Title: High CD169 monocyte/lymphocyte ratio reflects the immunophenotyping disruption and predicts oxygen need in COVID-19 patients

Authors:
Antonella Minutolo[1], Vita Petrone[1§], Marialaura Fanelli[1§], Marco Iannetta[2,3], Martina Giudice[1], Ines Ait Belkacem[4,5], Marta Zordan[2,3], Pietro Vitale[3], Guido Rasi[1], Paola Sinibaldi Vallebona[1,6], Loredana Sarmati[2,3], Massimo Andreoni[2,3], Fabrice Malergue[5], Emanuela Balestrieri[1], Sandro Grelli[1,7], Claudia Matteucci*[1]

Affiliations:
1. Department of Experimental Medicine, University of Rome Tor Vergata, Rome, 00133, Italy
2. Department of Systems Medicine, University of Rome Tor Vergata, Rome, 00133, Italy
3. Infectious Diseases Clinic, Policlinic of Tor Vergata, Rome, 00133, Italy
4. Aix Marseille Université, CNRS, INSERM, CIML, Centre d'Immunologie de Marseille-Luminy, Marseille, FRANCE
5. Department of Research and Development, Beckman Coulter Life Sciences-Immunotech, Marseille, 13009, France.
6. Institute of Translational Pharmacology, National Research Council, Rome, 00133, Italy
7. Virology Unit, Policlinic of Tor Vergata, Rome, 00133, Italy

(*) corresponding author
(§) equally contribute

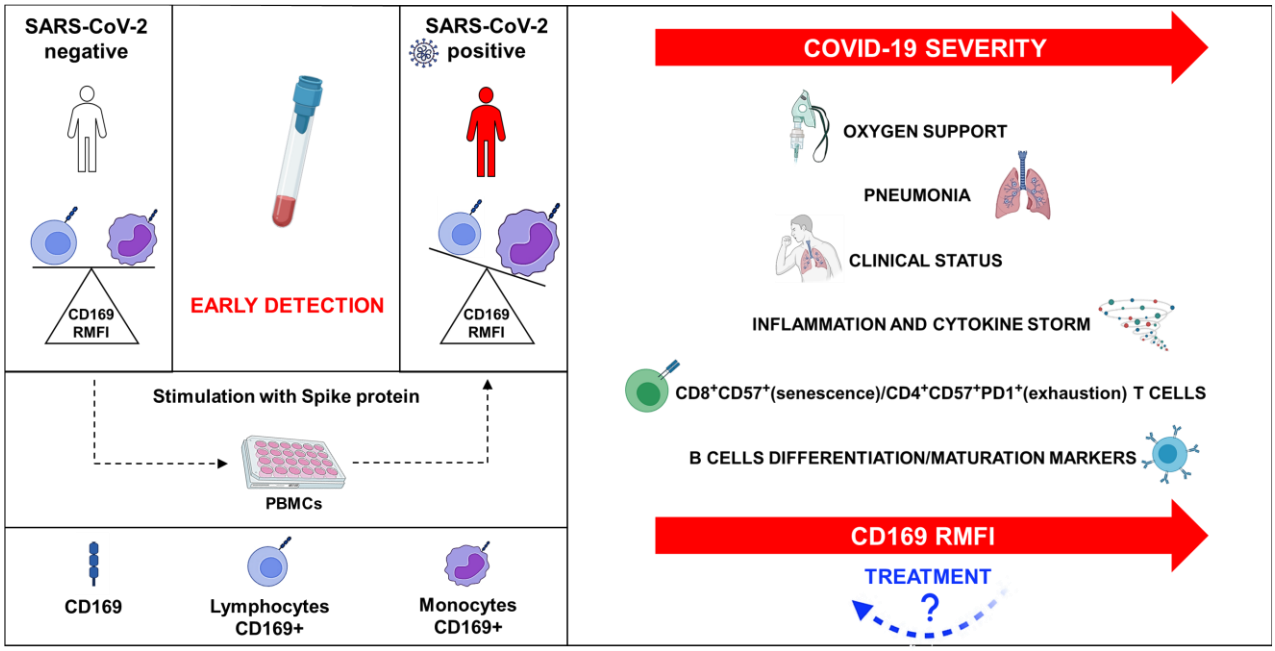
Abstract:

Background: The sialoadhesin CD169 has been found overexpressed in the blood of COVID-19 patients and identified as a biomarker in early disease. We have analysed CD169 in blood cells of COVID-19 patients to assess its role as predictive marker of disease progression and clinical outcome. **Methods:** The ratio of the median fluorescence intensity of CD169 between monocytes and lymphocytes (CD169 RMFI) was analysed by flow cytometry in blood samples of COVID-19 patients (COV) and healthy donors (HD) and correlated with immunophenotyping, inflammatory markers, cytokines mRNA expression, pulmonary involvement and disease progression. **Results:** CD169 RMFI increased in COV but not in HD, and correlated with T-cell differentiation and exhaustion markers as well as with B cells maturation and differentiation in COV. *In vitro* stimulation of PBMCs of HD with SARS-CoV-2 spike protein induced CD169 RMFI together with IL-6 and IL-10 gene expression. Likewise, CD169 RMFI correlated with blood cytokine mRNA levels, inflammatory markers, and pneumonia severity already in patients untreated at sampling, and reflected the respiratory outcome during hospitalization. **Conclusion:** Considering the immunological role of CD169 and its involvement during the infection and the progression of COVID-19, it could be considered as an early biomarker to evaluate disease progression and clinical outcome.

Keywords: cytokine storm, COVID-19, CD169, inflammation, respiratory outcome, T-cell exhaustion, COVID-19 therapy

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

Graphical abstract



Introduction

The coronavirus disease-19 (COVID-19) caused by severe acute respiratory coronavirus-2 (SARS-CoV-2) has led to a worldwide epidemic outbreak characterized by high morbidity and mortality. As a consequence of a derailed cellular and humoral immune response activation, numerous individuals develop persistent inflammation associated with a cytokine storm and diffuse organ involvement, mainly associated with the severe condition of COVID-19 patients such as acute respiratory distress syndrome (ARDS) [1]. Despite the best efforts on clinical practice, no standard therapeutic approach for COVID-19 patients has been yet established. This amplifies the need to identify early biomarkers to predict disease progression and guide personalized interventions.

In response to SARS-CoV-2 infection, host cells immediately produce cytokines including type I interferon (IFN-I that, in addition to carrying out its antiviral activity, also induces the expression of genes involved in limiting the viral spread. After antiviral cytokines are released, the sialoadhesin CD169 (also known as SIGLEC-1) is induced and expressed on the surface of myeloid lineage cells, such as dendritic cells and monocytes, [2,3]. In particular, two-fold upregulation of CD169 has been observed in monocytes (mCD169) exposed *in vitro* to IFNα [4].

Previous studies have demonstrated an important role of CD169/SIGLEC-1 in different viral infections, including those from Ebola virus and human immunodeficiency virus (HIV) [5-7]. Recently, it was suggested that SARS-CoV-2 infects macrophages, especially spleen and lymph node resident CD169 positive macrophages, and that this peculiar macrophage cell subpopulation plays a central role in mediating SARS-CoV-2 translocation [8]. Moreover, an increased expression of CD169 has been observed in monocytes from

COVID-19 patients. In observational studies conducted during the COVID-19 outbreak in France, mCD169 expression was also associated with the SARS-CoV-2 infection in patients at hospital admission underlining the importance as early infection biomarker [9-10].

To further investigate CD169 as a diagnostic and prognostic factor in SARS-CoV-2 infection and COVID-19 disease, CD169 RMFI was evaluated in COVID-19 patients hospitalized in the Policlinic of Tor Vergata in Rome, and correlated with their inflammatory and immunological status as well as respiratory outcome.

Results

Demographics and clinical classification of COVID-19 patients

We have analysed the clinical status at the enrolment and the respiratory outcome of 68 COVID-19 patients, who were hospitalized at the Policlinic of Tor Vergata between May and October 2020 (Table 1). The cohort of patients was divided into 5 groups, according to the patients' clinical features on hospital admission or study enrolment for those already hospitalized because of pre-existing diseases. Four (4) (5.8 %) patients showed no symptoms related to COVID-19 and were therefore referred to as asymptomatic (AS), although they were already hospitalized for pre-existing medical conditions. 15 (22.05%) were symptomatic with few clinical manifestations but at least one symptom including cough or fever, and therefore defined as paucisymptomatic (PS). 27 (39.70%) were classified as patients with mild symptoms (Mild); 12 (17.64%) with moderate symptoms (Mod); and 10 (14.70%) patients were categorized as severe (Sev). Overall, among the 68 COVID-19 patients enrolled, 43 (63.23%) showed radiological signs of SARS-CoV-2-related pneumonia, defined as monolateral interstitial pneumonia (MiP) in 6 cases (8.82%) and bilateral (BiP) in 37 cases (54.41%).

Concerning comorbidities, 51 (75%) patients showed the presence of at least one pre-existing chronic disease, with cardiovascular conditions being the most common comorbidity present (26 patients = 38.23%). At the sampling time, the patients had been hospitalized about 2 days, most of the patients were hospitalized from one day to 10 days (n=62), 6 patients were hospitalized from 10 days to 20 days and two were hospitalized for more than 20 days, with 32 (47.00%) of them being treated with antiviral and corticosteroid therapies while six died (fatality rate 8.82%). Patients did not show a reduction in the absolute lymphocyte counts at the time of sampling, although the relative counts tended to be at the lower limit of the normality range. Considering inflammatory markers and other parameters of coagulation, the levels of C-reactive protein (CRP), fibrinogen, D-dimers, blood urea nitrogen (BUN), aspartate transaminase (AST) or glutamic oxaloacetic transaminase (GOT), alanine transaminase (ALT), lactate dehydrogenase (LDH), lipase and amylase were increased on average, considering the overall cohort, although no differences were found among the five COVID-19 groups.

All the COVID-19 patients were evaluated for respiratory outcomes during the hospitalization period and divided into three groups: 38 (55.88%) patients who did not need oxygen therapy; 13 (19.11%) who were supported by a nasal cannula (NC) or Venturi masks (VMKs); and 17 (25.00%) who were supported by non-invasive ventilation (NIV), continuous positive airway pressure (C-PAP), and orotracheal intubation (OTI) for mechanical invasive ventilation.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

Table 1. Demographics and clinical classification of COVID-19 patients at enrolment and respiratory outcome

