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Research article

A cross-sectional study of laboratory parameters 5–6 months after the first COVID-19 infection

Taja Zore [a], Jasna Lojk [b], Katarina Reberšek [c], Elizabeta Božnar Alič [b], Urška Čegovnik Primožič [b], Alenka France Štiglic [b], Aleš Jerin [a,b], Irena Prodan Žitnik [a], Helena Podgornik [c], Nada Snoj [b], Barbara Ostanek [a], Gabriele Turel [d], Tatjana Lejko Zupanc [d], Janja Marc [a,b], Darko Černe [b,*]

[a] Department of Clinical Biochemistry, Faculty of Pharmacy, University of Ljubljana, Aškerčeva Cesta 7, 1000, Ljubljana, Slovenia
[b] Clinical Institute for Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana, Njegoševa Cesta 4, 1000, Ljubljana, Slovenia
[c] Department of Haematology, University Medical Centre Ljubljana, Zaloška Cesta 7, 1000, Ljubljana, Slovenia
[d] Department of Infectious Diseases, University Medical Centre Ljubljana, Japljeva Ulica 2, 1000, Ljubljana, Slovenia

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ABSTRACT

Objectives: Despite extensive study of COVID-19 disease, only a few studies also addressed the aftermath of the disease and potential long-term consequences. The aim of this study was to assess COVID-19 resolution through the cross-sectional analysis of an extensive range of haematological and biochemical laboratory parameters and to find potential markers still associated with disease severity 5-6-months post infection.

Methods: In this study, we analysed 92 routine biochemical, haematological and immunological parameters in 75 non-vaccinated patients 5–6 months after recorded first time SARS-CoV-2 infection without reinfection. Demographic and disease severity data were obtained through surveys.

Results: The majority of analysed parameters were within the normal reference intervals, however, statistically significant correlations with the disease severity were detected in 15 parameters: B lymphocytes, NK cells, interleukin (IL)-12, IL-1β, cortisol, ferritin, SARS-CoV-2 specific IgG and IgM antibodies, Na, Cl, creatinine, alkaline phosphatase, cholesterol, HbA1c and alpha 2 and beta 2 globulin fractions of the proteinogram.

Conclusions: Although most observed parameters returned to their normal reference intervals, significant correlations were still observed with disease severity, that could indicate either the pre-infection baseline state which affected disease outcome or minor remaining alterations in function of certain organs, pertaining their stress or damage during the acute phase of the disease.

1. Introduction

In early December 2019, the new coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected in Wuhan, central China. The disease rapidly spread through the world, causing a global pandemic with enormous economic and social crisis [1]. Four years later, the disease is still present, but it has become a part of our everyday life.

* Corresponding author.
E-mail address: darko.cerne@kclj.si (D. Černe).

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In this time, numerous reports and studies were performed, addressing pathogenesis, clinical characteristics, risk factors as well as potential treatments. It quickly became evident that COVID-19 is a multisystem disease which primarily affects lungs and can evolve into acute respiratory distress syndrome (ARDS), but can also cause acute kidney injury (AKI), liver, neuronal and cardiovascular damage [2]. The intense immune reaction that is triggered by SARS-CoV-2 infection is also reflected in abnormalities in laboratory measurements during the acute phase of the disease. These routine parameters have been intensively studied and several routine laboratory parameters have been identified as significantly changed in COVID-19 patients and some of them consistently correlated with disease severity [3,4]. C-reactive protein (CRP), D-dimer, neutrophil count, blood urea, creatinine, cardiac troponin I, lactate dehydrogenase (LDH), bilirubin, ferritin, and procalcitonin were increased in critical patients [3,5], while albumin, lymphocyte count and lymphocyte percentage were decreased [5]. Most severe-COVID-19 patients also showed dysregulated secretion of cytokine IL-6, but also IL-1β, IL-10, IL-2 and IL-8 [3]. Similarly, higher concentrations of essential trace elements, such as selenium, zinc and cooper, were associated with better outcomes and lower mortality [6], while higher concentrations of heavy metals, such as arsenic, cadmium, lead and mercury, were more frequently found in severe and deceased patients [7]. Certain parameters, as thrombocytopenia and decreased monocytes were mostly observed in fatal cases [8,9].

Much less attention has been given to parameters in recovering patients [5,10], which can provide important information on the disease resolution and long-term changes in organ function. Laboratory parameters have mostly been followed for a few months after discharge, when most of the parameters returned to their reference intervals and were considered normal [5]. Parameters stabilized in different time periods, from 2 weeks after disease onset to several months, depending on the study and observed parameter [8]. The longest follow-up period was presented by Liu and colleagues, who followed their patients for 12 months. Although they reported that most parameters resolved in this time, they also noticed that certain parameters, such as creatinine, urea, LDH and WBC could still change even 6 months after infection and individual patients still presented symptoms even 12 months after discharge [10]. Several questions regarding resolution of COVID-19 infection thus remain open.

