UNIVERSITÀ DEGLI STUDI DI MILANO

Sistema Socio Sanitario
Regione Lombardia
ASST Santi Paolo e Carlo

SARS-CoV-2 plasma viral load and adaptive immune response in COVID-19 patients

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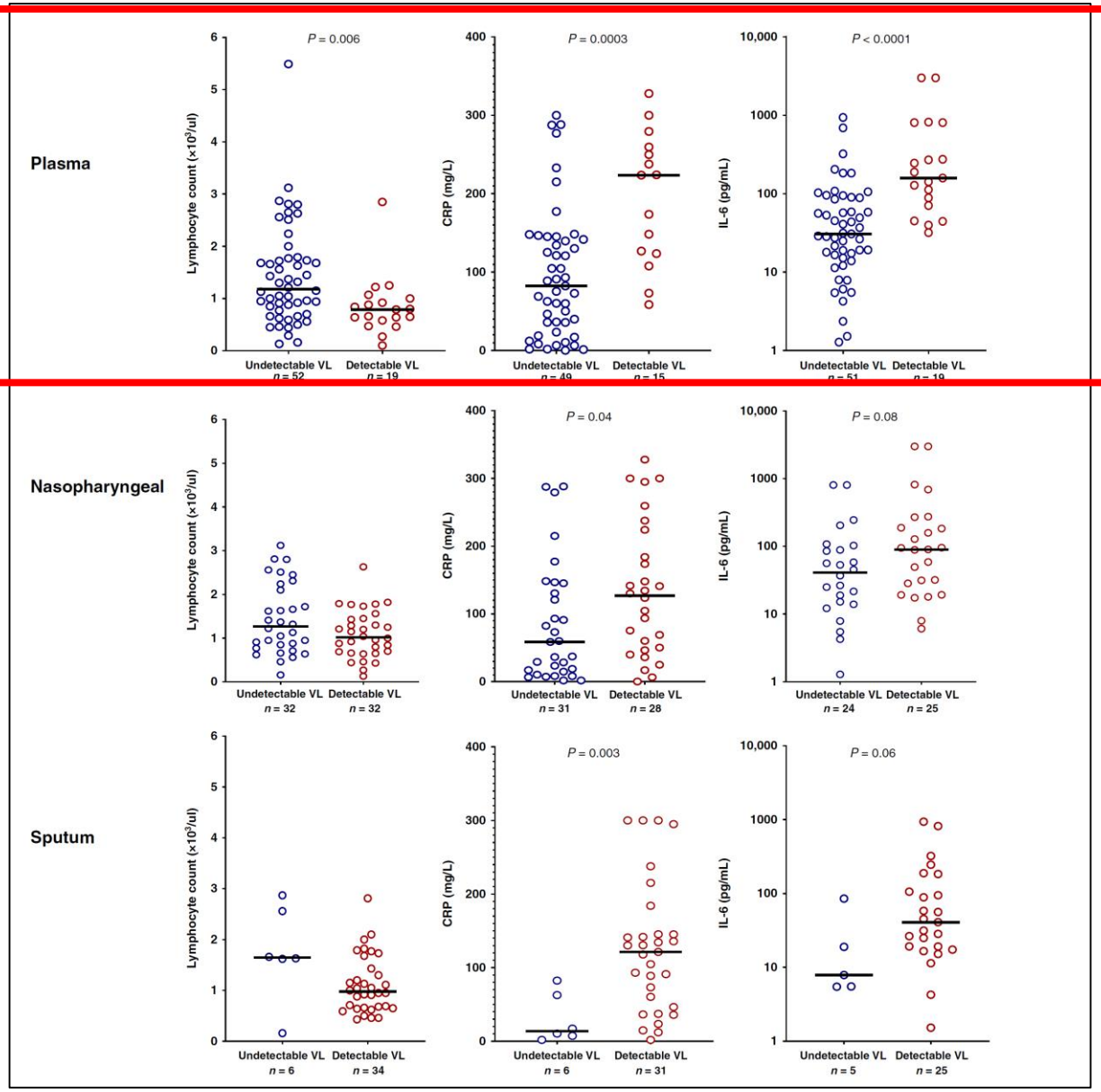
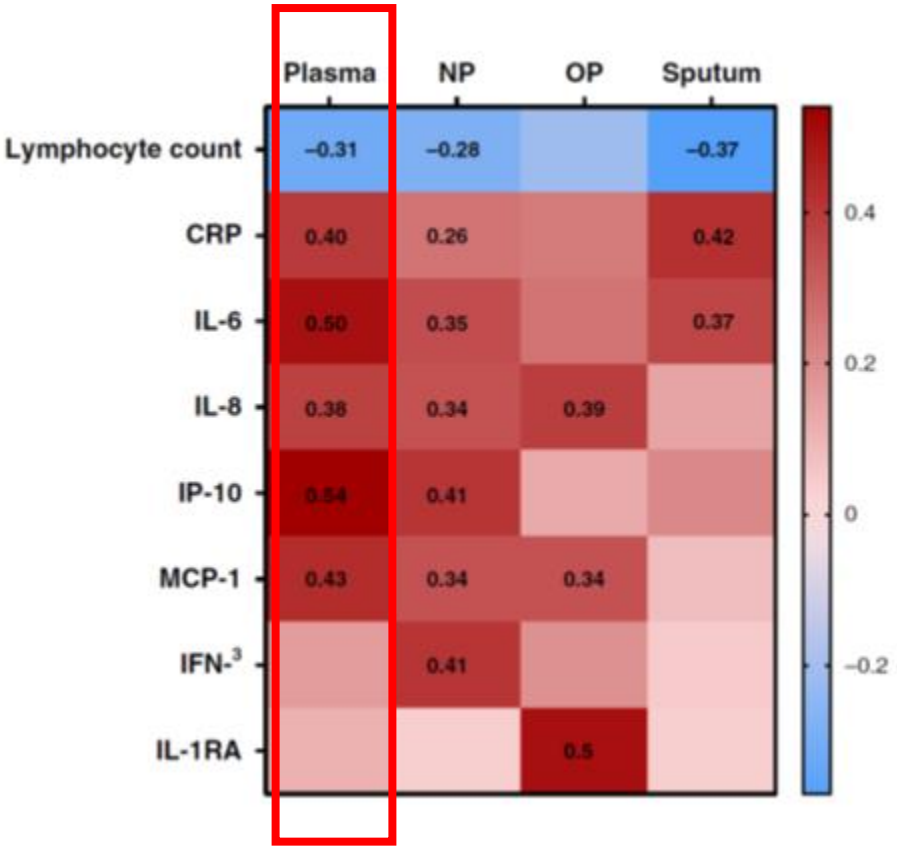
7 June 2021

