Uncontrolled innate and impaired adaptive immune responses are prognostic features of COVID-19 ARDS

Online supplement

Content

Methods

Non-COVID-19 ARDS patients

All patients with moderate/severe pneumonia-related ARDS were included consecutively with the following inclusion criteria: tracheal intubation and mechanical ventilation since less than 48 hours; pulmonary infection diagnosed less than 7 days before ICU admission ; bilateral pulmonary infiltrates on chest x-ray; a PaO_2/FiO_2 ratio ≤200 mm Hg with a positive end-expiratory pressure (PEEP) ≥5 cm H_2O. Non-inclusion criteria were as follows: age <18 years; pregnancy; chronic respiratory failure requiring long-term oxygen therapy; Child-Pugh C liver cirrhosis; lung fibrosis; immunosuppression (*i.e.*, HIV infection, active hematological malignancy or solid cancer receiving chemotherapy, corticosteroids therapy for more than 0.5 mg/kg/day since more than 4 weeks, organ transplant patients), SAPS II (Simplified Acute Physiology II score) >90, irreversible neurological disorders, patients with withholding/withdrawing of life-sustaining therapies and profound hypoxemia (PaO_2/FiO_2 <75 mm Hg). Seventy patients with non-COVID-19 ARDS were included during the study period, 36 of whom underwent at least two blood samples drawn at days 1-2 and days 4-6 time points, as depicted in the flow chart (Figure E1).

Study design and patients

ARDS patients received mechanical ventilation using a standardized protective ventilation strategy (1). Other treatments, including neuromuscular blocking agents, nitric oxide inhalation, prone positioning and venovenous extra-corporeal membrane oxygenation were administered depending on the severity of ARDS and according to National guidelines (2). The prevention of ventilator-associated pneumonia followed a multifaceted program (3). Sedation and mechanical ventilation weaning followed standardized protocols.

Data collection

Demographics, clinical and laboratory variables were recorded upon ICU admission, at samples collection time points and during ICU stay. Other recorded variables included the use of adjuvant therapies for ARDS, the need for hemodialysis or vasopressors, corticosteroids administration, and the number of organ failure-free days at day 28. The primary clinical endpoint of the study was day-28 mortality.

Flow cytometry analyses

Blood samples were collected within 48 hours of ICU admission (Days 1-2 sample) and 4 days thereafter (Days 4-6 sample) in EDTA tubes, shipped at room temperature and analyzed within

2 h. Immuno-staining was performed as follows: 100 μL of whole blood was incubated for 10 min at RT in the dark with the different combinations of the following conjugated-monoclonal antibodies: anti-CD4-PE (clone 13B8.2), anti-CD3-AA750 (clone UCHT1), anti-CD8-AA700 (B9.11), anti-CD38-PC5.5 (LS198-4-3) or isotype control, anti-CD279 (PD-1)-PC7 (clone PD1) or isotype control, anti-HLA-DR-PB (Immu-357) or isotype control, anti-CD14-ECD (RMO52) and CD45-Krome Orange (clone J33) (all reagents were from Beckman Coulter). Red-blood-cells were then lysed using VersaLyse Solution (Beckman Coulter) according to the manufacturer's instructions. Washed samples were immediately acquired on a 10-multicolor Navios flow cytometer (Beckman Coulter) and analyzed with the Kaluza 2.1 software (Beckman Coulter).

Independent T and B cell counts were performed using standardized procedures. For non COVID-19 patients, absolute cell counts were performed using FC500 cytometer (Beckman Coulter) and a routine bead-based single platform technology according to the manufacturer instructions (All reagents and materials were from Beckman Coulter). For COVID-19 patients, T and B cell counts were performed using a fully automated AQUIOS cytometer. Of note, the two lymphocytes counts methods have an excellent correlation and agreement.

Monocytes, and lymphocytes were first gated on a side scatter-area (SSC-A) versus CD45 (the leukocyte common antigen) flow cytometry dot plots. Monocytes were defined as Side Scatter (SS) intermediate, CD45+ and CD14+ cells (Figure E2). Expression of HLA-DR was then analyzed (Figure E2, upper panel). T CD8+ lymphocytes were identified as CD45+ CD3+ CD8+ cells within the CD45+ SS low lymphocyte gate. Expression of HLA-DR, CD38 and PD-1 was then analyzed on T CD8+ lymphocytes (Figure E2, lower panel).

Measurements of serum cytokine concentrations

Blood samples collected at days 1-2, days 4-6 and days 8-12 for patients who still had ARDS criteria were immediately centrifuged for storage at – 80°C. Cytokines were measured at distance in serum inactivated for 20 minutes at 56°C using Luminex® multiplex bead-based technology (R&D Systems, Minneapolis, MN. USA) and a Bio-Plex 200® instrument (BioRad, Hercules, CA. USA), according to the manufacturers' protocols on serum diluted to 1/2. Laboratory technician who performed the assays was blinded to all clinical data. The following cytokines/chemokines were evaluated CCL2/MCP-1, CCL4/MIP-1β, CCL19/MIP-3β, CD40L, CXCL10/IP-10, FGF-basic, G-CSF, GRZ-B, IFN-β, IL-1α, IL-1ra, IL-3, IL-5, IL-7, IL-10, IL-13, IL-17A, PD-L1, TNF-α, VEGF, CCL3/MIP-1α, CCL11/Eotaxin, CCL20/MIP-3α, CX3CL1/Fractalkine, EGF, Flt-3L, GM-CSF, IFN-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-

8/CXCL8, IL-12p70, IL-15, IL-33, TGF-α, TRAIL, CCL5/RANTES, IL-17E/IL-25. All samples, including those obtained from both COVID-19 and non-COVID-19 patients, were inactivated during 20 minutes at 56°C, as previously described (4). We analyzed cytokines/chemokines which displayed more than 80% of concentration values above the lower limit of quantification (n=19). For each of these analytes, extrapolated concentration values calculated by Bioplex Manager 6.1 sofware were taken into account and undetectable values were imputed to the lowest extrapolated concentration value.

Statistical analyses

Descriptive results are presented as means (±standard deviation [SD]) or medians (1^{st}-3^{rd} quartiles) for continuous variables, and as numbers with percentages for categorical variables. Log-transformation was applied to non-normally distributed variables, as assessed by graphical methods and the Shapiro-Wilk test. Boxplots were plotted to illustrate differences in cytokines levels according to time and COVID-19 status.

Bivariate correlation analyses between cytokines and COVID-19 status were conducted by computing Spearman and biserial correlation coefficients for continuous-continuous and binary-continuous variables correlations, respectively. A correlation network plot was built from those results to graphically illustrate relationships.