The aim of this study was to determine the cross-sectional state of laboratory parameters 5–6 months after the first-time SARS-CoV-2 infection in regards to disease severity through analysis of an extensive array of biochemical, haematological and immunological measurements. The detected differences in measurements distributions between groups gave us information on which parameters are involved in the disease severity, either as baseline differences, which affected the disease outcome, or as pertaining consequences of the disease severity per se.

2. Materials and methods

2.1. Ethics approval

This study was approved by Slovenian National Ethics Committee (0120–578/2020/6, 16. 3. 2021). Upon their inclusion into the study, all participants signed a written consent after a given detailed explanation of the study. All the measurements were performed in accordance with the national guidelines and international regulations. This study adhered to STROBE (The Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

2.1. Study design and participants

One hundred and five study participants were recruited between January and March of 2021, between 1 and 3 months after their infection with SARS-CoV-2, via the University Medical Centre Ljubljana (UMCL), Slovenia. Asymptomatic, mild and moderate patients were recruited as volunteers based on a call through the UMCL. We included all eligible volunteers who reached out to us with the intention to participate in the study. Critical patients, on the other hand, were invited by their medical doctors during their routine post-COVID-19 follow-ups. Only participants which met the following inclusion criteria were enrolled into the study: (1) not yet

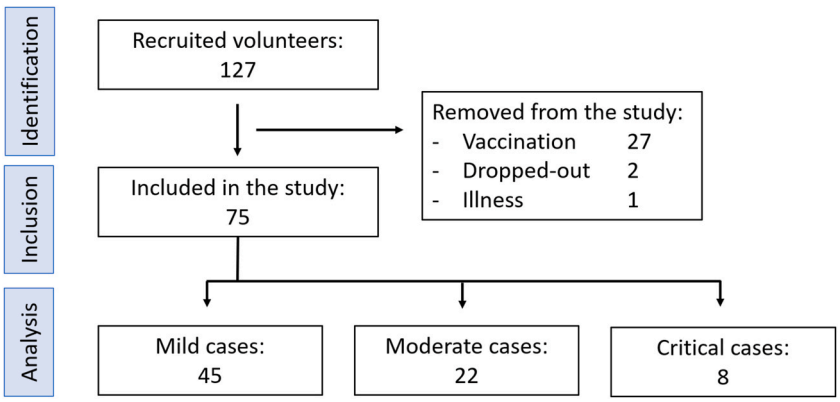

Fig. 1. Flow diagram of the study.

vaccinated, (2) PCR confirmed first time SARS-CoV-2 infection, (3) confirmed presence of anti-SARS-CoV-2 antibodies (against nucleocapsid protein), (4) no reinfection before blood collection, (5) with no known major comorbidities (diabetes, autoimmune diseases, heart diseases, cancers, neurological diseases) present and 5) at least 18-years old at the time of the inclusion. Out of the 105 recruited, 27 participants were removed from the study as they got vaccinated before the end of the 5-6-month study period, 2 participants requested to be removed from the study and one was ill (with a COVID-19 unrelated disease) at the time of sample collection. The remaining 75 participants followed through and their data was included in the final analysis (Fig. 1).

Upon their inclusion in this study, the participants were grouped according to their clinical manifestation of COVID-19 into three groups based on the COVID-19 Treatment Guidelines [11] by the National Institutes of Health (NIH) of the United States of America. Asymptomatic participants and participants with mild symptoms were classified as mild cases, those with moderate symptoms were classified as moderate cases, and those with critical illness were classified as critical cases. No participants with severe clinical manifestation of the disease were included in the study. None of the included critical patients was diagnosed with so called long-COVID at the 6-month follow-up by their doctors.

2.3. Data collection

At the time of the inclusion, a participant survey was conducted, which gave us the information about their age, gender, lifestyle, present comorbidities, and the clinical manifestation of COVID-19 disease. The blood samples for the study were collected 5–6 months after the infection with SARS-CoV-2. Whole blood was collected in 6 mL K_2EDTA BD Vacutainer® tubes (DB Life Sciences, MD, USA) for the analysis of differential blood count, lymphocyte subpopulations and glycated haemoglobin (HbA1c). Whole blood and plasma samples for trace elements were collected in 6 mL K_2EDTA BD Vacutainer® tubes for Trace Element Testing (DB Life Sciences, MD, USA). Serum was collected in 6 ml BD Vacutainer® Serum Tubes without separation gel (DB Life Sciences) and analysed immediately for other routine parameters. Serum, plasma, and whole blood samples for measurement of other analytes were aliquoted and stored at −80 °C until further use. Samples were anonymized before analysis and storage and the researchers performing the analysis and data

Table 1
Demographic and clinical characteristics of participant cohort. The data was gathered with participant survey at their inclusion in the study, in most cases 1–3 months after the infection with SARS-CoV-2. Median and IQR are shown for each group (mild, moderate and critical cases), statistical significance between all groups is noted in Total column.

