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Biomarkers of sequela in adult patients convalescing from COVID-19

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Biomarkers of sequela in adult patients convalescing from COVID-19

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Abstract

Different biomarkers for SARS-CoV-2 have been linked to detection, diagnosis, treatment, disease progression, and development of new drugs and vaccines. The objective of this research was to evaluate various hematological, biochemical, immunological, radiological and spirometric parameters in 20 adult patients convalescing from COVID-19 and their possible relationship with the clinical course of the disease. The frequencies of categorical variables were compared using the chi-square and Fisher's exact test. The levels of statistical significance were denoted in each figure legend. Two-dimensional clustering analysis was performed using MeV software from TIGR. The tests with P value of ≤ 0.05 were considered statistically significant. Most of the patients studied presented alterations in dissimilar laboratory, radiological and spirometric parameters, which were related to the clinical evolution of the disease. The results obtained show that certain hematological, biochemical, immunological and radiological parameters can be considered as biomarkers of sequela in adult COVID-19 patients, which allows their stratification, according to the degree of involvement or sequela, into three groups: I (mild degree of involvement or sequela), without lung lesions on computerized axial tomography (CT scan) and high values of IgG, C3 and hemoglobin, II (moderate degree of involvement or sequela), without lung lesions on CT scan, characterized by high levels of

CD3+/CD4+ T lymphocytes and the rest of the variables with low values and III (severe degree of involvement or sequela), with lung lesions on CT scan and high values of erythrocyte sedimentation rate, monocytes and neutrophils, associated with lymphopenia and decreased concentrations of IgG and C3.

Keywords: biomarkers, COVID-19, adult, convalescent, sequela.

1. Introduction

In December 2019, several patients with severe pneumonia were reported in Wuhan, Hubei province, China, which quickly spread throughout the country and became a pandemic (1). The World Health Organization (WHO) later named this disease COVID-19, from the English acronym Coronavirus disease 2019 and its causative agent, SARS-CoV-2.

The absence of prior immunity to this virus led to a rapid increase in infected patients worldwide and on January 30, 2020, the WHO declared a public health emergency with international repercussions (2).

This disease has a wide clinical spectrum. About 45% of those infected show no symptoms, 40% present mild clinical manifestations, 10% develop severe disease, and about 5% exhibit critical clinical states, characterized by severe respiratory failure, systemic shock, or multi-organ failure. Severe and critical states of the disease require admission to Intensive Care Units and some patients require mechanical ventilation (3-10).

Different investigations report the presence of clinical manifestations, morphological and functional alterations, weeks and even months after the resolution of SARS-CoV-2. Persistent signs and symptoms can affect multiple organs and systems. These depend on several factors, among which are: 1) pathophysiological mechanisms of the virus, 2) magnitude of the immune response triggered and 3) drugs and techniques used in the treatment of the disease. Some publications indicate that around 10% of patients may present clinical manifestations after three weeks of acquiring the infection and to a lesser extent, during several subsequent months, being more frequent in women, with a mean age of 45 years (11-16).

Post-COVID manifestations are divided into two categories: 1) subacute, which include symptoms and abnormalities that occur 4 to 12 weeks after infection, and 2) chronic or post-COVID-19 syndrome, the that gather symptoms and abnormalities that persist for 12 weeks or more after acquiring the infection and are not due to other causes (17,18). Dissimilar biomarkers have been described for SARS-CoV-2 related to detection, diagnosis, treatment, disease progression, and development of new drugs and vaccines (19, 20).

The objective of this research was to evaluate different hematological, biochemical and immunological parameters in a group of adult patients convalescing from COVID-19 and their possible relationship with the clinical course of the disease.

2. Materials and methods

2.1 Type of study. Selection of patients and healthy controls

A prospective and analytical study was carried out. The patients were recruited in a home investigation consultation by two specialists in Immunology, from the Institute of Hematology and Immunology “*José Manuel Ballester Santovenia*”, Havana, Cuba. The universe consisted of 49 adult patients who had previously presented COVID-19. Twenty patients older than 18 years of age, recovered from COVID-19, with negative PCR for SARS-CoV-2, 1 to 3 months after infection and informed consent signed by the patients, between the months of May and June 2020 were included. Those patients who received blood transfusion in the three months prior to the study, those who had immunomodulatory treatment in the last 30 days after the start of the investigation and with incomplete necessary data were excluded. No patient included in the investigation decided to abandon it. Currently, all patients are alive.

For the selection of controls, 60 adult subjects, with no previous history of COVID-19, with 60 years and more and signed written consent to participate in the study. Of the total, 40 subjects were excluded, with a history of acute infections in the last seven days prior to the completion of the complementary ones, acute allergic processes, decompensate chronic diseases, with immunomodulatory or immunosuppressive treatment and blood transfusions in

the last month. Finally, 20 “apparently healthy” subjects were included as controls, without distinction of sex or skin color, with a negative PCR for SARS-CoV-2.

2.2 Laboratory techniques and procedures

Different hematological, biochemical and immunological parameters were evaluated.

Hematological parameters included complete blood count: hemoglobin (11-16 g/L), hematocrit (0.34-51%), leukocytes ($4-10 \times 10^9/L$), neutrophils (50-70%), lymphocytes (20-40%), monocytes (3-10%), eosinophils (0.5-6%), basophils (0-1%) and platelets ($150-350 \times 10^9/L$), with the use of a hematological counter (Sysmex XS -1000i), erythrocyte sedimentation rate (ESR) (male: up to 10 mm/h and female: up to 20 mm/h, performed by the traditional Wintrobe method (21), ABO and anti-D blood group, for the hemagglutination technique (22) and complete coagulogram, with coagulometer equipment, Stago compac Max3 model.

Both the biochemical parameters, such as: quantitative C-reactive protein (CRP) (0-3 mg/L), total proteins (60-80 g/L), glycemia (4.2-6.11 mg/L), gamma glutamyl transferase (GGT) (0-55 U/L), glutamic oxaloacetic transaminase (TGO) (0-55 U/L), glutamic pyruvic transaminase (TGP) (0-55 U/L), creatinine (0-120 $\mu\text{mol/L}$), leukocyte alkaline phosphatase (ALP) (100-290 U/L) and uric acid (155-357 mmol/L), as well as the evaluation of the humoral immune response: serum immunoglobulins (Igs) M (0.4- 2.5 g/L), G (6.80-14.45 g/L) and A (0.7-3.7 g/L) and components C3 (0.8-1.4 g/L) and C4 (0.1-0.4 g/L) of the complement system, were executed by Inlab 240 technology (CPM).

The antibody response to SARS-CoV-2 in convalescents was evaluated by:

- *RBD-specific IgG ELISA assay* to detect antibodies against RBD-mFc as coating antigen. Plates were incubated with six two-fold serial dilutions of serum samples, starting in a wide range of dilution (1:100-1:5000). An anti-human-IgG:peroxidase conjugate was used. Experimental IgG titers were considered as the inverse of the highest serum dilution giving optical density (OD) values that were four-fold the value of the negative control serum.

- *Surrogate Virus Neutralization Assays (sVNA)* to detect antibody-mediated blockage of RBD: ACE2 interaction. Plates were coated with ACE2-hFc and

serial dilutions of sera were incubated with RBD-mFc (at a final concentration of 20 ng/mL). The RBD-Fcm that was not inhibited by polyclonal antibodies can bind to ACE2-Fch. Inhibition was calculated and expressed as a percentage according to the formula:

$$\text{Inhibition (\%)} = [1 - (A_{405\text{nm sample}}/A_{405\text{nm maximal recognition}})] \times 100.$$

Maximal recognition corresponds to RBD-mFc (20 ng/mL).

The determination of anti-hepatitis C and human immunodeficiency virus (HIV) antibodies and hepatitis B surface antigen was performed by the UMELISA Ultramicroanalytic System of the Immunoassay Center.

On the other hand, the evaluation of the cellular immune response was carried out in both patients and 20 healthy controls, using the flow cytometry technique (23), with a Gallios cytometer (Beckman Coulter, USA). Data analysis was performed with Kaluza software, version 1.2.

The populations of CD3+/CD4+ T lymphocytes (30.3-55.7%) and CD3+/CD8+ (13.2-42.9%), CD19+ B lymphocytes (5.4-49.5%), the CD3-/CD56+ natural killer (NK) cells (3.7-28%) and CD3+/CD56+ natural killer T cells (NKT) (0.9-20.1%).

The expression of CD38, HLA-DR and both cell activation antigens was also studied in T lymphocytes: CD3+/CD4+/CD38+/DR+ (0.3-1.0%), CD3+/CD4+/CD38-/DR- (50.9-71.8%), CD3+/CD4+/CD38-/DR+ (0.8-2.2%), CD3+/CD8+/CD38+/DR+ (0.9-4.2%), CD3+/CD8+/CD38-/DR- (20.8-48.2%) and CD3+/CD8+/CD38-/DR+ (1.2-8.1%); as well as the populations of naive CD3+/CD4+ T cells (CD45RA+) (34.8-70.3%) and memory (CD45RO+) (24.3-42.7%), the different populations of B cells: naive CD19+/IgM+/IgD+/CD27- (42.6-82.8%), memory CD19+/IgM+ /IgD- /CD27+ (0.9-9.1%) and post switched CD19+/IgM-/IgD-/CD27+ (14 -20.6%) and CD3-/CD19+/CD27+/CD38+ antibody-secreting cells (0.4-3.6%).

2.3 Data collection

The general data of each patient, the demographic characteristics (age, skin color and sex), comorbidities, clinical manifestations that characterized the disease, radiological studies: ultrasound and computerized axial tomography (CT scan) of the lung and spirometry were collected from the histories clinics of each patient and took a data collection sheet.

2.4 Bioethical aspects

The protocol for this research was approved by the Scientific Council and the Research Ethics Committee of the institution with number 5/2020, dated April 30, 2020. Ethical procedures were maintained regarding the confidentiality of the results obtained, as well as the absence of conflicts of interest among the researchers who participated in the study.