Demographics							
		Asymptomatic (AS)	**Paucisymptomatic (PS)**	**Mild (Mild)**	**Moderate (Mod)**	**Severe (Sev)**	**Total**
Number		4	15	27	12	10	68
Age (Mean±SD)		65±17	53±13	61±17	56±18	64±14	61±15
Sex (M/F)		3/1	9/6	19/7	10/2	8/2	49/18
Hospitalization (days^{+})		4±2	3±2	6±10	6±9	4±5	4.54±7.54
$^{+}$days between hospitalization and sampling							
Clinical status at enrolment and treatment							
		AS	**PS**	**Mild**	**Mod**	**Sev**	**Total**
Pneumonia	None*	1	4	7	0	0	12 (17.64%)
	P	1	1	6	5	0	13 (19.11%)
	MiP	0	6	0	0	0	6 (8.82%)
	BiP	2	4	14	7	10	37 (54.41%)
Comorbidities	None	3	3	4	0		10 (6.80%)
	Obesity	0	2	3	1	2	8 (11.76%)
	Diabetes	1	2	5	1	0	9 (13.23%)
	Cardiovascular	6	4	5	3	1	26 (38.23%)
	Others**	2	7	4	0	2	15 (66.60%)
Mortality		0	0	1	1	1	6/68 (8.82%)
Treatment	Antiviral & Corticosteroid	0	1	14	10	7	32 (47.00%)
* None =no pneumonia, P= no interstitial pneumonia, MiP= Monolateral or minimal interstitial pneumonia, BiP= bilateral interstitial pneumonia; ** other comorbidities or habits: solid organ replacement, gastrointestinal, smoke							
Blood Count							
		AS	**PS**	**Mild**	**Mod**	**Sev**	**Total**
Red blood cells (4.40-6.00) 10^6/µl		3.95±0.07	4.70±0.42	4.23±0.78	4.48±1.10	4.76±0.43	4.40±0.73
Haemoglobin (13.00–18.00) g/dl		10±1.36	13±1	12.24±2	12.27±2.65	13.68±2.10	**12.67±2.14**
White blood cells (4.30–10.80) 10^5/µl		3.51±1.43	4.77±1.69	7.18±3.22	6.78±3.91	7.15±1.28	6.37±2.92
Neutrophils	Abs count	2.5±1.25	2.80±1.61	5.0±3.68	5.0±2.31	5.83±2.55	4.52±2.9
	% (40–75)	68.6±17	56.05±1.31	67±14.1	67.85±13.59	**78.89±1.83**	66.42±16.0
Lymphocytes	Abs count	0.63±0.23	1.42±0.43	1.52±0.8	1.27±0.73	0.07±0.57	1.31±0.66
	% (20–45)	20.43±10.00	32.91±8.6	24±13	23.62±15.29	**14.59±2**	23.04±1.0
Monocytes	Abs count	0.25±0.18	0.43±0.015	0.46±0.2	0.52±0.3	0.43±0.40	0.45±0.11
	% (3.4–11)	8.26±5.7	9.78±3	6.8±2.19	8.24±4.34	6.12±2.6	6.89±2.60
Eosinophils	Abs count	0.09±0.13	0.07±0.08	0.06±0.08	0.05±0.02	0.013±0.3	0.06±0.05
	% (0–7)	2.3±3.7	0.04±1.06	2.5±2.19	0.67±0.35	1.0±3.60	1.90±3.62
Basophils	Abs count	0.01±0.01	0.018±0.01	0.02±0.075	0.028±0.01	0.014±0.01	0.02±0.00
	% (0–1.5)	0.23±0.2	0.44±0.28	0.36±0.26	0.55±0.10	0.19±0.07	0.20±0.17
Number in bold: value of the analysis outside the reference values							**Table 1 continued**

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

Table 1 continued

Respiratory Outcome***

	AS	PS	Mild	Mod	Sev	Total
None	4	15	19	0	0	38 (53.3%)
NC/VMK	0	0	8	5	0	13 (26.6%)
NIV/C-PAP/OTI	1	0	1	7	10	17 (20.0%)

*** None = No oxygen support, NC/VMK = Nasal cannula/Venturi mask, NIV/C-PAP = Non-invasive ventilation or OTI = invasive ventilation

Biochemicals

	AS	PS	Mild	Mod	Sev	Tot
Fibrinogen (200-400) mg/dl	341±144	460±171	495±53	520±122	527±1.3	**480±135**
Antithrombin III % (75-128)	91±15	100±3	104±17	106±14	99±1.1	101±19
D-dimers (0-500) ng/ml	1252±619	745±633	1018±603	2196±425	2196±432	**1133±911**
Glucose (70-100) mg/dl	101±25	101±32	133±72	122±43	112±21	**119±51**
BUN (15-40) mg/dl	147±71	41±28	39±19	50±26	65±43	**53±40**
AST (5-34) U/liter	40±29	27±11	41±43	40±29	81±6	**44±46**
ALT (0-55) U/liter	27±12	24±17	46±45	40±43	107±134	**48±63**
LDH (125-220) U/liter	456±172	234±71	342±304	295±135	438±166	**330±214**
Reactive C Protein (CRP) (0-5) mg/liter	34±34	25±22	43±50	79±71	49±43	**46±49**
Lipase	84±19	55±47	52±86	44±30	110±119	**63±64**
Amylase	161±113	70±32	54±30	61±33	110±71	**76±53**

Number in bold: value of the analysis outside the reference values

Flow cytometry analysis of CD169 expression in COVID19 patients and healthy donors

Sixty-eight patients hospitalized, tested positive for SARS-CoV-2 RNA (COV), were screened for CD169 expression, using flow cytometry and compared to 57 healthy donors (HD). The ratio of the MFI of CD169 between monocytes and lymphocytes (CD169 RMFI) was calculated as previously described [9-11]. **Figure 1A** compares the CD169 RMFI in the HD and COV groups, showing that the CD169 RMFI was higher in patients with COV compared to HDs (median 16.17 ± 30.23 and 2.01±0.70 respectively, $p<0.001$). The test accuracy of CD169 RMFI to distinguish the COVID-19 patients of our cohort from HD was studied via the receiver operating characteristic curve (ROC curve). With a cut-off of 3.01, the sensitivity and specificity at the optimal operating point were 97% and 80%, respectively, with an area under the ROC curve (AUC) of 0.950 ($p<0.001$).

As shown in **Figure 1B**, HD samples were confirmed negative at 94.73%. Among COV, 79% (n=68) were positive to the CD169 RMFI and 20.58% (n=14) were found to have a CD169 RMFI below the cut-off. As previously described, the Spearman correlation coefficient analysis revealed an inverse correlation between days of hospitalization and CD169 RMFI value, confirming the relevance of the RMFI CD169 expression for the diagnosis in the early phases of the infection (more than 10 days, compared to 4 days of the positive group, $p<0.001$, **Figure 1C**). Of the 14 false-negative samples, ten were from patients that had been hospitalized for more than 10 days before testing. The remaining four patients presented symptoms consistent with COVID-19 at the time of nasopharyngeal swab testing, but were not confirmed as SARS-CoV-2 positive after Real

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

Time PCR. Moreover, CD169 RMFI in COV was inversely correlated with the mRNA levels on N, E and RDP SARS-CoV2 genes (**Figure 1D**).

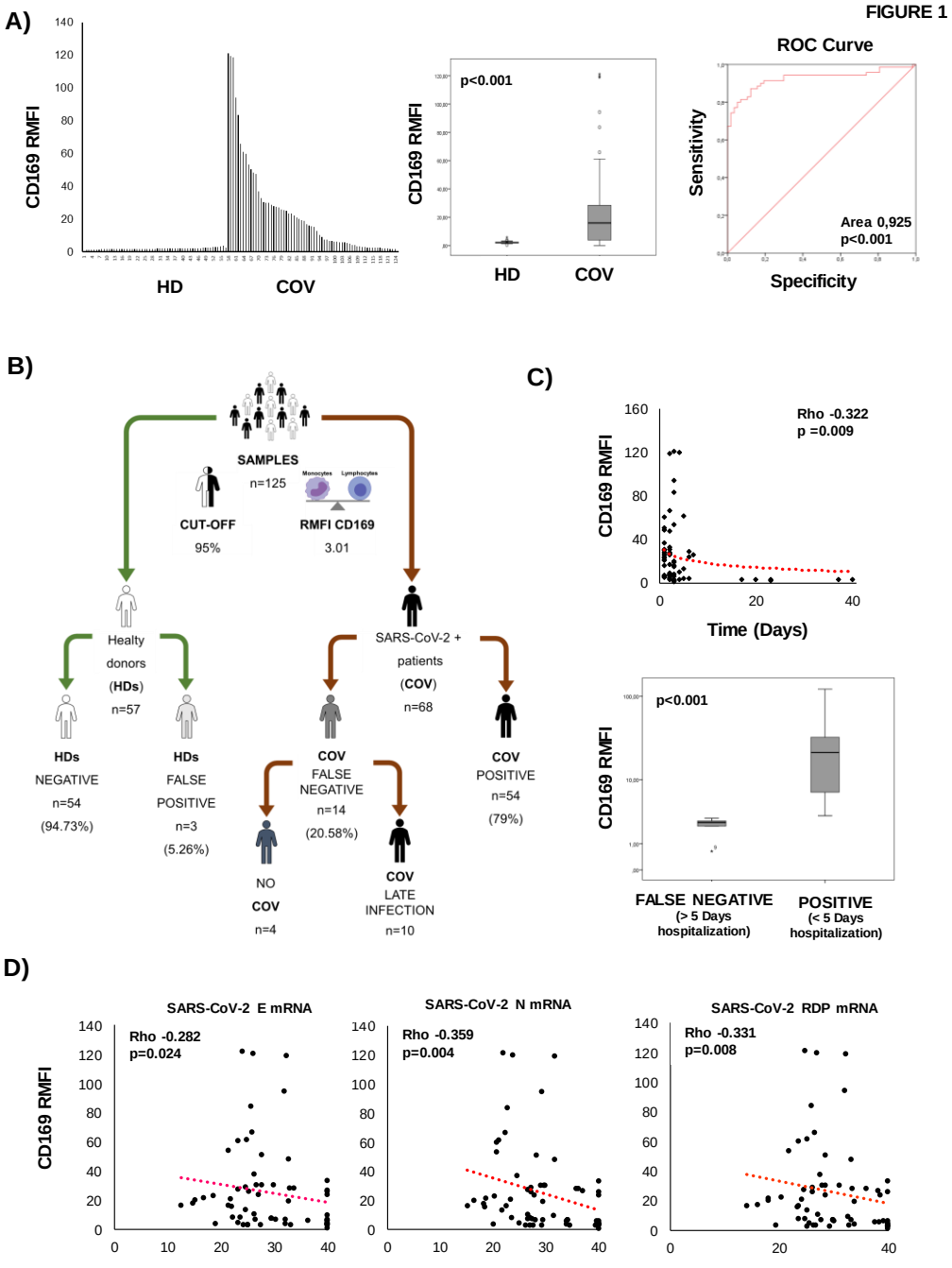


Figure 1. Flow cytometry analysis of CD169 to screen COVID-19 patients.
The ratio of the MFI of CD169 between monocytes and lymphocytes (RMFI) was used in the screening study. A) CD169 RMFI values in enrolled healthy donors (n=57) and COVID-19 patients (n=68); box plot of the analysed population; ROC curve for CD169 RMFI, the area under roc curve (AUC) is indicated. B) Workflow of the screening carried out on the CD169 expression in collaboration with Policlinic of Tor Vergata of Rome Foundation. C) Relationship between days of hospitalization and CD169 expression in patients hospitalized for less than 5 days and for more than 5 days before sampling. D) Scatter plot of SARS-CoV-2 mRNA genes

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

and CD169 RMFI in COVID-19 patients. Non-parametric Mann-Whitney test was used to compare groups and pairwise associations between continuous variables were tested through the Spearman correlation coefficient. Statistically significant values were considered when p ≤0.050.

CD169 RMFI correlates with biochemical parameters involved in COVID-19 severity and is associated to pneumonia

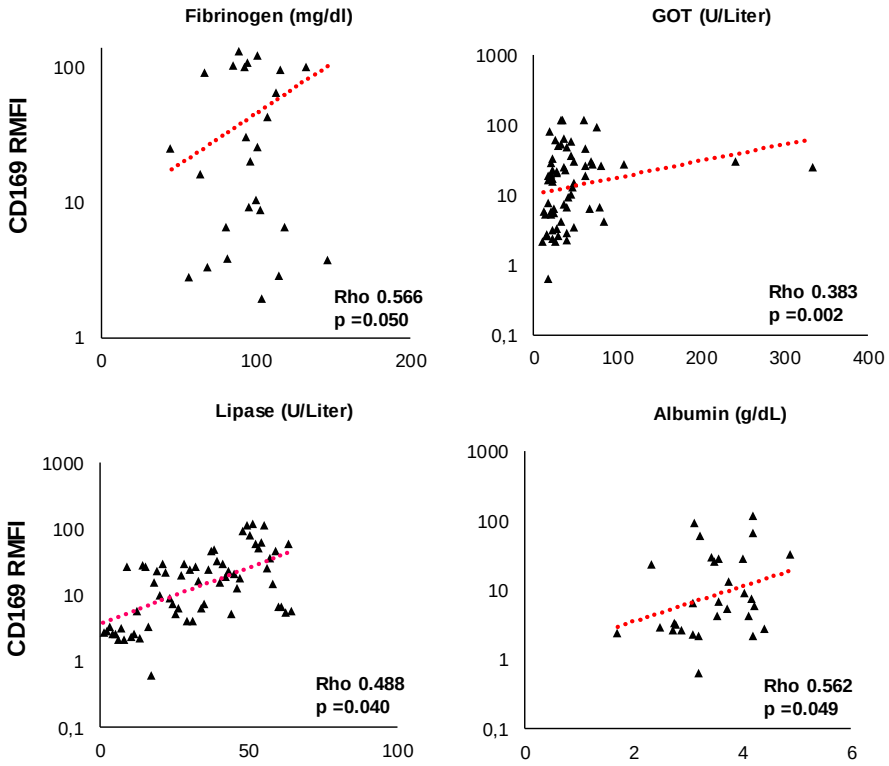
To evaluate the association of CD169 RMFI to the disease characteristic and evolution, the clinical parameters were analysed in true positive COV within day 5 of hospitalization (n=54). CD169 RMFI in COV patients was positively correlated with inflammatory markers and biochemical parameters associated with COVID-19 severity, such as fibrinogen, GOT, lipase and albumin (**Figure 2A**).