Characteristic		Mild cases	Moderate cases	Critical cases
	Total (n = 75)	n = 45	n = 22	n = 8
Age – year – median (IQR)	43 (36 54) P = 0.006	43 (35–53)	41 (36–52)	58 (50–64)
Gender (%)				
Male	15 (20.0)	4 (8.9)	4 (18.2)	7 (87.5)
Female	60 (80.0)	41 (91.1)	18 (81.8)	1 (12.5)
BMI – median (IQR)	25.7 (22.6–30.1) P = 0.013	24.5 (22.5–28.9)	25.3 (21.8–30.5)	30.9 (27.4–41.2)
BMI categories (%)				
Underweight	1 (1.3)	1 (2.2)	0	0
Normal weight	33 (44.0)	23 (51.1)	10 (45.5)	0
Overweight	22 (29.3)	12 (26.7)	6 (27.3)	4 (50)
Obesity	19 (25.3)	9 (20.0)	6 (27.3)	4 (50)
Smoking (%)				
No	56 (74.7)	36 (80.0)	16 (72.7)	4 (50)
Former smoker/occasionally	6 (8.0)	1 (2.2)	1 (4.5)	4 (50)
Yes	13 (17.3)	8 (17.8)	5 (22.7)	0
Comorbidities (%)				
Hypertension	1 (1.3)	0	1 (4.5)	0
High cholesterol	4 (5.3)	2 (4.4)	1 (4.5)	1 (12.5)
Different allergies	8 (10.7)	4 (8.9)	3 (13.6)	1 (12.5)
Hypothyroidism	6 (8)	4 (8.9)	2 (9.1)	0
Migraine	2 (2.7)	1 (2.2)	1 (4.5)	0
Fibromyalgia	1 (1.3)	0	1 (4.5)	0
Gastritis	2 (2.7)	0	2 (9.1)	0
Symptoms of COVID-19 (%)				
Cough	34 (45.3)	14 (31.1)	15 (68.2)	5 (62.5)
Malaise/Fatigue	56 (74.7)	31 (68.9)	19 (86.4)	6 (75.0)
Fever	39 (52.0)	17 (37.8)	15 (68.2)	7 (87.5)
Headache	46 (61.3)	23 (51.1)	19 (86.4)	4 (50.0)
Loss of smell	50 (66.7)	29 (64.4)	17 (77.3)	4 (50.0)
Loss of taste	47 (62.7)	27 (60.0)	17 (77.3)	23 (37.5)
Muscle pain	45 (60.0)	26 (57.8)	16 (72.7)	3 (37.5)
Shortness of breath	30 (40)	0	22 (100)	8 (100)
Sore throat	14 (18.7)	7 (15.6)	5 (22.7)	2 (25.0)

Abbreviations: IQR – interquartile range, BMI – body mass index.

collection were not aware of their designation. The list of measured analytes, analysers and methods/kits used for their measurement are shown in Supplementary Table S1.

2.4. Statistical analysis

IBM SPSS statistics 28 (IBM Corporation, Armonk, NY, USA) was used for the statistical analysis of the gathered data and GraphPad Prism version 8.0.1. (GraphPad Software, San Diego, California USA) was used for the graphical presentation of the results. Categorical variables were presented as frequency rates and percentages, and continuous variables (both normally and non-normally distributed) were presented as median and interquartile range (IRQ). The Shapiro-Wilk's test was used to verify the normality of distribution of continues variables. Depending on their distribution, continuous variables were analysed using one-way ANOVA analysis with Post Hoc test with Bonferroni correction or Kruskal Wallis Test with Bonferroni correction for multiple comparison between groups. Correlations between selected parameters were determined using Pearson's correlation test for normally distributed parameters and Spearman's rank correlation test as a nonparametric alternative. No multivariate analysis was performed on the data. P-value <0.05 was considered statistically significant. Statistical significance is displayed as follows: ns – not significant ($P > 0.05$); *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. Sample size varied because of the missing data as noted in the table footnotes and figure captions.

3. Results and discussion

3.1. Basic characteristics of the participants

The study population included 75 participants (60 females, 15 males) with PCR confirmed first time infection with SARS-CoV-2 and confirmed anti-SARS-CoV-2 antibodies that have not yet been vaccinated until sample collection (Fig. 1, Table 1). The median age of the participants was 43 years (21–79). According to the self-reported clinical manifestation of COVID-19, there were 45 participants in the mild case group, 22 in the moderate cases group and 8 in the critical cases group. There was a significant difference in gender ratio between critical case group (1 female, 7 males) and other two groups (41 females and 4 males in mild cases group and 18 females and 4 males in moderate cases group). We observed a significant increase in age ($P = 0.006$ between groups mild and critical and $P = 0.009$ between groups moderate and critical) and in BMI ($P = 0.010$ between groups mild and critical and $P = 0.047$ between groups moderate and critical) with increasing severity of disease (Fig. 2). Both parameters are well known risk factors for poor prognosis [12,13]. The most common symptoms at the initial stages of the disease (before manifestation of severe symptoms in critical cases) were fatigue, loss of smell and taste, headache and muscle pain (Table 1). Results of self-reported physical activity and nutritional habits are summarized in Table S7.