Background: SARS-CoV-2 viremia and clinical markers of disease severity

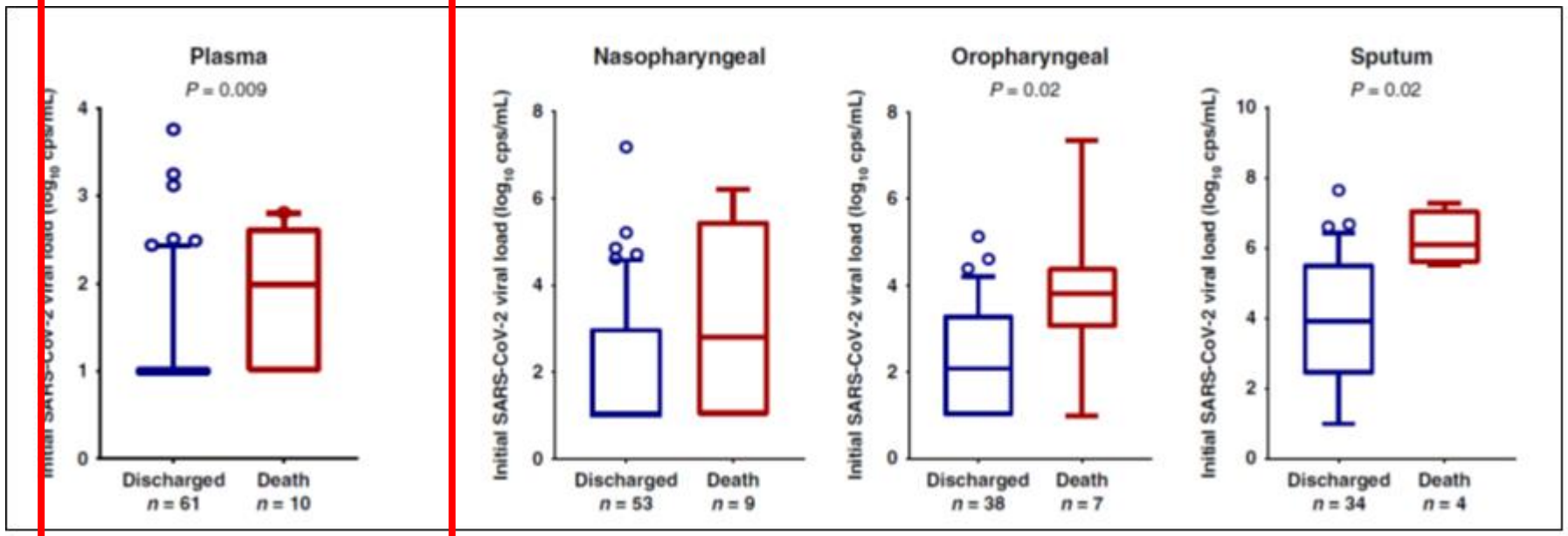


J. Fajnzylber et al, Nature Communications 2020



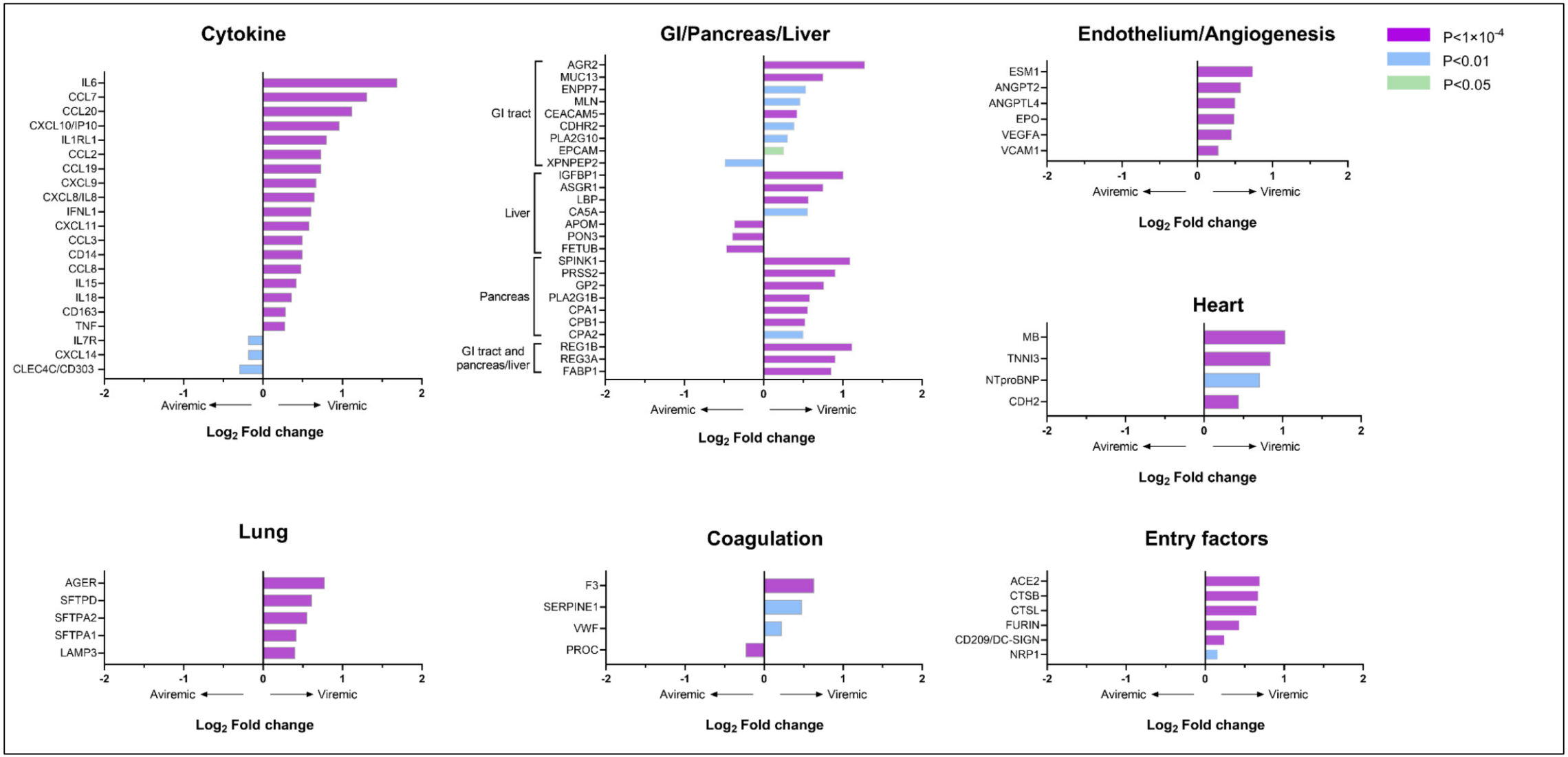
Y. Li et al, medRvix 2021
K. Hagman et al, Clinical Infectious Diseases 2020
K. A. Ederhardt et al, Viruses 2020
J. D. Jarhult et al, Nature Scientifi Reports
L. Chen et al, The Journal of Molecular Diagnostics 2021
L. Colagrossi et al, BMC Infectious Diseases 2021