Unadjusted between-groups comparisons between conditions (COVID-19 *versus* non-COVID-19) and outcome (alive *versus* dead at ICU day-28) were performed using Mann-Whitney tests for continuous variables, and Chi^2 or Fisher's exact tests for categorical variables, as appropriate. Within-groups comparisons between baseline and subsequent assessments were conducted using paired Wilcoxon signed ranks test. Association between cytokines, other covariates and final outcome were further assessed after systematically adjusting for age and SOFA score, using logistic regression (categorical variables) and linear regression modeling (continuous variables) to compute adjusted odds ratios (95% confidence interval) and adjusted means (±standard error), respectively.

Longitudinal analyses were finally performed to assess the temporal evolution of cytokines levels over a 12-day period using mixed effects linear regression models to account for the correlation between repeated data over time. Models included a COVID-19 status variable, a time variable and an interaction term COVID-19*time to test for differences in average cytokines levels between COVID-19 and non-COVID-19 patients, significant positive or negative trends over time (slopes), and differentiated trends over time between COVID-19 and ARDS patients.

Two-tailed p-values < 0.05 were considered statistically significant, applying Benjamini-Hochberg correction for test multiplicity in correlation analyses. Analyses were performed using Stata V16.0 statistical software (StataCorp, College Station, TX, USA), R 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria; *corrplot* and *qgraph* packages) and GraphPad Prism v8.0 (GraphPad Software, San Diego, CA, USA).

References

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Results

Table E1. **Results from longitudinal analysis by mixed effects linear regression model assessing the association between COVID-19 status, time elapsed since hospital admission and serum cytokines concentrations**

	COVID-19 status[a]	Time[b]	Interaction (COVID-19 status x time)[c]
IL-6 (log)	0,093	**<0.0001**	0,218
IL-1ra/IL-1F3 (log)	0,432	**<0.0001**	0,267
IL-8/CXCL8 (log)	**0,045**	0,337	0,111
IL-10	**<0.0001**	**0,005**	**0,004**
CXCL10/IP-10	0,082	**0,001**	0,201
GM-CSF	**0,001**	**0,001**	**0,045**
CCL2/MCP-1 (log)	0,068	**0,007**	0,924
CCL3/MIP-1a (log)	**0,007**	0,707	0,985
CCL4/MIP-1b (log)	0,615	0,103	0,266
CCL19/MIP-3b (log)	0,141	0,039	0,149
CCL5/RANTES	**0,033**	0,766	0,688
CCL20/MIP-3a (log)	**0,002**	0,176	**0,003**
EGF	**<0.0001**	0,794	0,289
CX3CL1/Fractalkine (log)	0,677	0,693	0,571
VEGF (log)	0,625	0,877	0,019
Flt3 (log)	0,756	0,218	0,397
IL-15 (log)	**0,022**	**<0.0001**	**0,031**
IL-7 (log)	0,169	0,798	0,241

Results are p-values from mixed effects linear regression model, testing for [a] differences in average cytokines levels between COVID-19+ and ARDS patients, [b] significant positive or negative trends over time (slopes), and [c] differentiated trends over time between COVID-19 and ARDS patients.

Table E2. **Results from longitudinal analysis by mixed effects linear regression model assessing the association between COVID-19 status, time elapsed since first symptoms of disease onset (COVID-19 or non-COVID-19) and serum cytokines concentrations.**

	COVID-19 status[a]	Time[b]	Interaction (COVID-19 status x time)[c]
IL-6 (log)	0,183	**0,001**	0,265
IL-1ra/IL-1F3 (log)	0,665	**0,002**	0,440
IL-8/CXCL8 (log)	0,082	0,243	0,122
IL-10	**<0.0001**	0,264	**<0.0001**
CXCL10/IP-10	**0,002**	0,098	**0,010**
GM-CSF	**<0.0001**	0,155	**0,002**
CCL2/MCP-1 (log)	0,065	0,061	0,809
CCL3/MIP-1a (log)	**0,028**	0,116	0,777
CCL4/MIP-1b (log)	0,195	0,281	0,983
CCL19/MIP-3b (log)	0,720	0,824	0,919
CCL5/RANTES	0,089	0,926	0,817
CCL20/MIP-3a (log)	**0,002**	0,101	**0,003**
EGF	**<0.0001**	0,381	0,689
CX3CL1/Fractalkine (log)	0,932	0,931	0,965
VEGF (log)	0,253	0,419	**0,015**
Flt3 (log)	0,910	0,177	0,335
IL-15 (log)	0,060	**<0.0001**	0,075
IL-7 (log)	0,058	0,835	0,079

Results are p-values from mixed effects linear regression model, testing for [a] differences in average cytokines levels between COVID-19+ and ARDS patients, [b] significant positive or negative trends over time (slopes), and [c] differentiated trends over time between COVID-19 and ARDS patients.

Table E3. Serum cytokines concentrations according to the microbiological etiology of acute respiratory distress syndrome (non-COVID-19 viral or bacterial/non-documented (ND) or COVID-19.

Serum cytokines concentrations (pg/mL)	**Non-COVID-19 (n=31)[a]**		**COVID-19 (n=35)[b]**	**Global p-value[c]**	**P-value for *Post-hoc* comparisons[b]**		
	Viral (n=10)	**Bacterial/ND (n=21)**			**Viral *vs* bacterial**	**Viral *vs* COVID-19**	**Bacterial *vs* COVID-19**
IL-6	298.4 [41.1-8796.1]	165.6 [87.8-430.6]	171.4 [81.2-333.5]	0,682			
IL-1-ra/IL-1F3	314.8 [119.9-764.4]	184.8 [113.6-320.9]	172.6 [134.4-336.6]	0,734			
IL-8/CXCL8	106.4 [60.6-147.2]	52.2 [40.8-97.4]	117.6 [68.0-398.2]	**0,010**	0,661	>0.99	**0,006**
IL-10	451.4 [219.6-595.4]	99.3 [59.4-201.1]	456.7 [348.8-534.7]	**<0.001**	**0,045**	>0.99	**<0.001**
CXCL10/IP-10	2214.9 [362.3-4048.5]	188.4 [101.3-450.8]	1911.3 [919.1-2555.6]	**<0.001**	**0,016**	>0.99	**<0.001**
GM-CSF	206.7 [83.9-299.7]	44.2 [21.7-74.4]	209.4 [158.4-233.8]	**<0.001**	**0,045**	>0.99	**<0.001**
CCL2/MCP-1	624.6 [406.8-3289.2]	649.7 [400.1-1145.1]	1473.4 [897.5-2798.0]	**0,012**	>0.99	0,202	**0,016**
CCL3/MIP-1a	17.3 [9.4-61.3]	22.5 [19.1-38.7]	53.5 [22.5-116.0]	**0,007**	>0.99	0,070	**0,018**
CCL4/MIP-1b	233.6 [142.5-397.8]	229.1 [112.6-278.1]	246.5 [179.2-397.8]	0,368			
CCL19/MIP-3b	195.8 [124.6-1089.0]	140.6 [99.8-206.7]	117.7 [78.2-213.6]	0,154			
CCL5/RANTES	27348.3 [21191.6-47176.3]	30490.5 [19437.4-45335.9]	45900.9 [29541.2-60859.8]	**0,030**	>0.99	0,122	0,082
CCL20/MIP-3a	17.2 [7.6-75.5]	19.9 [8.0-66.0]	9.2 [4.2-20.3]	**0,043**	>0.99	0,235	0,082
EGF	87.9 [50.8-158.2]	127.6 [71.1-244.8]	433.0 [290.1-604.0]	**<0.001**	0,549	**<0.001**	**<0.001**
CX3CL1/Fractalkine	1537.3 [1267.1-1948.8]	1156.6 [562.6-1386.9]	880.0 [783.1-1377.4]	0,075			
VEGF	1014.2 [712.0-1422.2]	882.9 [583.8-1602.8]	920.4 [628.6-1508.1]	0,962			
Flt-3L	101.7 [60.0-150.7]	98.7 [73.5-134.9]	112.2 [81.2-168.4]	0,624			
IL-15	6.9 [3.4-13.0]	5.7 [3.5-9.3]	5.0 [3.6-7.6]	0,440			
IL-7	16.3 [9.7-22.5]	13.4 [7.0-22.8]	13.4 [8.4-23.0]	0,778			