To analyse the association between CD169 RMFI and pneumonia status, the COV group was divided according to chest computed tomography (CT) images at the time of hospitalization and sampling: no pneumonia and non-interstitial pneumonia (None+P, n=10), monolateral or minimal interstitial pneumonia (MiP, n=7), bilateral or severe pneumonia (BiP, n=37). As shown in **Figure 2B**, a significant higher CD169 RMFI was observed in the group with bilateral interstitial pneumonia when compared to monolateral pneumonia (p=0.018) or compared to the None+P group (p=0.031).

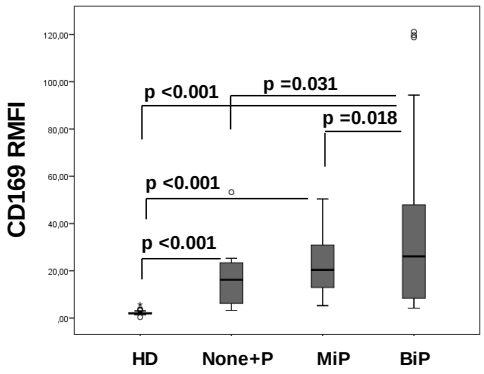
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

FIGURE 2

A) Inflammatory Biomarkers



B) Pneumonia



None+P: no pneumonia+ no interstitial pneumonia

MiP: monolateral or minimal interstitial pneumonia

BiP: bilateral interstitial pneumonia

Figure 2. Elevated CD169 RMFI correlates with inflammatory markers and is associated with pneumonia status in COVID-19 patients. *Scatter plot of (A) biochemical markers (X axis) and the CD169 RMFI (Y axis) in COVID-19 patients). The biochemical markers examined were fibrinogen, glutamate oxaloacetate transaminase (GOT), lipase and albumin. (For the albumin test, 9 patients, these analyses were not performed at the time of collection). (B) Patients were stratified into three groups based on pulmonary status and compared to HD (n=57): no pneumonia and non-interstitial pneumonia (None+P, n=10), mono-lateral or minimal interstitial pneumonia (MiP, n=7), bilateral or severe pneumonia (BiP, n=37). The CD169 RMFI was represented as a box plot in all the groups examined and statistical difference was shown. All the data were analysed by non-parametric Kruskal-Wallis test and Bonferroni's correction were used to compare groups and pairwise associations between continuous variables were tested through the Spearman correlation coefficient. Statistically significant values were considered when p ≤0.050.*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

CD169 RMFI correlates with the expression of pro-inflammatory cytokines in COVID-19 patients and is altered by therapy

To evaluate the effect of therapy on CD169 expression, patients were stratified based on the drug treatment received (antiviral and corticosteroids) or not (untreated) The group of patients under antiviral and corticosteroids therapy at the time of sampling (treated COV) showed significant lower expression of CD169 compared to untreated COV (p=0.033) (**Figure 3A)**. The expression of a selected group of cytokines was also analysed in blood samples of COV and HD patients by qRT-PCR (**Figure 3B**). IL-6, IL-10, IFNγ and TNFα expression was significantly higher in untreated COV patients than in HD, while in treated patients, IL-6 and IL-10 had significantly lower values than untreated patients (p=0.012 and p=0.001). Moreover, the Spearman analysis revealed a positive correlation of CD169 RMFI with IL-6 (Rho= 0.415, p=0.015) and with IL-10 (Rho= 0.488, p<0.001) in untreated COV, while an inverse correlation with IL-6 was observed in treated COV (Rho= 0.506, p<0.001). No significant correlation of CD169 RMFI with TNFα and INFγ expression levels was observed (**Figure 3B**).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

FIGURE 3

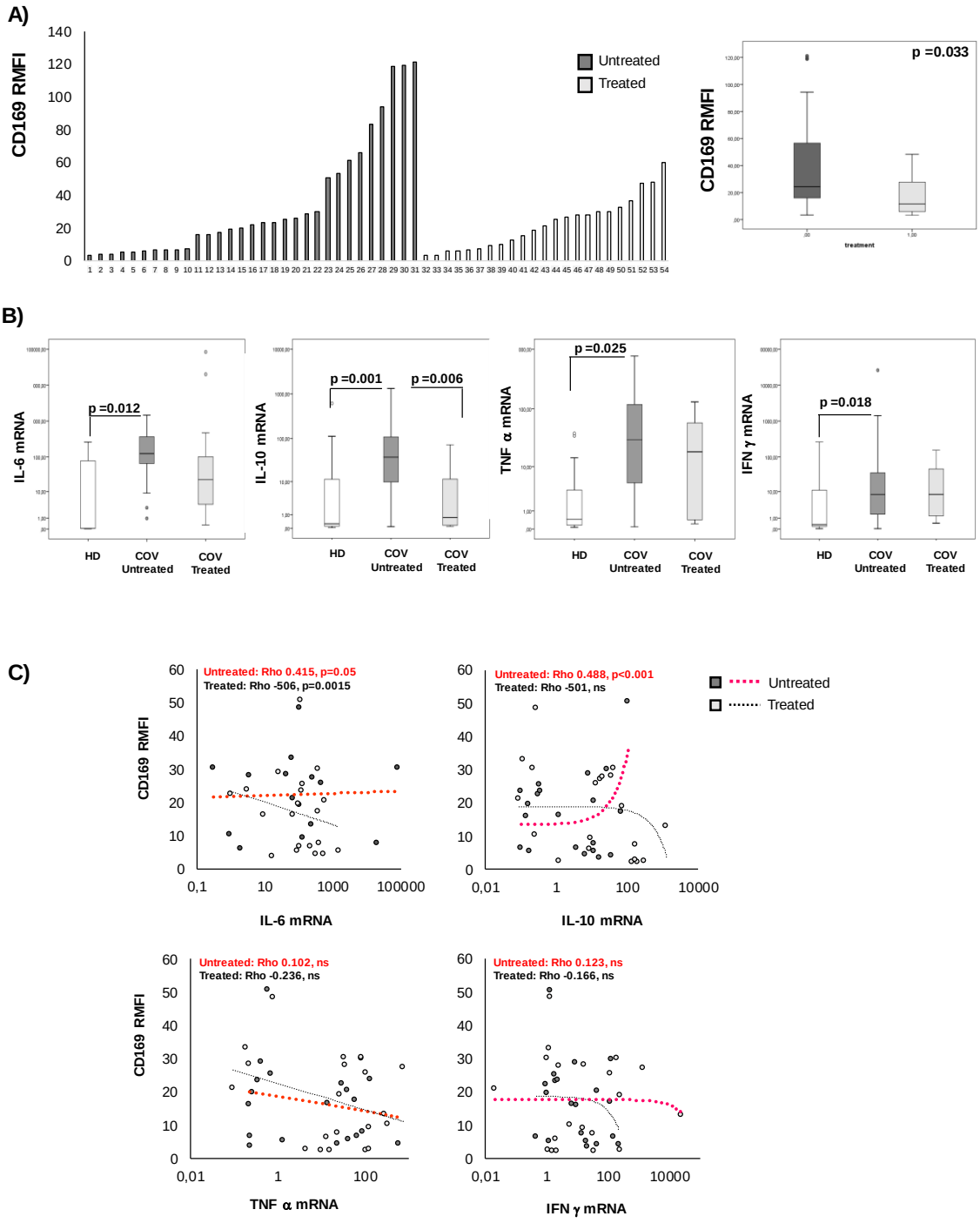


Figure 3. CD169 RMFI correlates with IL-6 and IL-10 in untreated COVID-19 patients. *Patients were stratified into two groups based on treatment with antiviral and corticosteroids at sampling (COV treated n=25 and COV untreated n=19) and represented in ascending order of CD169 RFMI (left panel A) and median ± SD of CD169 RMFI in treated vs. untreated COV patients was represented as box plot and statistical difference was shown. (B) IL-6, IL-10, IFNγ and TNFα mRNA expression in HD (white), COV untreated (grey) and COV treated (light grey) was represented as a box plots and statistical difference was shown. (C) Scatter plots of the cytokines expression (X-axis: IL-6, TNFα, IL-10, INFγ) obtained by qRT Real-time PCR and CD169 RMFI in COV untreated (grey dots) and COV treated (light grey dots) at sampling (X-axis). Non-parametric Kruskal-Wallis test and Bonferroni's correction was used to compare groups; pairwise associations between continuous variables were tested through the Spearman correlation coefficient. Statistically significant values were considered when p ≤0.050.*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

CD169 RMFI correlates with the expression of T lymphocytes differentiation and senescence/exhaustion markers in untreated and treated COVID-19 patients.

The analysis of T-lymphocyte cell phenotype confirmed a significant difference between important markers of differentiation and exhaustion of T cells in COV treated or untreated patients compared to HD (**Table 2)**.

Table 2. Flow cytometry analysis of the T cells subsets in healthy donors (HD) and in untreated /treated COVID-19 patients

		LYMPHO	MONO	NEUTRO	CD3	CD4	CD8	CD8+CD4+
HD	median	21.79	6.73	45.73	65.17	59.70	33.02	1.36
	SD	11.92	3.29	18.95	19.11	8.96	7.73	1.18
Untreated	median	**14.04**	5.53	**58.63**	**58.55**	**53.10**	30.36	1.25
	SD	8.39	4.08	19.97	20.88	18.63	12.16	1.08
Treated	median	**11.53***	6.77	**64.51**	**57.88**	**54.51**	**28.32**	**2.26**
	SD	7.63	3.70	19.18	18.68	18.22	11.43	2.21

		CD4CM	CD4NAIVE	CD4EM	CD4TEM	CD4CD57	CD4PD1	CD4PD1+CD57+
HD	median	50.66	38.47	50.66	0.89	5.74	8.69	0.90
	SD	13.08	11.56	13.08	1.85	6.21	14.81	1.59
Untreated	median	51.61	34.39	**11.94**	**2.03**	5.55	**16.93**	1.83
	SD	17.24	14.42	12.14	4.01	6.54	16.98	2.87
Treated	median	47.15	37.59	**11.84**	**3.42**	6.30	**17.21**	**3,29**
	SD	17.92	14.31	12.52	4.40	6.60	20.22	5.45

		CD8CM	CD8NAIVE	CD8EM	CD8TEM	CD8CD57	CD8PD1	CD8PD1+CD57+
HD	median	28.76	44.96	17.05	9.23	1.96	7.00	8.24
	SD	17.99	18.19	16.61	9.30	0.55	10.04	14.74
Untreated	median	**13.19**	**33.77**	**31.71**	**21.64**	**33.55**	8.77	6.27
	SD	10.07	20.19	21.92	17.64	21.59	9.85	8.21
Treated	median	20.75	**28.05**	**33.35**	**17.87**	**35.68****	9.69	8.73
	SD	12.48	18.89	22.62	15.15	20.09	14.72	9.47

Number in **bold** Significant differences by non-parametric Kruskal-Wallis and Bonferroni's correction vs HD;

** significant difference for untreated vs treated COV. Statistically significant values were considered when $p \leq 0.050$.

SD= Standard deviation

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

In CD4+ T cells, a decrease in effector memory (EM) subset and an increase in terminal effector memory (TEM) subset in both treated and untreated COV compared to HD was observed, and a significant increase in CD4+ cells expressing markers of senescence and exhaustion (CD57+/PD1+) only in treated. In the CD8+ T cells from COVID-19 patients, the decrease in central memory (CM) subset was significant only in untreated group in comparison to HDs, while an increase EM and TEM subset was present in both patient groups. The percentage of CD57+ positive cells increased significantly in COV patients compared to HD, and in treated compared to untreated COV.

