

**Multiplex array analysis of circulating cytokines and chemokines in COVID-19 patients during the first wave of the SARS-CoV-2 pandemic in Milan, Italy**

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**Table S1.** Concentrations of immune mediators in serum of SARS-CoV-2 positive (n=21) versus negative (n=9) patients. Data are provided as median and quartiles ranges (pg/mL).

Immune effector cell	Immune mediator	Type of mediator	SARS-CoV-2 negative	SARS-CoV-2 positive	p-value*
Th1	IFN- $\gamma$	Cytokine	15.15 (2.34-25.05)	21.21 (6.66-32.58)	0.4685
	IP-10 (CXCL10)	Chemokine	2026 (487-2087)	4669 (1849-5393)	0.0138
	TNF- $\alpha$	Cytokine	57.43 (47.41-67.10)	52.98 (40.27-62.67)	0.5811
Th2	IL-4	Cytokine	2.95 (2.34-4.08)	2.33 (1.79- 2.71)	0.8224
	IL-5	Cytokine	2.57 (0.94-4.87)	6.26 (0.94-10.55)	0.4574
	IL-9	Cytokine	82.72 (65.44-122.0)	125.01 (99.00-134.27)	0.0268
	IL-13	Cytokine	5.12 (2.79-6.67)	4.21 (3.48-5.28)	0.6501

	Eotaxin (CCL11)	Chemokine	74.72 (32.02-112.80)	100.50 (63.19-126.11)	0.0880
Th17	IL-6	Cytokine	18.39 (4.32-32.52)	22.44 (11.60-28.41)	0.1713
	IL-8 (CXCL8)	Cytokine	31.43 (10.56-44.85)	20.55 (16.25-24.22)	0.8270
	IL-17	Cytokine	26.09 (15.61-20.68)	18.81 (15.91-21.57)	0.9372
T reg	IL-10	Cytokine	28.28 (1.26-13.37)	7.91 (4.17-11.57)	0.5810
Broad spectrum	IL-1 $\beta$	Cytokine	1.84 (1.45-2.49)	1.84 (1.38- 2.00)	0.6651
	IL-1ra	Cytokine	1306 (556-2157)	1128 (398-1627)	0.4951
	IL-2	Cytokine	5.59 (5.02-6.63)	6.26 (5.02-7.75)	0.7104
	IL-7	Cytokine	30.33 (16.20-41.98)	34.51 (23.67-43.98)	0.1932
	IL-12(p70) (75)	Cytokine	3.51 (2.08-2.08)	4,11 (2.08-2.08)	0.8777
	IL-15		3.17 (3.17-3.17)	24.61 (3.17-3.17)	0.3859
	FGF basic	Trophic factor	36.85 (37.23-43.66)	50.09 (35.61-40.37)	0.1853
	G-CSF	Trophic factor	281.3 (148.7-378.3)	223.3 (147.0-246.6)	0.7462
	GM-CSF	Trophic factor	1.90 (0.59-.50)	2.27 (0.89-3.26)	0.5401
	MCP-1(MCAF) (53)		51.95 (20.97-74.84)	103.50 (48.12-137.18)	0.0372
	MIP-1 $\alpha$ (CCL3)	Chemokine	5.73 (2.23-7.68)	3.53 (2.34-3.57)	0.4172
	PDGF-bb	Trophic factor	3074 (1467-4740)	3726 (1990-5225)	0.3760
	MIP-1 $\beta$ (CCL4)	Chemokine	69.53 (51.75-98.24)	77.93 (58.49-89.41)	0.1259
	RANTES (CCL5)	Chemokine	8672 (5284-13207)	13480 (10405-17024)	0.0297
VEGF	Trophic factor	34.26 (1.20-69.26)	85.88 (1.20-179.50)	0.2361	