*Results are presented as median (1st-3rd quartiles); **bolded** results are statistically significant at the p<0.05 level; [a] Missing data, n=5; [b] Missing data, n=3; [c] p-values come from the Kruskal-Wallis test; [b] Post-hoc comparisons were performed when the global p-value was <0.05 and corrected for multiple testing using the Bonferroni correction.*

Table E4. **Clinical and biological variables associated with day-28 mortality in COVID-19 patients and non-COVID-19 patients**

	COVID-19 (n=38)			Non-COVID-19 (n=36)		
	Alive	**Dead**	**p-value[a]**	**Alive**	**Dead**	**p-value[a]**
	N=25	N=13		N=32	N=4	
Serum cytokines concentrations (pg/mL) [b]						
IL-6	129.2 [77.7-239.2]	219.9 [174.8-377.0]	0,070	161.2 [84.3-534.7]	457.1 [86.6-492.1]	0,738
IL-1-ra/IL-1F3	160.4 [124.9-275.3]	278.1 [142.0-685.5]	0,126	186.9 [112.4-418.9]	321.9 [142.6-443.6]	0,462
IL-8/CXCL8	155.0 [67.7-431.8]	111.6 [73.0-136.5]	0,303	58.1 [40.8-144.9]	97.4 [17.2-136.1]	0,789
IL-10	429.7 [304.6-493.3]	517.7 [473.8-580.8]	**0,013**	167.1 [84.5-549.3]	87.5 [8.4-138.7]	0,192
CXCL10/IP-10	1461.6 [793.9-2103.8]	2555.6 [1758.9-3284.1]	**0,017**	294.4 [153.4-3314.5]	166.8 [65.4-306.9]	0,229
GM-CSF	196.1 [146.4-220.6]	233.8 [213.5-272.3]	**0,005**	70.1 [34.1-259.1]	40.2 [4.4-54.5]	0,181
CCL2/MCP-1	1442.7 [876.0-2471.4]	2034.0 [1019.7-4020.7]	0,500	699.3 [403.5-1465.6]	500.2 [320.3-2720.8]	0,548
CCL3/MIP-1a	57.6 [22.5-149.0]	31.8 [21.0-84.7]	0,394	22.5 [13.8-43.6]	15.4 [9.4-38.7]	0,482
CCL4/MIP-1b	246.0 [184.7-379.0]	246.5 [179.2-469.5]	0,957	231.3 [142.5-298.0]	66.4 [66.4-316.6]	0,181
CCL19/MIP-3b	110.1 [77.7-159.3]	213.6 [102.1-402.5]	0,065	146.2 [112.1-267.2]	102.1 [63.5-719.6]	0,504
CCL5/RANTES	45606.7 [31139.0-69829.2]	45900.9 [28297.0-53720.2]	0,477	29748.1 [21068.9-46256.1]	7170.3 [1302.6-50869.9]	0,181
CCL20/MIP-3a	7.6 [4.0-13.5]	17.5 [6.4-28.5]	0,076	19.0 [7.9-82.8]	16.2 [5.9-66.0]	0,640
EGF	492.7 [324.3-619.6]	324.2 [240.6-433.0]	**0,033**	120.1 [70.1-188.9]	15.5 [1.5-325.1]	0,333
CX3CL1/Fractalkine	835.6 [718.6-1075.9]	1193.4 [950.9-1748.0]	**0,028**	1294.7 [617.1-1663.8]	935.4 [750.2-2576.9]	0,894
VEGF	874.2 [619.5-1363.4]	1361.4 [860.5-1668.6]	0,177	875.7 [659.7-1586.4]	1116.9 [373.0-1981.2]	0,947
Flt-3L	110.6 [76.6-166.7]	112.8 [103.8-170.4]	0,570	97.5 [70.6-139.0]	136.9 [97.4-277.2]	0,181
IL-15	4.5 [3.5-6.7]	6.9 [4.5-10.0]	0,126	6.0 [3.5-9.7]	6.8 [3.4-13.4]	0,815
IL-7	13.1 [8.1-24.2]	13.5 [9.3-23.0]	0,915	15.5 [9.3-23.4]	5.2 [2.5-22.8]	0,216
Other laboratory features						
RT-qPCR viral load, CT[c]	32.0 [29.5-35.0]	26.0 [20.7-28.6]	**<0.001**	-	-	-
T $CD4^+$ lymphocytes, /mm^3	308.0 [229.0-380.0]	212.0 [152.0-281.0]	0,186	335.0 [190.0-526.5]	457.5 [159.5-704.0]	0,940
T $CD8^+$ lymphocytes, /mm^3	111.0 [88.0-153.0]	142.0 [99.0-171.0]	0,469	179.0 [68.5-399.5]	211.5 [48.0-361.5]	0,669

B lymphocytes, /mm^3	435.0 [322.0-537.0]	323.0 [296.0-443.0]	0,364	199.5 [105.0-326.0]	217.0 [114.0-326.5]	0,880
NK cells, /mm^3	47.0 [31.0-76.0]	51.0 [28.0-85.0]	0,735	-	-	-
T CD4$^+$PD1$^+$ lymphocytes, %	20.4 [16.5-26.4]	23.6 [19.1-30.0]	0,317	24.9 [17.8-37.6]	28.1 [16.9-38.5]	0,836
T CD8$^+$PD1$^+$ lymphocytes, %	25.8 [16.5-42.8]	26.1 [14.7-39.8]	0,470	42.5 [26.3-50.8]	34.7 [19.7-50.6]	0,632
T CD8$^+$HLA-DR$^+$CD38$^+$ lymphocytes, %	9.8 [8.5-18.7]	8.9 [8.1-20.2]	0,854	12.4 [4.9-18.6]	9.0 [6.4-20.4]	0,821
HLA-DR$^+$ monocytes, %	68.3 [49.8-79.4]	81.4 [44.3-82.4]	0,508	28.5 [19.0-43.3]	40.3 [24.6-65.0]	0,406