The expression of CD169 was closely related to the immunological modifications, especially in untreated patients. The Spearman's analysis revealed a significant correlation between specific markers of differentiation and exhaustion of T cells and CD169 RMFI, as illustrated in **Figure 4** and **Table 3**. In CD3+CD4+ T cells from untreated COV, the CD169 RMFI was associated with a decrease in both CM and EM ($p=0.005$ and $p=0.050$ respectively) and with an increase in exhausted cells expressing CD57 and PD-1 markers ($p=0.038$). In treated COV the CD169 RMFI was associated to the decrease of EM ($p=0.025$), but positively correlated with naive compartment ($p=0.025$) and in TEM cells ($p=0.021$) (**Figure 4A**).

In CD3+CD8+ T cells from untreated COV the CD169 RMFI was associated with a decrease in naive cells ($p=0.038$) and positively correlated with EM ($p=0.016$) (**Figure 4B**). In treated COV the CD3+CD8+ CM cells negatively correlated with CD169RMFI ($p=0.050$) while a positive correlation with TEM cells was observed ($p=0.012$). Finally, the analysis also revealed CD169 RMFI positively correlated with CD57+ senescence marker in CD3+CD8+ T cells in untreated COV.

FIGURE 4

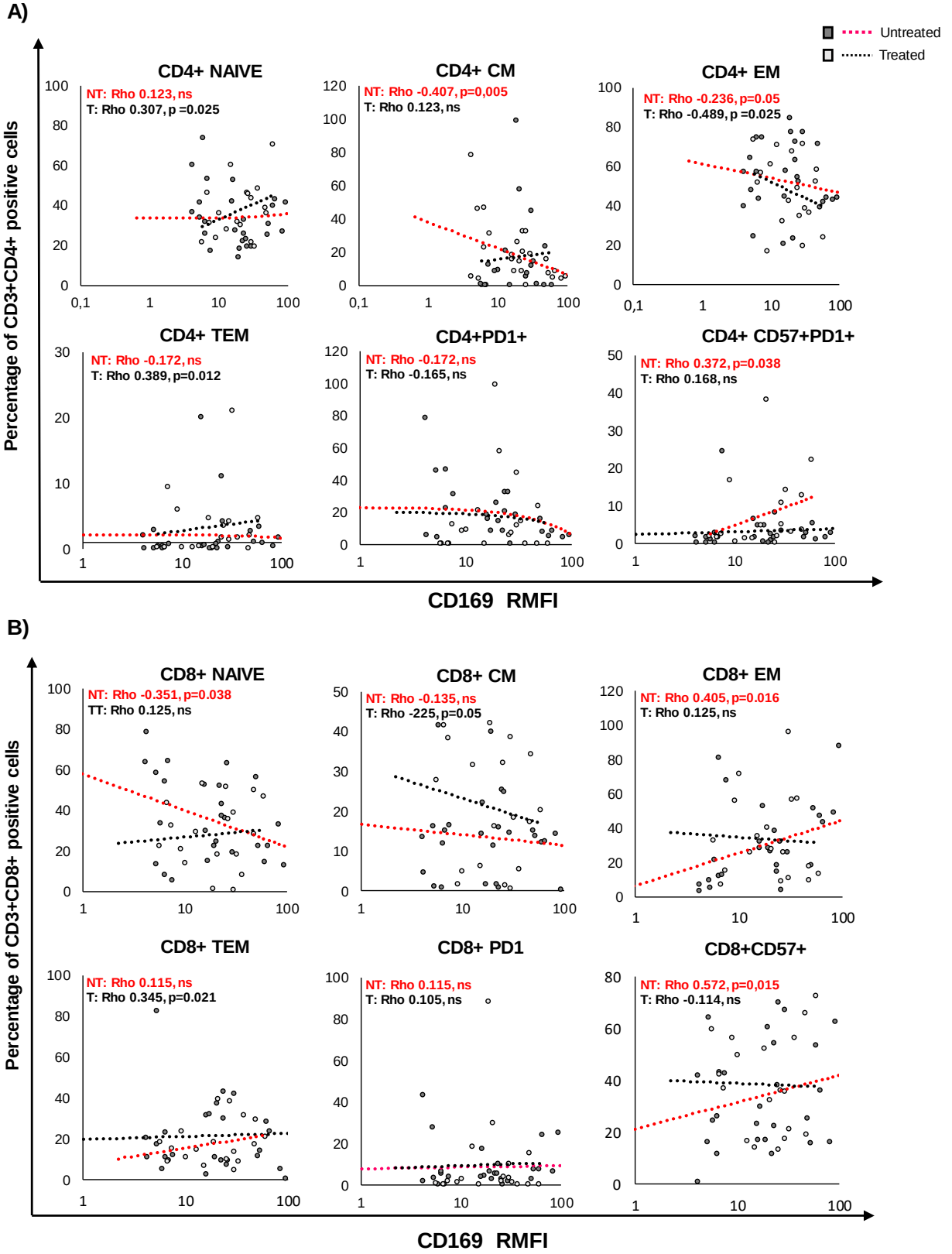


Figure 4. CD169 RMFI correlates with the expression of differentiation and senescence/exhaustion markers in T cells from COVID-19 patients.

Patients were stratified into two groups based on treatment at sampling with antiviral + corticosteroids (treated COV n=23) or untreated COV (n=31). A) Scatter plot of the CD169 RMFI (X axis) and the expression of markers of differentiation and senescence/exhaustion in CD3+CD4+ T cells (A) and CD3+CD8+ T cells (B) (Y axis) in

COVID-19 patients. The gating strategy to analyse markers related to differentiation, activation status, senescence, and exhaustion in T cells was provided by Beckman Coulter (Duraclone), specifically, naive (CCR7+CD45RA+CD28+CD27+), central memory (CM: CCR7−CD45RA+CD28+CD27+/−), effector memory (EM: CCR7−CD45RA−CD28+/−CD27+/−), terminal effector memory (TEM: CCR7−CD45RA+CD28−CD27+/−), PD1+ exhausted and CD57+ senescent T cells. Pairwise associations between continuous variables were tested through the Spearman correlation coefficient. Statistically significant values were considered when p ≤0.050.

Table 3: Spearman's correlation between CD169 RMFI and T cells differentiation and senescence/exhaustion markers in untreated and treated COVID-19 patients

	UNTREATED		TREATED	
	CD4	CD8	CD4	CD8
NAIVE				
CM				
EM				
TEM				
Senescent (CD57+)				
PD1+				
Exhausted (CD57 +PD1+)				
	CD169 RMFI		CD169 RMFI	

Rho negative, p<0.050= green;
Rho positive, p<0.050=red;
no difference, p>0.050= grey

CD169 RMFI correlates with the expression of differentiation and maturation markers in B cells from COVID-19 patients.

The analysis of B-lymphocytes cell phenotype revealed a significant difference between markers of differentiation and maturation of B cells in COV respect to HD (**Table 4)**. Among COV patients, in CD45+CD19+ B cells the percentage of positive Marginal B cells and naive B cells significantly decrease, in parallel with a decrease of the numbers of memory switched and non-switched B cells with significant increase of plasmablasts.

Table 4. Flow cytometry analysis of the B cells subsets in healthy donors and in untreated /treated COVID-19 patients

		Marginal	NaiveB	Unswitched	Switched memory	Plasmablasts
HD	median	16.05	53.36	37.52	35.44	17.01
	SD	20.50	22.62	19.32	15.75	1.85
Untreated	median	**8,52**	**43.21**	**33.89**	**24.10**	**19.11**
	SD	8.62	23.09	22.91	20.45	5,92
Treated	median	**6.50**	**41.59***	**31.49***	**25.71**	**20.77**
	SD	6.39	20.65	18.18	20,33	4.84

Number in **bold** Non-parametric Kruskal-Wallis and Bonferroni's correction in untreated COV or treated COV vs. HD ; * the same test in untreated vs. treated; Statistically significant values were considered when p ≤0.050

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

The Spearman analysis revealed a significant correlation between markers of differentiation and maturation of B cells and CD169 RMFI, as illustrated in **Figure 5.** In particular, in CD45+CD19+ B cells the expression of CD169 was associated with an increase in marginal B cells (p=0.025) and naive B cells (p=0.032) in untreated COV, while in treated group no correlation was observed. Switched B cells and plasmablasts negatively correlated with CD169 RMFI (p=0.025 and 0.010 respectively) in untreated COV as well as in treated COV patients. Moreover, a significant inverse correlation between the specific SARS-CoV-2 IgG analysed in sera and the RMFI of CD169 in both of patients group has been observed.

FIGURE 5

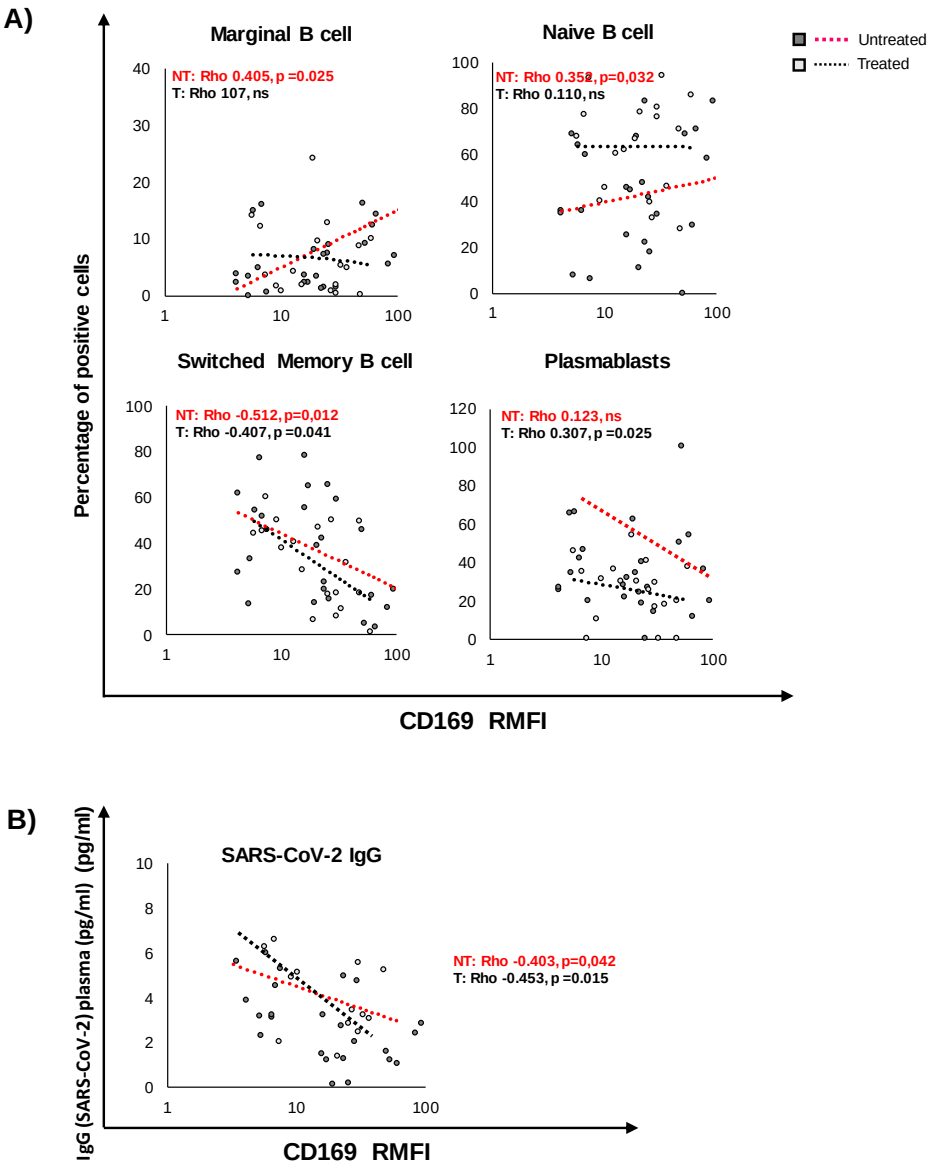


Figure 5. CD169 RMFI correlates with the expression of differentiation and maturation markers in B cells from COVID-19 patients and with SARS-CoV-2 IgG