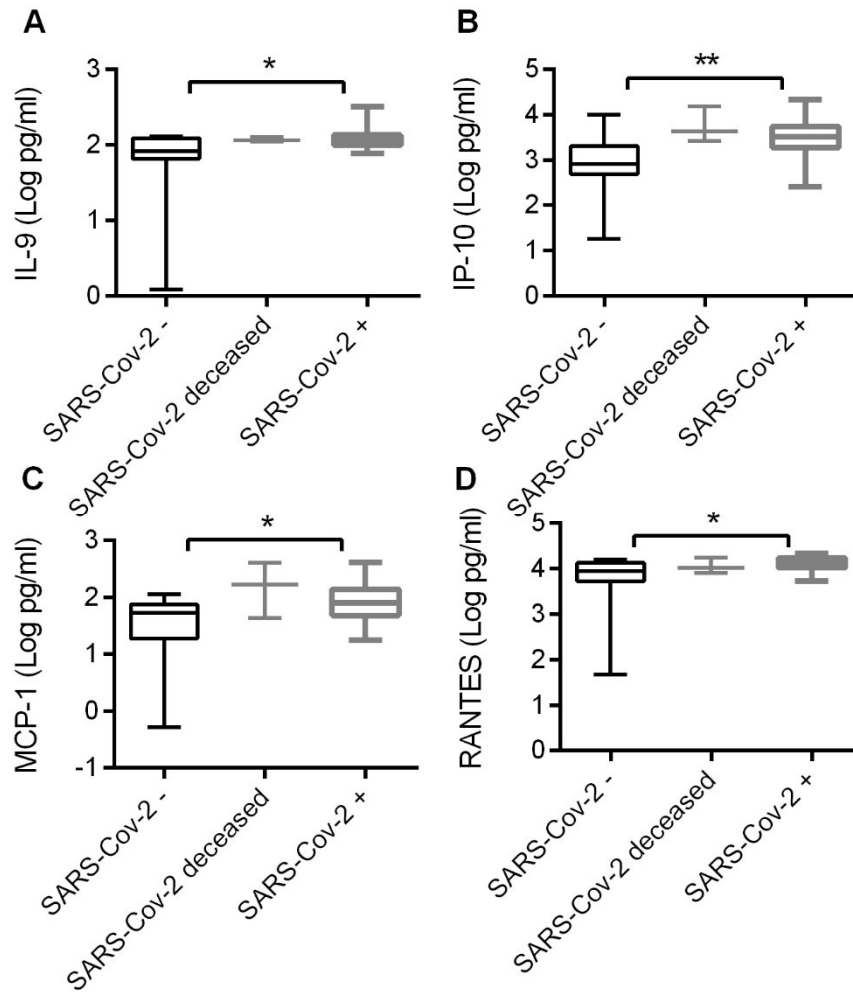
\*p-values were calculated on the log<sub>10</sub> (Log) data

**Table S2.** Concentrations of immune mediators in serum of SARS-CoV-2 positive (n=21) patients. Data are provided as median and quartiles ranges (pg/mL).