2.5 Statistical analysis

Categorical variables were summarized as frequencies and percentages, and continuous variables were described using median and interquartile ranges (IQR) values. To compare the continuous variables for different patient groups, non-parametric Mann-Whitney U or Kruskal Wallis test were used as appropriate. The frequencies of categorical variables were compared using the chi-square and Fisher's exact test. The levels of statistical significance were denoted in each figure legend. Pearson's correlation coefficient and completed linkage was used as distance metrics for calculation of the relationships between variables and patients. Two-dimensional clustering analysis was performed using MeV software from TIGR. Additional statistical analysis and graphs were generated and plotted using Statistic 7.0 and GraphPad Prism version 5.00 software (GraphPad Software, San Diego, CA, USA). The tests with P value of ≤ 0.05 were considered statistically significant.

To analyze the specific IgG antibody titers against SARS-CoV-2 and% inhibition, median, arithmetic mean and standard deviation were used, with a 95% confidence interval. Spearman's rank correlation was used to assess relationships among techniques used to evaluate the immune response.

3. Results

3.1 Demographic and clinical characteristics of adult patients recovered from COVID-19

Table 1 shows the demographic and clinical characteristics of the patients recovered from COVID-19, according to the evolution of the disease.

Of the total of patients studied, 8 (40%) had ages between 40 and 59 years and 6 (30%) between 19 and 39 and equal to or greater than 60 years, respectively. The distribution of the patients according to age and form of presentation of the disease showed that in the youngest, mild clinical manifestations predominated and, from the age of 40, moderate to critical.

A slight predominance of the female sex was found (55%). The women presented mainly moderate (4/20) and severe (5/20) manifestations of the disease, only 2/20 were critical. On the other hand, 3/20 men exhibited light and moderate expressions, respectively, 2/20 evolved to severe forms and 1/20 to critical.

In the studied sample, patients with white skin predominated (80%). They exhibited all the manifestations of the disease, with the majority presenting moderate or severe forms (11/16).

Comorbidities were found in 7 patients (85%), the majority (13) with moderate or severe clinical manifestations, the most frequent being bronchial asthma (45%) and arterial hypertension (35%). In 19 patients (95%) the disease presented with symptoms at the time of diagnosis, with a predominance of moderate and severe forms in 7 patients, respectively. In 13 patients (65%), no complications associated with COVID-19 were found. Respiratory distress associated with bronchopneumonia was the most frequent complication (15%).

3.2 Hematological and biochemical parameters of adult patients recovered from COVID-19

Table 2 shows the hematological and biochemical parameters studied in post-COVID-19 patients.

Mean values of hemoglobin, hematocrit, platelets and leukocytes of 12.8 g/L, 0.39%, $258 \times 10^9/L$ and $5.8 \times 10^9/L$ were obtained. Monocytes and eosinophils presented mean values of 2.4% and 1.07%, respectively. In 75% and 60% of the patients, decreased levels of monocytes and eosinophils were found, correspondingly. Lymphocytes and ESR showed mean values of 38.57% and 35.25 mm/h. These two parameters were increased in 45% and 70% of the patients. Platelets and basophils were normal in 100% of them. The coagulogram was normal in almost all (95%) of the patients.

When analyzing the behavior of the absolute values of eosinophils, monocytes, lymphocytes and ESR according to the form of presentation of the disease, it was found that the patients who had had the severe form of the disease still presented high blood counts during convalescence of eosinophils, monocytes and ESR. The patients who had a critical clinical state, in turn, showed the highest ESR values and lowest absolute lymphocyte counts (Figure 1).

Average normal concentrations of glycemia, total proteins, TGO, TGP, creatinine, alkaline phosphatase and uric acid were found (5.10 mg/L, 64.85 g/L, 23.03 U/L, 3458 U/L, 106.05 μ mol/L, 148.98 U/L and 258.55 mmol/L, respectively). High mean concentrations of quantitative CRP and GGT were found (7.72 mg/L and 62.75 U/L, respectively). Quantitative CRP and GGT were increased in 80% and 50% of the patients (Table 2).

Patients with bronchial asthma had the highest glycemetic values, although these were within normal parameters (Figure 2). A significant difference ($p=0.001$) was found when comparing blood glucose values between asthmatic and non-asthmatic patients. The patients who presented respiratory distress as a complication showed higher concentrations of ALP and lower lymphocyte counts (Figure 3). Significant differences were obtained when comparing ALP concentrations and lymphocyte counts between patients with respiratory distress and those without this complication ($p=0.038$ and $p=0.023$, respectively).

Of the total of patients studied, 10 (50%) corresponded to blood group A+, 4 (20%) to B+, 4 (20%) to O+ and only 2 (10%) had O-. Patients in group B+ showed the highest concentrations of quantitative CRP and leukocytes. A significant difference was obtained when comparing leukocyte values between patients with blood groups B+ and O+, $p=0.05$. Similarly, a significant difference was achieved when comparing quantitative CRP between groups B+ and O-, $p=0.02$ (Figure 4).

3.3 Immunological parameters of adult patients recovered from COVID-19

Anti-hepatitis C and HIV antibodies and hepatitis B surface antigen were negative in 100% of the patients.

Table 3 shows the Igs, C3, and C4 concentrations of recovered COVID-19 adult patients. Mean serum concentrations of IgG, IgM, IgA, C3 and C4 of 5.21 g/L, 0.55 g/L, 1.59 g/L, 0.46 g/L and 0.10 g/L were found, respectively. In 95% and 80% of the patients, decreased concentrations of C3 and IgG were found, respectively. Patients who had developed the most severe forms of the disease showed a tendency to present lower IgG and C3 values in convalescence (Figure 5).

Most of the COVID-19 convalescent patients studied (18/20, 90%) had specific IgG antibodies against the RBD protein. Of these, 5/18 (27.8%) showed high

titers of 12,800-40,444. Only two patients (10%) did not develop specific antibodies against this protein. Most of the convalescent patients (75%) showed % inhibition > 20 for a dilution 1: 100; median: 44.54, arithmetic mean: 44.92, SD: 33.53, CI: 29.22-60.61 (Figure 6 a). A positive correlation was found between the titers of specific IgG antibodies against RBD and their ability to inhibit the interaction between this protein and ACE2, Spearman r 0.9081 (Figure 6 b).

Figure 7 shows a great heterogeneity of expression of the immunological markers studied in both recovered patients and healthy controls.

Figure 8 a) shows the behavior of the total lymphocyte populations studied in patients recovered from COVID-19 and in healthy controls. Convalescent patients had lower percentages of naive CD3+/CD4+ T lymphocytes (CD45RA+) and memory (CD45RO+), CD19+ B lymphocytes and post-switched B lymphocytes (CD19+/IgD- /IgM- /CD27+) populations in relation to controls, without significant differences.

On the other hand, CD3-/CD56+ natural killer cells (NK), CD3+/CD56+ natural killer T cells (NKT) and CD3-/CD19+/CD27+/CD38+ plasma cells (PC) were decreased in patients when compared with healthy controls, with significant differences ($p=0.05$, $p=0.01$ and $p=0.00$, respectively). The populations of naive B lymphocytes (CD19+/IgM+/IgD+/CD27-) and IgM memory (CD19+/IgM+/IgD- /CD27+), CD3+/CD4+ T lymphocytes, with expression of activation markers CD38+/DR- and CD38+/DR+, respectively, and CD38+/DR- and CD38+/DR+ activated CD3+/CD8+ T lymphocytes showed higher percentages in convalescent patients compared to controls, without significant differences. The subpopulation of CD3+/CD8+ T lymphocytes was increased in most of the patients when compared with controls, with a significant difference ($p=0.04$).

Figure 8 b) shows the percentages of the lymphocyte subpopulations with statistical significance when comparing healthy patients and controls, these were: NK cells, CD3+/CD8+ T lymphocytes, NKT cells and PC. The patients who presented a lower number of CD19+ B lymphocytes, in turn, showed lower absolute counts of eosinophils, with a significant difference, $p=0.02$ (Figure 9).

3.4 Pulmonary and spirometric radiological parameters of adult patients recovered from COVID-19

Table 4 shows the results of the pulmonary and spirometric radiological studies of the adult patients recovered from COVID-19. Of the total number of patients studied, 55% presented an abnormal pattern compatible with COVID lesions on the lung ultrasound, this being more evident in seriously ill patients. In 44% of the patients, a positive lung CT scan was evidenced, with ground glass lesions, condensation and both, mainly in seriously ill patients. Moderate obstructive disorder predominated (15%) in spirometry.

3.5 Relationship between hematological, biochemical and immunological biomarkers in adult patients recovered from COVID-19

Figure 10 shows the two-dimensional grouping between the patients and the biomarkers studied.

This grouping allowed establishing different relationships between the variables studied. A first correspondence was established between hemoglobin and hematocrit and of both, with the naive CD19+/IgM+/IgD+/CD27- B cells. These three variables were also associated with CD3+/CD4+ T lymphocytes.

A second relationship was found between the global lymphocyte count and the CD3+/CD8+ T lymphocyte subpopulation with expression of the activation markers HLA-DR+ and CD38+, respectively, and of both, with TGO. For its part, glycemia was associated with CD19+ B lymphocytes. These five variables were related to each other. Total proteins were related to the CD3+/CD4+ T lymphocyte subpopulation that expressed the activation antigens HLA-DR+ and CD38+ and both, with the CD3+/CD56+ NKT cells.

It was also found an association of eosinophils with CD3-/CD56+ NK cells and of CD3-/CD19+/CD27+/CD38+ PC with CD3+/CD8+ T lymphocytes that expressed the CD38+ activation antigen. CD3+/CD4+/CD45RO+ memory T cells associated with post-witched CD19+/ IgD- /IgM-/CD27+ B cells and these with CD3+/CD8+ T cells. Platelets were associated with IgM, TGP with CD3+/CD8+ T lymphocytes expressing CD38+ and both with CD3+/CD4+ T lymphocytes expressing HLA-DR+. All of them with CD19+/IgM+/IgD- /CD27+ memory B cells.