Scatter plot of the CD169 RMFI (X axis) and the expression of markers of differentiation and maturation (A) in CD19 B cells of COVID-19 patients. The gating strategy to analyse markers related to differentiation, activation status, senescence, and exhaustion in T cells was provided by Beckman Coulter (Duraclone),

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

specifically, naive (CD45+CD19+CD27-IgD+), marginal (CD45+CD19+CD27+IgD+, unswitched memory (CD45+CD19+CD27+CD38-IgD+IgM+), Switched memory (CD45+CD19+CD27+CD38-IgD-IgM-) and Plasmablasts (CD45+CD19+CD27+CD38+IgD-IgM-). B) IgG specific for SARS-CoV-2 detected in sera *of COV patients at least one week after sampling. Pairwise associations between continuous variables were tested through the Spearman correlation coefficient. Values were considered statistically when p ≤0.050.*

Table 4: Spearman's correlation between CD169 RMFI and B cells differentiation markers in untreated and treated COVID-19 patients

	UNTREATED	TREATED
	CD19	CD19
Marginal		
Naive		
Unswitched		
Switched memory		
Plasmablasts		
	CD169 RMFI	

The CD169 RMFI reflects severity score and respiratory outcome of COVID-19 patients during hospitalization in relation with treatment at sampling

We then evaluated the CD169 RMFI in relation to clinical score and pneumonia and its predictive value according to oxygen need during hospitalization. As shown in **Figure 6A**, at all the clinical score the patients showed higher CD169 RMFI than HD. In untreated patients, the CD169 RMFI was found higher in symptomatic patients with significant difference compared to asymptomatic ($p<0.008$). Notably the treated patients showed no significant differences among the diverse disease scores, but was significantly different than HD ($p=0.042$). CD169 RMFI was markedly increased in untreated patients with bilateral interstitial pneumonia (BiP) compared to patients without pneumonia or no interstitial pneumonia ($p=0.045$) and to patients showing monolateral or minimal interstitial pneumonia (MiP) ($p=0.035$). The treated patients showed no significant differences when comparing the groups of patients with diverse radiological findings, but still significant after comparing BiP with HD ($p=0.015$) **Figure 6B**.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

FIGURE 6

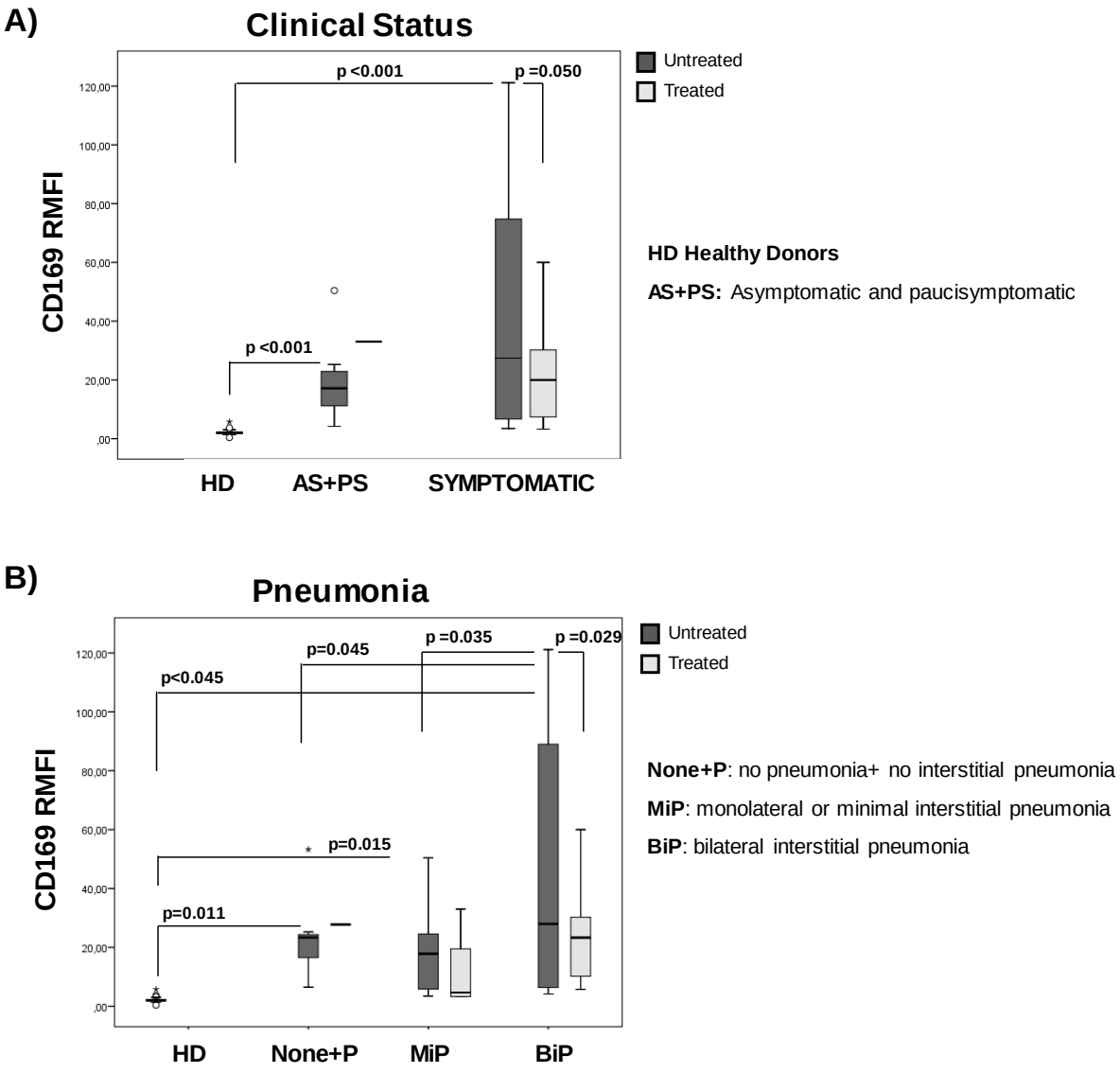


Figure 6. CD169 RMFI association with the severity of the disease and pulmonary involvement depends on the treatment at the time of sampling.

The COVID-19 patients (n=54) have been stratified respect to the clinical status in two groups: asymptomatic and pauci-symptomatic group (n=19), and symptomatic (Mild, n=17; moderate and severe, n=18) (A). Patients were also stratified into 3 groups based on pulmonary status (B) as previously described (Figure 2B). The CD169 RMFI was represented as box plots (grey box plots: patients positive for SARS-CoV-2 not treated at sampling (n=31), light grey box plots patients (n=23) treated with antiviral and corticosteroids at sampling); statistical difference was shown. Non-parametric Kruskal-Wallis test and Bonferroni's correction were used to compare groups and statistically significant values were considered when p ≤0.050.

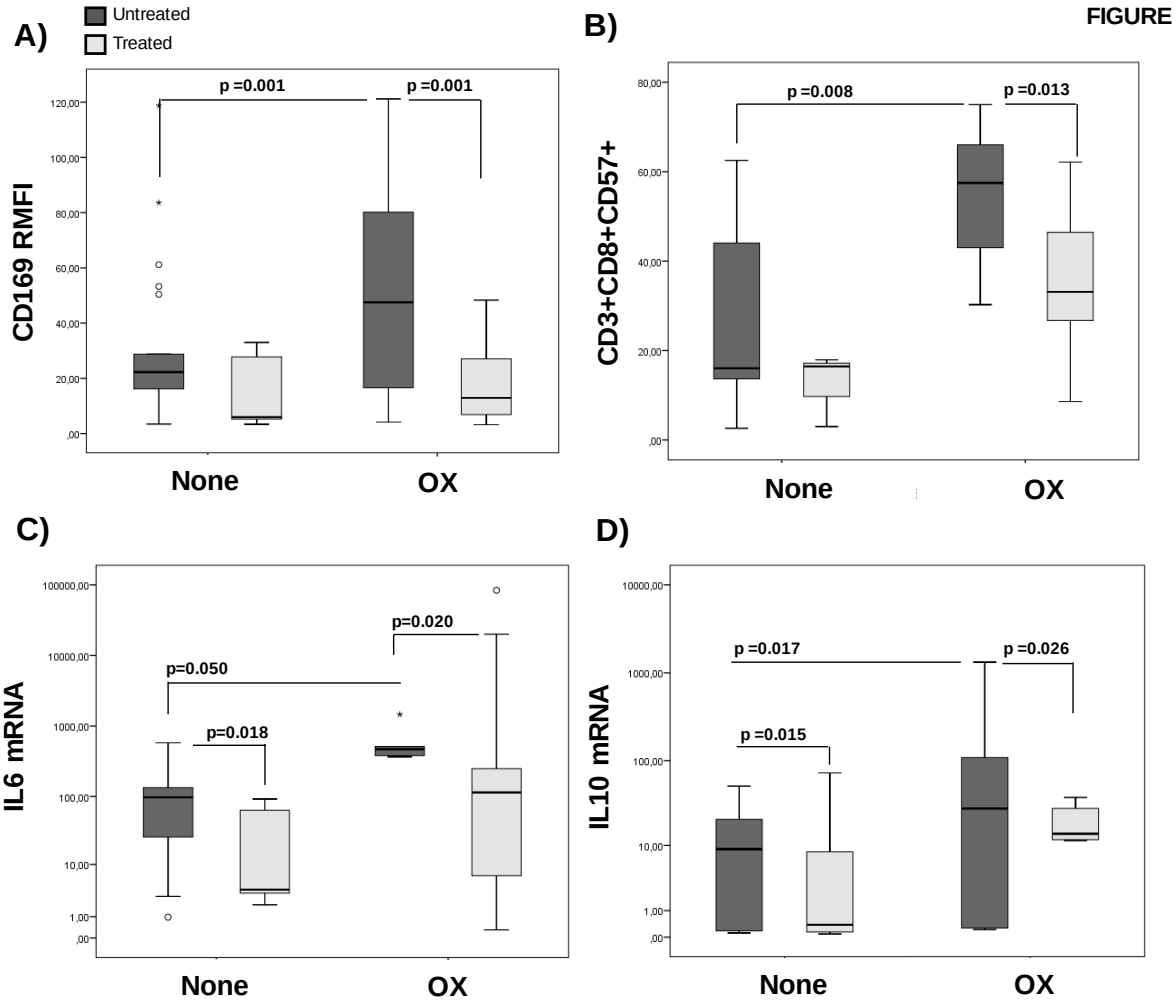
Using information on the requirement of oxygen supply during the course of the disease, the COVID-19 patients were divided into two categories representing the respiratory outcome: no oxygen support needed (None), or oxygen support (OX). As shown in **Figure 7A** the CD169 RMFI was found to be significantly higher in the OX group compared to the 'None (p=0.001) only in untreated patients at sampling, while no differences were observed in treated patients. Moreover, a statistically significant differences between the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

percentage of senescent CD8+ cells between treated and untreated patients within the OX and None groups were observed **Figure 7B.** In addition, a significant increase in the transcriptional levels of IL-6 (p=0.050) and IL-10 (p=0.017) was found across the respiratory categories in untreated group (**Figure 7C and 7D)**. The test accuracy of CD169 RMFI to predict the respiratory outcome in untreated or treated COVID-19 patients was studied by means of the receiver operating characteristic curve (ROC curve) to identify patients requiring respiratory support (**Figure 7E**). With a cut-off of 48.31, for the untreated group, the sensitivity and specificity at the optimal operating point were 89% and 80%, respectively, with an area under the ROC curve (AUC) of 0.879 ($p<0.001$).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