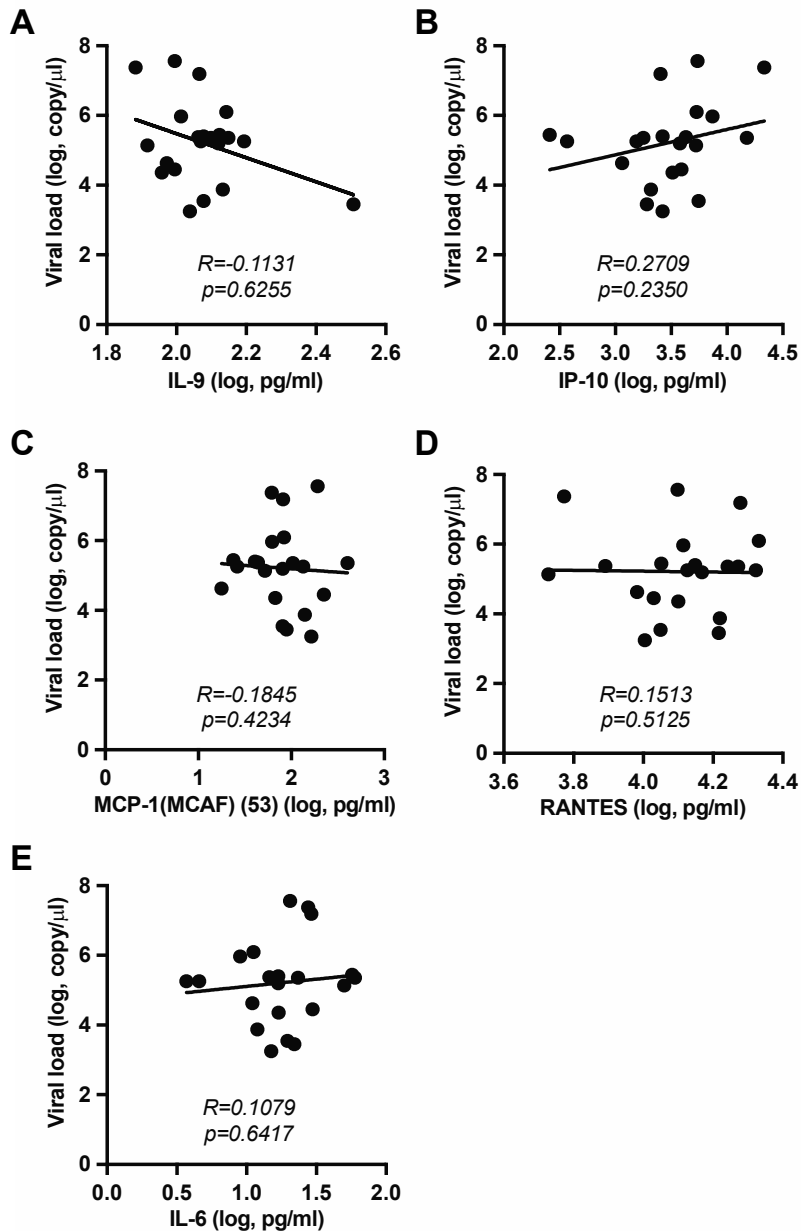
<b>Immune mediator</b>	<b>T0</b>	<b>T1</b>	<b>T2</b>
IL-1 $\beta$	1.78 (1.36-1.90)	1.82 (1.45-2.22)	2.23 (1.45-2.44)
IL-1ra	1012 (378.9-1446)	1148 (475.5-1416)	1835 (387.4-922.9)
IL-2	6.06 (5.02-6.86)	6.50 (5.02-6.86)	6.44 (5.02-7.75)
IL-4	2.31 (1.71-2.64)	2.63 (1.71-3.57)	3.48 (1.71-4.32)
IL-5	6.13 (0.94-8.12)	6.95 (0.94-9.38)	6.11 (0.94-9.38)
IL-6	22.69 (13.92-28.06)	15.75 (2.50-15.41)	16.28 (1.87-24.08)
IL-7	34.65 (25.61-43.42)	34.84 (28.13-43.98)	35.96 (23.03-47.85)
IL-8	20.72 (16.04-26.42)	23.66 (12.13-31.47)	68.61 (11.13-39.94)
IL-9	124.5 (99.00-132.7)	125.5 (105.2-137.8)	118.6 (99.00-133.8)
IL-10	7.93 (4.17-12.30)	7.88 (5.30-11.57)	6.03 (2.65-8.27)
IL-12(p70) (75)	3.31 (2.08-2.08)	2.89 (2.08-3.67)	3.57 (2.08-5.09)
IL-13	4.22 (3.48-5.44)	4.32 (3.14-4.80)	5.01 (3.48-6.37)
IL-15	26.87 (3.17-3.17)	3.17 (3.17-3.17)	63.84 (3.17-98.98)
IL-17	18.96 (16.21-21.57)	19.96 (18.00-21.57)	22.65 (15.60-25.10)
Eotaxin	100.50 (63.19-126.1)	111.5 (66.82-183.8)	122.7 (64.74-181.1)
FGF basic	51.28 (34.78-39.61)	51.93 (34.78-46.77)	42.63 (34.78-44.01)
G-CSF	215.7 (143.3-238.2)	267.9 (184.3-295.0)	263.1 (145.4-343.1)
GM-CSF	2.22 (0.64-3.43)	2.76 (1.98-3.43)	3.12 (0.49-4.57)
IFN- $\gamma$	20.60 (7.27-29.56)	26.63 (9.86-32.07)	31.54 (5.54-48.26)
IP-10	4751 (1918-5347)	1243 (393.4-1824)	906.9 (479.8-848.1)
MCP-1(MCAF) (53)	104.1 (43.9-140.2)	123.0 (31.55-127.6)	138.1 (34.41-168.2)
MIP-1 $\alpha$	3.34 (2.25-3.55)	4.36 (2.29-5.05)	3.90 (2.07-4.68)
PDGF-bb	3645 (2031-5200)	4578 (2567-5894)	3765 (2172-4638)
MIP-1 $\beta$	78.83 (59.41-89.25)	86.80 (68.97-92.93)	85.80 (78.72-92.12)
RANTES	13105 (10100-16604)	13011 (10325-16145)	12915 (10989-14716)
TNF- $\alpha$	51.93 (39.51-58.97)	51.85 (41.02-57.48)	55.26 (41.78-61.93)