Results are presented as median (1st-3rd quartiles); ***bolded*** *results are statistically significant at the p<0.05 level; [a] P-values comes from the Mann-Whitney test; [b] Missing data, n=3 for COVID-19 patients and n=5 for non-COVID-19 patients; [c] obtained from naso-pharyngeal swabs*

Table E5. **Clinical and biological variables associated with day-28 mortality in COVID-19 patients (n=38)** [a]

	Unadjusted analysis			*Adjusted analysis*[b]		
	Alive N=25	**Dead** N=13	**p-value**	**Alive** N=25	**Dead** N=13	**p-value**
Clinical features (categorical variables)	**N (%)**	**N (%)**		**Adjusted odds ratios (95% CI)**		
Male gender	22 (88%)	10 (77%)	0,392	0.28 (0.03;2.94)		0,289
Diabete	4 (16%)	8 (61%)	**0,009**	4.10 (0.67;25.00)		0,126
COPD	1 (4%)	4 (31%)	**0,038**	1.41 (0.09;21.49)		0,805
Chronic heart failure	1 (4%)	5 (38%)	**0,012**	13.12 (0.41;424.00)		0,147
Obesity	9 (36%)	5 (38%)	1,000	3.03 (0.39;23.59)		0,290
Smoker	9 (36%)	6 (46%)	0,728	0.64 (0.11;3.73)		0,620
ARDS severity (Berlin definition)			0.904			
Mild	6 (24%)	2 (15%)		1(ref)		0,582
Moderate	11 (44%)	7 (54%)		3.75 (0.31;45.39)		0,298
Severe	8 (32%)	4 (31%)		2.62 (0.22;30.97)		0,445
Clinical and general laboratory features (continuous variables)	**Mean (±SD)**	**Mean (±SD)**		**Adjusted mean (±SE)**	**Adjusted mean (±SE)**	**p-value**
ICU admission – intubation, days	2.38 (±5.95)	1.23 (±2.24)	0,578	2.36 (±1.12)	1.26 (±1.61)	0,606
Age, years	57.04 (±12.43)	68.15 (±10.37)	**0,007**	56.93 (±2.53)	68.36 (±3.55)	**0,017**
BMI, kg/cm^2	28.83 (±5.18)	29.82 (±5.01)	0,589	28.33 (±1.04)	30.76 (±1.49)	0,225
Temperature, °C	38.58 (±1.01)	38.42 (±1.11)	0,987	38.58 (±0.23)	38.42 (±0.33)	0,723
SOFA	7.0 (±2.8)	9.8 (±2.6)	**0,017**	7.1 (±0.6)	9.9 (±0.8)	**0,013**
SAPS II	39.4 (±13.2)	53.5 (±25.1)	0,054	45.6 (±3.0)	42.3 (±4.5)	0,579
PaO_2/FiO_2 ratio, mmHg	121.2 (±58.6)	361.3 (±761.0)	**0,036**	128.8 (±101.1)	356.0 (±145.2)	0,243
pH	7.41 (±0.07)	7.36 (±0.11)	0,206	7.39 (±0.02)	7.38 (±0.03)	0,840
$PaCO_2$, mm Hg	43.4 (±11.2)	43.5 (±10.1)	0,963	42.1 (±1.9)	43.0 (±2. 8)	0,815
Arterial blood lactates, mM	1.5 (±0.5)	1.5 (±0.5)	0,823	1.5 (±0.1)	1.5 (±0.2)	0,781

Creatinine, µmol/L	85.6 (±34.6)	210.4 (±287.4)	0,093	94.6 (±38.3)	191.3 (±55.0)	0,191
Bilirubin, µmol/L	12.3 (±9.2)	13.7 (±21.5)	0,345	14.8 (±3.0)	8. 7 (±4.2)	0,281
Prothrombin time, %	77.8 (±19.0)	80.0 (±10.3)	0,644	77.5 (±3.8)	81.6 (±5.3)	0,575
WBC counts, $10^3/mm^3$	7.72 (±2.52)	7.55 (±4.04)	0,302	7.79 (±0.70)	7.24 (±1.01)	0,684
Lymphocytes, $10^3/mm^3$	0.72 (±0.32)	0.67 (±0.30)	0,598	0.71 (±0.07)	0.70 (±0.10)	0,924
Monocytes, $10^3/mm^3$	0.38 (±0.22)	0.37 (±0.30)	0,389	0.39 (±0.06)	0.35 (±0.08)	0,670
Neutrophils, $10^3/mm^3$	6.39 (±2.51)	6.39 (±3.52)	0,460	6.37 (±0.65)	6.22 (±0.94)	0,907
Neutrophils-to-lymphocytes ratio	10.66 (±6.54)	12.52 (±9.73)	0,794	10.76 (±1.75)	11.98 (±2.51)	0,714
Serum cytokines concentrations (pg/mL)						
IL-6 (LOG)	4.91 (±1.00)	5.55 (±0.75)	0,070	4.99 (±0.20)	5.56 (±0.31)	0,168
IL-1-ra/IL-1F3 (LOG)	5.20 (±0.77)	5.74 (±0.81)	0,126	5.37 (±0.16)	5.52 (±0.24)	0,633
IL-8/CXCL8 (LOG)	5.25 (±1.20)	4.79 (±1.16)	0,303	5.42 (±0.26)	4.45 (±0.41)	0,073
IL-10	397.08 (±133.07)	503.68 (±116.54)	**0,013**	399.90 (±29.86)	502.18 (±46.03)	0,093
CXCL10/IP-10	1563.28 (±878.86)	2542.21 (±1025.40)	**0,017**	1613.27 (±213.95)	2487.20 (±329.87)	**0,047**
GM-CSF	179.12 (±60.60)	232.80 (±52.93)	**0,005**	179.08 (±13.63)	234.18 (±21.01)	**0,050**
CCL2/MCP-1 (LOG)	7.29 (±0.72)	7.51 (±0.81)	0,500	7.44 (±0.16)	7.24 (±0.25)	0,528
CCL3/MIP-1a (LOG)	4.10 (±1.16)	3.76 (±1.00)	0,394	4.17 (±0.24)	3.49 (±0.38)	0,170
CCL4/MIP-1b (LOG)	5.53 (±0.62)	5.56 (±0.53)	0,957	5.51 (±0.13)	5.52 (±0.21)	0,987
CCL19/MIP-3b (LOG)	4.72 (±0.59)	5.22 (±0.93)	0,065	4.83 (±0.15)	5.10 (±0.23)	0,381
CCL5/RANTES	54108.66 (±34724.68)	44143.23 (±20631.97)	0,477	57323.60 (±7103.50)	38981.52 (±10952.62)	0,201
CCL20/MIP-3a (LOG)	2.04 (±0.89)	2.69 (±1.09)	0,076	2.14 (±0.20)	2.45 (±0.30)	0,429
EGF	479.45 (±221.60)	336.62 (±129.05)	**0,033**	477.57 (±44.41)	363.71 (±68.48)	0,204
CX3CL1/Fractalkine (LOG)	6.80 (±0.41)	7.14 (±0.52)	**0,028**	6.86 (±0.10)	7.03 (±0.16)	0,384
VEGF (LOG)	6.75 (±0.60)	7.10 (±0.71)	0,177	6.85 (±0.13)	7.03 (±0.21)	0,492
Flt-3L (LOG)	4.71 (±0.53)	4.79 (±0.35)	0,570	4.82 (±0.10)	4.62 (±0.15)	0,328
IL-15 (LOG)	1.49 (±0.63)	1.72 (±0.74)	0,126	1.51 (±0.15)	1.68 (±0.23)	0,556
IL-7 (LOG)	2.64 (±0.75)	2.67 (±0.79)	0,915	2.64 (±0.16)	2.73 (±0.25)	0,798