FIGURE 7



None: no Oxygen support

OX: Oxygen support VMKs; NIV; C-PAP; OTI or mechanical invasive ventilation

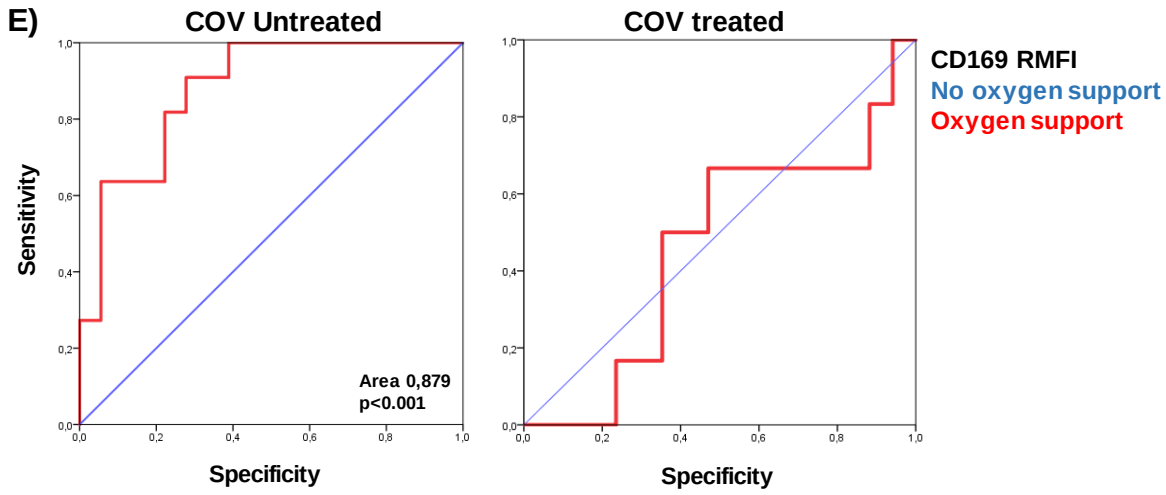


Figure 7 CD169 RMFI reflects respiratory outcome of untreated COVID-19 patients. *The COVID-19 patients (n=54) were stratified according to respiratory needs during hospitalization: no oxygen support needed (None; n=27) and oxygen support (OX; n=27) with nasal cannula or with ventimask (n=12) oxygen support by non-invasive ventilation, continuous positive airway pressure or orotracheal intubation (n=15).*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

(A) The CD169 RMFI were represented as box plots (grey box plots: patients positive for SARS-CoV-2 not treated at sampling (n=31), light grey box plots patients (n=23) treated with antiviral and corticosteroids at sampling).; The statistical differences are shown. (B) The percentage of CD8 senescent cells for the different treatments; (C) The levels of IL-6 for the different treatment group. (D) The levels of IL-10 expression (mRNA) for the different treatment groups. (E) ROC curve for CD169 RMFI in untreated or treated COV in respect to the oxygen support, the area under ROC curve (AUC) is indicated. Non-parametric Kruskal-Wallis test and Bonferroni's correction were used to compare groups and statistically significant values were considered when p ≤0.050.

SARS-CoV-2 Spike protein stimulation enhanced the RMFI CD169 in PBMCs from healthy donors.

To clarify whether CD169 was directly activated by SARS-CoV-2, PBMCs from seven healthy donors were stimulated *in vitro* for 24 hours with increasing concentration of the spike protein. The viral protein induced the expression of the activation marker HLA-DR in monocytes and the CD169 RMFI in a dose-depend manner (**Figure 8**). Also the expression of IL-6 and IL-10 was significantly higher after spike stimulation, and Spearman's analysis showed a positive correlation between CD169 RMFI and IL-6 and IL-10 expression confirming the data observed above in COV untreated patients (**Figure 3C**).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

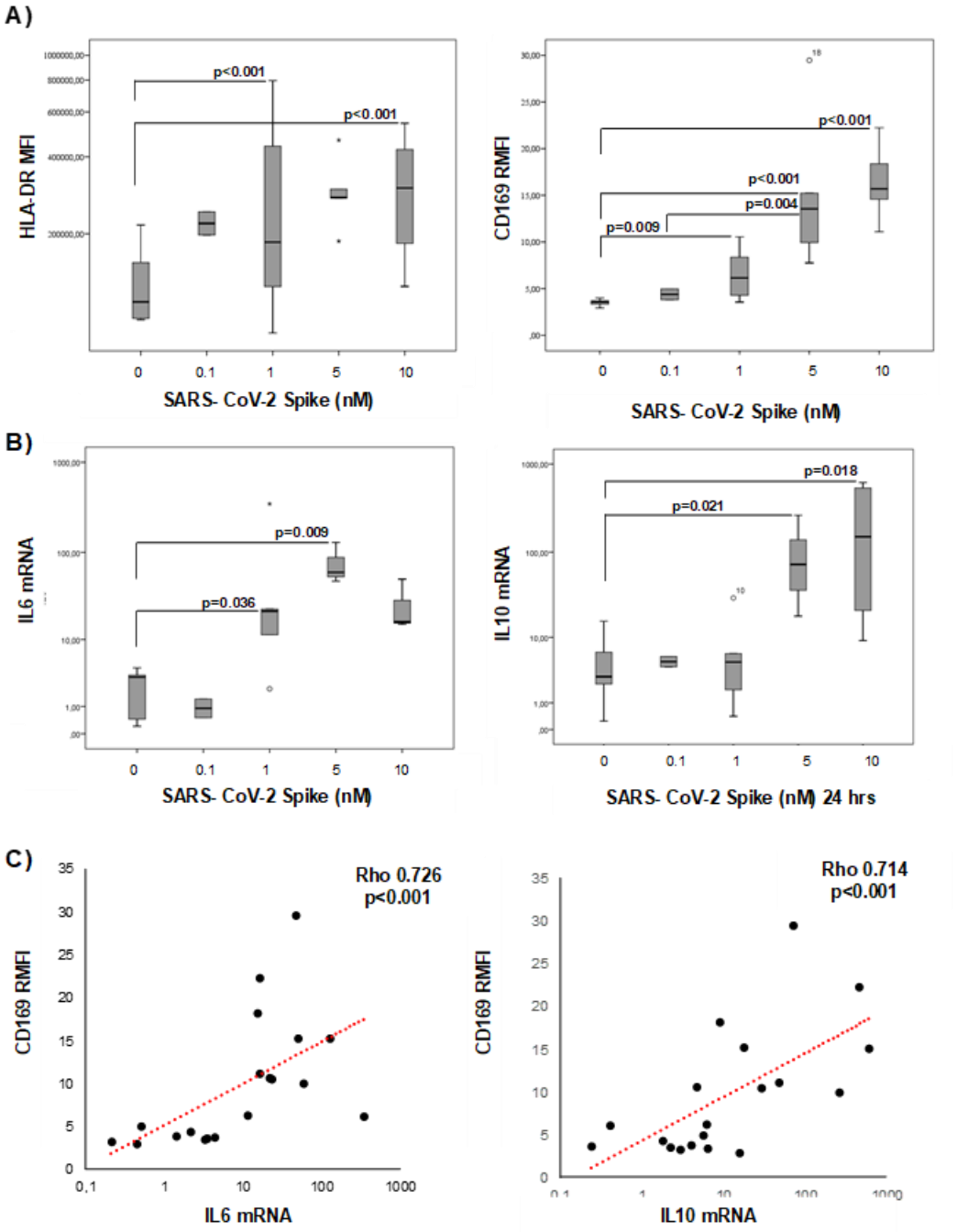


Figure 8. ***In vitro*** **stimulation of PBMCs from healthy donors with SARS-CoV-2 induces the CD169 RMFI, which correlates with the expression of IL-6 and IL-10.**

The HLA-DR MFI and CD169 RMFI in PBMCs from healthy donors stimulated in vitro *with different concentration (range 0-10 nM) of spike protein for 24 hours were represented as box plots; statistical difference was shown (A). IL-6 and IL-10 mRNA expression in the same samples were represented as box plots. (C) Scatter plot of the IL-6 and IL-10 mRNA expression (X-axis) and the CD169 RMFI (Y-axis) in COVID-19 patients. Non-parametric Kruskal-Wallis test and Bonferroni's correction were used to compare groups. Pairwise associations between continuous variables were tested through the Spearman correlation coefficient. Statistically significant values were considered when p ≤0.050.*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

Discussion

The role of CD169+ macrophages in immune regulation and human diseases has been widely reported [2]. In viral infections, CD169+ macrophages resident in lymphoid organs are the first cells that bind incoming pathogens and act as guardians to prevent their further spread. Circulating monocytes represent the mirror of the systemic immune response to infectious agent [11-13]. In SARS CoV-2 infection, myeloid cells have been described as responsible for the pathophysiology of the disease by contributing to local tissue damage and as potential producers of cytokines leading to the hyper-inflammatory state observed in severe COVID-19 [14-16]. Indeed, it was shown that CD169 expression was strongly increased on circulating monocytes from COVID-19 patients compared to those from healthy donors or patients with bacterial sepsis. Increased CD169 expression was also associated with an increase of other activation markers including CD64, CD68, and CD38 [9]. Moreover, CD169+ monocytes were present in high numbers in the early stages of the disease and in the group including milder cases, where the monocyte compartment consisted almost exclusively of CD169 clusters [17, 18].

Accordingly, with recent works [9-11], the analysis we carried out on whole blood taken from COVID-19 patients at the time on admission, diagnosed by detection of SARS-CoV-2 RNA in nasopharyngeal swabs via Real Time PCR, showed higher levels of CD169 RMFI compared to healthy donors, which correlated with SARS-CoV-2 RNA expression detected in swabs. Interestingly, the increased CD169 RMFI values strongly associated with various clinical and biological parameters, were related not only with the patient's status at admission but also with the progression of the disease.

Of note, a positive correlation of CD169 RMFI with the levels of several pro-inflammatory mediators predictive of severe COVID-19, such as fibrinogen, lipase, albumin and glutamate oxaloacetate transaminase and the associated cytokine storm [19-21], was found in our patient cohort.

To understand the role of the CD169 marker in natural SARS-CoV-2 infection and the influence that drugs could have on its expression, patients treated with antivirals and corticosteroid at the time of sampling were examined. The results showed that the distribution of CD169 expression was different in patients undergoing treatment than in untreated patients, with higher CD169 RMFI levels in the untreated group. In the latter group of patients, IL-6 and IL-10 mRNA expression levels were also higher. Interestingly, we found CD169 RMFI was strongly correlated with IL-6 and IL-10 mRNA levels, which are known to be associated with poor outcomes [22,23]. CD169+ macrophages play a central role in mediating SARS-CoV-2 translocation in spleens and lymph nodes and thus contribute to viral replication and spread and resulting cytokine storm [8,24]. Interestingly, in *in vitro* experiment viral Spike protein triggers CD169 RMFI in a dose dependent manner and enhanced the IL-6 and IL-10 gene transcription in PBMCs from HD, confirming the results obtained in COVID-19 patients.

The unbalance in the levels of immune-activation and immune-suppression is crucial in the loss of host defence against SARS-CoV-2 infection [25-27] and changes in the expression of some immune cells and factors is considered as predictors of severity/mortality in COVID-19 patients [28]. Particularly, we analysed the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

association between CD169 expression in the early phase of SARS-CoV-2 infection and T and B cells immune response. CD169 RMFI was closely correlated with changes in differentiation markers in both CD4+ and CD8+ T cells from COV patients, especially in untreated patients, inversely correlating with the expression of CM and EM cells. Moreover, CD169 RMFI directly correlated with exhaustion markers (CD57+PD1+) in CD4+ T cells. In CD8+ T cells, CD169 RMFI was associated with the decrease of naive and increase of EM cells. Notably, CD169 RMFI correlated positively with the percentages of CD8+ CD57+ senescent lymphocytes. It has been demonstrated the presence of CD169+ macrophages in tumor tissue in correlation with CD57+CD8+ T cells or NK cells infiltration [29]. The CD57 marker was used to assess functional immunodeficiency in several diseases, suggesting that positive cells do not proliferate despite the preserved ability to secrete cytokines after activation [30]. COVID-19 patients have increased amounts of CD8+ T cells expressing CD57, which is considered a key marker of senescence and is associated both to human aging and chronic infections also in association with others endogenous markers recently described [31,32]. We also found an association of CD169 RMFI with PD1 expression, a crucial molecule for the induction and maintenance of peripheral tolerance, and of T cells stability and integrity. Indeed, the PD1/PD-L1 axis mediates potent inhibitory signals to block proliferation and function of T effector cells, inhibiting antiviral immunity [33].