Background: SARS-CoV-2 viremia and mortality



J. Fajnzylber et al, Nature Communications 2020

Background: SARS-CoV-2 viremia and tissue damage



Y. Li et al, medRvix 2021

Research question: what is the link between SARS-CoV-2 viremia and adaptive immunity?

Materials and methods

- 54 hospitalized COVID-19 patients with pneumonia studied within the first 10 days from disease onset
- Plasmatic SARS-COV-2 viral load quantification (copies/ml): RT-PCR
- Plasmatic cytokines quantification (pg/ml): MACSPlex Cytokine 12 kit (Miltenyi)
- T cell activation immunophenotype evaluation: HLA-DR+CD38+ T cells (flow cytometry)
- T cell degranulation immunophenotype evaluation: Granzyme B+/Perforin+ T cells (flow cytometry)

- SARS-CoV-2 specific T cell response: CD4/CD8/IFNg/IL2/TNFa/IL4/IL17 (flow cytometry)

- SARS-CoV-2 specific Ab production:
  - Total IgG vs S1/S2 (Liaison SARS-CoV-2 S1/S2 IgG, DiaSorin)
  - Total Ig vs RBD (home-made ELISA, IFOM collaboration)

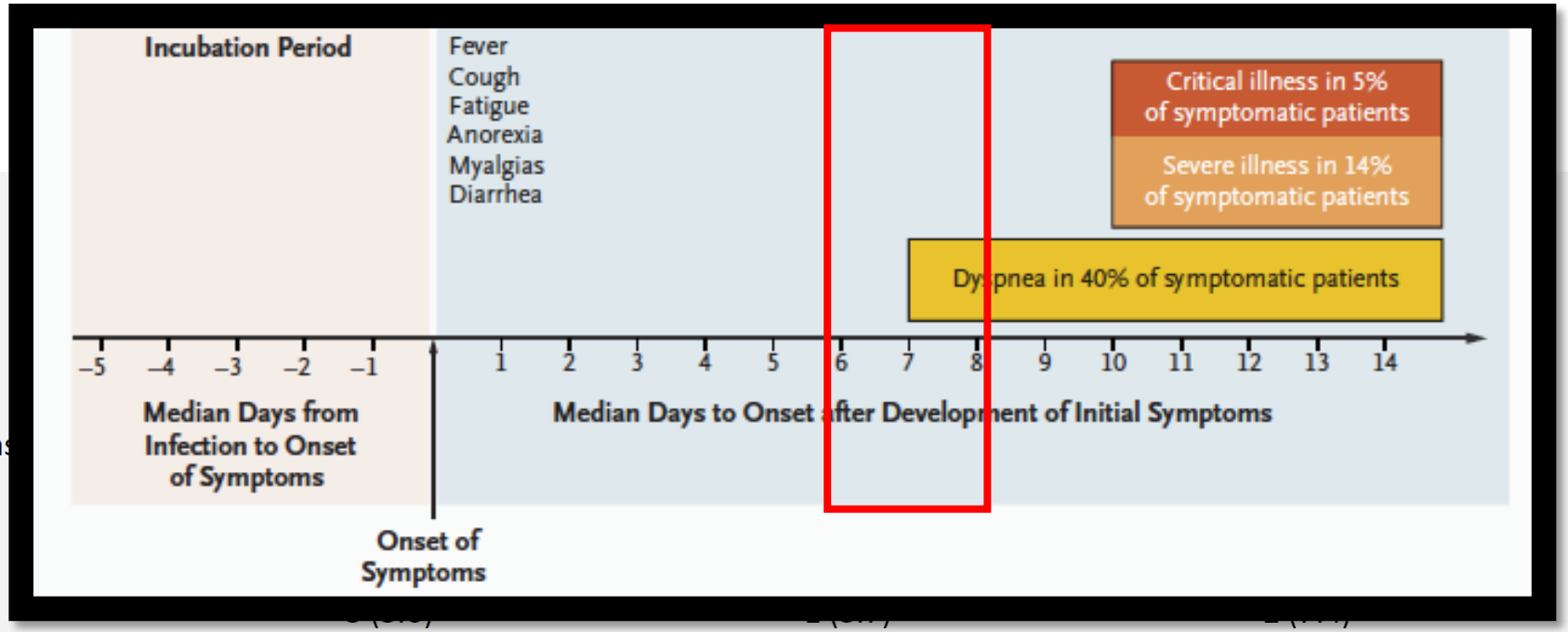
- Statistical analyses: non-parametric two-tailed Mann-Whitney U and Spearman correlation test.