Other laboratory features						
RT-qPCR viral load[b], cycle threshold	31.68 (±4.70)	25.27 (±5.23)	**0,002**	31.31 (±1.09)	25.71 (±1.56)	**0,010**
T CD4+ lymphocytes, /mm^3	315.44 (±161.20)	258.00 (±160.72)	0,186	324.17 (±35.58)	242.45 (±51.12)	0,233
T CD8+ lymphocytes, /mm^3 (LOG)	4.73 (±0.41)	4.90 (±0.52)	0,469	4.76 (±0.09)	4.84 (±0.13)	0,636
B lymphocytes, /mm^3	439.36 (±191.64)	419.69 (±251.42)	0,364	448.93 (±46.07)	403.05 (±66.19)	0,602
NK cells, /mm^3 (LOG)	3.93 (±0.62)	4.00 (±0.77)	0,735	3.91 (±0.15)	4.10 (±0.21)	0,521
T CD4+ PD1+ lymphocytes, % (LOG)	3.02 (±0.34)	3.17 (±0.49)	0,317	3.02 (±0.09)	3.13 (±0.12)	0,486
T CD8+ PD1+ lymphocytes, % (LOG)	3.25 (±0.49)	3.06 (±0.67)	0,470	3.30 (±0.12)	2.93 (±0.17)	0,099
T CD8+ HLA-DR+ CD38+ lymphocytes, % (LOG)	2.47 (±0.62)	2.44 (±0.78)	0,854	2.56 (±0.15)	2.29 (±0.21)	0,338
HLA-DR+ monocytes, % (LOG)	64.04 (±19.64)	64.54 (±28.41)	0,508	65.87 (±5.15)	63.27 (±7.40)	0,792

Results are presented as median (1st-3rd quartiles) or means±standard deviation (SD) or ±standard error (SE). as appropriate; ***bolded*** *results are statistically significant at the p<0.05 level; [a]Results from linear regression modeling (continuous variables) or logistic regression modeling adjusting for age and SOFA score; [b]obtained from naso-pharyngeal swabs*

Figure E1.

Flow chart of patients with non-COVID-19 acute respiratory distress syndrome (ARDS) included between January 2014 and December 2018. CRD: chronic respiratory disease; LTO: long-term oxygenotherapy; OTI: oro-tracheal intubation; WH/WD of LST: withholding/withdrawal of life-sustaining therapies.

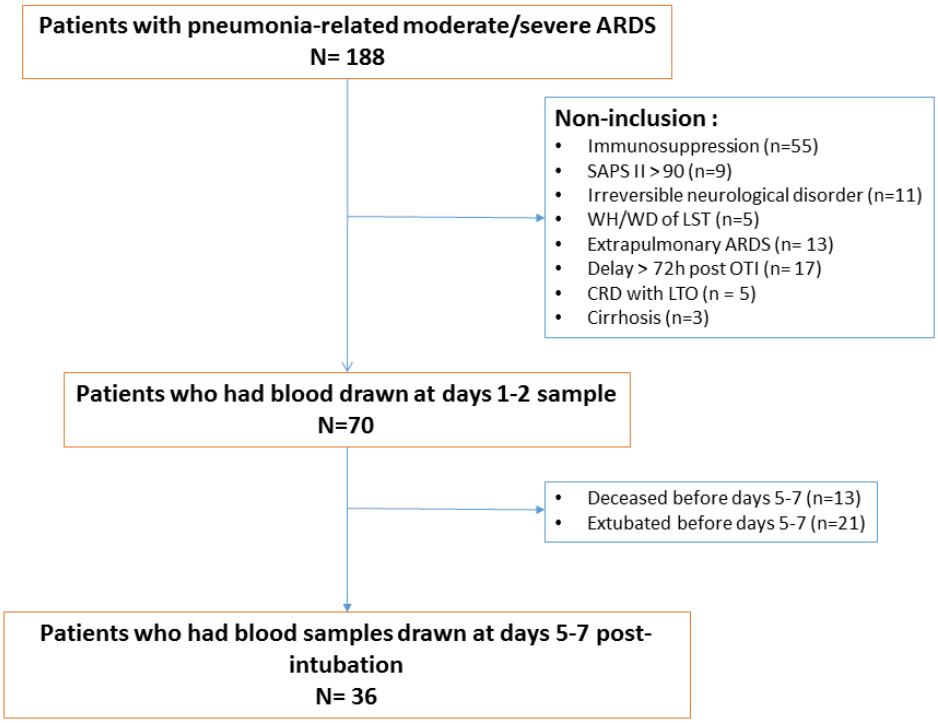


Figure E2.

Flow cytometry gating strategies. Monocytes, and lymphocytes were first gated on a side scatter-area (SSC-A) versus CD45 (the leukocyte common antigen) flow cytometry dot plots. Monocytes were defined as Side Scatter (SS) intermediate, CD45+ and CD14+ cells (Figure E2). Expression of HLA-DR was then analyzed (upper panel). T CD8+ lymphocytes were identified as CD45+ CD3+ CD8+ cells within the CD45+ SS low lymphocyte gate. Expression of HLA-DR, CD38 and PD-1 was then analyzed on T CD8+ lymphocytes (lower panel).