CD169 RMFI value was inversely correlated with the presence of specific SARS CoV-2 IgG in serum, confirming the data of previous studies [9,10]. Novelty, by the characterization of B cell compartment, we demonstrated the CD169 RMFI in untreated patients correlated directly with the percentage of naive and marginal B cells, while inversely with plasmablasts and switched B cells in all patients. These results reinforce the spatial temporal dynamic of CD169 macrophages activation and B cell response in driving antibody production. Moreover, alterations in the B-cell compartment due to SARS-CoV-2 infection may emphasize the effort of the immune system to counterbalance lymphopenia by increasing transient B-cells and plasmablasts [34,35].

Our data demonstrate that CD169 is a valuable early marker associated with SARS-CoV-2 infection and COVID-19 severity mainly in patients who have not yet received treatments. CD169 expression is recognized as a sensitive biomarker for other diseases such as multiple sclerosis in which CD169 cells promote neuro-inflammation [36] and systemic lupus erythematosus [37]. In these diseases, the highest levels of CD169 expression were observed in patients not receiving drugs, such as glucocorticoids or hydroxychloroquine, that decrease interferon production through inhibition of toll-like receptors in plasmacytoid dendritic cells [38]. Recently, a lower circulating counts of CD169+ monocytes have been described in severe COVID-19 patients than in mild ones [39]. Conversely, we observed increased CD169 expression in moderate and severe patients and those with extensive interstitial pneumonia that did not receive any treatment, but was not confirmed in treated patients. Moreover, we have also highlighted the close association between CD169 RMFI and the respiratory outcome, suggesting CD169 RMFI as specific marker for patients who need respiratory support during their hospitalization, ,thus underlining both a diagnostic and predictive value to this marker.

Thus, the early detection of CD169 on macrophages along with T and B lymphocytes immunophenotyping could provide a reliable early measure of immune status that may be useful to assess COVID-19 disease progression especially in patients not treated with corticosteroids and antivirals at sampling.

Materials and Methods

Patients & controls.

Sixty-eight patients with positive SARS-CoV-2 RT-PCR were enrolled in an open study by the Clinic of infectious Diseases, Departments of System Medicine, University of Rome "Tor Vergata" and Policlinic of Tor Vergata (PTV) of Rome Foundation. Ethical approval for the collection and use of human samples was obtained from the ethical committee of "Tor Vergata" Hospital, COronaVIrus Disease: Safety and Efficacy of Experimental Treatment (COVID_SEET prot.7562/2020, 9th April 2020, experimental register 46.20). Blood samples from healthy donors (n=57, HDs) were obtained from individuals attending the local blood Transfusion Unit of PTV that referred to the Virology Unit of PTV for screening. All HDs and COVID patients provided written informed consent. The diagnosis of SARS-CoV-2 infection has been done by the Virology Unit of PTV using Allplex (SeeGene, Inc) 2019-nCoV multiplex Real-time PCR assay, according to manufacturer instructions. All clinical data were collected as reported in Table 1.

In vitro stimulation with spike protein

Peripheral blood mononuclear cells (PBMCs) from EDTA blood samples of all the individuals enrolled in the study were isolated by density gradient centrifugation (Pancoll human) and collected immediately after the separation. PBMCs were cultured at density of 0.25 x 10^6 in RPMI 1640 (PAN-Biotech) enriched with 2mM of L-glutamine, 100 U/ml of penicillin, 0.1 mg/ml of streptomycin, 12% fetal bovine serum in presence of human recombinant interleukin-2 (IL-2), 20 U/ml (all from Sigma, MO, USA). PBMCs were exposed for 24 hours at 37 °C in 5% CO_2 to 0.1-1-5-10 nM SARS-CoV-2 S protein, active trimer (ACROBiosystems, San Jose, CA). After incubation, samples were recovered and analysed by flow cytometry and Real time PCR. Each culture condition was done in duplicate.

Flow cytometry for CD169 expression and immunophenotyping analysis.

10 µl of EDTA blood sample was simultaneously lysed with 500 µl of Versalyse lysing solution (Beckman Coulter) and stained with CD64-CD169/infections dried custom mixture, composed of anti-CD169-phycoerythrin (PE) (clone 7-239) and anti-CD64-Pacific Blue (PB) and HLA-DR (APC) (clone 22) (Beckman Coulter.

The DuraClone IM T-cell subsets tube and B cell subset tube from Beckman Coulter were used to analyse differentiation and exhaustion markers. Stained cells were then washed with Dulbecco's phosphate buffered saline (PBS, PAN-Biotech). The stained cells were examined via CytoFLEX (Beckman Coulter) and data were analysed by the CytExpert 2.2 software (Beckman Coulter). The gating strategy was reported in **Figure S1** as

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 31 May 2021 doi:10.20944/preprints202105.0731.v1

previously described [8]. Results were expressed as the percentage of positive cells or median intensity of fluorescence (MFI).

RNA extraction from blood samples.

Blood samples were centrifuged at 800 g for 8 minutes and washed with PBS two times. The obtained pellets were treated with 150 µl of Red Blood lysing buffer (GRiSP, Lda) for 5 minutes at room temperature for two times to remove red cells. After washing with PBS, the pellets were resuspended in 400 µl of R1 buffer of RNA (Grisp) and dithiothreitol (DTT, Merck, Germany) 1 mM and incubated at room temperature for 5 minutes. Next samples were mixed with ethanol 70% and transfer on RNA mini spin column, according to manufacturer instruction (Total RNA extraction kit blood, Grisp). Treatment with DNase I "in column" at room temperature for 15 minutes ensures removal of contaminating DNA. RNA samples were evaluated by Nanodrop DS 11(DeNovix, USA), showing 260/280 ratio of about 2.0 and a concentration ranging from 10 to 100 ng/µl.

Quantitative RT-Real time PCR

DNase treated RNA (100 ng) was reverse-transcribed into cDNA using Improm-II Reverse Transcription System (Promega, Fitchburg, Wisconsin, USA) and oligo dT, according to the manufacturer's protocol; controls without template and another without enzyme were included in each RT reaction. An amount of 2.5 ng of initial RNA in RT reaction has been used to quantitatively evaluate genes, and the gene expression of the IL-6, IL-10, TNF-α, INF-γ, by Real-time PCR (all primer pair used have been listed in Table 5) [40-41]. The assays were performed in a Bio-Rad instrument (CFX96 Real-Time System, Bio-Rad, Hercules, California, USA) using SYBR Green chemistry (Fast QPCR Master Mix, Smobio, Taiwan). Each sample was analysed in triplicate and a negative control (no template reaction) was included in each experiment, to check for contamination. The expression of the housekeeping gene beta-glucuronidase (GUSB) in healthy donors was used to normalize the results. Each experiment was completed with a melting curve analysis to confirm the specificity of amplification and the lack of any non-specific product and primer dimer. Quantification was performed using the threshold cycle (Ct) comparative method: the relative expression was calculated as follows:$2^{-[\Delta Ct\ (sample) - \Delta Ct\ (calibrator)]} = 2^{-\Delta\Delta Ct}$, where ΔCt (sample) = [Ct (target gene) − Ct (housekeeping gene)] and the ΔCt (calibrator) was the mean of ΔCt of all HD samples.

TABLE 5. Primer pair sequences used in the Real Time-PCR analysis

GENE		FORWARD PRIMER (5'→3')	REVERSE PRIMER (5'→3')
#GUSB	NM_000181	CAGTTCCCTCCAGCTTCAATG	ACCCAGCCGACAAAATGC
#IL-6	NM_000600.3	TGCAATAACCACCCCTGACC	ATTTGCCGAAGAGCCCTCAG
#IL-10	NM_000572.2	ACATCAAGGCGCATGTGAAC	CACGGCCTTGCTCTTGTTTT
#TNFα	NM_000594.3	CCCGAGTGACAAGCCTGTAG	TGAGGTACAGGCCCTCTGAT
#INFγ	NM_000619.2	TCAGCTCTGCATCGTTTTGG	GTTCCATTATCCGCTACATCTGAA

Statistical analysis.

Statistical analysis of group-wise expression levels was performed through nonparametric Mann Whitney test in case of 2-independent samples or Kruskal Wallis test and Bonferroni's correction in case of n-independent samples. Pairwise associations between continuous variables were tested through the Spearman correlation coefficient. Statistical significant comparisons were considered when $p \leq 0.050$. Data analyses were performed using the SPSS statistical software system (version 23.0 for Windows, USA).

Supplementary Materials:

Figure S1 Gating strategy for the flow cytometry analysis of CD169 expression in blood cells of COV and HD samples.

Funding: No specific funding was received for this work. The study was supported by Beckman Coulter providing material and reagents for CD169 analysis and by facilities from University of Rome "Tor Vergata"

Acknowledgments

The authors would like to thank Joe Olechno for helpful discussions and linguistic revision, and Beckman Coulter for giving reagents used in the study. This private company had no role in the study design, or collection and interpretation of the clinical data.

Author Contributions:

Conceptualization, Antonella Minutolo and Claudia Matteucci; **Data curation**, Antonella Minutolo, Vita Petrone, Marialaura Fanelli, Marco Iannetta, Loredana Sarmati, Emanuela Balestrieri, and Fabrice Malergue; **Formal analysis**, Antonella Minutolo, Vita Petrone, Marco Iannetta, Ines Ait Belkacem and Emanuela Balestrieri; **Investigation**, Antonella Minutolo, Vita Petrone, Marialaura Fanelli, Marco Iannetta, Martina Giudice, Ines Ait Belkacem, Marta Zordan, Pietro Vitale, Loredana Sarmati and Sandro Grelli; **Methodology**, Fabrice Malergue and Claudia Matteucci; **Project administration**, Matteucci Claudia; **Resources**, Marco Iannetta, Marta Zordan, Pietro Vitale, Loredana Sarmati, Massimo Andreoni, Sandro Grelli and Claudia Matteucci; **Supervision**, Antonella Minutolo and Claudia Matteucci; **Validation**, Antonella Minutolo, Vita Petrone, Marialaura Fanelli and Emanuela Balestrieri; **Visualization**, Vita Petrone and Marialaura Fanelli; **Writing – original draft**, Antonella Minutolo, Vita Petrone, Marialaura Fanelli and Claudia Matteucci; **Writing – review & editing**, Marco Iannetta, Guido Rasi, Paola Sinibaldi-Vallebona, Loredana Sarmati, Massimo Andreoni, Emanuela Balestrieri, Sandro Grelli and Claudia Matteucci.

All authors will be informed about each step of manuscript processing including submission, revision, revision reminder, etc. via emails from our system or assigned Assistant Editor.

Institutional Review Board Statement: Ethical approval for the collection and use of human samples was obtained from the ethical committee of "Tor Vergata" Hospital, COronaVIrus Disease: Safety and Efficacy of

Experimental Treatment (COVID_SEET prot.7562/2020, 9th April 2020, experimental register 46.20). Blood samples from healthy donors (n=57, HDs) were obtained from individuals attending the local blood Transfusion Unit of Policlinic of "Tor Vergata" in Rome that referred to the Virology Unit of PTV for screening. All HDs and COV patients provided written informed consent.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The other authors have nothing to disclose. Fabrice Malergue and Inès Ait Belkacem are Beckman Coulter employees. All private companies had no role in the study design, or collection and interpretation of the clinical data.

