

DYNAMICS OF IMMUNOLOGICAL AND BIOCHEMICAL
MARKERS DEPENDS ON THE SEVERITY AND TIME
AFTER SYMPTOMS ONSET OF COVID-19 – SINGLE
CENTRE ANALYSIS

Anastasiya Mihaylova[#], Spaska Lesichkova*, Maria Atanasova**,
Julieta Hristova***, Veneta Grigorova****, Nevena Gesheva,
Dobrin Svinarov***, Milena Velizarova***, Elisaveta Naumova*

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Abstract

More than three years after the first wave of COVID-19, the biomarkers responsible for the course of this disease continue to be debated. Moreover, the new condition, long COVID, poses many unsolved questions. Therefore, we decided to perform a complex analysis of immune and biochemical parameters in patients with different severity of COVID-19. Eighty-five adult patients with RT-PCR confirmed SARS-CoV-2 infection hospitalized in the period April–November, 2020, were enrolled in the study. Extended immunological and biochemical parameters were analyzed in different time points after symptoms onset of COVID-19. Our results demonstrated that severe patients present with increased CRP, ferritin, D-dimer and NLR, decreased counts of lymphocytes, total T lymphocytes and major T-cell subsets, increased proportions of activated T cells, Tregs and Bregs. Markers of immune response dysfunction combined with hematological, inflammatory and coagulation indicators may be more informative for predicting severe COVID-19. Moreover, the changes and dynamics of immunological and biochemical biomarkers depend on the severity and time after COVID-19 symptoms onset.

Key words: COVID-19, severity, biomarkers

[#]Corresponding author.

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Introduction. Coronavirus disease (COVID-19) due to the infection with the novel coronavirus SARS-CoV-2, manifests with varying clinical presentations ranging from asymptomatic and mild illness to severe interstitial pneumonia and respiratory failure with even fatal issue. To date, a huge number of publications have reported aberrant immune reactivity, and elevated inflammation- and coagulation-associated markers in COVID-19 patients, data from which have been summarized in a number of reviews and meta-analyses [1-8]. Additionally, dysregulated immune responses may contribute to long-term effects from the infection, known as Long COVID or Post-COVID Conditions [9].

Therefore, we hypothesized that a complex analysis of immune and biochemical parameters in patients with different severity of COVID-19 would contribute to identify biomarkers explaining the differences in clinical course of the disease and complement other well-known prognostic factors.

Material and methods. Patients. Eighty-five patients (50 male, 35 female, median age 59, range: 25–84 years) with RT-PCR confirmed SARS-CoV-2 infection, hospitalized in the period April–November, 2020, were enrolled in the study. The main clinical manifestations included fever, chills, malaise, cough, smell or taste abnormalities. The patients were categorized by disease severity into three groups: mild, moderate and severe, according to the criteria of Centers for Disease Control and Prevention [10], and treated as per standardized institutional protocols. Risk factors for a severe COVID-19 were identified in 56/85 (65.9%) of the patients: cardiovascular diseases ($n = 51$; 60.0%), metabolic syndrome ($n = 10$; 11.8%), diabetes mellitus ($n = 4$; 0.45%), other chronic diseases (asthma, chronic lung disease and chronic renal failure – $n = 9$; 10.5%). Additionally, patients were classified according to the time post-onset of symptoms (PoS): up to 2 weeks (median 9 days, IQR 7–10 days) and after 2 weeks (median 19 days, IQR 16–22 days). The study was approved by the institution's ethics committee and all patients signed an informed consent.

Clinical laboratory tests. Complete blood count with differential, D-dimer, CRP, ferritin, LDH were tested using routine analytical methods.

Immunophenotyping. Lymphocyte subset analysis was performed by multicolour flow cytometry using standard whole blood staining with combinations of monoclonal antibodies labelled with different fluorochromes. Acquisition and analysis were done with a FACSCanto II flow cytometer and FACSDiva software (Becton Dickinson, USA). The analysis was based on gating of lymphocytes and subsequent determination of major lymphocyte subpopulations by combinations of lineage-specific markers. To analyze CD4+, CD8+ and B-cell subsets, additional gates were defined using combinations of surface markers specific for a particular subpopulation. The data are presented as percentage (%) and absolute count (cells/ μ L).

Serum levels of *IgG*, *IgA*, *IgM*, and *C3* and *C4* fractions of complement were measured by immunoturbidimetry (SPAPLUS, Binding Site, UK).