Creatinine was associated with uric acid and both with the C4 component of complement. Total leukocytes were related to quantitative CRP and both, to the global neutrophil count. The monocytes with the ESR and these, in turn, with the subpopulation of naive CD3+/CD4+/CD45RA+ T lymphocytes. Finally,

basophils were associated with the liver enzymes GGT and ALP, and all of them with IgA.

The titers of specific antibodies against RBD (Tab) were related to the percentage of inhibition of the same or the ability to neutralize (Nab) both were associated with platelets and all of them, with basophils.

This multivariate analysis made it possible to identify three groups of COVID-19 convalescents. **Group I** (mild degree of involvement or sequela), which included patients 1, 4, 9, 10, 11, 12, 13 and 14, with negative lung CT scan and relatively high values of IgG, C3 and hemoglobin. **Group II** (moderate degree of involvement or sequela), represented by patients 3, 5, 6, 7, 8, 17 and 18, characterized by negative lung CT scan, high percentages of CD3+/CD4+ T lymphocytes and the rest of the variables with relatively low levels. **Group III** (severe degree of involvement or sequela), expressed by patients 2, 15, 16, 19 and 20 and characterized by having positive lung CT scan with ESR, monocytes and high neutrophils, associated with lymphopenia and decreased IgG and C3 concentrations, respectively.

4. Discussion

4.1 Demographic characteristics and comorbidities influence the clinical course of recovered adult COVID-19

In this study an average age of 49.5 years and a range of 21 to 74 years were found, with predominance (12 for 60%) of 50 and more years of age. In a series of 41 patients, Huang C et al (24) found a similar median age of 49 years with a range between 41 and 58 years. He R (25) in 204 patients also found an equivalent mean age, with a range of 34 to 67 years. The patients in this last series who were between 52 and 74 years old, presented the most severe clinical manifestations in relation to the younger ones ($p < 0.001$). Liu J (26) found in 40 patients, an average age of 48.7 being the severely ill those older than 59 years. Bo X et al. (27) found in 187 patients a higher average age, 62 years with a range between 48.5 and 70 years.

In this research, the female sex predominated (55%). These results were similar to those found by He R (25) in 61.3% of the patients studied. For their part, other researchers reported a predominance of the male sex. Quin Ch (28) found

it in 52% of a total of 452 patients with a higher proportion in severe cases (54.2%) and Bo X (27) in 55.1% of 187 patients.

In this study, most of the patients who progressed to severe and critical forms were women, which did not correspond to what was reported by most authors (27-29). In general, higher mortality from COVID-19 is achieved in men. Bienvenu L (29) reported higher percentages (59-75%) of deaths from COVID-19 in men. Similarly, in the context of SARS-CoV (2002-04) men had a higher mortality than women (21.9% vs 13.2%), respectively. By MERS-CoV in the years 2012, 2015 and 2018, there were more deaths among men (30). Klein SL (31) reported that there are immunological differences related to sex, which contribute to a lower or greater susceptibility to infections specifically, against viruses. Men infected by SARS-CoV-2 have higher circulating concentrations of ACE-2, which could explain the differences in the severity of the disease, according to sex (29). The immune system of women has a greater reactivity with great ability to produce antibodies, maintain a type I response mediated by interferon (IFN) and greater activity to present antigens by monocytes (32,33). The predominance of white skin in this research corresponded to the highest proportion that exists in the Cuban population of whites in relation to non-whites (34). However, several studies report that non-white patients are more susceptible to acquiring SARS-CoV-2 and having an unfavorable clinical course in relation to whites (35). This could be related to the higher frequency in non-whites of certain comorbidities, such as: heart disease, arterial hypertension and cerebrovascular disorders, certain social behaviors and sociocultural differences (36,37).

In this research, the white-skinned patients evolved mainly to the severe form of the disease and presented as the most frequent comorbidity bronchial asthma, which, associated with the higher glycemetic figures demonstrated in these patients, could explain the difference with the results of other investigations. Different authors (24,25,27) report that the presence of comorbidities is related to the appearance of complications, torpid evolution and death, which is enhanced in the advanced ages of life. Among the most frequent are: cardiovascular diseases, high blood pressure, diabetes mellitus, chronic lung disease, bronchial asthma and cancer, among others.

In this study, the moderate and severe forms of the disease predominated. Bo X et al. (27) reported in their series of patients a higher frequency of the moderate (42.8%) and critical (33.2%) forms and respiratory distress is one of the most frequent complications in older adults with associated comorbidities. In 27 published studies, 759 patients with this complication were reported (38). In this investigation, respiratory distress associated with bronchopneumonia was the most frequent complication.

A connection has been found between several genes associated with bronchial asthma, asthma-related metabolites, and host genes for SARS-CoV-2. Genes encoding IRAK3 and ADRB2 are associated with bronchial asthma and are in turn targets of SARS-CoV-2. These proteins are highly expressed in asthma patients and in human bronchial epithelial cells infected by SARS-CoV-2 (38). An association between bronchial asthma endotypes and greater or lesser expression of ACE2 in bronchial epithelial cells is described. The high Th2 type or atopic asthma, presents a low expression of ACE2 and a lower susceptibility to both infection and severity by SARS-CoV-2, while the low Th2 type or non-atopic asthma exhibits high susceptibility and evolution to conditions severe and critical. In the latter endotype, a higher Th1/Th17 activity is involved, with release by bronchial epithelial cells of IL-33, IL-6, IL-23, INF- γ and TNF- α in response to various irritants, with resulting infiltration of neutrophils. The low Th2 type is more common in women older than 35 years of age. On the other hand, in low Th2 asthmatics, but smokers and with chronic obstructive pulmonary disease, the expression of ACE2 increases, thus being more susceptible to acquire SARS-CoV-2 and to evolve torpidly (39).

In older adults with associated comorbidities, COVID-19 generally progresses to the severe or critical form. These patients constitute a risk group due to the presence of several factors: higher expression of ACE-2, lower quality of the immune response product of immunosenescence with accumulation of memory cells, loss in the expression of costimulatory molecules CD27/CD28, presence of pathological T cells with self-reactive potential, changes in the function of primary and secondary lymphoid organs, alterations in the number and composition of B-cell compartments, and an unmodulated inflammatory response (33).

4.2 Different hematological and biochemical parameters are biomarkers of recovery in adult COVID-19 patients

Qin Ch et al. (40) found a mean of 0.4% and 0.0% monocytes and eosinophils in 452 infected patients, respectively, without significant differences between severe and non-severe patients. In the study presented, recovered patients showed higher averages of monocytes and eosinophils (2.43% and 1.07%, respectively), which could be related to the persistence of the inflammatory state and the high number of allergic patients from this series. In patients with severe and critical manifestations, inflammatory monocytes with the CD14+/CD16+ phenotype favor the state of hyperinflammation by mediating the pro-inflammatory cytokine storm (41).

Qin Ch (40) found a mean number of lymphocytes of 17.5% with a significant difference between severe and non-severe patients (14.1% vs 21.4%, $p < 0.001$). Liu J (26) found lymphopenia in 11/13 (84.6%) of infected patients with severe and critical manifestations of COVID-19. In this study, the recovered patients had a higher mean number of lymphocytes (38.57%). Similar to what was found by these authors, patients who had presented critical states during infection with respiratory distress, showed lower absolute counts of lymphocytes in the state of recovery from the disease.

In patients with COVID-19, lymphopenia is considered a predictive indicator of the evolution of the disease to severity. This decrease may be related to: 1) the direct cytopathic action of the virus on infected lymphocytic cells through ACE-2 expressed in lymphocytes, 2) the destruction of lymphoid organs by the virus, with the consequent loss of cells lymphoid, 3) the generation of the cytokine storm that can lead to apoptosis of lymphocytes or a blockage of lymphopoiesis, 4) metabolic alterations induced by viral infection that lead to the generation of molecules that result in lymphoid cell depletion, 5) consumption of activated lymphocytes during the infection stage and 6) differences in lymphocyte settlement due to lymphocyte infiltration in different organs and tissues, outside of the peripheral blood (42-44).

Quin Ch (28) found a mean ESR value of 31.5 mm/h, with no significant difference between the severe and non-severe infected ($p = 0.123$). In this study, recovered patients presented a slightly higher mean ESR (35.2 mm/h), being higher in those patients who had critical forms of the disease.

Qin Ch (40) found mean CRP concentrations of 44.1 mg/L (15.5-93.5 mg/L), these being lower in non-severe patients in relation to severe ones, with significant differences (33.2 vs 57.9 mg/L, $p < 0.001$). Zhu Z et al. (45) also found high concentrations of CRP in patients who progressed to severe and critical forms of the disease, similar to this study, that in 80% of the recovered patients presented high concentrations of quantitative CRP. These results suggest that this protein not only constitutes a prognostic biomarker of the evolution to serious states of the disease but also that it could constitute an indicator of the persistence of the inflammatory state in the convalescent stage (46-48).

Half of the patients in this study had increased serum GGT concentrations. Elevated concentrations of this enzyme are observed in hepatobiliary disorders especially with cholestasis, as well as by stimulation of certain drugs. The liver is the vital metabolic organ par excellence, with numerous physiological functions, in immunity and the detoxification of xenobiotics. This organ is highly sensitive to infections caused by viruses, bacteria and other pathogens (47). ACE-2 is also expressed in hepatocytes, which are a target for virus entry (49). Between 14-20% of COVID-19 patients, elevations of liver enzymes and bilirubin were reported from the early stages of the infection, being more evident in severe patients (50,51). Recently, moderate areas of microvascular steatosis were found in biopsies of patients infected with SARS-CoV-2. Several factors may be involved in liver damage: 1) the direct action of the virus on hepatocytes, 2) the dysregulated immune response and 3) the hepatotoxic effect of the drugs used (52).