VEGF	75.41 (1.20-179.5)	118.2 (1.20-201.6)	105.6 (1.20-175.0)
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T0 = first day of hospital admission; T1 = intermediate time; T2 = last day of hospital admission.



**Figure S1.** Cytokine and chemokine levels in SARS-CoV-2-negative subjects, deceased SARS-CoV-2-positive patients, and SARS-CoV-2-positive patients who recovered. Cytokines and chemokines (A: IL-9; B: IP-10; C: MCP-1; D: RANTES) were measured in serum samples using multiplex immunoassays and analyzed in relation to SARS-CoV-2 infection status. Data are presented as log<sub>10</sub> (Log) of concentrations (picograms per milliliter, pg/ml). Statistical analyses were performed using an unpaired t-test. \*p<0.05; \*\*p<0.01.



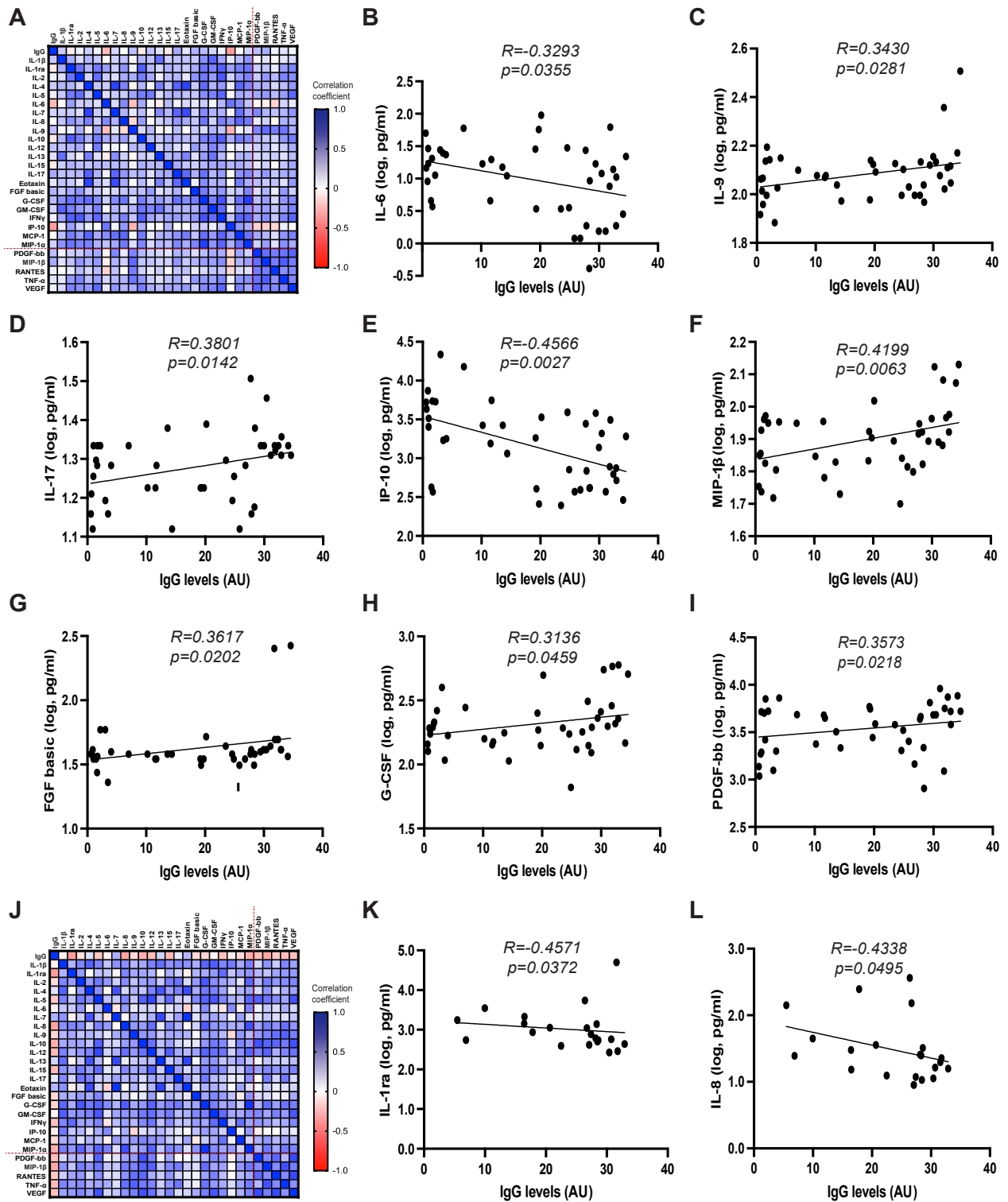
**Figure S2.** Correlation analysis between viral load and serum cytokines/chemokines levels. Each scatter plot represents individual patient data points, assessing the relationship between viral load detected in nasal swabs and serum levels of specific cytokines/chemokines (A: IL-9; B: IP-10; C: MCP-1; D: RANTES; E: IL-6). The Spearman correlation coefficient (R) and the associated p-value are displayed on each graph, with a fitted line illustrating the correlation trend. No significant correlation was observed across the cytokines/chemokines tested.

**Table S3.** Anti-Spike IgG levels in the sera of SARS-CoV-2 positive patients by semi-quantitative ELISA. Data are provided as median with quartile ranges.

<b>SARS-CoV-2 positive patients IgG (ratio)*</b>		
