Recovery of systemic hyperinflammation in patients with severe SARS-CoV-2 infection

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Supplemental Table S1. Baseline characteristics of patient population at study entry

Parameters	All Patients (n=23)
Clinical characteristics	
Age. y	69 (55-79)
Male	12 (52.2)
BMI (kg/m²)	27 (25.7-31.5)
ARDS	
- mild	15 (65.2)
- moderate	2 (8.7)
- severe	4 (17.4)
Horovitz Index (HI)	
- HI > 300 mmHg	12 (52.2)
- HI 201 - 300 mmHg	6 (26.1)
- HI 101 - 200 mmHg	4 (17.4)
- HI ≤ 100 mmHg	1 (4.3)
High flow 0_2 Therapy	4 (17.4)
Mechanical ventilation	5 (21.7)
Vasopressor	4 (17.4)
Lymphocyte count at Nadir (1000/µl)	0.7 (0.6-1)
Bacterial Co-Infection	10 (43.5)
Dialysis	1 (4.3)
Acute hepatic injury	2 (8.7)
Cardiovascular risk factors	
Arterial hypertension	17 (73.9)
Dyslipidemia	11 (47.8)
Diabetes mellitus	7 (30.4)
Current smokers	4 (17.4)
Obesity	9 (39.1)
Atrial fibrillation	3 (13)
CAD	6 (26.1)
- 1 vessel	1 (4.3)
- 2 vessels	2 (8.7)
- 3 vessels	3 (13)
Chronic kidney disease	1 (4.3)
Asthma	3 (13)
COPD	1 (4.3)
Congestive heart failure	1 (4.3)
Malignoma	1 (4.3)
Liver disease	1 (4.3)
Prior IS	4 (17.4)

Concomitant cardiac medication at study entry	
Oral anticoagulation	4 (17.4)
ACE-I or ARB	9 (39.1)
MRA	4 (17.4)
Diuretics	4 (17.4)
Calcium channel blockers	4 (17.4)
Beta blockers	13 (56.5)
Statins	10 (43.5)
ASA	8 (34.8)
P2Y12 inhibitors	2 (8.7)
Concomitant cardiac medication at hospital discharge	
Oral anticoagulation	1 (4.3)
ACE-I or ARB	12 (52.2)
MRA	2 (8.7)
Diuretics	4 (17.4)
Calcium channel blockers	3 (13)
Beta blockers	10 (43.5)
Statins	11 (47.8)
ASA	9 (39.1)
P2Y12 inhibitors	1 (4.3)

Values are n (%) or are given as median and interquartile range (IQR). ACE - Angiotensin Converting Enzyme. Afib – atrial fibrillation. ALT – alanine amino-tranferase. ARB – Angiotensin II Receptor Blockers**.** ARDS – Acute respiratory distress syndrome. ASA – Acetylsalicylic acid. AST – aspartate-aminotransferase. BMI – body mass index. CAD – coronary artery disease. CK – creatinine kinase. CRP – C-reactive protein. GFR-MDRD – glomerular filtration rate. Hb – hemoglobin. hs TNI – high sensitive Troponin I. HI – Horowitz ratio (P/F ratio). INR – international normalized ratio. LDH – lactate dehydrogenase. MRA - mineralocorticoid receptor antagonists. NT-pro-BNP – N-terminal pro- brain natriuretic peptide. Pap Sys – pulmonary arterial pressure systolic. PCT – procalcitonin. PTT – partial thromboplastin time. SARS-CoV-2 – severe acute respiratory syndrome coronavirus-2