SARS-CoV-2 viremia: study population

Demographic and clinical characteristics	**All COVID-19 patients (n=54)**	**Viremic COVID-19 patients (n=27)**	**Aviremic COVID-19 patients (n=27)**	***p*-value viremic *vs* aviremic COVID-19 patients°**
Sex, n (%)				1
Male	39 (72.2)	20 (74.1)	19 (70.4)	
Female	15 (27.8)	7 (25.9)	8 (29.6)	
Age, years, median (IQR)	64 (52.50–75.25)	67 (55–78)	63 (45–71)	0.166
Ethnicity, n (%)				
Caucasian	46 (82.2)	24 (88.9)	22 (81.5)	0.704
Maghrebi/Middle Eastern	1 (1.9)	1 (3.7)	0 (0)	1
South Asian	1 (1.9)	1 (3.7)	0 (0)	1
Latin American	6 (11.1)	1 (3.7)	5 (18.5)	0.192
Comorbidities, n (%)				
Any comorbidity	29 (53.7)	16 (59.3)	13 (48.2)	0.586
Hypertension	20 (37)	10 (37)	10 (37)	1
Myocardial infarction	8 (14.8)	6 (22.2)	2 (7.4)	0.250
Cardiac arrhythmia	3 (5.6)	1 (3.7)	2 (7.4)	1
COPD	1 (1.9)	1 (3.7)	0 (0)	1
Asthma	2 (3.7)	0 (0)	2 (7.4)	0.491
Peptic ulcer	2 (3.7)	0 (0)	2 (7.4)	0.491
Chronic kidney disease	2 (3.7)	2 (7.4)	0 (0)	0.496
Diabetes	14 (25.9)	9 (33.3)	5 (18.5)	0.352
Stroke	3 (5.6)	1 (3.7)	2 (7.4)	1
Dementia	1 (1.9)	0 (0)	1 (3.7)	1
Cancer	2 (3.7)	2 (7.4)	0 (0)	0.496
Rheumatologic disease	1 (1.9)	0 (0)	1 (3.7)	1

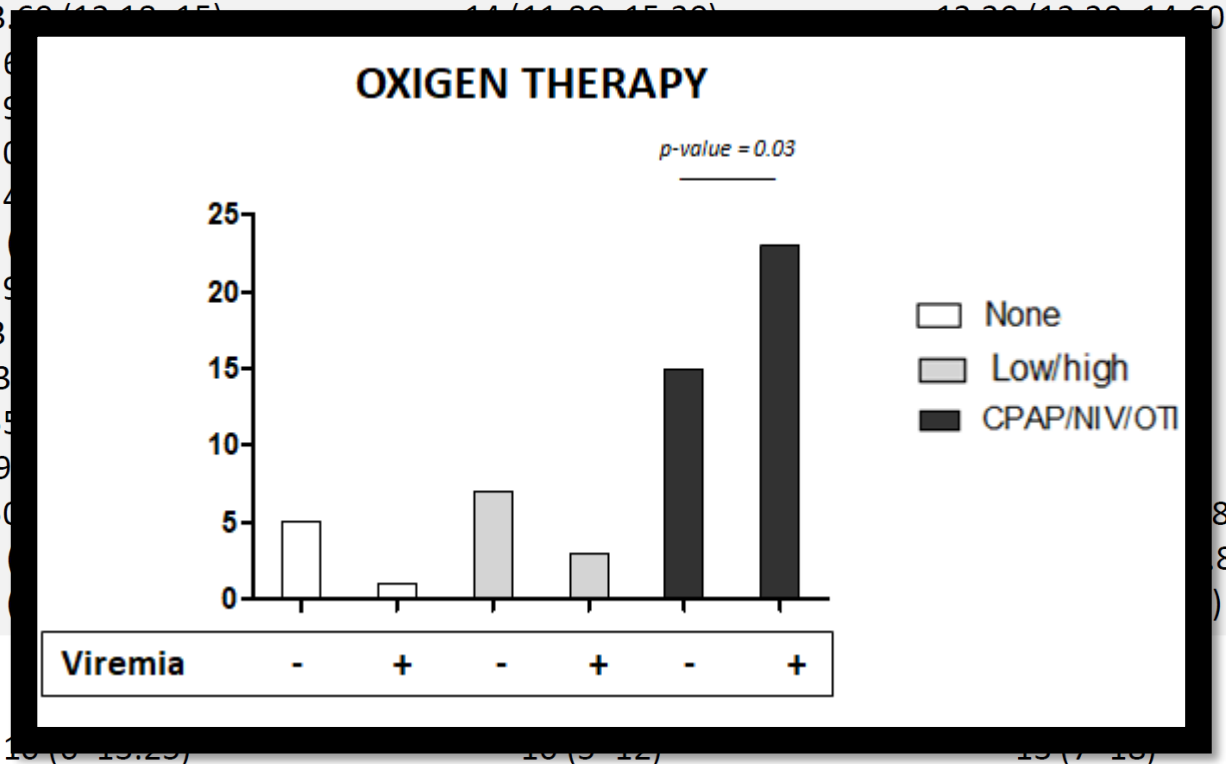
SARS-CoV-2 viremia: study population

BMI, median (IQR)	26.70 (23.98–29.45)	26.40 (25.70–27.14)	27.10 (22.95–31.73)	0.776
Smoke, n (%)				
Current smoker				1
Former smoker				0.744
Never smoker				**0.028 (*)**
Unknown				
Symptoms at admission, n				
Fever				1
Arthromyalgia				1
Chest pain				0.1
Cough				1
Dyspnea				1
Gastrointestinal symptoms				1
Nausea/vomiting				1
Abdominal pain				1
Diarrhea				1
Syncope				1
Headache	1 (1.9)	0 (0)	1 (3.7)	1
Anosmia/dysgeusia	10 (18.5)	6 (22.2)	4 (14.8)	0.728
Duration of symptoms before hospitalization, days, median (IQR)	7 (4–10)	7 (4–10)	6 (4–14)	0.41
Radiological pulmonary infiltrates, n (%)				1
Bilateral	50 (92.6)	25 (92.6)	25 (92.6)	
Monolateral	4 (7.4)	2 (7.4)	2 (7.4)	
P_aO_2/F_iO_2 nadir, median (IQR)	188.5 (142–304.5)	157 (111–276)	225 (154–310)	0.143