3.2. Laboratory results

The majority of measured parameters were within the normal reference intervals 5–6 months after infection, as was also reported by other longitudinal studies [5,8,10]. Despite that, in our study we still observed significant differences in certain parameters based on the disease severity.

3.2.1. Haematology

Routine haematological analysis showed that the values of all parameters were within the normal reference intervals (Table 2). Despite indicated trends (e.g., increased RBC, MON, EOS, NEU with increasing disease severity), no significant differences in values between groups were observed. Such increase in MON was also observed previously 1–2 months [14] and up to 12 months after infection [10].

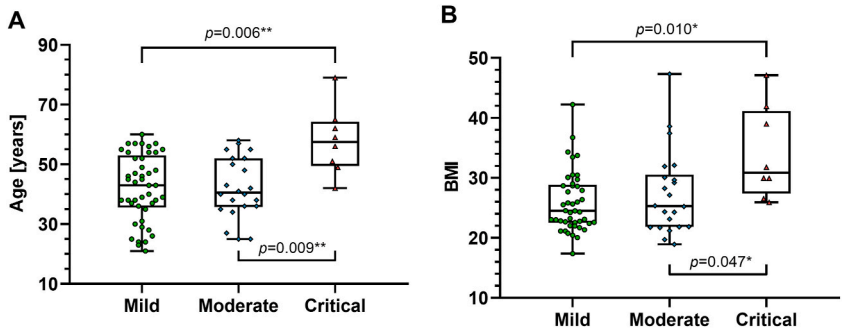

Fig. 2. Statistically significant correlations in demographic data based on COVID-19 disease severity were detected for A) age and B) body mass index (BMI) distribution. Median and interquartile range (IRQ) are shown for 45 mild, 22 moderate and 8 critical cases. P values indicate differences between groups (mild, moderate, and critical cases). Data was analysed using Kruskal Wallis test with Post Hoc test and Bonferroni correction.

Table 2
Comparison of haematological parameters between groups with different clinical manifestation of COVID-19 (mild, moderate and critical cases) determined 5–6 months after first-time infection.

Haematological parameter	Reference interval	Median (IQR)			
		Mild cases (n = 40)	Moderate cases (n = 21)	Critical cases (n = 8)	P value
WBC (10^9/L)	4.0–10.0	6.15 (4.88–7.53)	6.50 (5.35–7.60)	7.40 (6.55–8.18)	0.328
RBC (10^{12}/L)					
Male[a]	4.50–5.50	5.06 (4.94–5.39)	5.04 (4.86–5.75)	5.18 (4.83–5.38)	0.861
Female[b]	3.80–4.80	4.47 (4.24–4.68)	4.44 (4.17–4.65)	5.01	0.250
Hb (g/L)					
Male[a]	130–170	153.0 (145.5–155.3)	153.0 (151.5–171.0)	150.0 (145.0–157.0)	0.722
Female[b]	120–150	137.5 (128.5–143.0)	137.0 (125.5–142.0)	145.0	0.456
Ht (%)					
Male	0.400–0.500	0.45 (0.43–0.45)	0.44 (0.43–0.49)	0.44 (0.42–0.47)	0.978
Female[b]	0.360–0.460	0.40 (0.38–0.41)	0.40 (0.37–0.41)	0.43	0.365
MCV[c] (fL)	83.0–101.0	89.15 (86.40–91.78)	89.20 (87.15–91.25)	87.15 (84.70–90.78)	0.564
MCH[c] (pg)	27.0–32.0	30.35 (29.20–31.55)	30.60 (29.75–31.55)	30.00 (28.80–30.28)	0.172
MCHC[c] (g/L)	315–345	341.5 (337.8–346.0)	344.0 (339.5–349.0)	334.5 (333.3–347.0)	0.113
RDW (%)	11.6–14.0	13.40 (12.80–14.08)	13.30 (13.00–14.20)	13.60 (12.95–14.83)	0.795
PLT (10^9/L)	150–410	244.0 (187.3–298.0)	220.0 (194.0–264.0)	249.5 (190.0–269.5)	0.705
MPV (fL)	7.8–11.0	8.95 (8.43–9.63)	8.90 (8.40–9.60)	8.85 (8.15–9.88)	0.985
NEU (10^9/L)	1.50–7.40	3.30 (2.73–4.48)	3.60 (2.95–4.65)	4.00 (3.50–5.13)	0.583
LYM (10^9/L)	1.10–3.5	1.80 (1.43–2.30)	1.90 (1.60–3.00)	2.10 (1.68–2.43)	0.199
MON (10^9/L)	0.21–0.92	0.40 (0.36–0.60)	0.50 (040–0.65)	0.60 (0.53–0.85)	0.054
EOS (10^9/L)	0.02–0.67	0.10 (0.10–020)	0.10 (0.10–0.15)	0.15 (0.10–0.28)	0.433
BASO (10^9/L)	0.00–0.13	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.1 (0.0–0.1)	0.378
NEU (%)	40.0–80.0	57.9 (50.8–62.2)	57.5 (45.7–64.0)	55.9 (50.8–64.2)	0.944
LYM (%)	20.0–40.0	30.0 (23.9–36.3)	30.5 (26.4–43.9)	32.3 (26.1–36.5)	0.680
MON (%)	2.0–10.0	7.5 (6.1–9.6)	7.4 (6.6–8.7)	9.3 (7.9–12.0)	0.132
EOS (%)	1.0–6.0	2.1 (1.4–3.5)	1.7 (1.0–2.7)	2.3 (1.7–3.2)	0.382
BASO[d] (%)	0.0–2.0	0.8 (0.5–1.0)	0.6 (0.5–0.8)	0.8 (0.6–1.0)	0.329