Supplemental Table S2. Adverse events during follow up

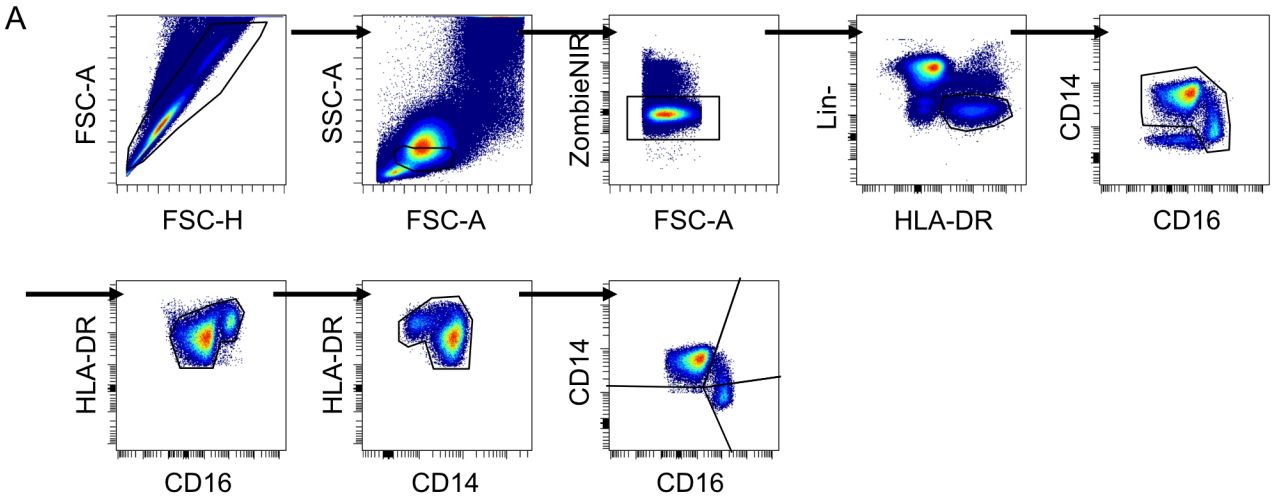
Parameters	All Patients, N=23
Adverse events	
- Death	0 (0)
- Myocardial infarction	1 (4.3)
- ECLS/ECMO	0 (0)
- Stroke	0 (0)
- Thromboembolism	0 (0)
- Bleeding	0 (0)
- Re-hospitalization	0 (0)

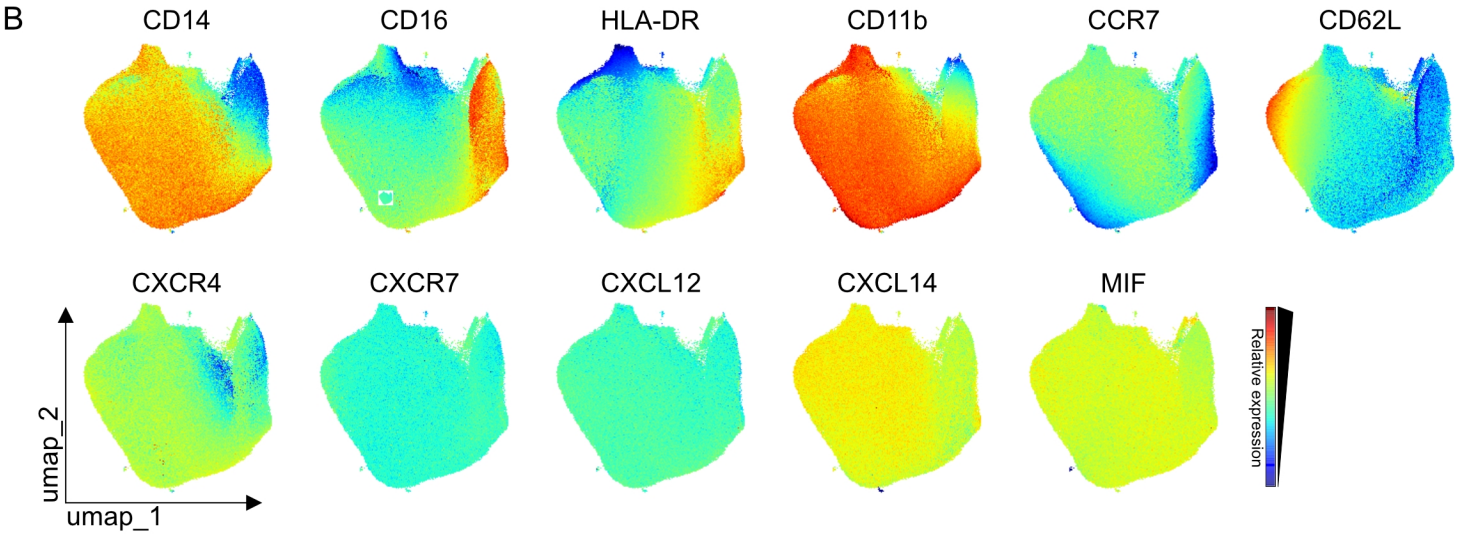
Values are n (%) or are given as median and interquartile range (IQR). ACE – Angiotensin Converting Enzyme. Afib – atrial fibrillation. ALT – alanine amino-tranferase. ARB - Angiotensin II Receptor Blockers**.** ASA – Acetylsalicylic acid. AST – aspartate-aminotransferase. BMI – body mass index. CAD – coronary artery disease. CK – creatinine kinase. CRP – C-reactive protein. GFR-MDRD – glomerular filtration rate. Hb – hemoglobin. hs TNI – high sensitive Troponin I. INR – international normalized ratio. LDH – lactate dehydrogenase. NT-pro-BNP – N-terminal pro- brain natriuretic peptide. Pap Sys – pulmonary arterial pressure systolic. PCT – procalcitonin. PTT – partial thromboplastin time. SARS-CoV-2 – severe acute respiratory syndrome coronavirus-2