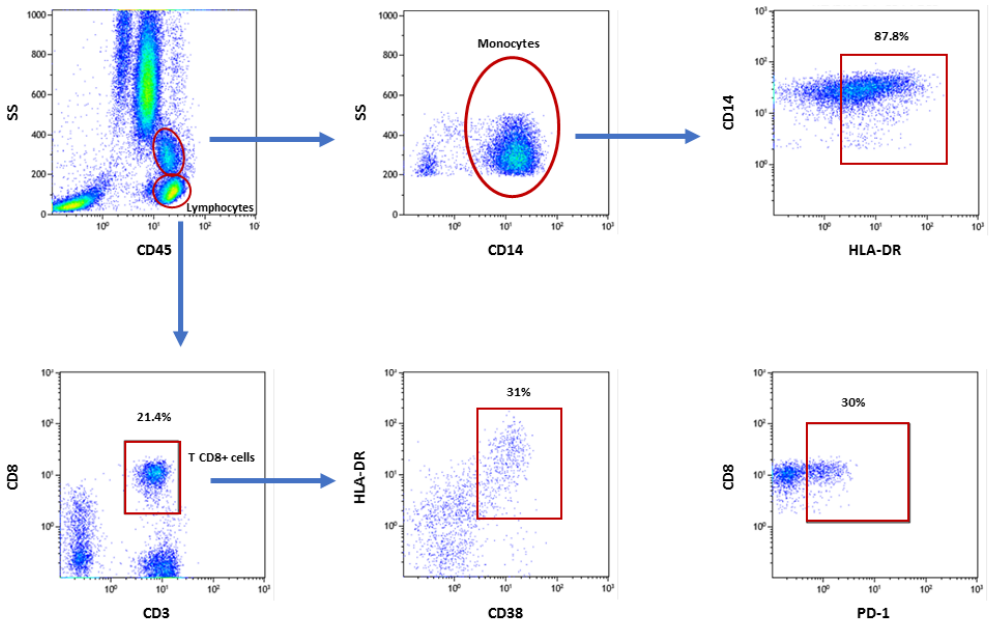


Figure E3.

Flow cytometry analysis of lymphocytes subsets and monocytes in COVID-19 (sky blue) and non-COVID-19 patients with viral (red) or bacterial/non-documented (ND) acute respiratory distress syndrome at days 1-2 of intensive care unit admission. A) Blood T CD4+ lymphocytes counts; There was no significant effect of ARDS group by the Kruskal-Wallis test (p=0.454); B) Blood T CD8+ lymphocytes counts; There was a significant effect of ARDS group by the Kruskal-Wallis test (p=0.021) but no significant between group differences on *post-hoc* comparisons; C) Blood B (CD19+) lymphocytes counts; There was a significant effect of ARDS group by the Kruskal-Wallis test (p=0.007) and a significant between group (viral *vs* COVID-19) difference on *post-hoc* comparisons (p-value comes from the Dunn's test); D) Percentage of T CD8+ CD38+ HLA-DR+ lymphocytes; There was no significant effect of ARDS group by the Kruskal-Wallis test (p=0.850); E) Percentage of T CD8+ PD1+ lymphocytes; There was a significant effect of ARDS group by the Kruskal-Wallis test (p=0.004) and a significant between group (viral *vs* COVID-19) difference on *post-hoc* comparisons (p-value comes from the Dunn's test); F) Percentage of HLA-DR+ monocytes; There was a significant effect of ARDS group by the Kruskal-Wallis test (p<0.0001) and a significant between group (viral *vs* bacterial/ND and bacterial/ND vs COVID-19) difference on *post-hoc* comparisons (p-value comes from the Dunn's test); Horizontal lines indicate the median value and the 1st and 3rd tertiles.

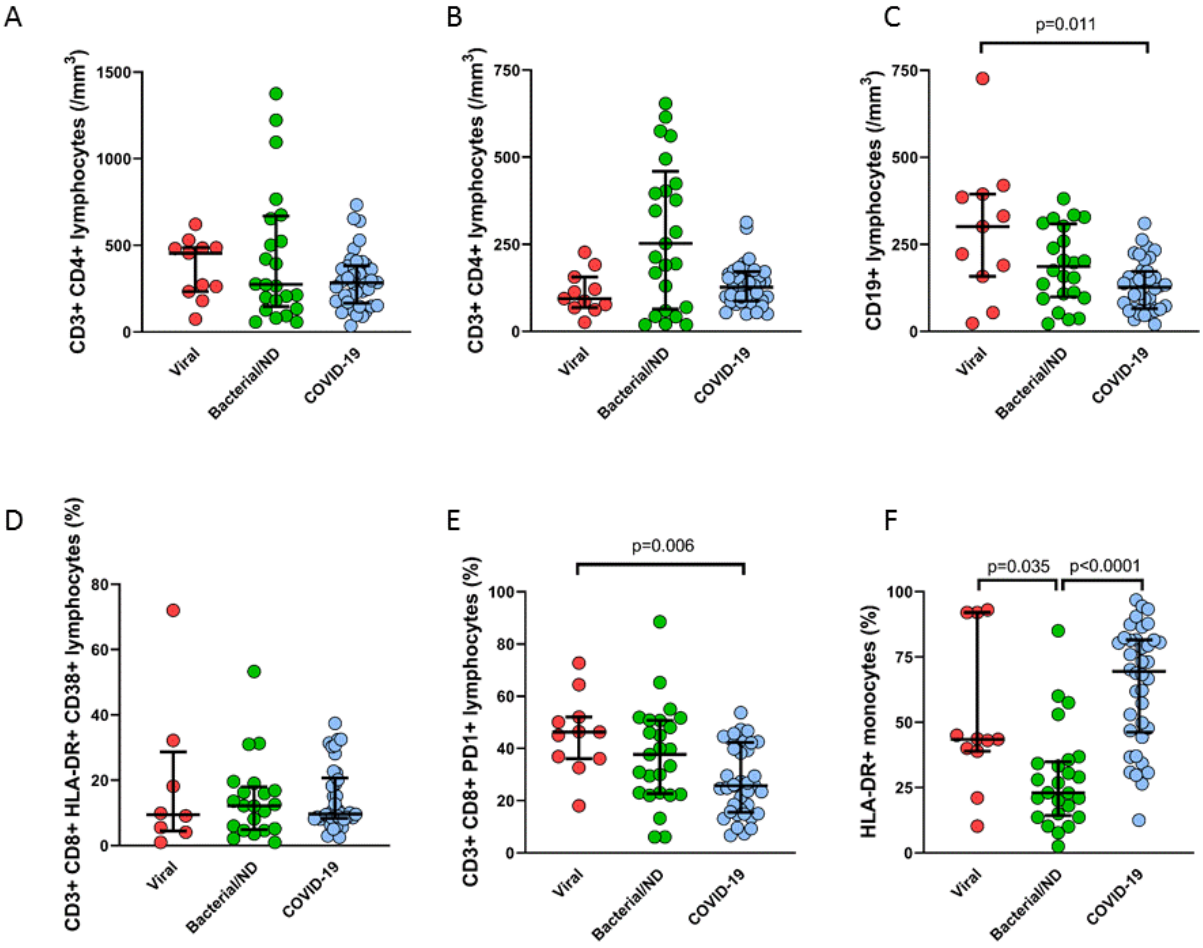


Figure E4.

