15 (58.03–65.97)	76.60 (58.10–81.20)	58.30 (5.33–68.40)	.24
$CD86^+$	2277.0 (2084.0–2424.0)	210.5 (194.5–2259.25)	2329.0 (2196.0–2536.0)	2324.5 (224.25–2603.5)	.04
Macrophages ($CD11b^+CD14^{++}$), % of PBMCs	12.80 (1.10–14.80)	1.20 (8.20–12.10)	12.60 (1.40–15.10)	14.60 (1.86–18.27)	.30
MFI in macrophages					
$CD80^+$	256.0 (186.0–363.0)	247.0 (183.5–323.0)	287.0 (193.5–341.5)	212.0 (173.8–321.5)	.92
$CD86^+$	120.0 (1046.0–1356.5)	1113.0 (101.5–1275.0)	119.0 (919.5–1502.0)	1236.5 (114.3–140.8)	.48
MDSCs, % of PBMCs					
Total ($CD11b^+CD33^+HLA\text{-}DR^-$)	1.61 (0.78–2.21)	1.36 (0.78–1.50)	2.33 (1.22–2.50)	1.85 (1.49–2.10)	.30
PMN ($CD11b^+CD33^+HLA\text{-}DR^-$ $CD14^-CD15^+$)	0.16 (0.05–0.23)	0.17 (0.12–0.58)	0.51 (0.24–0.81)	0.15 (0.07–0.33)	.13
MO ($CD11b^+CD33^+HLA\text{-}DR^-CD14^+CD15^-$)	0.05 (0.03–0.08)	0.05 (0.03–0.07)	0.05 (0.03–0.15)	0.07 (0.05–0.08)	.59
PMN/MO MDSC ratio	3.27 (1.54–6.59)	5.94 (1.87–9.29)	7.25 (4.78–1.55)	2.33 (1.64–4.70)	.34

Abbreviations: APs, asymptomatic patients; BMI, body mass index; DCs, dendritic cells; IQR, interquartile range; MDSCs, myeloid-derived suppressor cells; MFI, mean fluorescence intensity; MO, mononuclear; MPs, patients with mild/moderate coronavirus disease 2019 (COVID-19); NK, natural killer; NKT, natural killer T; PBMCs, peripheral blood mononuclear cells; PMN, polymorphonuclear; SD, standard deviation; SPs, patients with severe/critical COVID-19.

[a]Data represent median (IQR) value unless otherwise specified.

[b]*P* values were determined with Kruskal-Wallis test for continuous variables, unless otherwise noted, and with χ^2 or Fisher exact test for all categorical variables. False discovery rate (FDR) correction was performed at the FDR <0.05 significance threshold for comparison of these variables, but all were >0.05.

[c]*P* value determined with χ^2 test.

[d]BMI calculated as weight in kilograms divided by height in meters squared.

[e]*P* value determined with 1-way analysis of variance.