Supplemental Table S3. Inflammatory cytokines and chemokines

LEGENDPlex Inflammation Panel 1	Function	Source
IL-1ß	Immune activation; induces an inflammatory response	(Parkin and Cohen 2001)
IFN-a2	Immune activation and modulation	(Parkin and Cohen 2001)
IFN-y	Immune activation and modulation	(Parkin and Cohen 2001)
TNF-a	Stimulated generalized immune activation as well as tumor necrosis	(Parkin and Cohen 2001)
CCL2	Attracts monocytes and memory T cells to inflammatory sites	(Parkin and Cohen 2001)
IL-6	Promotes B cell growth and antibody production, induces acute phase response	(Parkin and Cohen 2001)
IL-8	Chemoattractant	(Parkin and Cohen 2001)
IL-10	Inhibits the production of IFN-α, IL-1, IL-6, TNF- α, and stops antigen presentation	(Parkin and Cohen 2001)
IL-12p70	Augments T helper 1 responses and induces IFN-γ	(Parkin and Cohen 2001)
IL-18	Recruits monocytes and T lymphocytes. Synergist with IL-12 in the induction of IFN- γ production and inhibition of angiogenesis	(Arend *et al.* 2008)
IL-23	Differentiation and stabilization of T helper cells 17	(Schinocca *et al.* 2021)
IL-33	Traditional cytokine and as a nuclear factor regulating gene transcription, induces Th2 cytokine production	(Miller 2011)
LEGENDPlex Proinflammatory Chemokine Panel	**Function**	**Source**
CCL2	Attracts monocytes and memory T cells to inflammatory sites	(Parkin and Cohen 2001)
CCL5	Attracts monocytes, T cells, and eosinophils	(Parkin and Cohen 2001)
CXCL10	Chemotaxis, induction of apoptosis, regulation of cell growth and mediation of angiostatic effects	(Liu *et al.* 2011)
CCL11	Eosinophil and basophil migration	(Griffith *et al.* 2014)
CCL17	Th2 responses, Th2 cell migration, Treg, lung and skin homing	(Griffith *et al.* 2014)
CCL3	Attracts monocytes and T cells	(Parkin and Cohen 2001)
CCL4	Attracts monocytes and CD8+ T cells	(Parkin and Cohen 2001)
CXCL9	Th1 response; Th1, CD8, NK trafficking	(Griffith *et al.* 2014)
CCL20	Th17 responses; B cell and DC homing to gut-associated lymphoid tissue	(Griffith *et al.* 2014)
CXCL5	Neutrophil trafficking	(Griffith *et al.* 2014)
CXCL1	Neutrophil trafficking	(Griffith *et al.* 2014)
CXCL11	Linage development of T-regulatory-1 cells	(Karin 2020)
CXCL8	Attracts neutrophils, naive T cells	(Parkin and Cohen 2001)