Evolution of serum concentrations of cytokines over time in patients with COVID-19 (thick red lines) and non-COVID-19 (thick blue lines) acute respiratory distress syndrome. The y-axis represents serum concentrations expressed in (log) ng/mL. Individual trajectories of COVID-19 (thin red lines) and non-COVID-19 (thin blue lines) patients are represented in the background; The x-axis represents the time elapsed since symptoms onset.

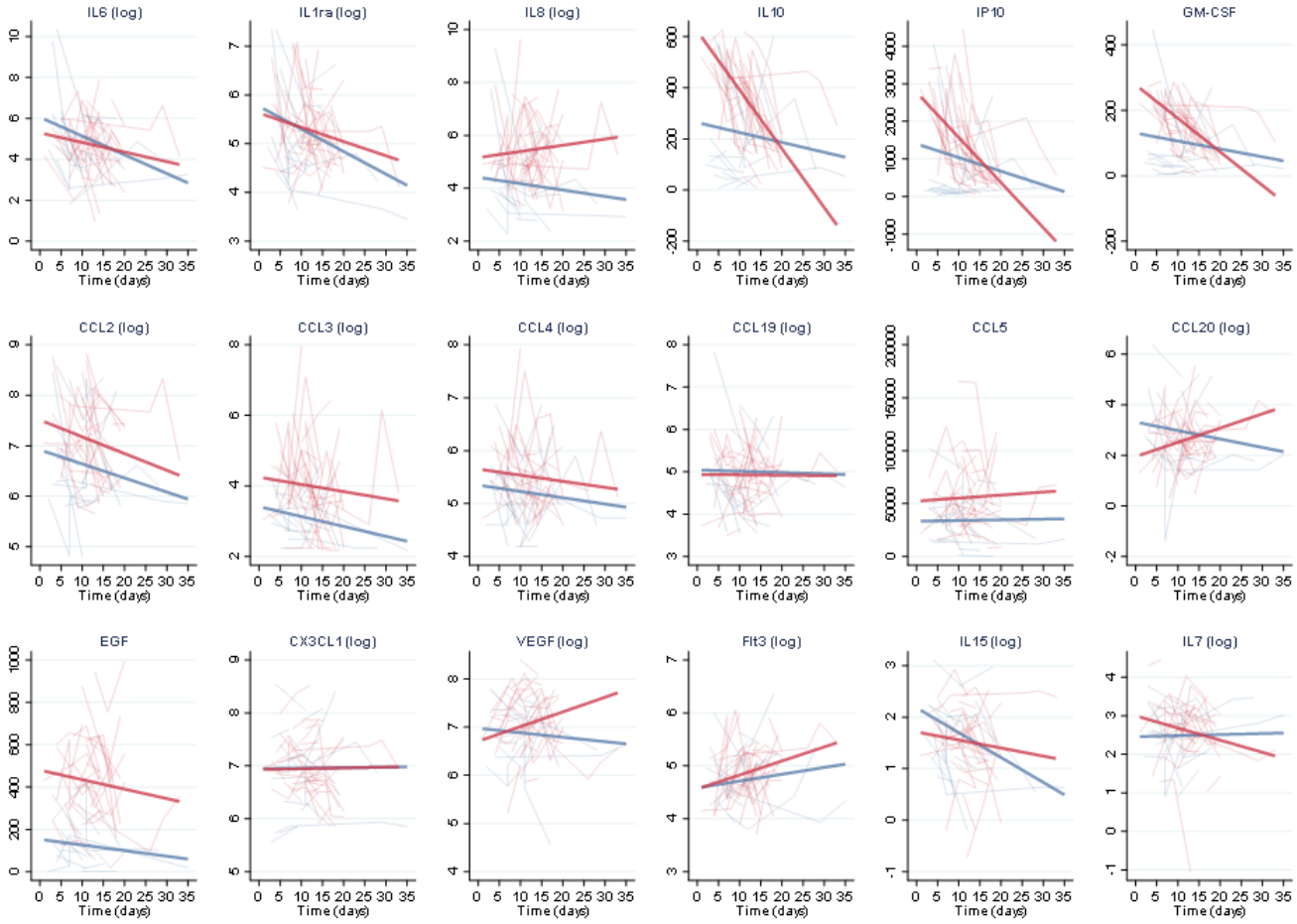
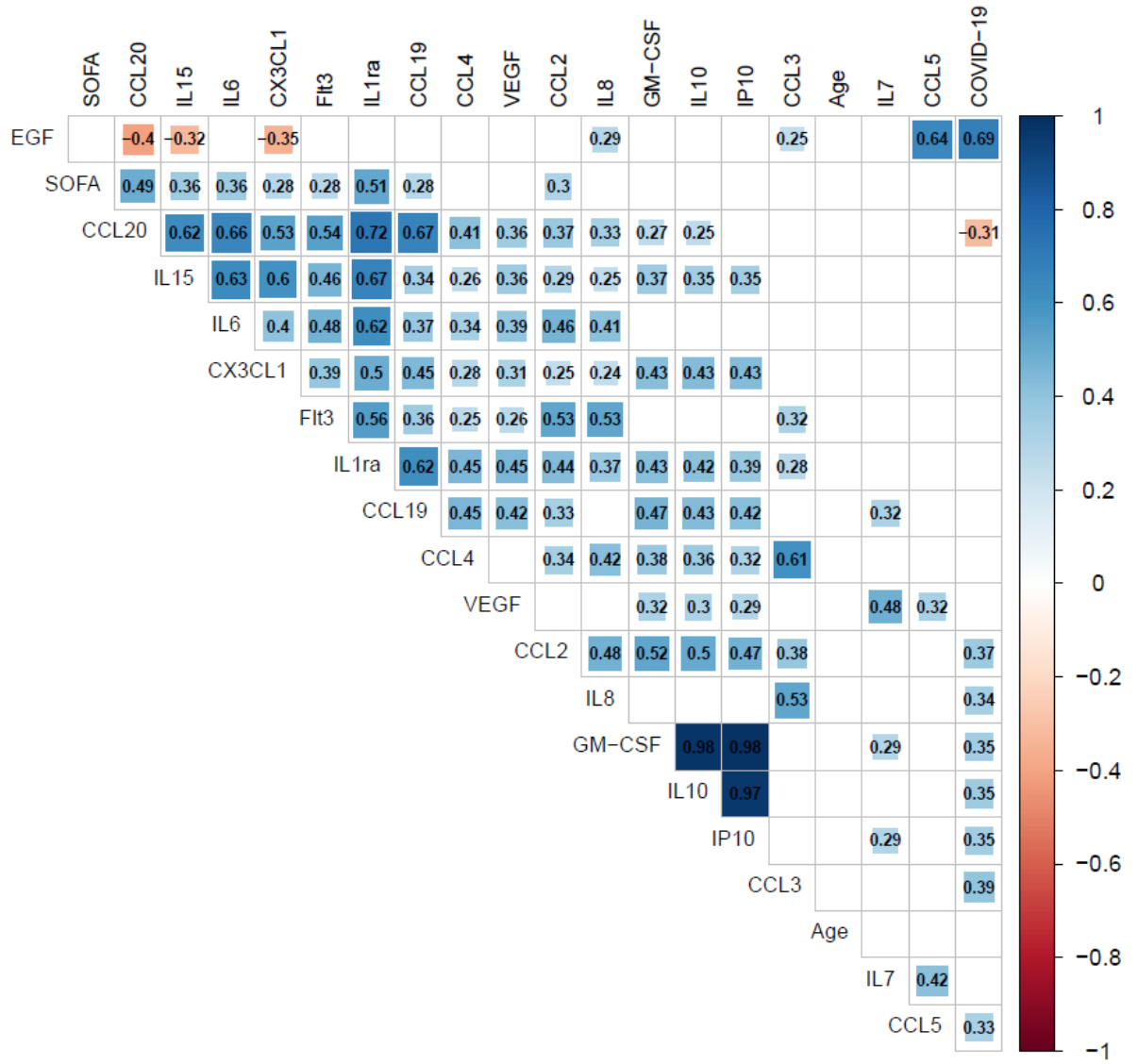


