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BRIEF REPORT

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Relationship between lymphocyte subsets values and C-reactive protein in COVID-19 patients

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Abstract

We enrolled 33 patients with COVID-19 (23 men and 10 women; age 59 ± 15; males, n = 23; females, n = 10) admitted to the Department of Infectious Diseases of Grande Ospedale Metropolitano "Bianchi-Melacrino-Morelli" of Reggio Calabria, Italy, between March and May 2020. Whole blood samples were collected before the start of therapeutic treatment using all virus spread containment measures. Sample preparation protocols were designed in order to minimize operators direct specimen's manipulation. On univariate analysis, circulating levels of CRP were strongly and inversely related to CD3+ (rho = −0.77, $p < 0.001$), CD3+4+ (rho = −0.74, $p < 0.001$), and CD3+8+ (rho = −0.66, $p = 0.001$) implying that the shared variances between absolute values T cells and CRP ranged from 44 to 59%. Of note, the strength of these associations was higher in patients with relatively lower (below the median value) white blood cells (WBC) as compared to those with WBC above the median value. CRP also correlated with NK bright (rho = −0.56, $p = 0.005$) but failed to be related with CD19+ (rho = −0.38, $p = 0.07$), CD4+/CD8+ ratio (rho = 0.03, $p = 0.89$), CD16+ CD56+ (rho = −0.18, $p = 0.43$), and NKdim (rho = −0.15, $p = 0.49$). Lymphocyte subsets alteration monitoring in COVID-19 positive patients may be a valid aid to control treatment efficacy of therapy and to choose better clinical approach. In particular, the negative correlation between CD3+, CD3+CD4+, CD3+CD8+ T cells values and CRP could be a useful tool to predict patient's response to therapy, particularly in patients with relatively lower WBC.

KEYWORDS
COVID-19, C-reactive protein, inflammation, SARS-CoV-2

COVID-19 affects both innate and adaptive host immune responses, but mechanisms mediating viral response are largely unknown [1–3]. In a cross sectional study, in a series of symptomatic patients with documented COVID-19 infection, we investigated the relationship between circulating levels of T, B, and NK cells and a biomarker of inflammation such as C-reactive protein (CRP). We enrolled 33 patients (23 men and 10 women; age 59 ± 15; males, n = 23; females, n = 10—see Table 1) admitted to the Department of Infectious Diseases of Grande Ospedale Metropolitano "Bianchi-Melacrino-Morelli" of Reggio Calabria, Italy, between March and May 2020.

The components of the "GOM-COVID-19 Working Group" are given in the Acknowledgments section.

1 | MATERIALS AND METHODS

The study received approval by the Ethical Committee of our institution and informed consent was obtained from each participant. Whole blood samples were collected before the start of therapeutic treatment using all virus spread containment measures. Sample preparation

TABLE 1 Main clinical characteristics of patients included into the study

Variables	
Age (years)	59 ± 15
Hypertension (%)	66%
Dementia (%)	24%
Chronic renal failure (%)	24%
Obesity (%)	18%
Type 2 diabetes (%)	33%
Chronic Obstructive Pulmonary Disease (%)	21%
Cancer (%)	21%
Heart failure (%)	33%
Male/female	23/10
CRP (mg/L)	8 (3–44)
IL-6 (pg/ml)	8 (4.5–27.5)
Hemoglobin (g/dl)	13.0 ± 1.9
White blood cells (10^3/μl)	6.50 (4.88–8.44)
Neutrophils (10^3/μl)	4.26 (2.62–6.02)
Lymphocytes (10^3/μl)	1.50 (1.24–1.94)
CD19+ (cell/μl)	150 (90–229)
CD3+ (cell/μl)	1033 (751–1394)
CD3+4+ (cell/μl)	681 (494–893)
CD3+8+ (cell/μl)	298 (185–490)
CD4+/CD8+ (%)	2 (1–3)
CD16+CD56+ (cell/μl)	220 (121–317)
NK-DIM (cell/μl)	290 (176–363)
NK-Bright (cell/μl)	5 (2–8)

Note: Data are mean ± SD, median, and inter-quartile range or absolute number, as appropriate.

protocols were designed in order to minimize operators direct specimen's manipulation. We provided double samples analysis: (a) standard panel connected with BD FACS Canto software that allows to analyze mature B, T and NK lymphocyte subsets through a mixture of monoclonal antibodies (CD3 FITC /CD16+CD56+ PE/CD45 PERCP-Cy5/CD4 PeCy7/CD19 APC/CD8 APC-Cy7) and BD Trucount Tubes, each with a calibrated number of fluorescent beads for absolute counts of lymphocyte subsets; (b) optimized panel (CD4 FITC/CD56 PE/CD8 PERCP-Cy5/CD19 PeCy7/CD HLA-DR APC/CD16 APC-H7/CD3V450/CD45V500) connected with BD FACS Diva software for a deeply study of natural killer (NK) cells in order to subdivide NK into three compartments: NK CD56 bright (CD56++/CD16+/−), producing cytokines, NK CD56dim (CD56+CD16++), with cytotoxic activity and NK CD56-CD16+, that increase in chronicle viral infections Sample's analysis was performed by BD FACS Canto II using Facs Diva software (version 6.1.3). In the first protocol, we used BD Multitest 6 color TBNK Kit with BD Trucount Tube. This IVD test has received the CE mark for an expanded clinical application of a test assess immune function in patients with COVID-19. In the second protocol, we prepared manually moAbs subsets tubes in order to subdivide NK into three compartments (NK CD56bright CD56++/CD16+/−, NK CD56dim, and NK CD56−CD16+). The multicolor panel contained following moAbs provided by Becton Dickinson: CD4 (FITC)/CD56(PE)/CD8 (PERCP-Cy5)/CD19 (PeCy7)/CD HLA-DR (APC)/CD16 (APC-H7)/CD3(V450)/CD45(V500).