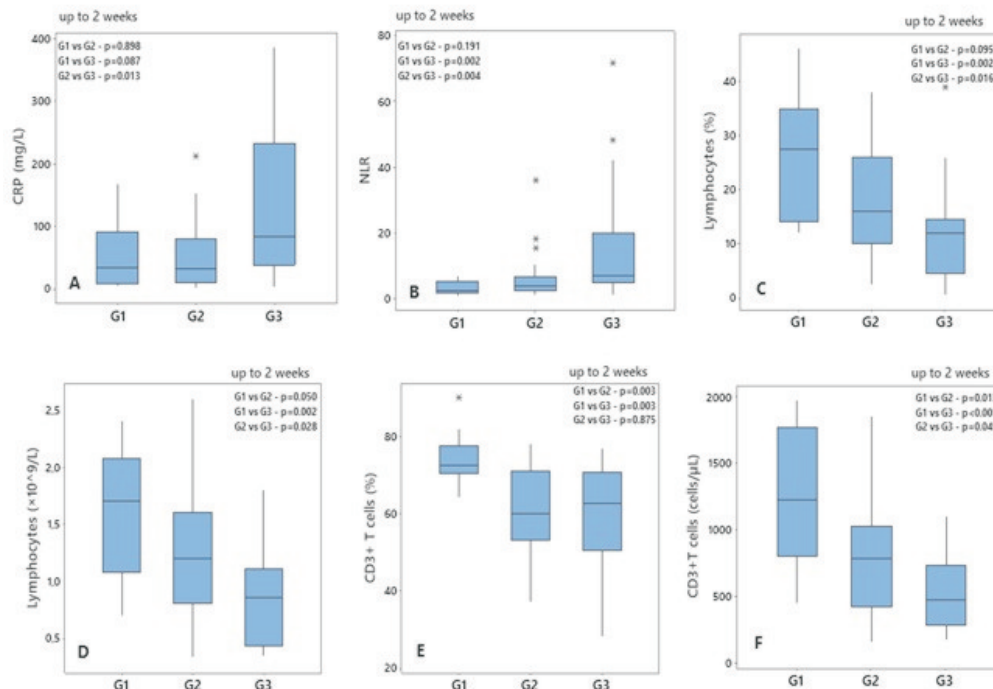


Fig. 1. Biochemical and immune parameters showing statistically significant changes up to 2 weeks post COVID-19 symptoms onset. Data are presented as median, IQR (boxes) and p values; G1 (group 1) – mild, G2 (group 2) – moderate, G3 (group 3) – severe COVID-19

Statistical methods. For continuous variables, the relationship with disease severity was examined with the Kruskal–Wallis test. Comparisons between 2 groups were performed with the Mann–Whitney test. Data are presented with median and interquartile range – IQR (25th percentile – Q1 and 75th percentile – Q3). Analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, NY, USA). A p -value < 0.05 was considered statistically significant.

Results. During the first two weeks PoS, CRP was sharply elevated in the severe compared with the moderate cohort (Fig. 1A). Significantly increased neutrophil-to-lymphocyte ratio (NLR) and significant lymphopenia were found in severe compared with mild and moderate COVID-19 (Fig. 1B, C, D).

The total (CD3+) T cells (Fig. 1E, F), helper-inducer (CD4+) and suppressor-cytotoxic (CD8+) subsets counts (Fig. 2A, B) were markedly lower in moderate and severe disease versus mild form with the most noticeable decrease in severe patients. T cell activation status, assessed by HLA-DR and CD38 expression, demonstrated the lowest number of CD8+HLA-DR+T cells in severe disease with a significant difference compared to moderate disease (Fig. 2C).