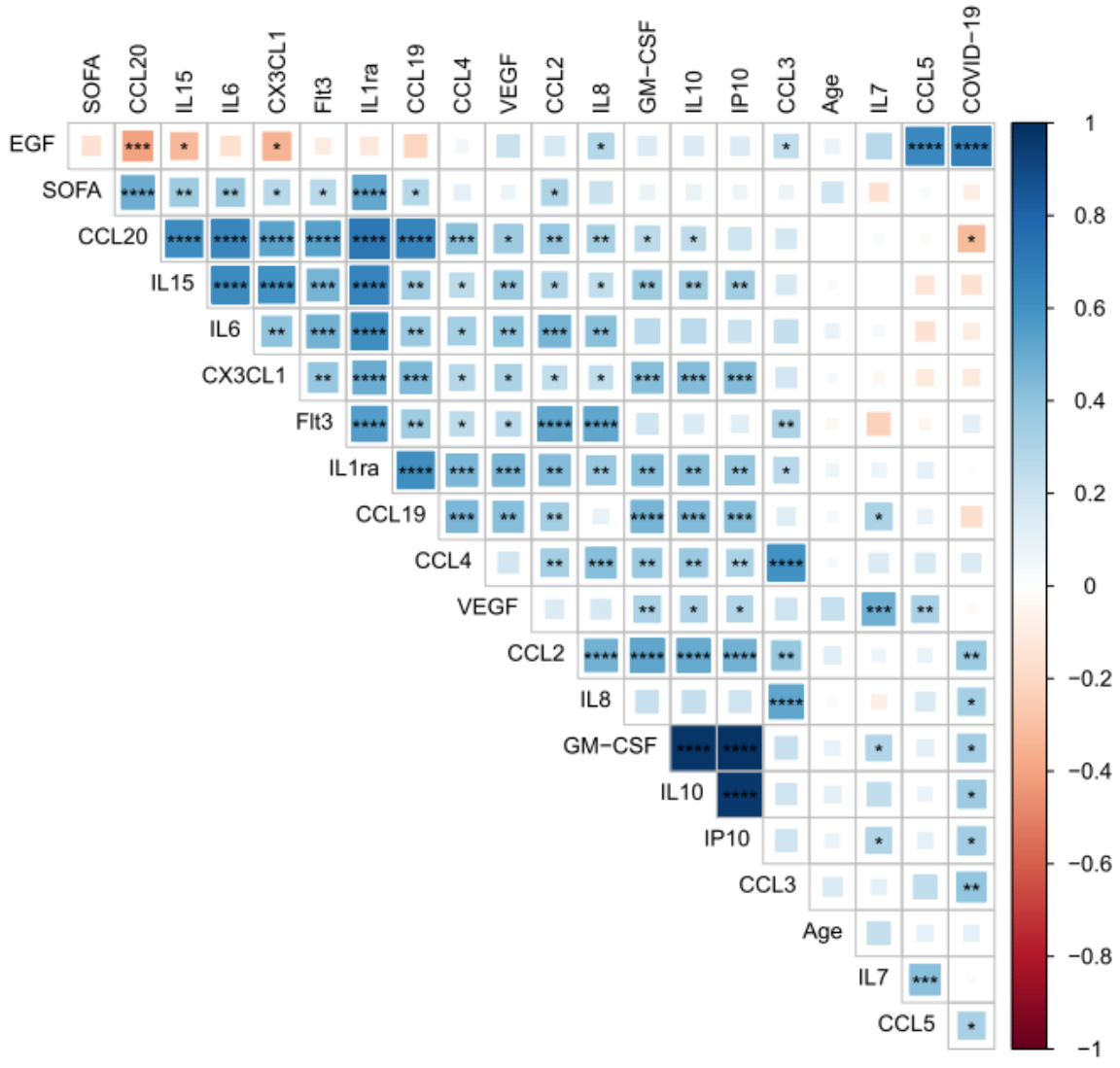
Figure E5.

Correlation matrix of serum cytokines concentrations, age and SOFA score in patients with COVID-19 (n=38) and non-COVID-19 (n=36) ARDS. A) Spearman and biserial correlation coefficients are provided for continuous-continuous and binary-continuous variables correlations, respectively, with positive (blue) and negative (red) correlation coefficients indicating statistical significance at the p<0.05 level after Benjamini-Hochberg correction for test multiplicity; B) Spearman and biserial correlation coefficients are provided for continuous-continuous and binary-continuous variables correlations, respectively, with p-values indicated as follows: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

A.



B.


SOFA
CCL20
IL15
IL6
CX3CL1
Flt3
IL1ra
CCL19
CCL4
VEGF
CCL2
IL8
GM−CSF
IL10
IP10
CCL3
Age
IL7
CCL5
COVID−19
EGF
SOFA
CCL20
IL15
IL6
CX3CL1
Flt3
IL1ra
CCL19
CCL4
VEGF
CCL2
IL8
GM−CSF
IL10
IP10
CCL3
Age
IL7
CCL5
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1