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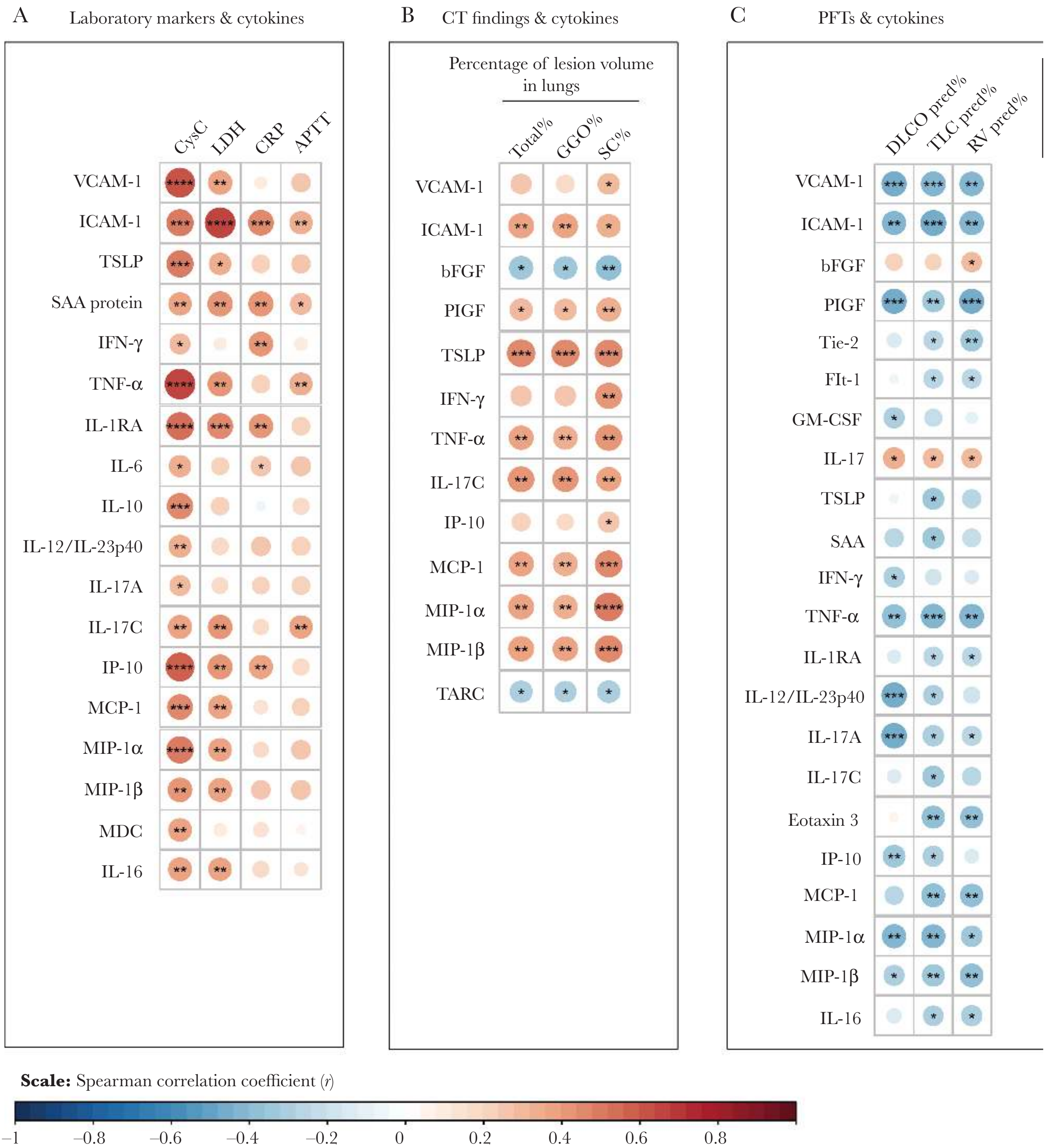

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Figure 5. Correlation between cytokines and abnormal clinical features in patients recovered from coronavirus disease 2019 (COVID-19). Correlation matrices of cytokines and abnormal clinical features are shown for 55 patients recovered from COVID-19, including 19 patients with severe/critical COVID-19 (SPs) 20 patients with mild/moderate COVID-19 (MPs), and 16 asymptomatic patients (APs). *A,* Spearman correlation of cytokines with laboratory markers (cystatin C [CysC], lactate dehydrogenase [LDH], C-reactive protein [CRP], and activated partial thromboplastin time [APTT]). *B,* Spearman correlation of cytokines with residual computed tomographic (CT) abnormities (total lesion, ground-glass opacity [GGO], and solid component [SC] percentages). *C,* Spearman correlation of cytokines with pulmonary function tests (PFT) results, including diffusing capacity of the lung for carbon monoxide (DLCO), total lung capacity (TLC), and residual volume (RV) predicted percentages). These correlations were calculated for 55 patients recovered from COVID-19 pooled as a single group, using the value of each variable for each patient. Only cytokines with significant correlations were displayed. Significance was determined using 2-tailed Spearman correlation analysis, and the correlation coefficients were visualized by means of color intensity and dot size. **P* < .05; †*P* < .01; ‡*P* < .001. Abbreviations: bFGF, basic fibroblast growth factor; Flt-1, vascular endothelial growth factor receptor 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule 1; IFN, interferon; IL-1RA (etc), interleukin 1RA (etc); IP, IFN-γ–inducible protein; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; PIGF, placental growth factor; SAA, serum amyloid A; TARC, thymus activation–regulated chemokine; Tie-2, tyrosine receptor kinase in the endothelium 2; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