Data was analysed using ANOVA or Kruskal Wallis test with Post Hoc test and Bonferroni correction. P values below 0.05 indicate at least one group is significantly different from the other two (mild, moderate, and critical cases). Abbreviations: IQR – interquartile range, WBC – white blood cells, RBC – red blood cells, Hb – haemoglobin, Ht – haematocrit, MCV – mean corpuscular volume, MCHC – mean corpuscular haemoglobin concentration, RDW – red blood cell distribution width, PLT – platelet, MPV – mean platelet volume, NEU – neutrophils, LYM – lymphocytes, MON – monocytes, EOS – eosinophils, BASO - basophils.

[a] Mild cases n = 4, Moderate cases n = 4, Critical cases n = 7.
[b] Mild cases n = 34, Moderate cases n = 17, Critical cases n = 1.
[c] Mild cases n = 38, Moderate cases n = 21, Critical cases n = 8.
[d] Mild cases n = 39, Moderate cases n = 21, Critical cases n = 8.

3.2.2. *Lymphocyte subsets*

All absolute counts of lymphocyte subsets were within the normal reference interval and no significant changes were observed between patients' groups except for percentage of B lymphocytes and NK cells (Table S2). For B lymphocytes, lower percentage was measured in the critical cases group in comparison to mild cases group (P = 0.032) (Fig. 3A), whereas the values in mild and moderate group were similar. We also detected higher percentage of NK cells (Fig. 3B) in critical cases group versus moderate cases group (P = 0.006). The percentage of NK cells was higher in critical cases group versus mild cases group, although not significantly (P = 0.093). This is in concordance with literature data, which indicate that long-term recovery of B lymphocytes and NK cells, requiring more than 3 months [15] and in our case more than 6 months, is expected.

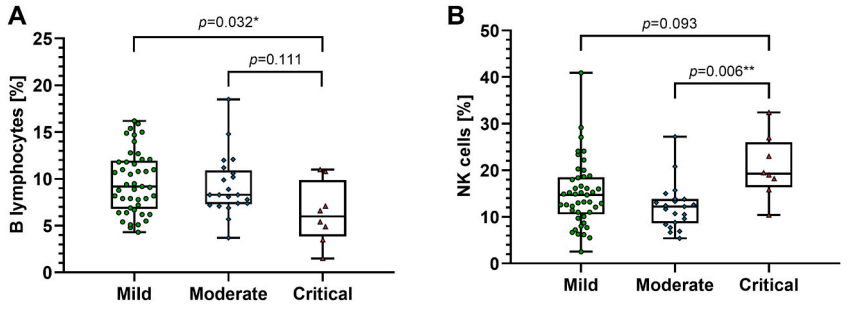

Fig. 3. Significant changes between groups with different clinical manifestation of COVID-19 5–6 months after the disease were detected in A) B lymphocytes and B) NK cells. Median and interquartile range (IRQ) are shown for 45 mild, 21 moderate and 8 critical cases. Data was analysed using ANOVA (B lymphocytes) or Kruskal Wallis test (NK cells) with Post Hoc test and Bonferroni correction.

3.2.3. Inflammatory markers

Routine markers of inflammation CRP, ferritin, IL-6 and the stress hormone cortisol were all within the normal reference interval (Table S3). Most measurements of CRP in mild and moderate patients were below detection level of the method (<5 mg/L) but could be detected in 5 out of 8 critical patients (P = 0.003). Ferritin was increased in male critical patients (man; P = 0.040) compared to moderate group, while cortisol levels were decreased (mild–critical: P = 0.032; moderate–critical: P = 0.031) (Fig. 4).

High ferritin is a well-known marker of poor prognosis during active disease [16,17], but was also shown to be elevated in COVID-19 pneumonia patients 3–6 months after discharge [18]. During the acute disease, ferritin is believed to be increased as a consequence of pulmonary tissue damage and macrophage activation [19], but has also been previously positively correlated with age [20] and obesity [21], both being well known COVID-19 comorbidities.