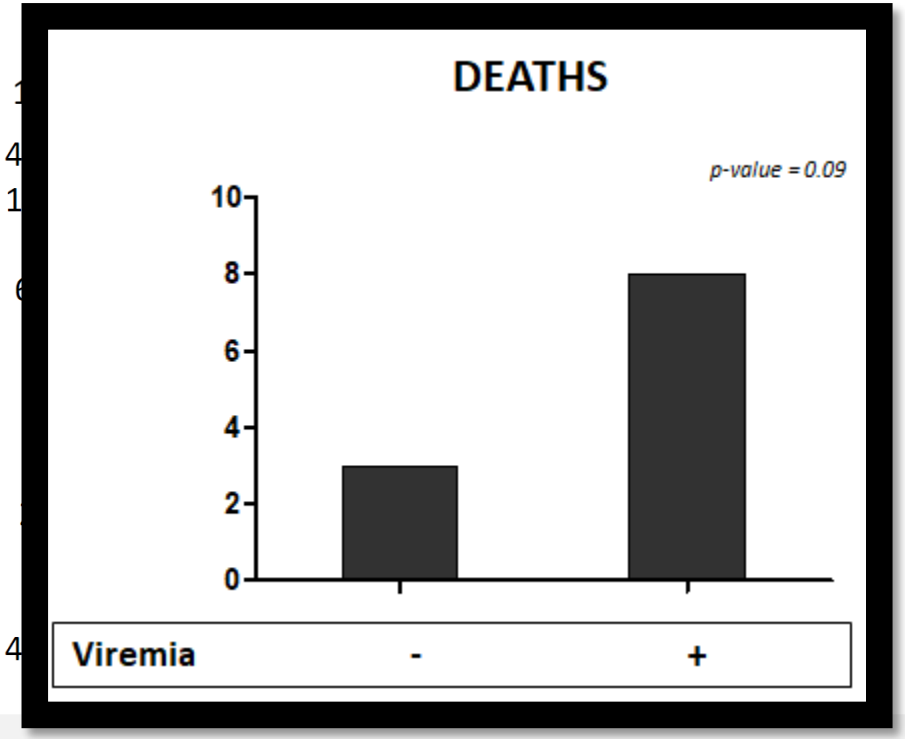
SARS-CoV-2 viremia: study population

Blood exams upon admission, median (IQR)				
Hemoglobin, g/dL	13	14	13	0.2
Leucocytes, $10^3/\mu L$	6.6			0.468
Neutrophils, $10^3/\mu L$	4.9			0.276
Lymphocytes, $10^3/\mu L$	1.0			0.299
Monocytes, $10^3/\mu L$	0.4			0.288
Platelets, $10^3/\mu L$	209			0.247
Creatinine, mg/dL	0.9			**0.028 (*)**
AST, U/L	43			0.908
ALT, U/L	3			0.666
CRP, mg/L	69.55			0.162
Ferritin, ng/mL	629			0.662
D-dimer, ng/mL	378.50		8)	0.811
LDH, U/L	301		80)	0.193
CPK, U/L	85		)	**0.013 (*)**
Time between symptoms onset and biological samples collection, days, median (IQR)				**0.016 (*)**
Maximum oxygen therapy, n (%)				
None	6 (11.1)	1 (3.7)	5 (18.5)	0.192
Low/high-flow systems	10 (18.5)	3 (11.1)	7 (25.9)	0.293
CPAP/NIV/OTI	38 (70.4)	23 (85.2)	15 (55.6)	**0.035 (*)**