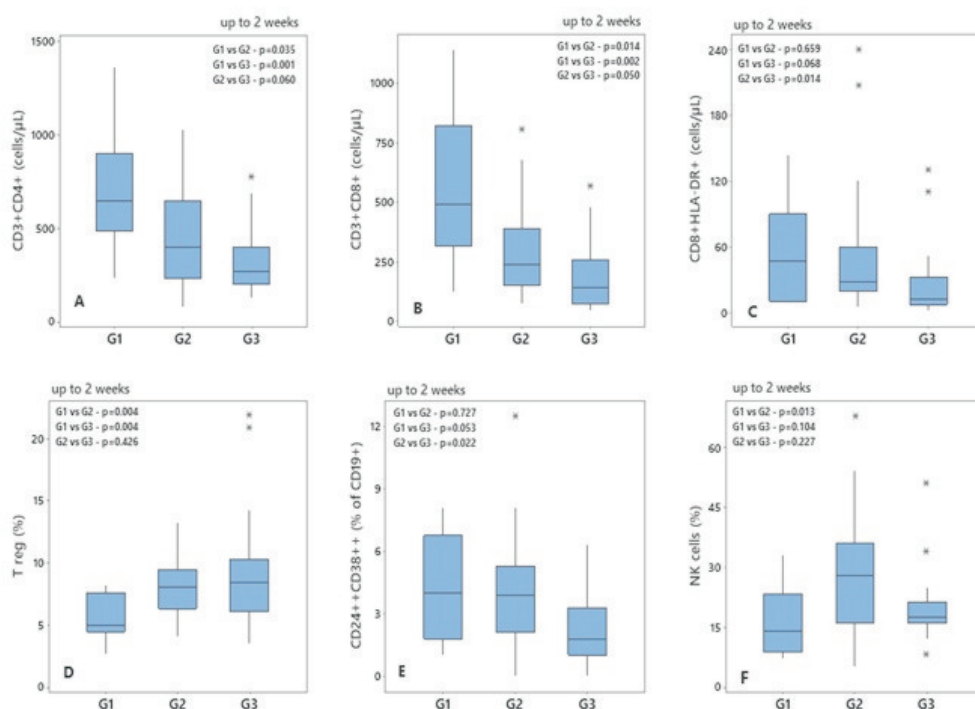


Fig. 2. Immune parameters showing statistically significant changes up to 2 weeks after COVID-19 symptoms onset. Data are presented as median, IQR (boxes) and p values; G1 (group 1) – mild, G2 (group 2) – moderate, G3 (group 3) – severe COVID-19

Significantly increased relative frequency of Tregs (CD4+CD25+CD127^{low}) was established for moderate and severe compared to mild COVID-19 (Fig. 2D). Alterations in the B-cell compartment were demonstrated by the lowest values of CD24++CD38++ subset (% of CD19+) in severe compared with moderate disease (Fig. 2E). Higher percentages of NK cells were observed in moderate versus mild disease (Fig. 2F). The only significant difference in humoral immunity was found for total IgA, demonstrated as increased levels ($p = 0.023$) in mild (median 3.50 g/L, IQR 2.25–5.5 g/L) compared to moderate (median 2.0 g/L, IQR 1.7–3.0 g/L) disease stages.

During weeks 3–4 PoS in addition to CRP, an increase of D-dimer and ferritin was found with the clinical worsening (Fig. 3A, B, C). The lowest lymphocyte, CD3+ and CD4+ T cell counts persisted in severe compared to moderate and mild disease (Fig. 3D, E, F). A decrease ($p = 0.007$) was also noticed for NK cell counts (CD3-CD16&56+) in severe (median 198 cells/ μ L, IQR 90–252 cells/ μ L) compared to moderate (median 342 cells/ μ L, IQR 210–505 cells/ μ L) cases.

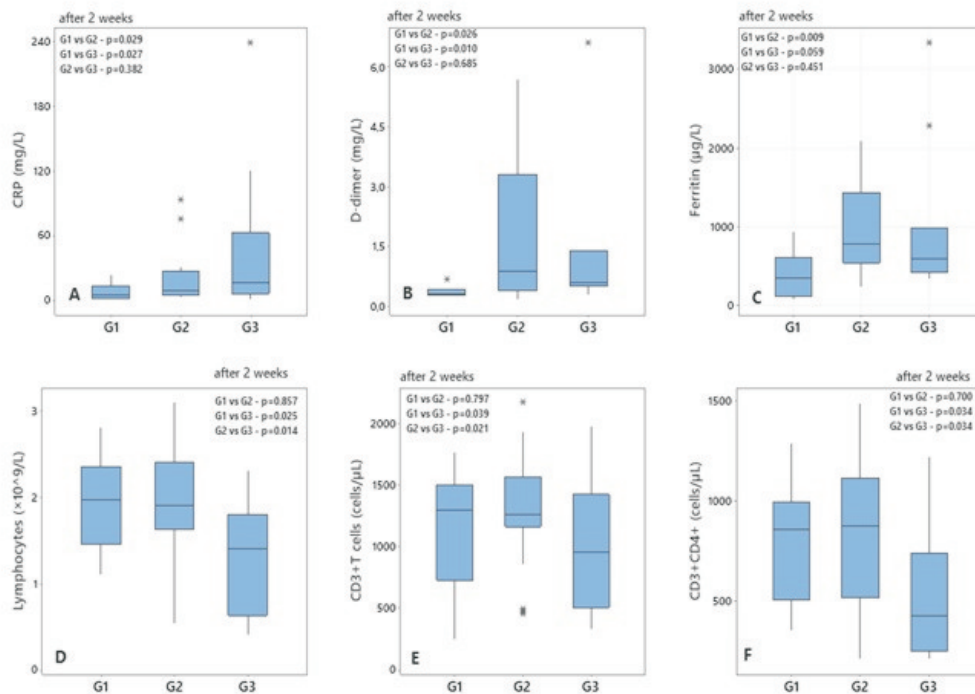


Fig. 3. Biochemical and immune parameters showing statistically significant changes after 2 weeks post COVID-19 symptoms onset. Data are presented as median, IQR (boxes) and p values; G1 (group 1) – mild, G2 (group 2) – moderate, G3 (group 3) – severe COVID-19

CD8+HLA-DR+ and CD8+CD38+ activated T cells percentages were significantly increased in moderate (CD8+HLA-DR+ – median 4.0%, IQR 2.4–5.0%, $p=0.04$; CD8+CD38+ – median 19.0%, IQR 12.0–22.0%, $p=0.012$) and severe (CD8+HLA-DR+ – median 4.25%, IQR 3.0–7.0%, $p=0.009$; CD8+CD38+ – median 14.5%, IQR 8.75–18.6%, $p=0.025$) compared with those in mild disease (CD8+HLA-DR+ – median 2.0%, IQR 1.3–3.0%; CD8+CD38+ – median 9.0%, IQR 5.5–12.0%).