These protocols allowed us to study CD3+, CD4+ and CD8+ T lymphocytes and CD19+ B lymphocytes percentage and absolute counts, CD4+/CD8+ ratio that represent an excellent indicator of patient's immune system condition and NK CD16+ CD56+.

2 | RESULTS

On univariate analysis, circulating levels of CRP were strongly and inversely related to CD3+ (rho = −0.77, $p < 0.001$), CD3+4+ (rho = −0.74, $p < .001$) and CD3+8+ (rho = −0.66, $p = 0.001$) implying that the shared variances between absolute values T cells and CRP ranged from 44 to 59%. Of note, the strength of these associations was higher in patients with relatively lower (below the median value) WBC as compared to those with WBC above the median value (see Figure 1). CRP also correlated with NK bright (rho = −0.56, $p = 0.005$) and the clinical staging of the disease (rho = 0.43, $p = 0.04$) but failed to be related with CD19+ (rho = −0.38, $p = 0.07$), CD4+/CD8+ ratio (rho = 0.03, $p = 0.89$), CD16+ CD56+ (rho = −0.18, $p = 0.43$), and NKdim (rho = −0.15, $p = 0.49$; see Figure 2).

Lymphocyte subsets alteration monitoring in COVID-19 positive patients may be a valid aid to control treatment efficacy of therapy and to choose better clinical approach. The strong associations among CD3+, CD3+4+, and CD3+8+ with CRP could represent the basis to plan a prospective study to assess whether these biomarkers can be useful to predict the response to therapy. For interested readers, other results on the same topic are reported elsewhere [4–7].

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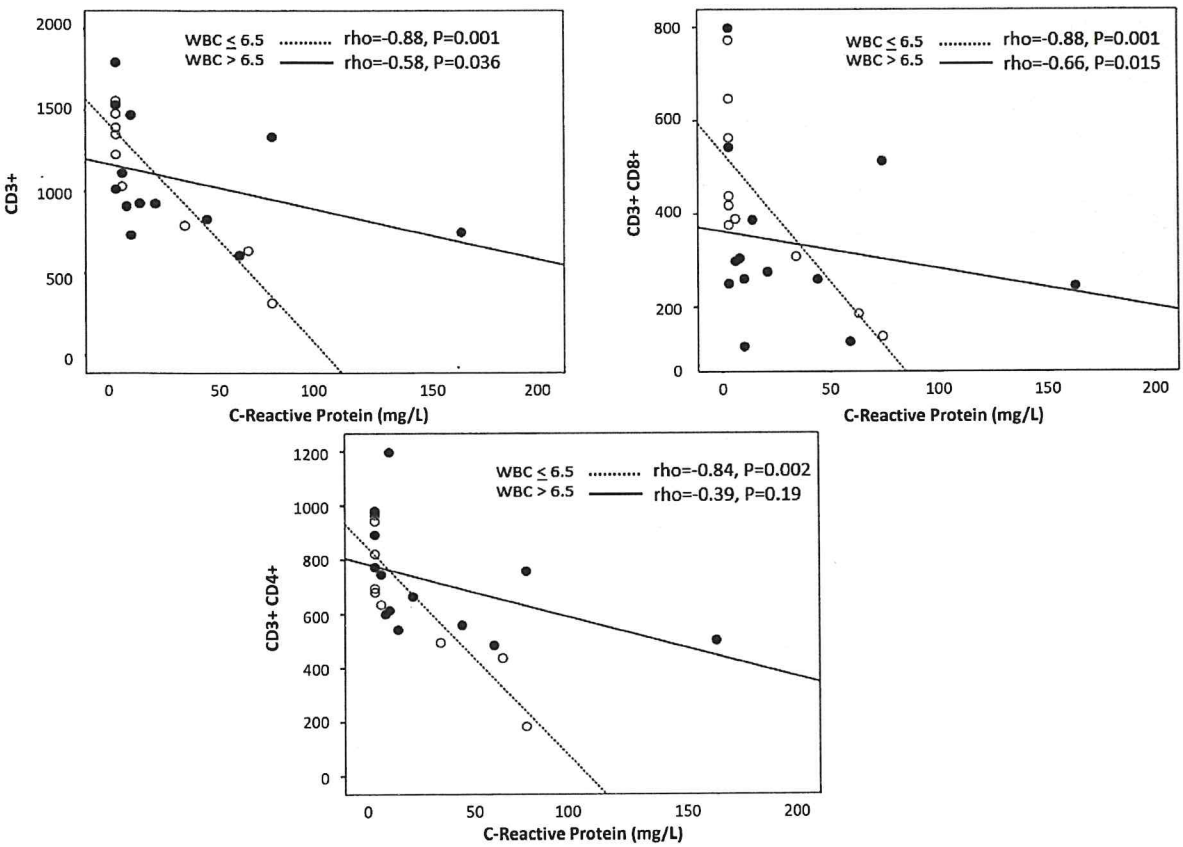