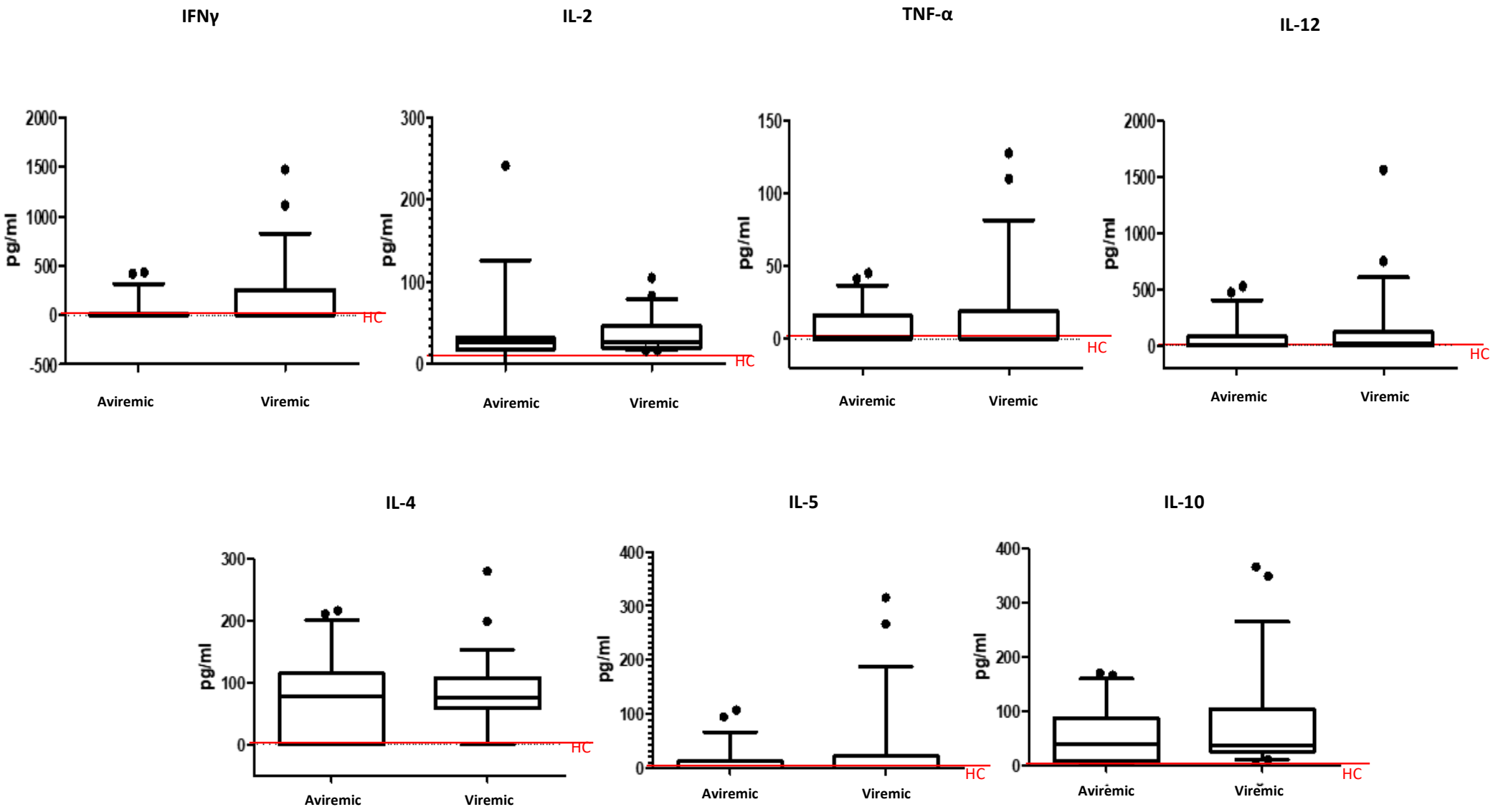


SARS-CoV-2 viremia: study population

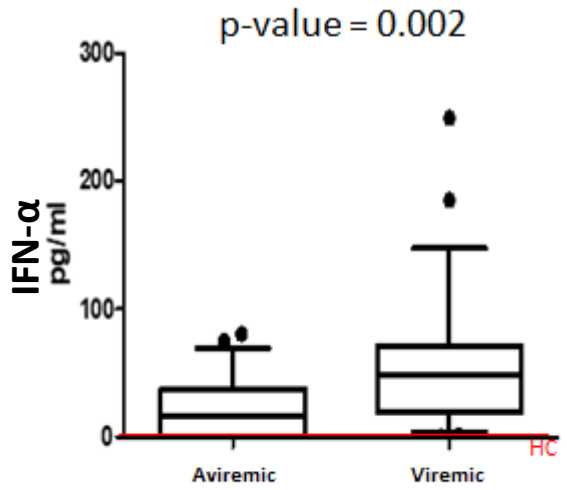
Medical therapy, n (%)				
LPV/r or DRV/c	1		7 (25.9)	0.766
Hydroxychloroquine	4		22 (81.5)	0.526
Azithromycin	1		10 (37)	0.773
Remdesivir			0 (0)	0.496
Tocilizumab	6		3 (11.1)	1
Sarilumab			1 (3.7)	1
Baricitinib			0 (0)	1
Ruxolitinib			1 (3.7)	1
Anakinra			0 (0)	1
Steroids			8 (29.6)	0.398
Celecoxib			1 (3.7)	0.1
Interferon beta 1a			0 (0)	1
Heparin prophylaxis	4		20 (74.1)	1
Hyperimmune plasma			0 (0)	0.236
Duration of hospitalization, days, median (IQR)	14.50 (6–25)	20 (8–28)	13 (6 –22)	0.426
Outcome, n (%)				0.0994
Death	12 (22.2)	9 (33.3)	3 (11.1)	
Dismissal	42 (77.8)	18 (66.7)	24 (88.9)	



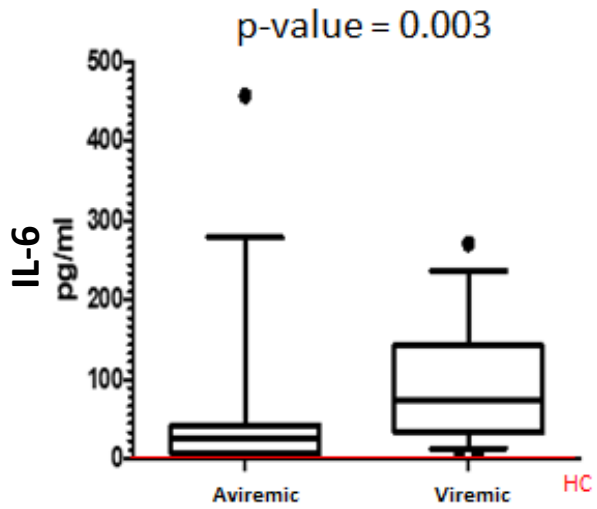
SARS-CoV-2 viremia: plasmatic cytokines quantification



SARS-CoV-2 viremia: plasmatic cytokines quantification



Correlation:
r=0.3
p-value=0.02



Correlation:
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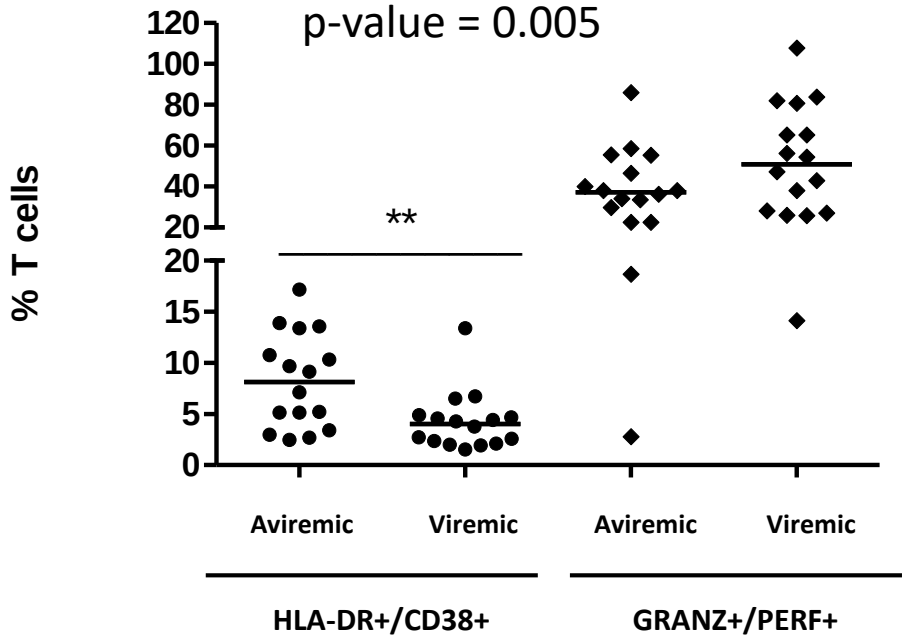
p-value=0.003