<b>T0 (n=21)#</b>	<b>T1 (n=18)#</b>	<b>T2 (n=21)#</b>
10.6713 (0.5895-34.5606)	26.4332 (5.4797-34.0751)	22.9372 (1.4682-32.9017)

\*Measurements were conducted using a commercial indirect ELISA colorimetric kit (ab275300, Abcam), according to the manufacturer's instructions. After determining the validity of the Positive and Negative Controls relative to the Calibrator value, the values of the samples were compared to the Calibrator to generate a ratio. Ratios equal to or greater than 0.9 were considered positive, while ratios of 0.8 or less were considered negative. Ratios between 0.8 and 0.9 were classified as equivocal, indicating indeterminate results.

#T0 = first day of hospital admission; T1 = intermediate time; T2 = last day of hospital admission.



**Figure S3.** Correlations between serum cytokines/chemokines and anti-SARS-CoV-2 IgG antibodies during distinct hospitalization periods. The samples are divided into early (from day 1 to 14, A-I) and late (from day 15 to 7 weeks, J-L) response periods. Heat maps represent Spearman's correlation coefficients for cytokines/chemokines and IgG antibody titers in early (A) and late (J) response samples. Scatter plots show the correlations between



IgG levels and individual cytokines/chemokines in early (B-I) and late response samples (K-L). Each plot includes individual data points with a fitted line indicating the correlation trend. Only significant correlations are shown ( $p < 0.05$ ). R: correlation coefficient.