FIGURE 1 Relationship between CRP with CD3+, CD3+CD4+, CD3+CD8+ T cells values separately in patients with WBC below and above the median value

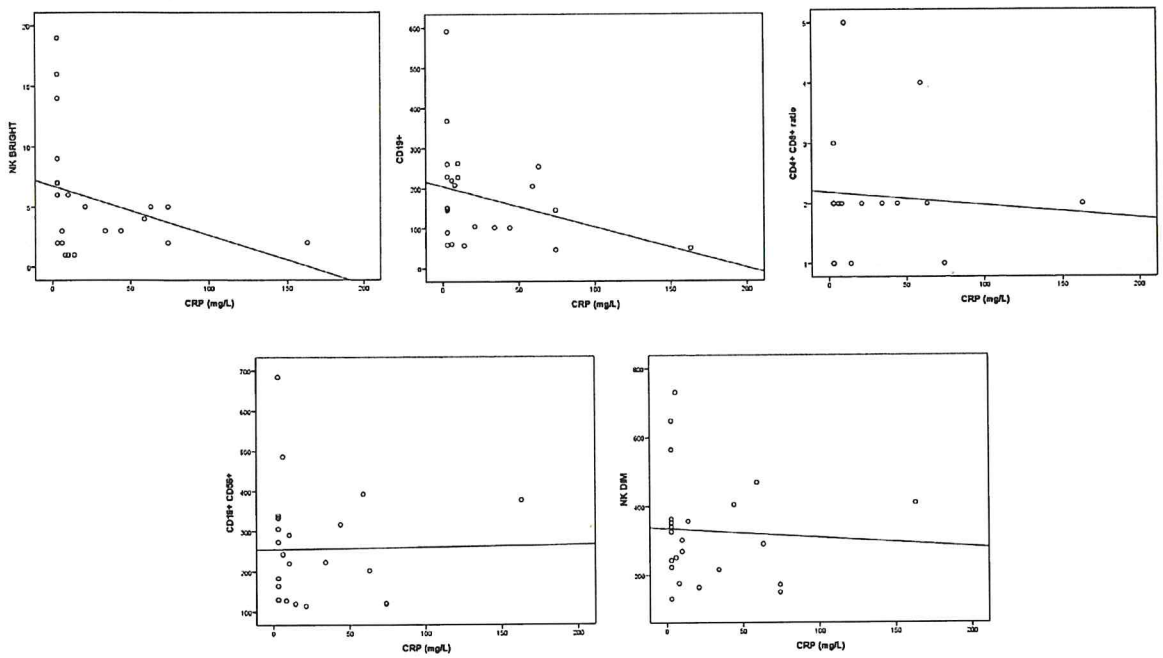

FIGURE 2 Association of CRP with NK bright (rho = −0.56, p = 0.005), CD19+ (rho = −0.38, p = 0.07), CD4+/CD8+ ratio (rho = 0.03, p = 0.89), CD16+ CD56+ (rho = −0.18, p = 0.43), and NKdim (rho = −0.15, p = 0.49)

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AUTHOR CONTRIBUTIONS

Carmelo Mangano and **Bianca Oliva:** Conceptualization; data curation; formal analysis; investigation; methodology; resources; software; supervision; validation; writing-original draft; writing-review & editing.

CONFLICT OF INTEREST

No conflict of interest is related to the article.

REFERENCES

1. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell. 2020;181(7):1489-1501.e15.
2. Weiskopf D, Schmitz KS, Raadsen MP, Grifoni A, Okba NMA, Endeman H, et al. Phenotype of SARS-CoV-2-specific T-cells in COVID-19 patients with acute respiratory distress syndrome. medRxiv. 2020:2020.04.11.20062349.
3. Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, et al. Presence of SARS-CoV-2 reactive T cells in COVID-19 patients and healthy donors. medRxiv. 2020:2020.04.17.20061440.
4. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: current state of the science. Immunity. 2020;52(6):910–41.
5. Liang J, Nong S, Jiang L, et al. Correlations of disease severity and age with hematology parameter variations in patients with COVID-19 pre- and post-treatment. J Clin Lab Anal. 2020;35(1):e23609.
6. Gan J, Li J, Li S, Yang C. Leucocyte subsets effectively predict the clinical outcome of patients With COVID-19 pneumonia: A retrospective case-control study. Front Public Health. 2020;8:299.
7. Wang L. C-reactive protein levels in the early stage of COVID-19. Med Mal Infect. 2020 Jun;50(4):332–4. https://doi.org/10.1016/j.medmal.2020.03.007.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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