Proportions of Immune Cells in PBMCs Among Patients Recovered From COVID-19

Several plasma cytokines remained at abnormal levels at 3 months after hospital discharge in patients recovered from COVID-19, especially in recovered SPs, which prompted consideration of immune cell recovery. We explored the proportions of 6 types of immune cells (T, NK, NKT, DC, macrophage, and MDSC) by isolating PBMCs from whole blood and phenotypically analyzed them with flow cytometry (Supplementary Figure 2). As shown in Table 2, a total of 51 age-, sex-, and BMI-matched subjects were analyzed (20 recovered SPs, 14 MPs, 9 APs, and 8 HCs). The proportions of total $CD3^+$, $CD4^+$, and $CD8^+$ T cells, NK cells, and NKT cells were slightly higher in recovered SPs at 3 months after discharge, with the ratio of $CD4^+/CD8^+$ T cells relatively lower than 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 patients recovered from COVID-19 and HCs. The mean fluorescence intensities (MFIs) of CD80 and CD86 in DCs were comparable across the 4 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 patients recovered from COVID-19 and HCs. Within the MDSC lineage, no significant differences were found in the frequencies of total, polymorphonuclear (PMN) , and mononuclear MDSCs between patients recovered from COVID-19 and HCs (all $P > .05$).

We further divided the patients recovered from COVID-19 into 2 groups according to the DLCO percentage and CT findings. However, none of the immune cells exhibited significant differences between the groups with normal versus abnormal DLCO percentage (Supplementary Figure 3), while the proportions of $CD4^+$ T cells and PMN MDSCs were significantly lower in the abnormal than in the normal CT group (Supplementary Figure 4).

Correlations Between Cytokine Profiles and Abnormal Clinical Features in Patients Recovered From COVID-19

Based on the above findings, we examined the potential associations between cytokine profiles and abnormal clinical features by using Spearman correlation analysis in patients recovered from COVID-19. Variables with significant correlations are shown in Figure 5 and Supplementary Tables 4–6. Mainly, class 1, 3, and 4 cytokines were significantly correlated with the indicated laboratory findings, residual CT abnormalities, and pulmonary function test. The vascular injury factors VCAM-1 and ICAM-1, the inflammatory cytokines TSLP, SAA protein, TNF-α, IL-1RA, and IL-17C, and the chemokines IP-10, MCP-1, MIP-1α, MIP-1β, and IL-16 were positively correlated withcystatin C and lactate dehydrogenase, which are indicators of organ damage (Figure 5A). Meanwhile, levels of cytokines, including 2 vascular injury/repair factors (ICAM-1 and PlGF), 3 inflammatory cytokines (TSLP, TNF-α, and IL-17C), and 3 chemokines (MCP-1, MIP-1α, and MIP-1β), were positively correlated with ground-glass opacity percentage and solid component percentage on CT images, whereas bFGF (tissue repair) and TARC (which 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 (Figures 1B and 4C). Furthermore, 3 vascular injury/repair factors (VCAM-1, ICAM-1, and PlGF), 3 inflammatory cytokines (TNF-α, IL-12p40, and IL-17A), and 3 chemokines (IP-10, MIP-1α, and MIP-1β) showed significant negative relationships with DLCO predictedpercentage and total lung capacity predicted percentage 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 patients recovered from COVID-19 at 3 months after hospital discharge.

DISCUSSION

The results of this study showed persisting respiratory sequelae (reduced lung volume, diffusion capacity disorder, and chest CT abnormalities) in patients recovered from COVID-19 at 3 months after discharge, more frequently and more conspicuous in recovered SPs than in 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 up-regulated in patients recovered from COVID-19, 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 patients recovered from COVID-19 and HCs, whereas in patients recovered from COVID-19, the proportion of $CD4^+$ T cells was significantly lower among those with abnormal than in those with normal CT findings. In addition, cytokines, such as VCAM-1 and ICAM-1 (class 1 cytokines), TNF-α (a class 3 cytokine); and IP-10, MCP-1, MIP-1α, and MIP-1β (class 4 cytokines) were positively correlated with all of the above abnormal clinical features observed in patients recovered from COVID-19.