Bronchial asthma is a chronic respiratory disorder that is registered by the Center for Disease Control (CDC) as one of the risk factors for the highest mortality in patients infected with SARS-CoV-2. Adult asthmatic patients between the ages of 18 and 49 have a higher risk of being infected by this virus than asthmatic children (27.3% vs 1.7%). Both the exacerbation of asthma and the SARS-CoV-2 infection itself cause bronchial hyperactivity and eosinophilic hyperinflammation (53).

The CDC and the WHO do not recommend the use of oral steroids in COVID-19 because they decrease the elimination of the virus and increase the risk of secondary complications. However, many international organizations guide the

use of these drugs in asthmatic patients, with the aim of reducing the expression of ACE-2, normally increased in them (54). Also, they constitute an anti-inflammatory drug used in therapeutic protocols internationally and in Cuba. 45% of the patients in this study were asthmatic. These presented higher concentrations of glycemia in relation to non-asthmatics, which could be related to the stimulation by the virus of the release of hyperglycemic hormones (glucocorticoids and catecholamines) as well as the use of steroidal drugs in this group of patients (55).

Respiratory distress is the main complication associated with COVID-19. The lungs have a high activity of the renin-angiotensin system- aldosterone (RAAS) as well as a high expression of ACE-2 in Type II alveolar cells (AT2), essential for maintaining homeostasis. AT2 cells synthesize lung surfactant that is essential to maintain low air surface tension and fluid interface in the alveolus, preventing lung collapse. Both the disruption of the ACE/ACE-2 physiological balance and the local over-activation of the RAAS occur due to infection with the SARS-CoV-2 virus, which increases vascular permeability and favors pulmonary edema. ACE-2 also directs the up-regulation of genes involved in pulmonary fibrosis. The RAAS is a hemodynamic and biological system that performs important functions in the regulation of blood pressure, sodium and potassium concentrations, cell proliferation, fibrosis and oxidative stress (56,57).

In this study, patients with respiratory distress had the highest levels of ALP. Elevated levels of this enzyme may indicate hepatic cholestasis. The liver synthesizes angiotensinogen and renin that is produced by the juxtaglomerular apparatus and converts angiotensinogen into angiotensin I. Subsequently, ACE converts angiotensin I into angiotensin II. The latter binds to its receptor, promotes vasodilation, reduces inflammation, cell proliferation, hypertrophy and fibrosis. ACE-2 is also expressed in the hepatocyte, so the liver is another target organ for SARS-CoV-2 (50,51). A significant relationship between liver dysfunction and COVID-19 mortality has been reported by several researchers (50-52,56). For this reason, continuous monitoring of liver enzyme concentrations is suggested in critically ill and critically ill patients.

The ABO blood group is another factor that affects the susceptibility of individuals to acquire COVID-19. Zhao et al. (58) found a relationship between

ABO blood group and SARS-CoV-2 infection. These authors demonstrated that individuals with blood group A had a higher risk of suffering from COVID-19. Individuals of blood group O presented the lowest susceptibility which could be explained by the presence of antibodies in the serum anti-blood group with cross-acting neutralizing activity against SARS-CoV-2, whose protein S has a structural similarity to blood group antigens.

In the Cuban population, blood group O is the most frequent (49.03%) followed by groups A, B and AB (36.28; 11.20 and 3.09%, respectively), being group B the most frequent, in individuals with black skin (17.13%) (59).

In this study, half of the patients had blood group A+. Four patients belonged to group B+, three were white-skinned and one mixed-race. They presented high leukocyte counts and higher serum concentrations of quantitative CRP. A p of 0.05 was found when comparing leukocyte values between patients with blood groups B+ and O+ and a significant difference ($p = 0.02$) when comparing quantitative CRP between patients with blood groups B+ and O- (Figure 4).

In patients with blood group O+, natural anti-A and anti-B antibodies have been shown to be protective biomarkers, rather than blood group O itself. For their part, patients with blood group B+ present high concentrations of anti-group A antibodies, which could trigger an unmodulated immune response and favor inflammatory processes (60,61).

4.3 Different cellular and humoral immunological parameters can be considered as recovery biomarkers in adult COVID-19 patients

Qin Ch (28) found mean serum IgG and C3 concentrations of 11.75 g/L and 0.88 g/L in patients infected by SARS-CoV-2, respectively, with no differences between severe and non-severe patients ($p = 0.551$ and $p = 0.942$). Jing L (62) also in infected patients, found similar mean concentrations of IgG (11.1 g/L) and C3 (0.80 g/L).

In this study, the mean IgG and C3 concentrations in recovered patients were lower than those reported by other investigators (5.21 g / L and 0.46 g / L, respectively).

In patients infected with SARS-CoV-2, antibodies constitute an important defense mechanism against the virus. First, antibodies are produced against nucleocapsid proteins (N) and from the fourth to eighth days, antibodies against protein S appear. Patients with severe clinical manifestations have higher IgG

concentrations and higher total antibody titers, which suggests the possible use of this antibody to neutralize the entry of the virus into cells and induce a severe inflammatory response. In seriously ill patients, high concentrations of the IgA isotype have also been found. The humoral immune response, especially the production of neutralizing antibodies, has an important protective role by limiting infection to the late phase and preventing reinfections in the future (26, 63,64). In a similar way to that reported by other authors (63,64) in this study a positive correlation was found between specific IgG titers against RBD and their inhibition capacity when interacting with ACE2.

The complement system also participates in antiviral defense. The deposit of the C3b component on the surface of the virus can block the viral proteins necessary for its entry into the host cell and thus neutralize it (65). On the other hand, specific antibodies against SARS-CoV-2 activate the classical complement pathway, promote lysis by the membrane attack complex and finally, form immune complexes that can be eliminated by the mononuclear-phagocytic system.

In patients infected by SARS-CoV-2 with severe and critical forms of the disease generally, the highest concentrations of IgG and C3 are found (26,63, 64). In some patients, activation of the complement system can contribute to endothelial cell damage, promote thrombosis and intravascular coagulation phenomena, and cause multisystemic organ failure (66). In most of the patients in this study, decreased concentrations of total IgG and C3 were found, mainly in those who had severe and critical forms of the disease, which could be explained by a higher consumption of these serum proteins during the phase of activation, at the time of infection and which is still evident, in the recovery stage of the disease.

When comparing the immunological markers between convalescent patients and controls, a great heterogeneity of their expression was found in the "apparently healthy" subjects. This behavior in the expression diversity of lymphocyte populations could be related to the age variable since the controls were older adults. The phenomenon of "immunosenescence" characterized by alterations in the composition and function of the T and B lymphocyte compartments is described in these individuals. On the other hand, there may

have been comorbidities, without clinical translation, not reported by the subjects, as possible biological modifiers.

The integrated participation of the cellular components of the innate and adaptive immune response is essential for the recognition, processing, presentation and elimination of infectious antigens, in particular SARS-CoV-2. (67).

NK and NKT cells are components of the innate immune response. In this study, recovered patients had a decrease in both types of cells in relation to healthy controls. The decrease in NK cells in these patients was also reported by Wan and Zeng (68,69). NK cells are specialized in killing tumor cells and virus-infected cells. They have Fc receptors for IgG and complement. Their interaction with the target cells occurs in two ways: 1) direct cell-cell interaction with the release of granzymes and perforins and 2) through the Ig molecule (antibody-dependent cytotoxicity). The end result of both pathways is programmed cell death by apoptosis (70).

For their part, NKT cells participate in the rejection of tumors, early protection against microbial and viral pathogens, as well as in the control of autoimmune diseases (71). NKTs are activated by two fundamental mechanisms: 1) direct recognition of the antigen through its receptor and 2) indirect recognition, due to expansion by cytokines (IL-12 and IL-18) produced by antigen-presenting cells: dendritic cells, neutrophils and macrophages (72). In this study, recovered patients had lower percentages of NKT cells than controls, which indicate their activation. These cells show great plasticity by performing functions of cytotoxicity and regulation of the inflammatory state (72).

The cellular adaptive immune response is also essential in the elimination of the SARS-CoV-2 virus. It is made up of different cellular components, among which are: CD3+/CD4+ T lymphocytes, CD3+/CD8+ T lymphocytes and B lymphocytes (73).

Specific CD3+/CD8+ T lymphocytes lyses infected cells that express SARS-CoV-2 fragments attached to MHC class I molecules on their membranes, while CD3+/CD4+ lymphocytes promote the production of specific antibodies against the virus by cooperating with dendritic cells and macrophages in the activation of B lymphocytes, for their differentiation into plasma cells (74). During infection caused by SARS-CoV-2, a highly heterogeneous CD3+/CD4+ T cell-mediated

response occurs when compared with recovered patients and healthy controls (75,76). Neidleman J (77) reported that specific CD3+/CD4+ T lymphocytes represent only about 0.01% of the total.

In this study, recovered patients had higher percentages of total CD3+/CD4+ and CD3+/CD8+ T lymphocytes when compared to healthy controls, with a significant difference in the cytotoxic cell population.

CD3+/CD8+ T lymphocytes are essential for the elimination of virus-infected cells. In patients infected by SARS-CoV-2, specific CD3+/CD8+ T cells have been detected against the virus, which are related to a favorable evolution of the disease. Sette A (78) reported that in patients infected with severe and critical forms of the disease, total CD3+/CD8+ T lymphocytes specific to the SARS-CoV-2 virus presented lower percentages than in those with mild and moderate forms.