Supplemental Figure S1

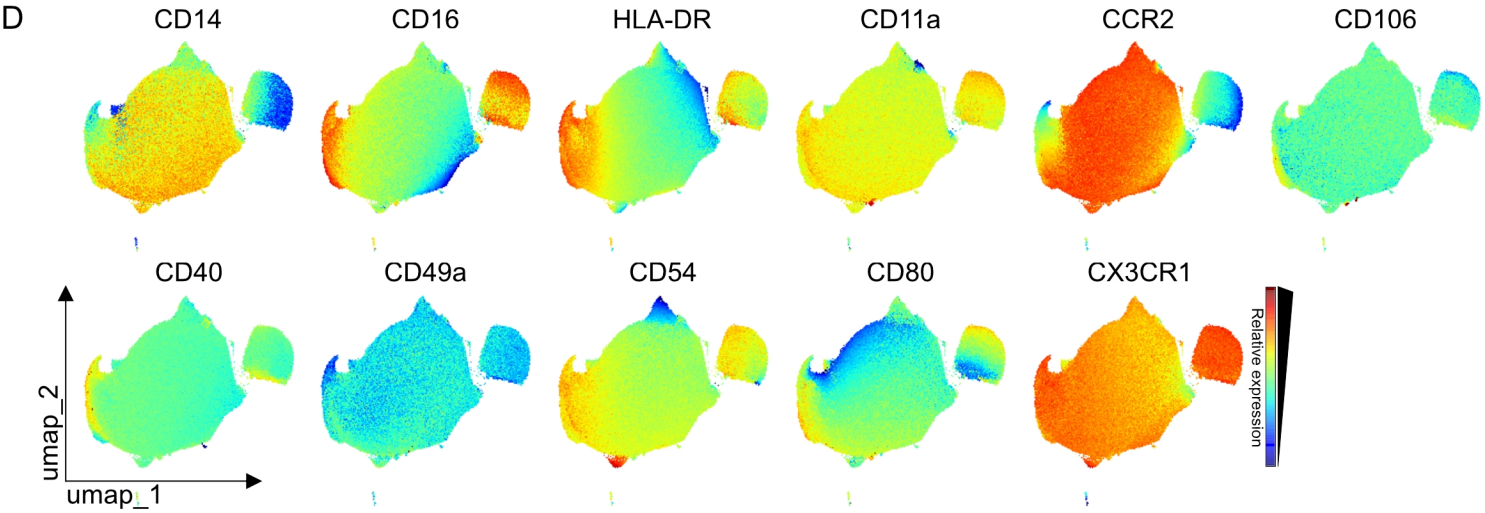




C

	CD14	CD16	HLA-DR	CD11b	CCR7	CD62L	CXCR4	CXCR7	CXCL12	CXCL14	MIF
Healthy Control	3.57	2.13	3.56	4.98	1.84	1.14	2.73	2.3	1.87	1.83	3.93
Acute Infection	3.56	2.1	3.51	4.99	1.92	1.08	2.81	2.3	1.88	1.86	3.93
Post-Infection	3.57	2.09	3.49	4.98	1.89	1.03	2.81	2.32	1.85	1.85	3.92

Min
Max



E

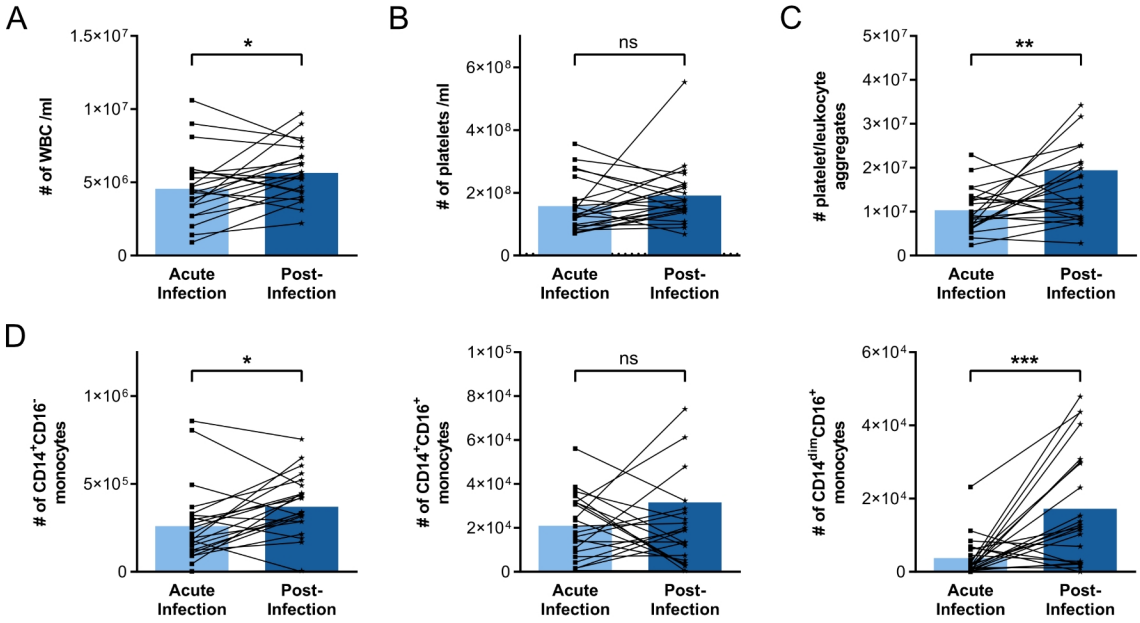
	CD14	CD16	HLA-DR	CD11a	CCR2	CD106	CD40	CD49a	CD54	CD80	CX3CR1
Post-Infection	3.19	2.37	3.31	4.13	5.33	1.05	1.92	0.67	3.34	0.99	4.43
Acute Infection	3.21	2.35	3.48	4.13	5.31	1.02	1.93	0.59	3.31	0.92	4.44
Healthy Control	3.2	2.37	3.41	4.15	5.3	1	1.89	0.68	3.38	0.88	4.47