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 Middle Eastern respiratory syndrome coronavirus [19, 20]. COVID-19 usually involves a cytokine storm, a phlogistic phenomenon caused by positive feedback loops that regulate cytokine production and overwhelm counterregulatory mechanisms [21]. Several inflammatory cytokines (eg, SAA protein, TNF-α, IL-6, and IL-17), chemokines (IP-10, MIP-1α, and MIP-1β),

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and vascular injury factors (ICAM-1 and VCAM-1) have been widely reported to be significantly elevated in the acute phase of COVID-19 [2, 22–24]. In our study, at 3 months after discharge, the levels of cytokines and chemokines related to hyperinflammatory response, including SAA protein, TNF-α, IL-17A, IL-17D, eotaxin, eotaxin 3, IP-10, MCP-1, MIP-1α, MIP-1β, and MDC, remained elevated in recovered SPs; the exception was IL-7 (a T-cell growth–promoting factor), which had a decreased level. Unexpectedly, factors related to vascular injury and angiogenesis, such as VCAM-1, ICAM-1, Tie-2, and PlGF, were significantly elevated in SPs; as proinflammatory 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 (2 vascular injury factors) were significantly negatively correlated with DLCO predicted percentage of pulmonary function, suggesting that the reduction in DLCO predicted percentage in patients recovered from COVID-19 may be caused by endothelial cell activation, leading to disturbance of alveolar-capillary gas exchange. However, compared with those in HCs, IL-7 levels were significantly decreased in recovered COVID-19 SPs unlike in MPs and APs and were positively correlated with DLCO predicted percentage, indicating the protective role of IL-7 in improving clinical outcomes. The ex vivo administration of IL-7 reportedly restored T-cell IFN-γ production in patients with COVID-19 [26], and current evidence has favored the effective role and safety of IL-7 in improving T-cell immunity among patients with critical COVID-19 [27, 28]. IL-7 therapy may help improve ongoing immune disorders in patients recovered from COVID-19, 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 SPs but were also positively correlated with all abnormal clinical features (residual CT abnormalities and impaired pulmonary function) observed 3 months after discharge in patients recovered from COVID-19. 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, apossibility that 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 the current study, we found that immune cells, including DCs, macrophages and their CD80 and CD86 MFI, as well as NK and NKT cells, T cells (total, $CD4^+$, and $CD8^+$), and MDSCs (total, PMN, and mononuclear), had returned to normal levels 3 months after discharge in patients recovered from COVID-19. These data were consistent with those of a 2020 study [8] reporting numbers of $CD4^+$ and $CD8^+$ T cells, B cells, and NK cells in patients recovered from COVID-19 that were comparable 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 stemlike memory phenotype, even in the absence of detectable humoral responses. Thus, the numbers of most immune cells had returned to normal 3 months after hospital discharge in patients recovered from COVID-19, and their function began to shift toward protective immunity against reinfection.

The current study has several limitations. First, the sample size was limited. Second, this cross-sectional study focused only on intermediate-term follow-up findings (3 months after discharge). Third, we did not assess the functional capabilities of SARS-CoV-2–specific immune cells or monitor antibody titers in convalescent individuals. Finally, we did not evaluate related damage to the cardiovascular system, although heart is an important target organ in vascular injury.

In conclusion, we found that vascular injury, aberrant proinflammatory cytokine and chemokine levels, and abnormal clinical features persisted in patients recovered from COVID-19 at 3 months after hospital discharge, especially in recovered SPs compared with MPs and APs. These findings raise concerns regarding ongoing aberrant cytokine–mediated organ damage in some patients recovered from COVID-19, especially survivors of severe/critical disease. Whether these findings 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 SPs. These 3 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 cytokine profiles and their correlation with clinical sequelae in patients recovered from COVID-19 of different disease severities 3 months after discharge, which may improve our understanding of the full spectrum of COVID-19 and provide guidance for long-term rehabilitation in recovered patients.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Notes

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Data availability. Anonymized clinical and laboratory test data are available on request, subject to an internal review by M. Z. and Y. J. 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 Y. J. (whuhjy@126.com).

Author contributions. M. Z. and Y. J. designed the study. M. Z., Z. Y., J. X., S. W., T. L., Y. L., F.Y., Z. W., and G. Y. collected the clinical data and information based on the follow-up protocols. M. Z, Z. Y., S. W., and T. L. collected the peripheral blood samples. M. Z., Z. Y., and T. L. performed the multiplex-cytokine assays and flow cytometry. M. Z., Z. Y., and J. X. summarized and checked all data. M. Z., Z. Y., and K. W. conducted the statistical analysis and produced all article figures. The manuscript was drafted by M. Z. and Z. Y. and critically revised by M. Z., J. Z., and Y. J.; all authors approved the final submission.

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