Rothan H (74) found in patients with severe clinical manifestations a decrease in naive T cells (CD3+/CD4+/CD27+/CD45RA+) as well as an increase in memory T cells (CD3+/CD4+/CD27+/CD45RO+). In this investigation, recovered patients had decreased percentages of both naive (CD45RA+) and memory (CD45RO+) CD3+/CD4+ T cell populations, relative to healthy controls. For the maintenance of an efficient immune response, there must be an adequate balance between the virgin and memory T cell populations (75). An investigation carried out at the University of Pennsylvania, in the USA, included the immunophenotypic evaluation of six subtypes of CD3+/CD4+ T lymphocytes: naive (CD45RA+/CD27+/CCR7+/CD95-), central memory (MC) with phenotype CD45RA-/CD27+/CCR7+, effector memory ME1 (CD45RA-/CD27+/CCR7-), ME2 (CD45RA-/CD27-/CCR7+) and ME3 (CD45RA-/CD27-/CCR7-) and central memory RA+(MCRA) with phenotype (CD45RA+/CD27-/CCR7-) in 149 infected adult patients, 46 recovered patients and 70 healthy controls. These studies revealed a relative loss of naive CD4+ T cells and an increase in ME2 and MCRA cells in infected adults, when compared to controls (79).

In this study, convalescent patients had a higher number of activated CD3+/CD4+ and CD3+/CD8+ T lymphocytes that expressed CD38+ and HLA-DR+ antigens, respectively, in relation to healthy controls. It has been reported that in patients infected by SARS-CoV-2, CD3+/CD4+ and CD3+/CD8+ T lymphocytes

are rapidly activated, become pathogenic and generate colony-stimulating factors for monocytes and macrophages (80). These researchers found co-expression of the HLA-DR+ and CD38+ markers in non-virgin cells mainly, in effector memory populations and correlated it with an increase in the expression of the marker KI67+ and a marked production of interferon gamma.

It has been reported that patients with a persistent immune response of CD3+/CD4+/DR+/CD38+ T lymphocytes have a higher frequency of complications related to bleeding disorders and increased serum ferritin concentration. In a large number of patients studied, the decrease in CD3+/CD8+ T lymphocyte populations correlates with that of neutrophils and total leukocytes, suggesting a relationship between the activation of these cells and lymphopenia. In severely infected patients, lymphocyte depletion is greater for CD8+ T cells than for CD4+ T cells (81).

The B lymphocyte response in COVID-19 patients occurs in conjunction with the activation of follicular helper T lymphocytes, about a week after the onset of symptoms. It has been reported that these patients show alterations in several populations of B lymphocytes, mainly in IgM memory cells (CD19+/IgM+/IgD-/CD27+) and post-“switched” cells (CD19+/IgM-/IgD-/CD27+) . In contrast, CD27-/IgD- B cells and CD27+/CD38+/CD138+ plasmablasts are generally increased. In some patients, plasmablasts represent more than 30% of circulating B cells, similar to what occurs in other viral infections, such as dengue and Ebola (79,81).

The patients recovered from this investigation had higher percentages of total virgin and IgM memory B lymphocytes, and lower percentages of plasmablasts, in relation to healthy controls.

Different researchers have reported that the production of antibodies in patients infected by SARS-CoV-2 is not related to a higher proportion of plasmablasts.

The lack of this equality suggests that the large plasmablast response seems to be generated more against the whole SARS antigen than against the spike and to be directed by inflammation, being non-specific and of low affinity (79,81).

The persistence in convalescent patients of total IgM memory B lymphocytes could be explained by the recent infection by SARS-CoV-2, while the decrease in plasmablasts, due to their generation and activation, with the consequent production of antibodies and possible migration to the germinal center.

In this study, recovered patients who had fewer CD19+ B lymphocytes showed lower absolute eosinophil counts. In patients infected by SARS-CoV-2, lymphopenia associated with eosinopenia constitute two hematological parameters involved both in the diagnosis and in the evolution to severe and critical forms of the disease. In a study by Li Q et al. (82) it was found that eosinopenia showed 74.7% sensitivity and 68.7% specificity and was associated with elevated quantitative CRP.

4.4 Certain pulmonary radiological lesions and spirometric alterations characterize adult patients recovered from COVID-19

A large number of patients recovered from this study (44%) showed ground glass lesions, condensation, and both on lung CT scan. Obstructive and ventilatory disorders were found in spirometric studies of some of the patients studied. Similar results were reported by other authors (5,6,9,83,84).

4.5 Different relationships between hematological, biochemical and immunological biomarkers are evidenced in adult patients recovered from COVID-19

An association was found between hemoglobin, hematocrit, naïve B cells and CD3+/CD4+ T lymphocytes. In states of anemia where hemoglobin and hematocrit are decreased, the collaboration of CD3+/CD4+ T lymphocytes is disturbed and as a consequence, the secretion of antibodies.

In patients infected with SARS-CoV-2 and in recovered, total and specific CD3+/CD8+ T lymphocytes are activated to eliminate the virus. Lymphopenia is associated with disease progression. In the activation phase lymphocytes express cell activation antigens (DR+/CD38+) and in some patients, depletion markers (PD-1, CTLA-4, TIM-3, NKG2A and Tim-3). CD3+/CD8+ T lymphocytes can produce interferon gamma, which activates macrophages and neutrophils that promote the release of multiple pro-inflammatory cytokines among them, interleukin 6, the classic JAK pathway is activated and these, in turn, of the STAT. This enzymatic stimulation induces the activation of immune cells that can cause damage to the hepatocytes, with repercussion in the elevation of liver enzymes.

Different interactions are established between the components of the innate immune system (monocytes, eosinophils, basophils, neutrophils, NK and NKT cells and complement components) and the adaptive immune system (B and T

lymphocytes) in anti-viral defense. The processing and presentation of viral antigens by antigen-presenting cells to T lymphocytes, the contacts between the cells and the vascular endothelium, the release of cytokines that promote cell activation, proliferation and differentiation, as well as the generation of effectors and memory cells.

When the immune response is deficient or poorly modulated the disease progresses to serious and critical states, being responsible for multi-organ damage, which is maintained in the convalescent stage. In patients who presented severe disease, a poor response was found mediated by NK cells and CD3⁺/CD8⁺ T lymphocytes, activation of humoral immunity from antibodies and complement that promote the inflammatory state, as well as a decline in certain negative regulatory immune signals that aggravate and they perpetuate it, activate platelets and stimulate the coagulation system (74).

Prolonged residual effects of COVID-19 on the innate and adaptive immune system have been reported by different researchers. Results similar to those found in this investigation were described by other working groups. Such is the case, in the research presented, of the decrease in NK and NKT cells, total B lymphocytes and plasma cells, as well as the increase in total activated CD3⁺CD8⁺ T lymphocytes. In relation to the percentage of total virgin B cells, dissimilar results were found. In our investigation, these showed higher values compared to the controls, while in studies carried out by other authors, decreased figures were shown for these populations. These differences could be due to different causes, such as: dissimilar periods of evaluation of the immunological parameters, genetic characteristics of the patients studied, among others (85-90).

The fact that in the post-COVID-19 period there are states of immunosuppression in patients indicates the presence of chronic activation that may be caused by: viral persistence, permanence of inflammation, autoimmunity phenomena due to cross-reactive antigens, as well as by repair of tissue damage. An excessive immune response can lead to marked immunosuppression, persistent inflammation and catabolic syndrome, this being more evident in patients with severe and critical forms during the infection (85-90).

In this study, a large part of the patients studied presented alterations in different laboratory, radiological and spirometric parameters, which were related to the clinical evolution of the disease.

The identification of negative and positive associations between the different variables studied allowed convalescent patients to be stratified into three prognostic groups, according to the degree of involvement or sequela: mild, moderate and severe. **Group I** (mild degree of involvement or sequela), without pulmonary lesions on CT scan and elevated levels of IgG, C3 and hemoglobin, **Group II** (moderate degree of involvement or sequela), without lung lesions on CT scan, characterized by high levels of CD3+/CD4+ and the rest of the variables with relatively low levels and **Group III** T lymphocytes (severe degree of involvement or sequela), with pulmonary lesions on CT scan and high ESR, monocyte and neutrophil values, associated with lymphopenia and decreased IgG and C3 concentrations.

The results of this research show that certain hematological, biochemical, immunological and radiological parameters could be considered as biomarkers of sequela in adult COVID-19 patients, which allows them to be stratified, according to the degree of involvement or sequela, into three groups: (I) light, (II) moderate and (III) severe.

Limitaciones de la investigación y consideraciones finales

The authors consider that a limitation of this research was the small sample size. However, with the multivariate analysis through a bioinformatics program, it was possible to reach interesting conclusions and identify three groups of convalescent COVID-19 patients, which presented a variable degree of affection or sequela. However, in order to reach more solid conclusions and generalize these preliminary results considered as predictive, it will be necessary: to study a greater number of adult patients convalescing from COVID-19, to carry out evaluations of the same parameters studied in several periods of time (longitudinal study), compare the results obtained with those of other research groups and extrapolate them to the pediatric population.

The authors suggest that patients identified with a higher degree of involvement or sequela should receive personalized follow-up in specialty consultations.