SARS-CoV-2 viremia: T cell activation and degranulation phenotype



Correlation:
r = -0.3
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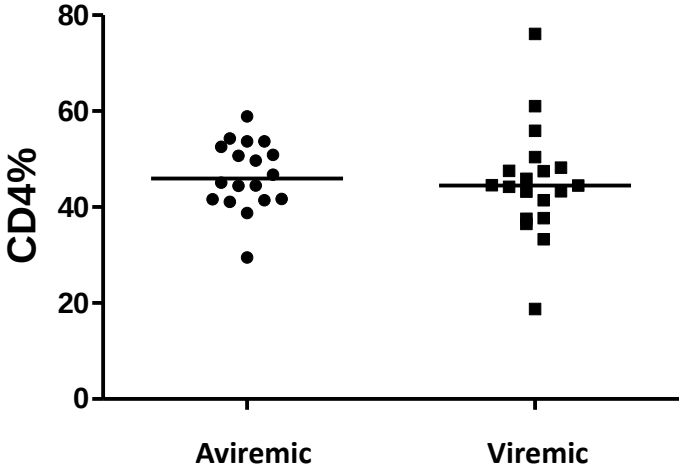
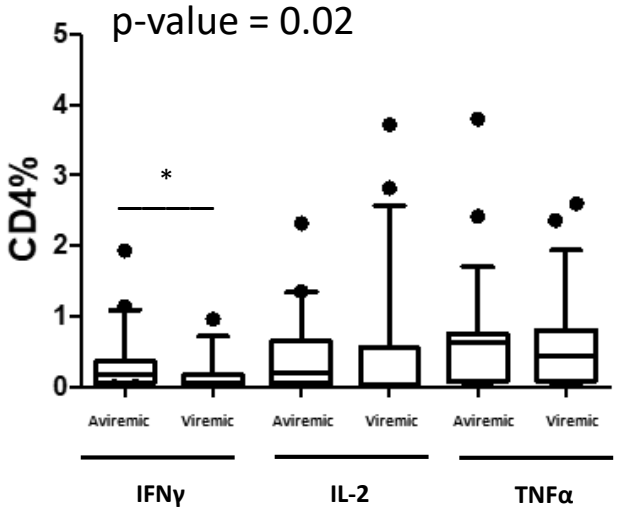
SARS-CoV-2 viremia: SARS-CoV-2 specific CD4 T cell response

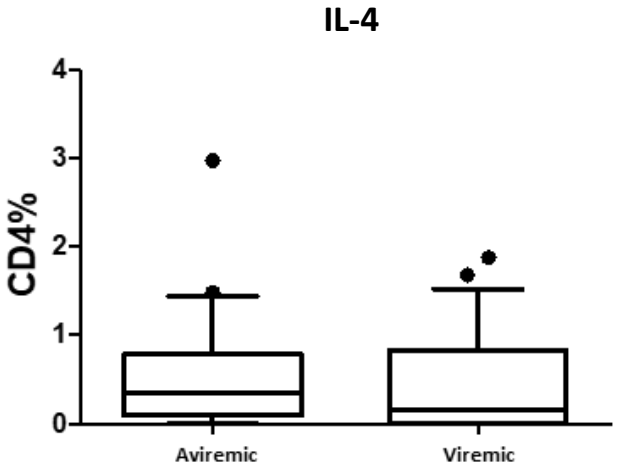
PBMCs stimulated with SARS-CoV-2 peptide pool (S, N and M)