Min
Max

Supplemental Figure S1: Gating strategy of human monocytes and UMAP analysis of surface marker expression.

(A) One representative gating strategy of human monocytes is shown. Gating strategy was used for both monocyte flow cytometry panel. All samples were gated as follows: singlets/ leukocytes/ CD14CD16 cells/ HLA-DR+CD16/ HLA-DR+CD14/ CD14CD16. Classical monocytes were characterized as $CD14^{+}CD16^{-}$, intermediate as $CD14^{+}CD16^{+}$ and non-classical as $CD14^{dim}CD16^{+}$. **(B)** Blots representing median marker expression of monocyte flow cytometry panel 1 as overlay of all patient samples. UMAP analysis was performed. **(C)** Heatmap of median expression of analyzed markers of monocyte flow cytometry panel 1. Patients are clustered into healthy control, acute infection and post-infection. **(D)** Blots representing median marker expression of monocyte flow cytometry panel 2 as overlay of all patient samples. UMAP analysis was performed. **(E)** Heatmap of median expression of analyzed markers of monocyte flow cytometry panel 2. Patients are clustered into healthy control, acute infection and post-infection. **(A-E)** Plots were generated using OMIQ data analysis software.

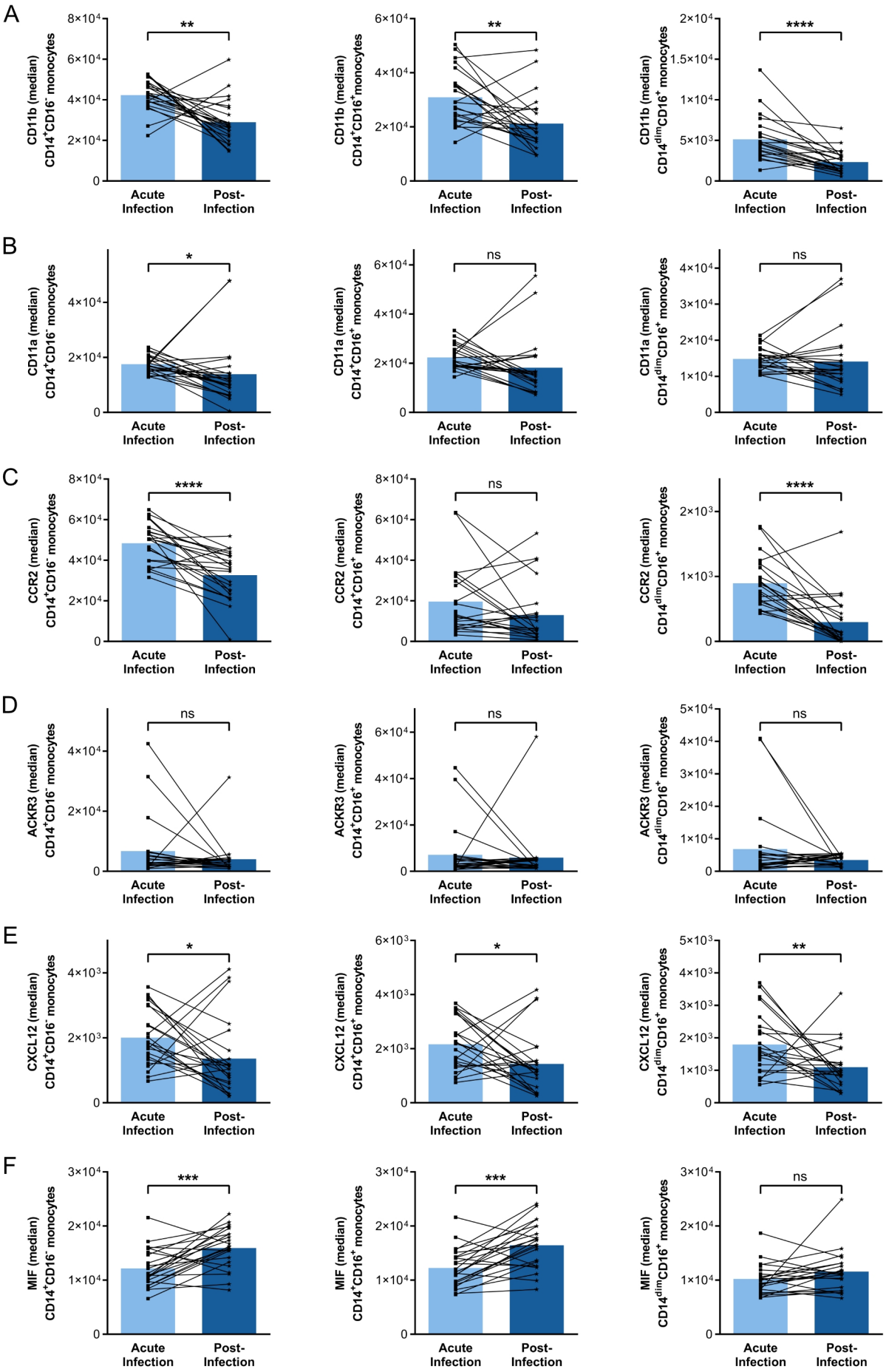
Supplemental Figure S2



Supplemental Figure S2: Cell count of monocytes, platelets and platelet/leukocyte aggregates in comparison of acute and post SARS-CoV-2 infection.