Journal Pre-proof

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Table1. Demographics and clinical characteristics of adult patients recovered from COVID-19 according to evolution of disease

Demographics and clinical characteristics	Mild (n=3)	Moderate (n=7)	Severe (n=7)	Critical (n=3)	Total n=20(%)
Age (range)					
19-39	3	2	1	0	6(30)
40-59	0	3	3	2	8(40)
≥ 60	0	2	3	1	6(30)
Sex					
Female	0	4	5	2	11 (55)
Male	3	3	2	1	9 (45)
Ethnicity					
White	2	5	6	3	16 (80)
Black	0	0	1	0	1 (5)
Mestizo	1	2	0	0	3 (15)
Comorbidities					
Bronchial asthma	1	5	3	0	9 (45)
Hypertension	0	2	4	1	7 (35)
Diabetes mellitus	0	0	1	0	1 (5)
Allergy	0	1	1	1	3 (15)
Neoplasms	0	1	1	0	2 (10)
Others	0	1	1	1	3 (15)
Clinical presentation					
Symptomatic	2	7	7	3	19 (95)
Asymptomatic	1	0	0	0	1 (5)
Complications					
Distress+bronchopneumonia	0	0	1	2	3 (15)
Bronchopneumonia	0	0	1	0	1 (5)
Distress	0	0	0	1	1 (5)
Dehydration+ respiratory	0	0	1	0	1 (5)
Alkalosis					

n=number of patients

Table 2. Hematological and biochemical parameters in adult's convalescent COVID-19 patients

Parameters	Decreased n (%)	Increased n (%)	Normal n (%)
<i>Hematological</i>			
Hemoglobin	3 (15)	1 (5)	16 (80)
Hematocrit	4 (20)	0	16 (80)
Leukocytes	3 (15)	0	17 (85)
Neutrophils	5 (25)	0	15 (75)
Lymphocytes	0	9 (45)	11 (55)
Monocytes	15 (75)	1 (5)	4 (20)
Eosinophils	12 (60)	0	8 (40)
Basophils	0	0	20 (100)
Platelets	0	0	20 (100)
ESR	0	14 (70)	6 (30)
Coagulogram	0	1 (5)	19 (95)
<i>Biochemical</i>			
CRPquantitative	0	16 (80)	4 (20)
Glycemia	1 (5)	1 (5)	18 (90)
Total Protein	5 (25)	1 (5)	14 (70)
GGT	0	10 (50)	10 (50)
TGO	0	2 (10)	18 (90)
TGP	0	3 (15)	17 (85)
Creatinine	0	4 (20)	16 (80)
LAP	2 (10)	0	18 (90)
Aciduric	3 (15)	2 (10)	15 (75)

n=number of patients, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, GGT: gamma glutamil transferasa, TGO: transaminasa glutámica oxalacética, TGP: transaminasa glutámica pirúvica, LAP: leukocyte alkaline phosphatase

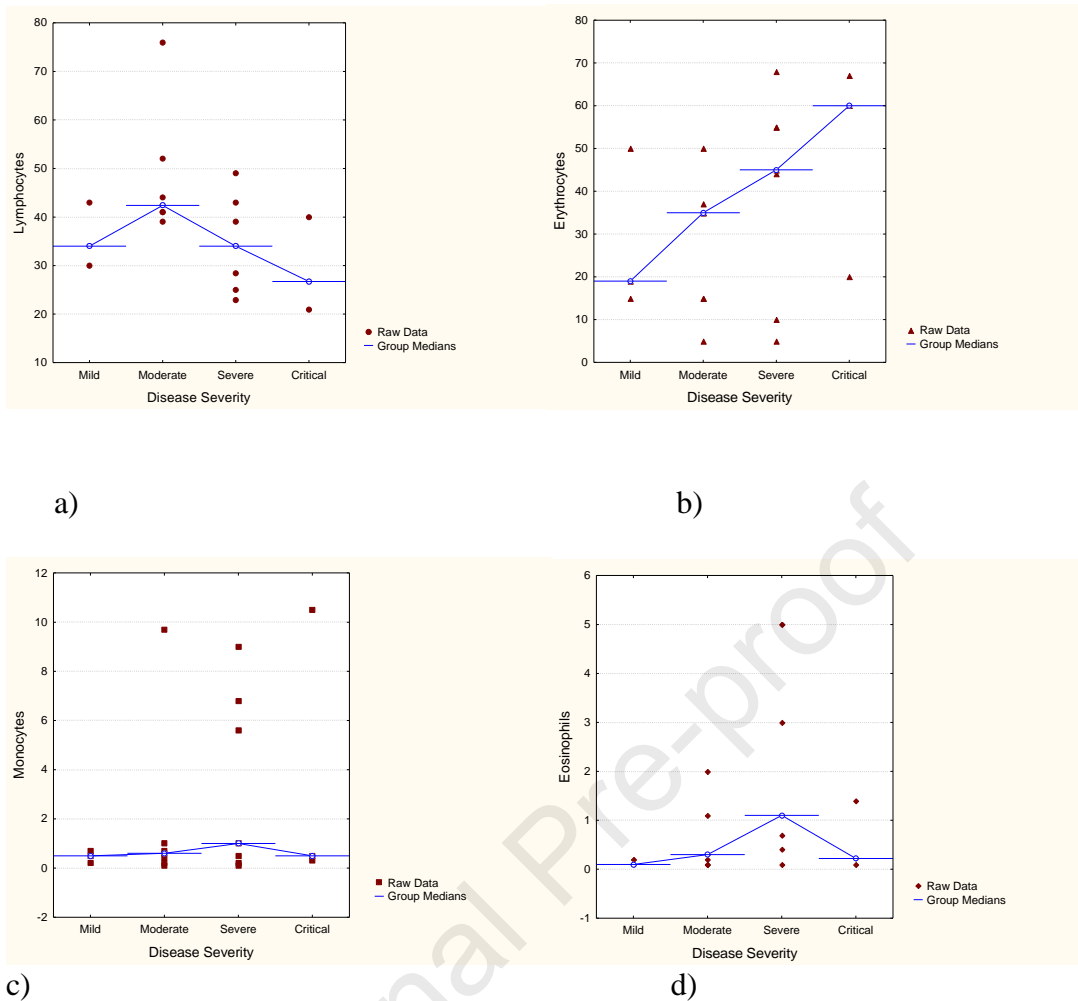


Figure 1. Absolute values of lymphocytes, erythrocyte sedimentation rate (ESR), monocytes and eosinophils in adult's COVID-19 convalescent patients grouped by disease severity (mild, moderate, severe and critical). It is appreciated that recovered patients who developed critical forms of the disease showed a tendency to have lower lymphocytes and increased ESR values. In severe cases patients showed higher eosinophil counts. None of the variables showed a statistical difference between the four levels of classification used for the disease ($p > 0.05$, using Kruskal Wallis test). Although, when we compare most severe patients (severe+critical) versus non-severe (mild+moderate), found a statistical significant difference in lymphocytes counts levels ($p = 0.03$ non-parametric Mann-Whitney U Test).

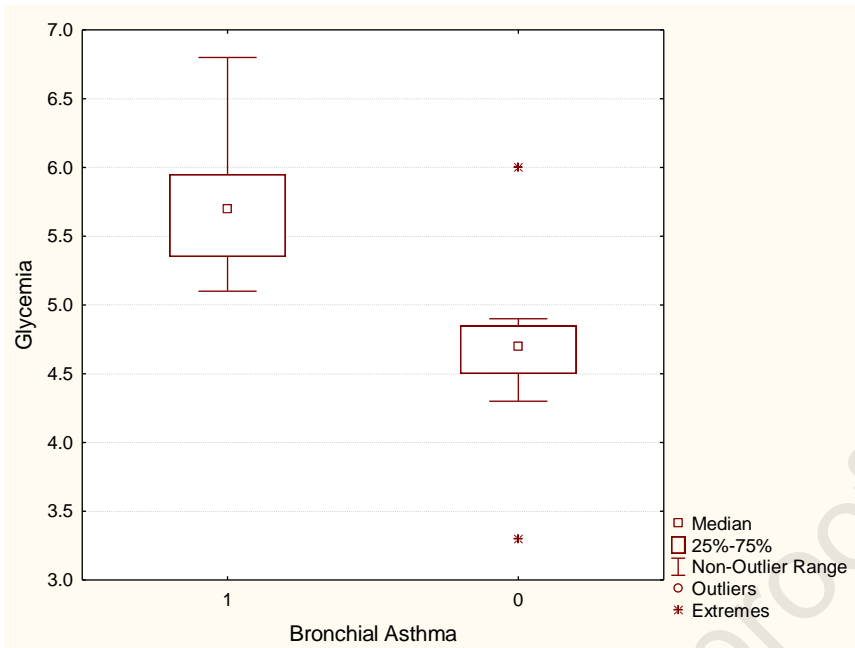


Figure 2. Absolute glycemia levels in asthmatic (1) and non-asthmatic (0) adult COVID-19 convalescent patients. Asthma patients showed higher glycemia values ($p=0.0005$, according to Mann-Whitney U test).

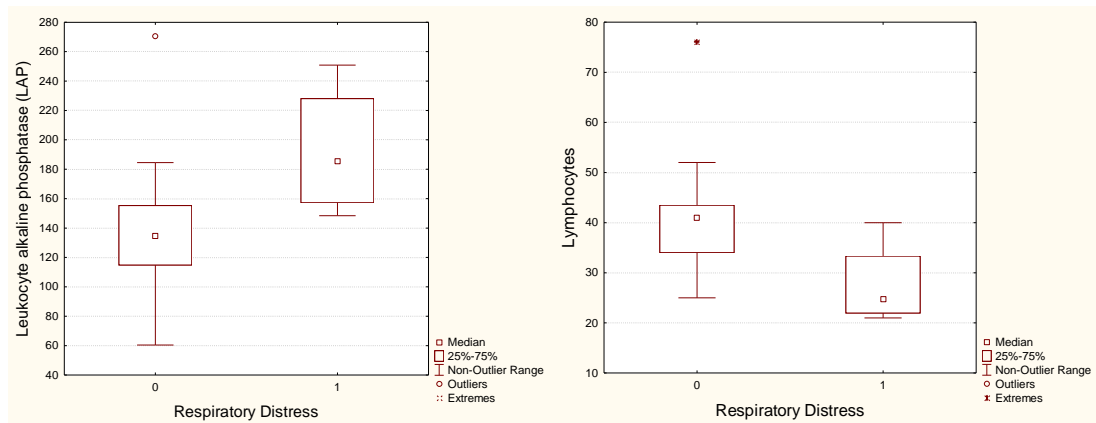


Figure 3. Leukocyte alkaline phosphatase concentration and absolute lymphocyte counts in COVID-19 convalescent patients without (0) and with (1) respiratory distress. Respiratory distress patients show higher levels of alkaline phosphatase and lower lymphocyte counts ($p=0.04$ y $p=0.02$, respectively; using Mann-Whitney U test).