Th1 response

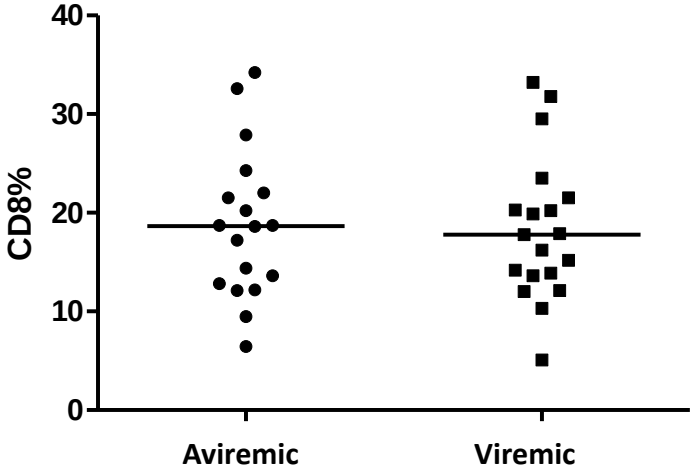


Th2 response

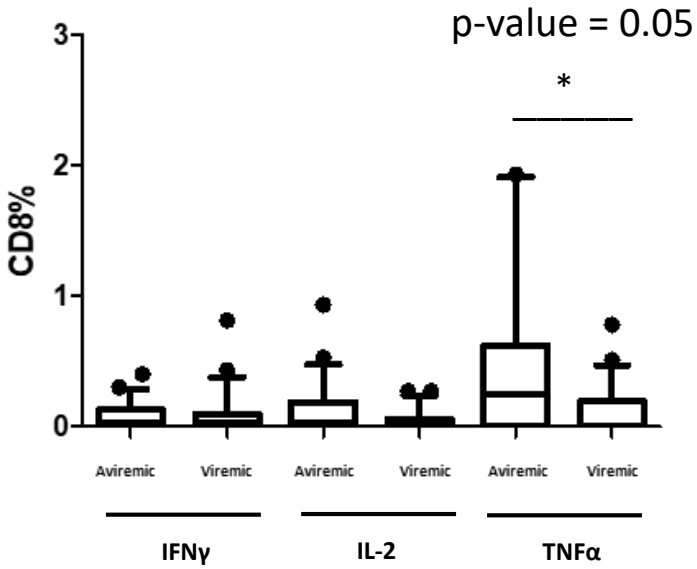


SARS-CoV-2 viremia: SARS-CoV-2 specific CD8 T cell response

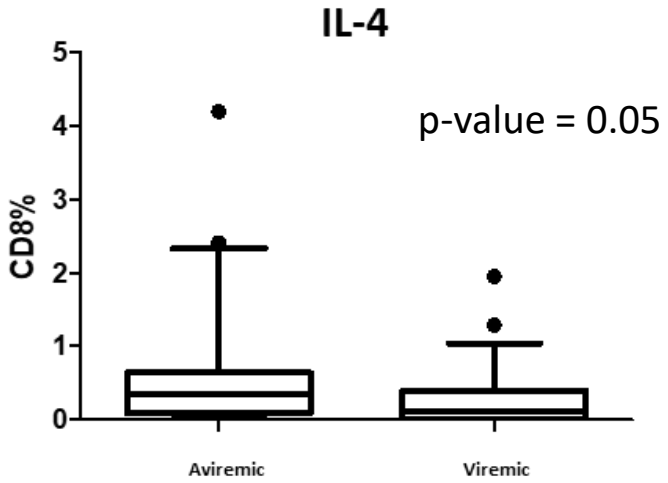
PBMCs stimulated with SARS-CoV-2 peptide pool (S, N and M)



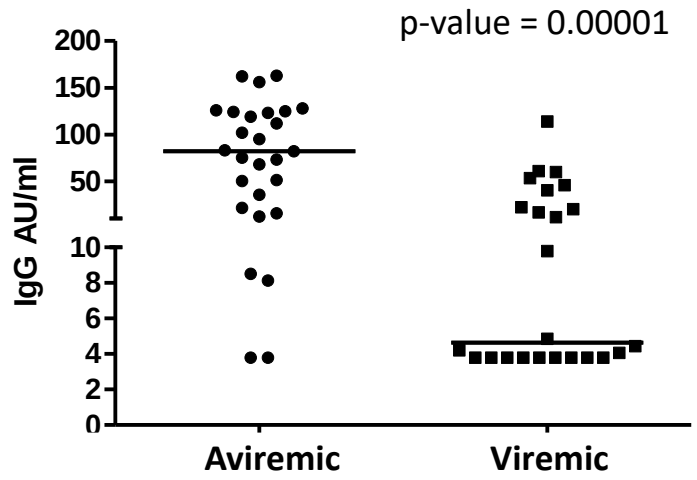
Th1 response



Th2 response



SARS-CoV-2 viremia: SARS-CoV-2 specific humoral response



Correlation:
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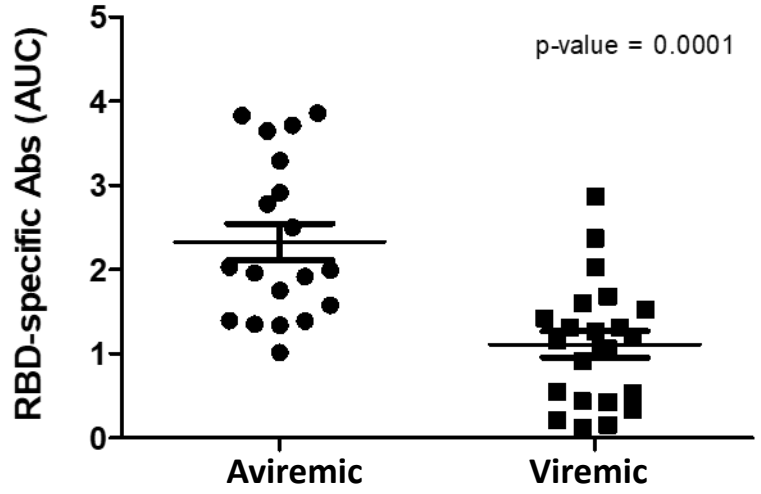

Correlation:
r = -0.6
p-value = 0.00001

SARS-CoV-2 viremia: conclusions

COVID-19 patients with pneumonia and detectable SARS-COV-2 plasma viremia display higher circulating IFNα and IL-6, and yet lower T-lymphocyte activation and SARS-COV-2-specific IFNγ+CD4 T cells, TNFα+CD8 and IL-4+CD8 T cells. Likewise viremic patients presented lower spike and RBD-specific Ig.

Taken together these data suggest higher pro-inflammatory milieu in viremic COVID-19 patients in the backdrop of reduced T-cell and humoral response.

Two non mutually-exclusive hypotheses:

1. Does a higher viremia prevent the establishment of a functional SARS-CoV-2 specific multi-layered immune response?
2. Does an *ab initium* impaired immune response not properly control the infection resulting in detectable plasmatic viral load?

Acknowledgements

Department of Health Sciences - Clinic of Infectious Diseases - San Paolo Hospital - University of Milan, Italy

- **Valeria Bono**
- Matteo Augello
- Camilla Tincati
- Mohamad Hadla
- Vaibhav Yellenki
- Antonella d'Arminio Monforte
- Giulia Marchetti

Department of Health Science - San Paolo Hospital - University of Milan, Italy

- Elisa Borghi

IFOM – The FIRC Institute of Molecular Oncology - Milan, Italy

- Federica Mainoldi
- Stefano Casola

San Paolo and San Carlo Hospitals – "COVID-19" wards – Milan, Italy

- All Staff
- Patients and their families

Funding: *Fondazione Cariplo/Fondazione Umberto Veronesi/Regione Lombardia*