Eight early inflammatory markers (CCL2/JE/MCP-1, CXCL10/IP-10/CRG-2, IL-8/CXCL8, IL-10, IL-12 p70, IL-1 β/IL-1F2 and IL-2) were determined in a smaller pool of samples and interestingly, several were increased in moderate cases, but not in mild or critical cases (Table S3). Among those, a significant difference was detected in IL-12 and IL-1β (Fig. 4). Only IL-10 showed a consistent increase with diseases severity, which was not statistically significant (Table S3). IL-12, IL-10 and MCP-1 were previously associated with more severe disease [22,23] and MCP-1 levels were increased six months following the infection regardless of the presence of long-term post COVID-19 condition [24]. In our study, most measurements of early inflammatory markers were higher in moderate cases compared to critical (Table S3), which might me due to the small number of subjects.

The results of immunoglobulin measurement (Table S4) showed that the values of IgG, IgA, and C3 were inside the normal reference interval and the difference in their concentration between groups was not statistically significant. In addition, the concentration of SARS-CoV-2 specific IgG/IgM antibodies (Fig. 5) was higher in critical group compared to mild (P < 0.001) and moderate (P = 0.008) group, as is frequently reported in the literature [25,26].

3.2.4. Biochemical serum markers

Serum parameter analysis showed that predictably, most measured parameters were within the normal reference interval (Table 3). Above the reference intervals were only measurements of Na (median 148.0 mmol/L; reference interval: 135–145), Cl (median 109.0 mmol/L; reference interval: 95–105) and total cholesterol (median 6.65 mmol/L; reference interval: <5.0) in critical patients' group (Fig. 6). All these parameters were also higher in critical patients compared to mild (Na: P = 0.003; Cl: P = 0.013; cholesterol: P < 0.001) and moderate patients compared to mild (Na: P = 0.030; cholesterol: P = 0.002) cases. An increase in critical patients was observed also for creatinine (mild–critical: P = 0.009), ALP (mild–critical: P = 0.024; moderate–critical: P = 0.030) and HbA1c (mild–critical: P = 0.001; moderate–critical: P < 0.001) (Fig. 6). A trend of increasing values in critical patients was detected for serum urea, bilirubin, ALT and iron levels, but the differences were not significant.

These markers are mostly related to renal function and lifestyle. Reduced renal function is indicated by increased Na, Cl and creatinine in critical patients, as well as increased serum urea concentration, which however was not statistically significant (Table 3).

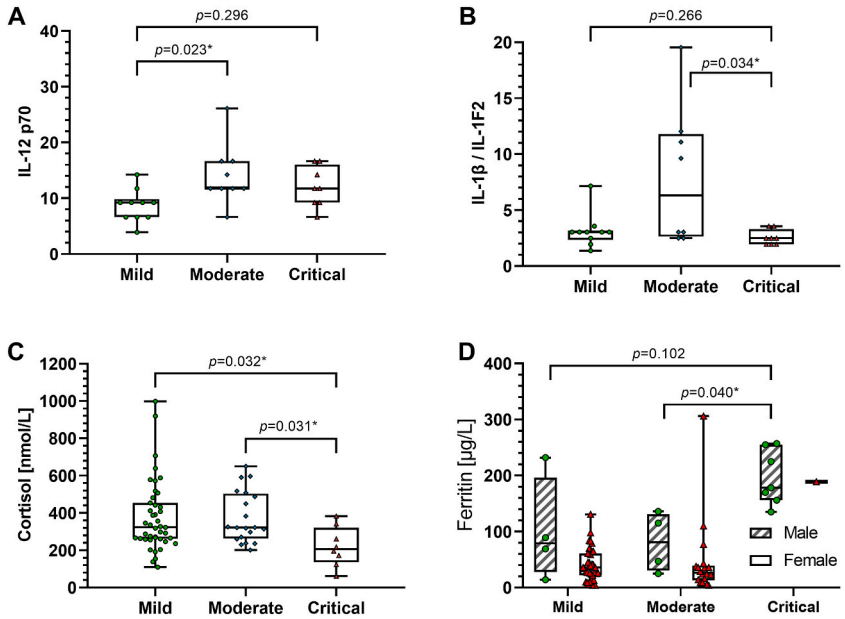

Fig. 4. Significant differences between patients with different COVID-19 severity (mild, moderate, critical) 5–6 months after the disease were detected for cytokines (A) Interleukin (IL)-12 p70, (B) IL-1β/IL-1F2 as well as (C) cortisol and (D) ferritin. Median and interquartile range (IRQ) are shown for 10 mild, 9 moderate and 8 critical cases for IL-12 and IL-1β and for ferritin for 4 mild, 4 moderate and 7 critical male patients and 37 mild, 18 moderate and 1 critical female patient. Cortisol was determined in 45 mild, 22 moderate and 8 severe cases. Data was analysed using ANOVA or Kruskal Wallis test with Post Hoc test and Bonferroni correction.