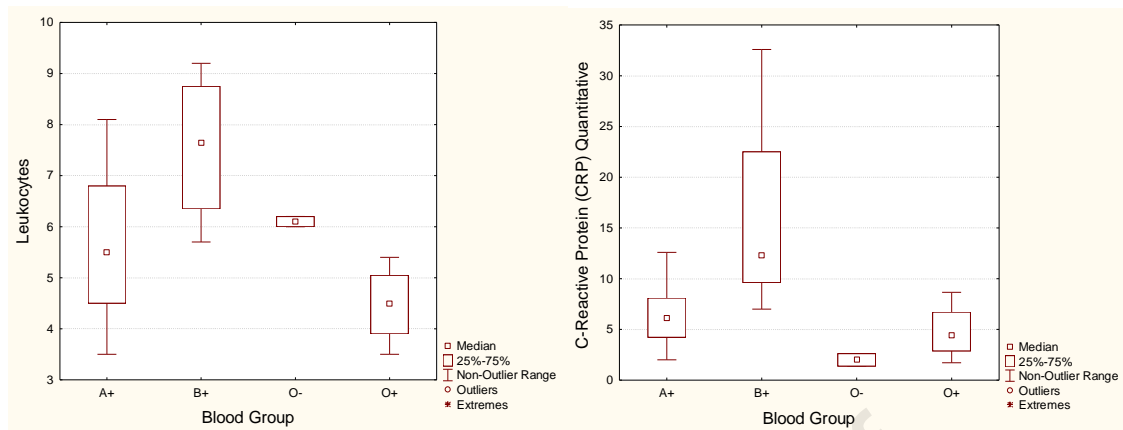


Figure 4. Absolute leukocyte count and quantitative C-reactive protein levels in COVID-19 convalescent patients grouped by blood type (left) or Rh factor (right). Patients with B+ blood type show higher leukocyte counts and higher concentrations of C-reactive protein (CRP) ($p=0.05$ and $p=0.02$ respectively, according to Kruskal-Wallis multiple test). Statistical significance was achieved when we compared leukocyte levels between B+ and O+ blood types ($p=0.05$) and when we compared Quantitative CRP levels between B+ and O- blood types ($p=0.02$)

Table 3. Serum concentrations of Immunoglobulins, C3 and C4 in adult COVID-19 convalescent patients

Serum concentrations	Decreased n (%)	Increased n (%)	Normal n (%)
<i>Immunoglobulin (Ig)</i>			
IgG	16 (80)	0	4 (20)
IgM	6 (30)	0	14 (70)
IgA	0	0	20 (100)
<i>Complement</i>			
C3	19 (95)	0	1 (5)
C4	3 (15)	0	17 (85)

n=number of patients

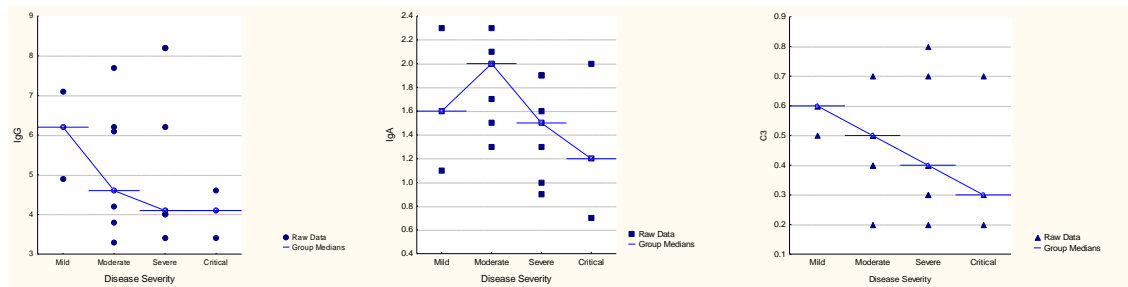


Figure 5. Serum immunoglobulin (Ig) G, (Ig) A and C3 complement concentrations in COVID-19 convalescent patients according to disease severity (mild, moderate, severe and critical). Patients in the critical level of severity showed a tendency to lower median concentrations of IgG, IgA and C3 complement but the differences are not statistically significant respect this classification of COVID-19 disease ($p > 0.05$, using Kruskal Wallis test). We found that COVID-19 convalescent patients classified as severe or critical have significant lower IgA than patients with non-severe disease (mild or moderate) ($p = 0.03$, using non-parametric Mann-Whitney U Test).

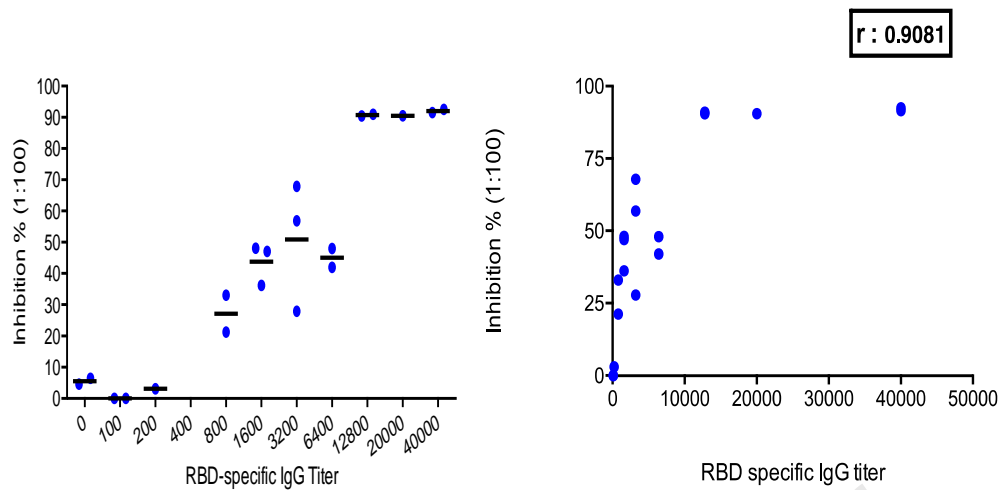


Figure 6. A) Specific IgG antibody titers against SARS-CoV-2 RBD and % inhibition of RBD: hACE2 from convalescent patients. B) Correlation between RBD-specific IgG antibody titers and % RBD inhibition. A positive correlation was found between specific IgG titers against RBD and % RBD inhibition: hACE2, $r = 0.9081$ ($n = 20$). The correlations were analyzed by Spearman correlation test.

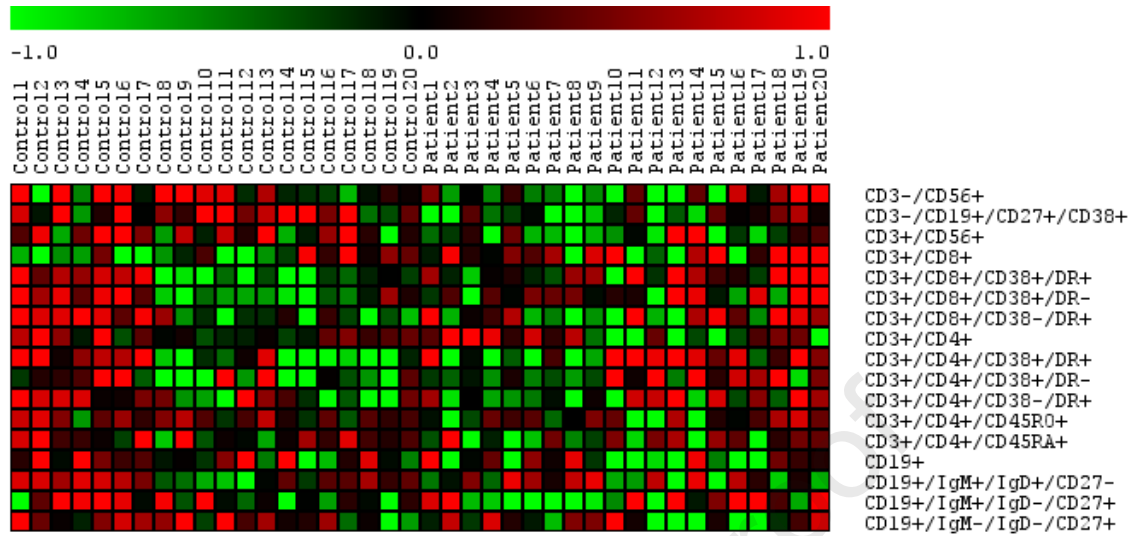
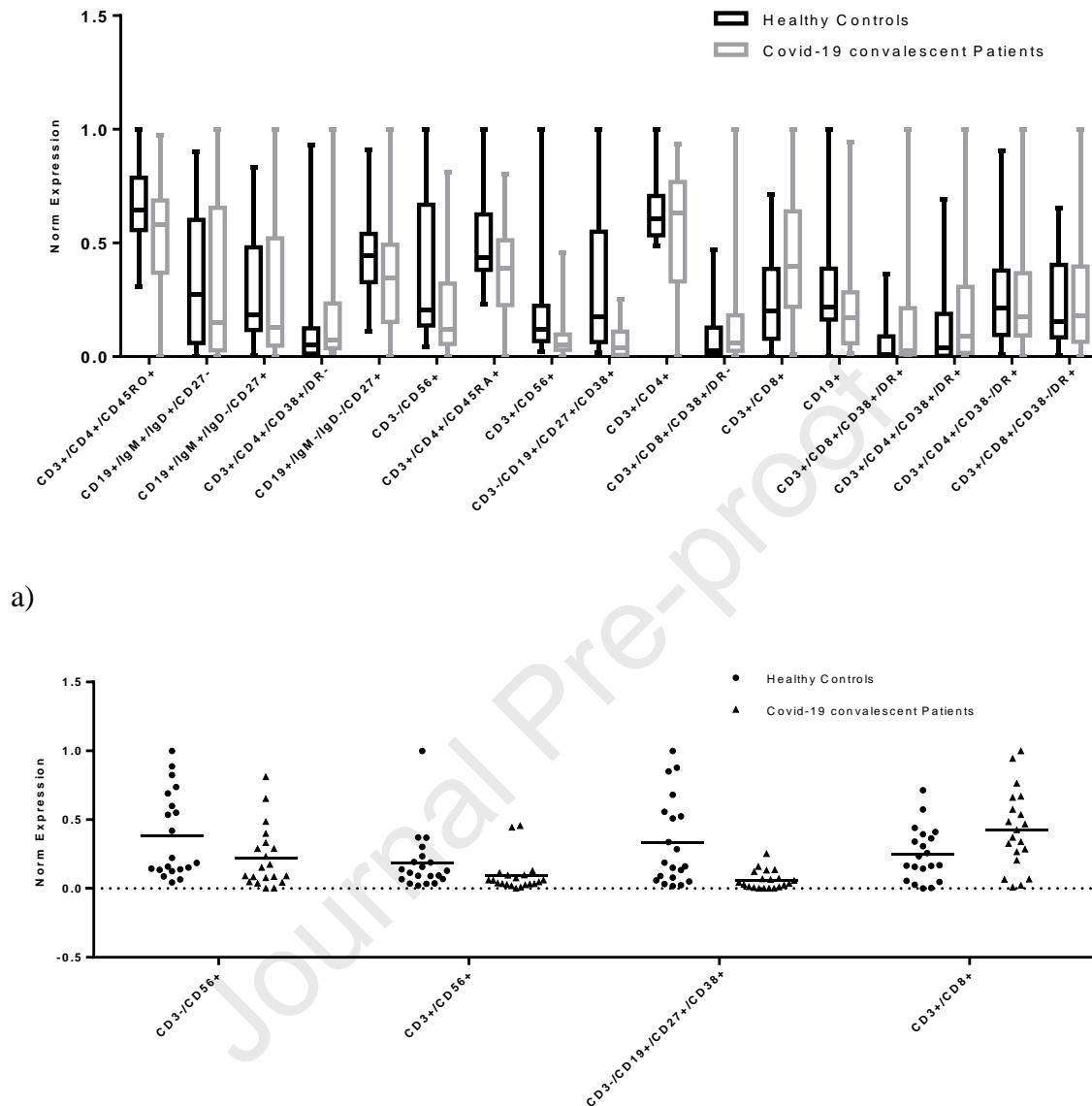