Discussion. Associations of increased CRP, a nonspecific but sensitive indicator of systemic inflammation, ferritin (another nonspecific acute phase reactant) and D-dimer (a coagulation parameter) with poor prognosis have been reported in SARS-Cov-2 infected patients since the beginning of the pandemic [4–8]. Our data show that CRP is one of the first laboratory parameters increasing with worsening of COVID-19. After two weeks PoS, elevated ferritin and D-dimer emerged as additional markers associated with more severe disease course. Lymphopenia along with decreased CD3+, CD4+, CD8+ T and NK cell counts, and increased NLR that are proportional to disease severity are a prominent feature in patients with COVID-19 [1–6]. Our results support these findings and additionally demon-

strate that these alterations are more profound during the first two weeks PoS. As for T-cell activation markers, conflicting results have been reported [4,11–13]. The observed by us gradual increase in proportions of CD8+ T cells expressing HLA-DR or CD38 with disease worsening is consistent with other studies [11,12] and reflects the involvement of activated T cells in the progression of COVID-19. SARS-Cov-2 infection has also been associated with perturbations in Treg homeostasis. Although most authors reported decreased Tregs correlating with disease progression [14], the present study revealed increased Treg proportions with the clinical worsening. Our results are consistent with data of GALVÁN-PEÑA et al. [15], who suggested that increasing of these cells may have an effect similar to that in tumours by overcurtailing the antiviral response during the severe disease phase. Another finding in our study is the significantly decreased percentage of CD24++CD38++ B cells in severe COVID-19 that may contribute to the immune imbalance and poor prognosis. Although this phenotype generally defines transitional B cells, CD19+CD24hiCD38hi B cells have been described to exhibit a negative regulatory function in healthy individuals [16]. Decreased proportion and number of CD24++CD38++ regulatory B cells (Bregs) were reported in sepsis [17] as predictive factors for poor septic outcome. An additional observation in our study was a significant increase in total IgA 1–2 weeks PoS associated with severe disease, which is also reported in the few studies [3,18] on total immunoglobulins in COVID-19. We have addressed the possibility that elevated total IgA in our group of severe patients may reflect higher levels of SARS-Cov-2-specific IgA antibodies reported early in the course of COVID-19. Our results demonstrated that severe patients present with increased CRP, ferritin, D-dimer and NLR, decreased lymphocyte, total T lymphocyte and major T cell subset counts, increased proportion of activated T cells, Tregs and Bregs. Parameters of immune response dysfunction combined with hematological, inflammatory and coagulation indicators may be more informative for predicting a severe course of COVID-19.

Conclusion. The present complex study showed that changes and dynamics of immunological and biochemical markers depend on the severity and time after symptoms onset of COVID-19. A new challenge for doctors, patients and scientists is the Long COVID, a new condition with many unanswered questions. In this regard, the present study can serve as a basis for further research.

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*Clinic of Clinical Immunology
with Stem Cell Bank
University Hospital “Alexandrovskia”
1 St. Georgi Sofiiski St
1431 Sofia, Bulgaria
e-mail: mihaylova_ap@abv.bg
n_gesheva@hotmail.com*

**Clinic of Clinical Immunology
with Stem Cell Bank
University Hospital “Alexandrovskia”
Department of Clinical Immunology
Medical University – Sofia
1 St. Georgi Sofiiski St
1431 Sofia, Bulgaria
e-mail: s_lesichkova@abv.bg
naumovaej@gmail.com*

***Clinic of anesthesiology,
resuscitation and intensive care
University Hospital “St. Anna”
1 Dimitar Mollov St
1750 Sofia, Bulgaria
e-mail: m.atanassova@abv.bg*

****University Hospital “Alexandrovskia”
Department of Clinical Laboratory
Faculty of Medicine
Medical University – Sofia
1 St. Georgi Sofiiski St
1431 Sofia, Bulgaria
e-mail: julieta_sd@yahoo.com
dsvinarov@yahoo.com
milena.velizarova@gmail.com*

*****Department of Cardiovascular Surgery
Medical Faculty
Sofia University “St. Kliment Ohridski”
1 Kozyak St
1407 Sofia, Bulgaria
e-mail: grigorveny@yahoo.com*