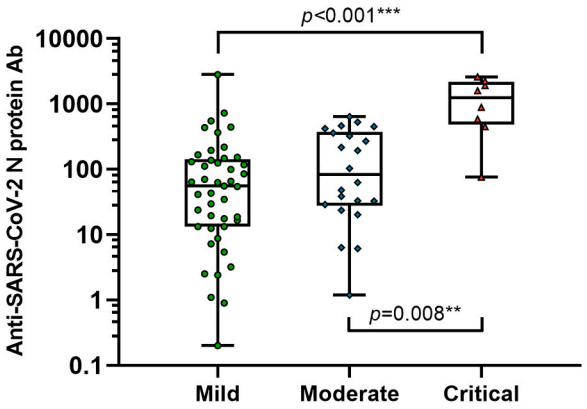

Fig. 5. Specific antibodies against nucleocapsid proteins of SARS-CoV-2 were statistically different between patients with different COVID-19 severity (mild, moderate, critical) 5–6 months after the disease. Median and interquartile range (IRQ) are shown for 42 mild, 22 moderate and 8 critical cases. Data was analysed using Kruskal Wallis test with Post Hoc test and Bonferroni correction.

Table 3
Comparison of serum biochemical parameters between groups with different clinical manifestation of COVID-19 (mild, moderate and critical cases) determined 5–6 months after first-time infection.

Biochemical parameters	Reference interval	Median (IQR)			
		Mild cases (n = 45)	Moderate cases (n = 22)	Critical cases (n = 8)	P value
K (mmol/L)	3.8–5.5	4.40 (4.20–4.75)	4.40 (4.20–4.60)	4.65 (4.40–4.88)	0.226
Na (mmol/L)	135–145	141.0 (139.0–144.0)	142.5 (139.8–145.3)	148.0 (144.0–151.0)	0.004**
Cl (mmol/L)	95–105	105.0 (104.0–107.0)	106.0 (104.8–108.0)	109.0 (106.5–110.8)	0.015*
Ca (mmol/L)	2.10–2.60	2.35 (2.26–2.43)	2.31 (2.26–2.37)	2.33 (2.32–2.48)	0.454
Pi (mmol/L)	0.84–1.45	1.18 (1.02–1.33)	1.25 (1.15–1.33)	1.04 (0.97–1.37)	0.429
Mg (mmol/L)	0.60–1.10	0.78 (0.75–0.83)	0.80 (0.78–0.83)	0.82 (0.79–0.83)	0.240
Urea (mmol/L)	2.8–7.5	5.10 (3.90–6.15)	5.40 (4.28–6.00)	6.25 (5.00–6.85)	0.095
Creatinine (μmol/L)	44–97	73.0 (64.5–79.0)	75.0 (65.5–85.3)	88.5 (80.8–93.5)	0.012*
oGFR (CKD-EPI) (mL/min)		90.00 (78.50–95.00)	95.00 (78.75–95.00)	80.50 (79.25–85.75)	0.343
Bilirubin (μmol/L)	3–22	9.0 (7.0–12.0)	9.0 (5.8–11.0)	11.5 (9.0–15.0)	0.142
ALP (μkat/L)	0.72–1.92 (M) 0.55–1.64 (F)	0.92 (0.73–1.05)	0.90 (0.67–1.03)	1.34 (0.98–1.52)	0.022*
AST (μkat/L)	<0.58 (M) <0.52 (F)	0.41 (0.34–0.50)	0.39 (0.33–0.48)	0.47 (0.36–0.56)	0.666
ALT (μkat/L)	<0.77 (M) <0.57 (F)	0.21 (0.18–0.31)	0.19 (0.15–0.30)	0.33 (0.22–0.54)	0.128
Troponin I (hs) (ng/L)					
Male[a]	<58	<3	3.00 (<3–6.00)	<3 (<3–5.00)	0.138
Female[b]	<40	<3	<3 (<3–4.25)	7.0	0.526
LDH[c] (μkat/L)	<4.13 (M) <4.12 (F)	2.64 (2.41–2.76)	2.69 (2.31–3.01)	2.97 (2.57–3.46)	0.390
Iron (III) (μmol/L)	10.7–28.6	13.9 (10.45–19.40)	15.65 (9.93–19.45)	19.30 (13.70–21.53)	0.394
Cholesterol (mmol/L)	<5.0	5.10 (4.35–5.70)	4.95 (4.60–5.83)	6.65 (5.85–6.80)	<0.001***
HDL-cholesterol (mmol/L)	>1.0	1.60 (1.30–1.80)	1.50 (1.28–1.90)	1.30 (1.03–1.55)	0.133
Lp(a)[d] (mg/L)	<300	40.0 (40.0–193.8)	40.0 (40.0–176.8)	111.0 (40.0–214.3)	0.623
Testosterone (nmol/L) (M) [a]	8.8–30.6	16.25 (6.96–25.08)	16.64 (8.61–23.49)	11.36 (8.88–21.65)	0.881
Alpha-1-antitripsin (g/L)	0.9–2.0	1.40 (1.30–1.53)	1.40 (1.18–1.60)	1.40 (1.20–1.60)	0.862
HbA1c[e] (%)	<6	5.20 (5.00–5.40)	5.15 (5.00–5.43)	5.75 (5.28–6.13)	<0.001***

Data was analysed using ANOVA or Kruskal Wallis test with Post Hoc test and Bonferroni correction. P values below 0.05 indicate at least one group is significantly different from the other two (mild, moderate, and critical cases). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
Abbreviations: IQR – interquartile range, Pi – inorganic phosphate, M – male, F – female, ALP – alkaline phosphatase, AST – aspartate aminotransferase, ALT – alkaline aminotransferase, LDH – lactate dehydrogenase, HDL-cholesterol – high-density lipoprotein cholesterol, Lp(a) – lipoprotein (a).