Figure 7. Heatmap of immunological markers evaluated in healthy controls and convalescent COVID-19 patients. A high heterogeneity of the immunological markers studied is observed in convalescent as well as in healthy individuals. Red and green color levels represent higher and lower levels, respectively, of each marker evaluated.



b)

Figure 8. Behavior of immunological markers in adult's convalescent COVID-19 patients and healthy controls.

Expression of: a) all evaluated markers and b) statistically significant markers. Convalescent patients show lower levels of CD3-/CD56+ natural killer cells, CD3+/CD56+ cytolytic lymphocytes and CD3-/CD19+/CD27+/CD38+ plasmatic cells, as well as an increase of CD3+/CD8+ T lymphocyte subpopulations when compared to healthy individuals, with statistical significant differences ($p=0.05$, $p=0.01$, $p=0.0004$ and $p=0.04$, respectively; using non-parametric Mann Whitney U test).

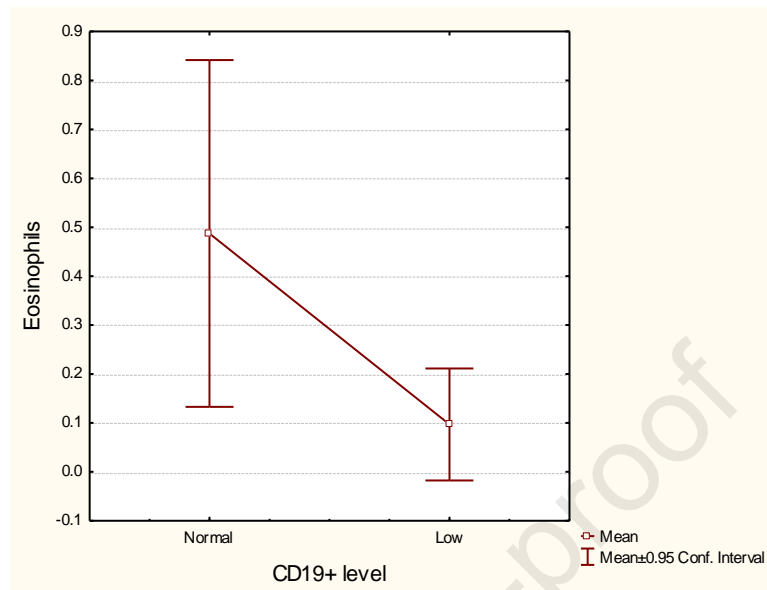


Figure 9. Eosinophil absolute counts according to CD19+ B lymphocyte. Patients with lower levels of CD19+ B lymphocyte show lower eosinophil absolute counts ($p=0.02$, using Chi-Square test).

Table 4. Pulmonary and spirometric radiological studies of adult patients recovered from COVID-19 according to disease severity

Pulmonary and spirometric radiological	Mild n=3	Moderate n=7	Severe (n=7)	Critical (n=3)	Total n=20 (%)
Lung ultrasound					
Normal	1	5	2	1	9 (45)
Abnormal	2	2	5	2	11 (55)
Computed axial tomography					
Negative	3	6	1	1	11 (55)
Positive	0	1	6	2	9 (45)
Spirometry					
Moderate obstructive disorder	1	1	0	1	3 (15)
Severe obstructive disorder	0	0	0	1	1 (5)
Non-obstructive ventilatory disorder	0	1	1	0	2 (10)
Uncooperative patient	0	0	2	0	2 (10)
Normal	2	5	4	1	12 (60)

n: número de pacientes

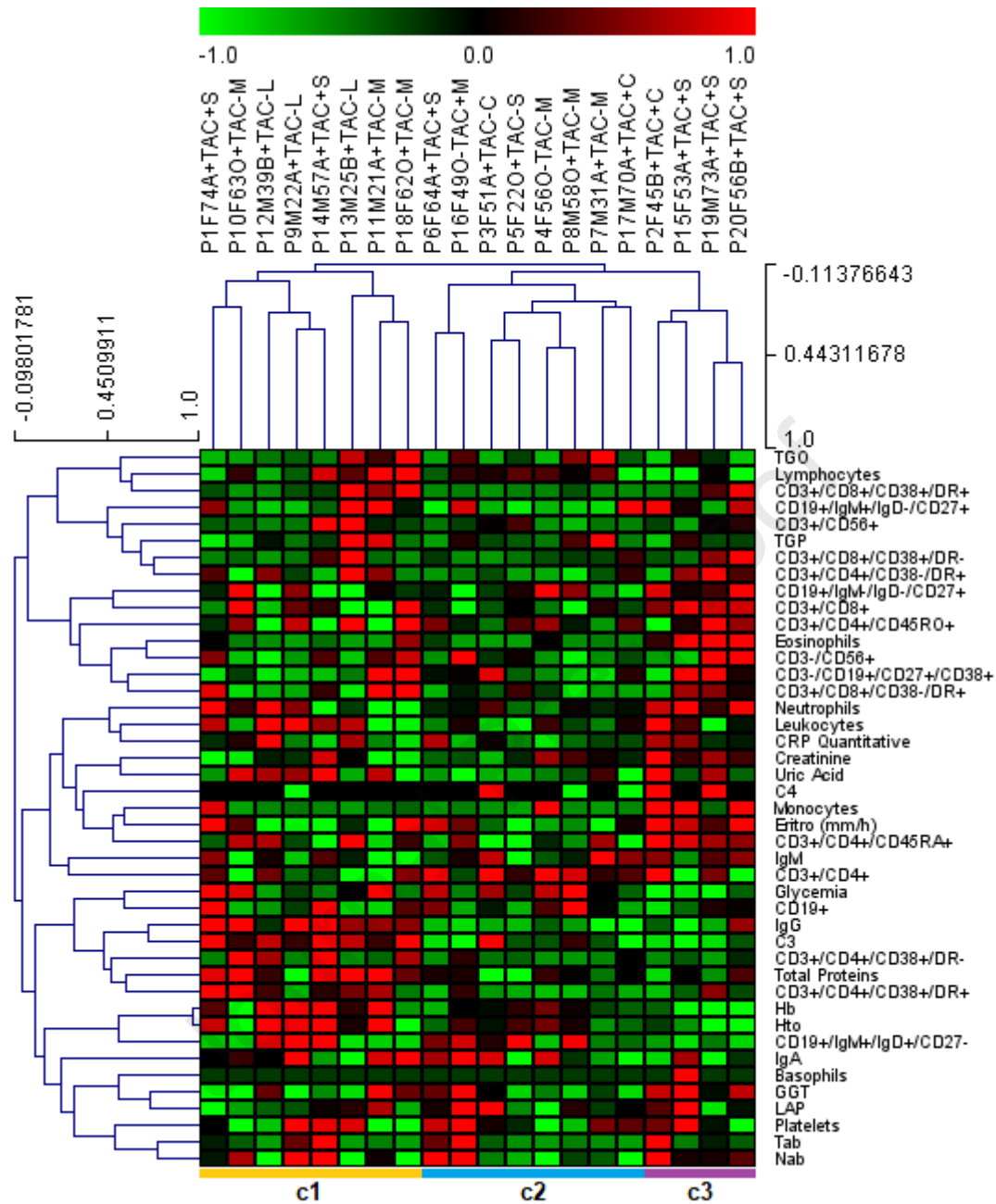


Figure 10. Two-dimensional clustering of hematological, biochemical and immunological data evaluated in adult patients recovered from COVID-19. A bi-dimensional clustering shows the correlation between variables and patient profiles. Three main clusters of COVID-19 convalescents are clearly identified, which are labeled c1, c2 and c3 at the bottom of the heatmap (highlighted in orange, blue and violet, respectively).

Competing Interests: No conflicts of interest are declared between the participating institutions and researchers.

Declarations of interest: None

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