References:

1. Tay, M.Z.; Poh, C.M.; Rénia, L.; MacAry, P.A.; Ng, L.F.P. The trinity of COVID-19: immunity, inflammation and intervention. *Nat. Rev. Immunol.* **2020**, *20(6)*, 363-374.
2. Liu, Y.; Xia, Y.; Qiu, C.H. Functions of CD169 positive macrophages in human diseases. *Biomed. Rep.* **2021**, *14(2)*, 26.
3. Klaas, M.; Crocker, P.R. Sialoadhesin in recognition of self and non-self. *Semin Immunopathol.* **2012**, *34(3)*, 353-64.
4. Bourgoin, P.; Biéchelé, G.; Ait Belkacem, I.; Morange, P.E.; Malergue, F. Role of the interferons in CD64 and CD169 expressions in whole blood: Relevance in the balance between viral- or bacterial-oriented immune responses. *Immun. Inflamm. Dis*. **2020**, *8(1)*, 106-123.
5. Pino, M.; Erkizia, I.; Benet, S.; Erikson, E.; Fernández-Figueras, M.T.; Guerrero, D.; Dalmau, J.; Ouchi, D.; Rausell, A.; Ciuffi, A.; et al. HIV-1 immune activation induces Siglec-1 expression and enhances viral trans-infection in blood and tissue myeloid cells. *Retrovirology* **2015**, 12:37.
6. Rempel, H.; Calosing, C., Sun, B.; Pulliam, L. Sialoadhesin expressed on IFN-induced monocytes binds HIV-1 and enhances infectivity. *PLoS One* **2008**, *3(4):* e1967.
7. Perez-Zsolt, D.; Erkizia, I.; Pino, M.; García-Gallo, M.; Martin, M.T.; Benet, S.; Chojnacki, J.; Fernàndez-Figueras, M.T.; Guerrero, D.; Urrea, V.; et al. Anti-Siglec-1 antibodies block Ebola viral uptake and decrease cytoplasmic viral entry. *Nat. Microbiol*. **2019**, *4(9)*,1558–70.
8. Feng, Z.; Diao B.; Wang, R.; Wang, G.; Wang, C.; Tan, Y.; Liu, L.; Wang, C.; Liu, Y.; Liu, Y.; et al The Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Directly Decimates Human Spleens and Lymph Nodes. Preprint at *medRxiv* **2020**, https://doi.org/10.1101/2020.03.27.20045427 (2020).
9 Ortillon, M.; Coudereau, R.; Cour, M.; Rimmelè, T.; Godignon, M.; Gossez, M.; Yonis, H.; Argaud, L.; Lukaszewicz, A.C.; Venet, F.; et al. Monocyte CD169 expression in COVID-19 patients upon intensive care unit admission. *Cytometry A.* **2021**, doi: 10.1002/cyto.a.24315.

10 Bedin, A.S.; Makinson, A.; Picot, M.C.; Mennechet, F.; Melergue, F.; Pisoni, A.; Nyiramigisha, E.; Montagnier, L.; Bollore, K.; Debiesse, S.; et al. Monocyte CD169 Expression as a Biomarker in the Early Diagnosis of Coronavirus Disease 2019. *J. Infect. Dis.* **2021**, *223(4)*, 562-567.

11 Bourgoin, P.; Soliveres, T.; Barbaresi, A.; Londou, A.; Ait Belkacem, I.; Arnoux, I.; Bernot, D.; Loosveld, M.; Morange, P.E.; et al. CD169 and CD64 could help differentiate bacterial from CoVID-19 or other viral infections in the Emergency Department. *Cytometry A.* **2021**, doi: 10.1002/cyto.a.24314. Online ahead of print.

12 Xu, H.C.; Huang, J.; Khairnar, V.; Duhan, V.; Pandyra, A.A.; Grusdat, M.; Shinde, P.; Mcllwain, D.R.; Maney, S.K.; Gommerman, J.; et al. Deficiency of the B cell-activating factor receptor results in limited CD169+ macrophage function during viral infection. *J. Virol.* **2015**, *89(9)*, 4748-4759.

13 Frederico, B.; Chao, B.; Lawler, C.; May, J.S.; Stevenson, P.G. Subcapsular sinus macrophages limit acute gammaherpesvirus dissemination. *J. Gen. Virol*. **2015,** *96(8)*, 2314-2327.

14 McKechnie, J.L.; Blish, C.A. The Innate Immune System: Fighting on the Front Lines or Fanning the Flames of COVID-19? *Cell Host Microbe*. **2020**, *27(6)*, 863–869.

15 Merad, M.; Martin, J.C. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. *Nat. Rev. Immunol.* **2020**, *20(6)*, 355–362.

16 Vabret, N.; Britton, G.J.; Gruber, C.; Hegde, S.; Kim, J.; Kuksin, M.; Levantovsky, R.; Malle, L.; Moreira, A.; Park, M.D.; et al. Immunology of COVID-19: Current State of Science. *Immunity* **2020**, *52(6):* 910–941.

17 Chevrier, S.; Zurbuchen, Y.; Cervia, C.; Adamo, S. ; Raeber, M.E. ; de Souza, N. ; Sivapatham, S.; Jacobs, A.; Bachli, E.; Rudiger, A.; et al. A distinct innate immune signature marks progression from mild to severe COVID-19. *Cell. Rep. Med*. **2020**, *2(1):* 100166.

18 Martineza, F.O.; Combes, T.W.; Orsenigo, F.; Gordon, S. Monocyte activation in systemic Covid-19 infection: Assay and rationale. *EBioMedicine* **2020**, 59:102964.

19 Di Micco, P.; Russo, V.; Carannante, N.; Imparato, M.; Cardillo, G.; Lodigiani, C. Prognostic Value of Fibrinogen among COVID-19 Patients Admitted to an Emergency Department: An Italian Cohort Study. *J. Clin. Med.* **2020**, *9(12):* 4134.

20 Barlass, U.; Wiliams, B.; Dhana, K.; Adnan, D.; Shahab R.K.; Mahdavinia, M.; Bishehsari, F. Marked Elevation of Lipase in COVID-19 Disease: A Cohort Study. *Clin. Transl. Gastroenterol*. **2020**, *11(7):* e00215.

21 Kulkarni, A.V.; Kumar, P.; Tevethia, H.V.; Premkumar, M.; Arab, J.P.; Candia, R.; Talukdar, R.; Sharma, M.; Qu, X.; Rao, P.N.; et al. Systematic review with meta-analysis: liver manifestations and outcomes in COVID-19. *Aliment. Pharmacol. Ther*. **2020**, *52(4)*: 584-599.

22 Ji, P.; Zhu, J.; Zhong, Z.; Li, H.; Pang, J.; Li, B.; Zhang, J. Association of elevated inflammatory markers and severe COVID-19: A meta-analysis. *Medicine (Baltimore)* **2020**, *99(47)*: e23315.

23 Han, H.; Ma, Q.; Li, C.; Liu, R.; Zhao, L.; Wang, W.; Zhang, P.; Liu, X.; Gao, G.; Liu, F.; et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerg. Microbes Infect.* **2020**, *9(1)*: 1123–1130.

24 Wang, W.; Ye, L.; Ye, L.; Li, B.; Gao, B.; Zeng, Y.; Kong, L.; Fang, X.; Zheng, H.; Wu, Z.; et al. Up-regulation of IL-6 and TNF-alpha induced by SARS-coronavirus spike protein in murine macrophages via NF-kappaB pathway. *Virus Res*. **2007**, *128(1-2):* 1-8.

25 Shah, V.K.; Firmal, P.; Alam, A.; Ganguly, D.; Chattopadhyay, S. Overview of Immune Response During SARS-CoV-2 Infection: Lessons From the Past. *Front. Immunol*. **2020**, *11*:1949.

26 Chen, Z.; Wherry, E.J. T cell responses in patients with COVID-19. *Nat. Rev. Immunol*. **2020**, *20(9)*:529–536.

27 De Candia, P.; Prattichizzo, F.; Garavelli, S.; Matarese, G. T Cells: Warriors of SARS-CoV-2 Infection. Trends Immunol. **2021**, *42(1)*:18-30.

28 Sami, R.; Fathi, F.; Eskandari, N., Ahmadi, M.; ArefNezhad, R.; Motedayyen, H. Characterizing the immune responses of those who survived or succumbed to COVID-19: Can immunological signatures predict outcome? *Cytokine* **2021**, *140*:155439.

29 Ohnishi, K.; Yamaguchi, M.; Erdenebaatar, C.; Saito, F.; Tashiro, H.; Katabuchi, H.; Takeya, M.; Komohara, Y. Prognostic significance of CD169-positive lymph node sinus macrophages in patients with endometrial carcinoma. *Cancer Sci*. **2016**, *107(6)*: 846-852.

30 Focosi, D.; Bestagno, M.; Burrone, O.; Petrini, M. CD57+ T lymphocytes and functional immune deficiency. *J. Leukoc. Biol.* **2010**, *87(1)*:107–116.

31 Pinti, M.; Appay, V.; Campisi, J.; Frasca, D.; Fülöp, T.; Sauce, D.; Larbi, A.; Weinberger, B.; Cossarizza, A. Aging of the immune system: focus on inflammation and vaccination. *Eur. J. Immunol.* **2016**, *46(10)*: 2286–2301.

32 Balestrieri, E.; Minutolo, A.; Petrone, V.; Fanelli, M.; Iannetta, M.; Malagnino, V.; Zordan, M.; Vitale, P.; Charvet, B,; et al. Evidence of the pathogenic HERV-W envelope expression in T lymphocytes in association with the respiratory outcome of COVID-19 patients. *EbioMedicine*. **2021**, *66*:103341.

33 Chamoto, K.; Al-Habsi, M.; Honjo,,T. Role of PD-1 in immunity and diseases. *Curr. Top. Microbiol. Immunol*. **2017**, *410*:75-97.

34 De Biasi, S.; Lo Tartaro, D.; Meschiari, M.; Gibellini, L.; Bellinazzi, C.; Borella, R.; Fidanza, L.; Mattioli, M.; Paolini, A.; Gozzi, L.; et al. Expansion of plasmablasts and loss of memory B cells in peripheral blood from COVID-19 patients with pneumonia. *Eur. J. Immunol.* **2020**, *50(9):* 1283-1294.

35 Sosa-Hernández, V.A.; Torres-Ruìz, J.; Cervantes-Dìaz, R.; Romero-Ramìrez, S.; Pàeaz-Franco, J. C.; Meza-Sànchez, D.E.; Juàrez-Vega, G.; Pèrez-Fragoso, A.; Ortiz-Navarrete, V.; Ponce-de-Leon, A.; et al. B Cell Subsets as Severity-Associated Signatures in COVID-19 Patients. *Front. Immunol.* **2020**, *11*:611004.

36 Bogie, J.F.; Boelen, E.; Louagie, E.; Delputte, P.; Elewaut, D.; van Horssen, J.; Hendriks, J.J.; Hellings, N. CD169 is a marker for highly pathogenic phagocytes in multiple sclerosis. *Mult. Scler.* **2018**, *24(3)*:290-300.

37 Von Stuckrad, S.L.; Klotsche, J.; Biesen, R.; Lieber, M.; Thumfart, J.; Meisel, C.; Unterwalder, N.; Kallinich, T. SIGLEC1 (CD169) is a sensitive biomarker for the deterioration of the clinical course in childhood systemic lupus erythematosus. *Lupus* **2020**, *29(14):*1914-1925.

38 Kuznik, A.; Bencina, M.; Svajger, U.; Jeras, M.; Rozman, B.; Jerala, R. Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. *J. Immunol.* **2011**, *186(8):* 4794–4804.

39 Doehn, JM.; Tabeling, C.; Biesen, R.; Saccomanno, J.; Madlung, E.; Pappe, E.; Gabriel, F.; Kurth, F.; Meisel, C.; Corman, V. M.; et al. CD169/SIGLEC1 is expressed on circulating monocytes in COVID-19 and expression levels are associated with disease severity. *Infection* **2021**, *Apr 6:*1-6.

40 Balestrieri, E.; Cipriani, C.; Matteucci, C.; Benvenuto, A.; Coniglio, A.; Argaw-Denboba, A.; Toschi, N.; Bucci, I.; Miele, M.T.; Grelli, S.; et al. Children with autism spectrum disorder and their mothers share abnormal expression of selected endogenous retroviruses families and cytokines. *Front. Immunol.* **2019**, *10:* 2244.

41 Balestrieri, E.; Arpino, C; Matteucci, C; Sorrentino, R.; Pica, F.; Alessandrelli, R.; Coniglio, A.; Paolo, Curatolo P.; Rezza, G.; Macciardi, F.; et al. HERVs Expression in Autism Spectrum Disorders. *PLoS One* **2012**, *7(11)*: e48831.