Flow cytometry analysis of 23 SARS-CoV-2 infected patients at acute stage and 3 months post-infection was performed in whole blood for phenotyping of systemic monocytes, platelets and platelet/leukocyte aggregates. Lines represent coherent patients. Following cell counts were measured: **(A)** WBC/ml **(B)** platelets/ml **(C)** platelet/leukocyte aggregates and **(D)** $CD14^{+}CD16^{-}$, $CD14^{+}CD16^{+}$ and $CD14^{dim}CD16^{+}$ monocytes. Plotted: Median with interquartile range (IQR); Statistics: Mann-Whitney U test. For this analysis a p-value ≤ 0.050 was considered significant, indicated by *, p-value ≤ 0.010 indicated by **, p-value ≤ 0.001 indicated by *** and p-value ≤ 0.0001 indicated by ****.

Supplemental Figure S3



Supplemental Figure S3: Activation and migration surface marker on monocyte subsets comparing acute and post SARS-CoV-2 infection.

23 SARS-CoV-2 infected patients at acute stage and 3 months post-infection were analyzed by flow cytometry staining in whole blood. Lines represent coherent patients. Plots representing surface marker expression of $CD14^{+}CD16^{-}$, $CD14^{+}CD16^{+}$ and $CD14^{dim}CD16^{+}$ monocytes: **(A)** median of CD11b **(B)** median of CD11a **(C)** median of CCR2 and **(D)** median of ACKR3. Following marker were stained intracellularly: **(E)** median of CXCL12 **(F)** median of MIF. Plotted: Median with interquartile range (IQR); Statistics: Mann-Whitney U test. For this analysis a p-value ≤ 0.050 was considered significant, indicated by *, p-value ≤ 0.010 indicated by **, p-value ≤ 0.001 indicated by *** and p-value ≤ 0.0001 indicated by ****.