[a] Mild cases n = 4, Moderate cases n = 4, Critical cases n = 7.
[b] Mild cases n = 37, Moderate cases n = 18, Critical cases n = 1.
[c] Mild cases n = 11, Moderate cases n = 10, Critical cases n = 8.
[d] Mild cases n = 43, Moderate cases n = 20, Critical case n = 8.
[e] HbA1c was measured in the whole blood; Mild cases n = 40, Moderate cases n = 22, Critical cases n = 8.

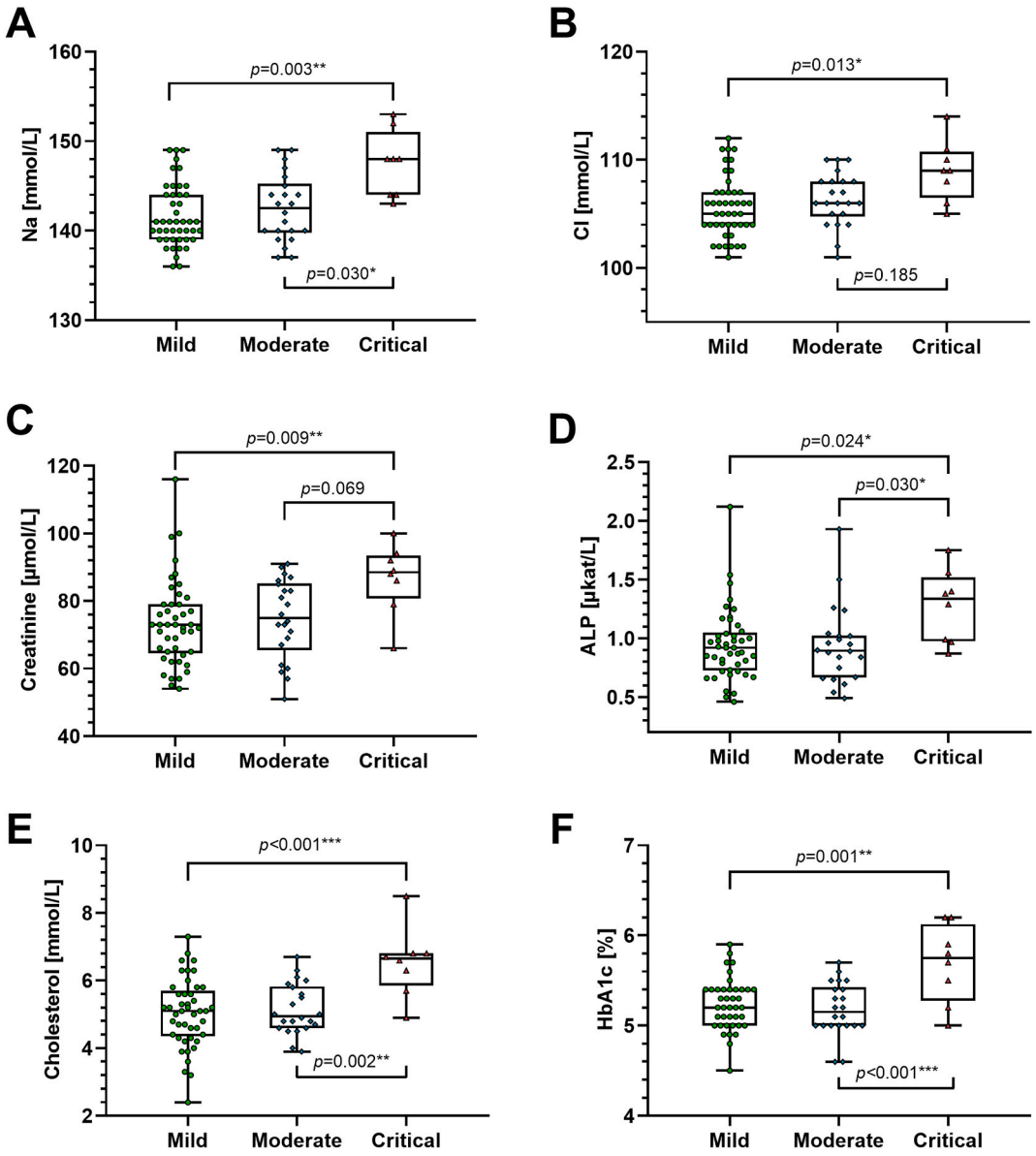

Fig. 6. Distribution of measured quantities of A) sodium (Na), B) chloride (Cl), C) creatinine, D) alkaline phosphatase (ALP), E) total cholesterol and F) glycated haemoglobin HbA1C in patients different COVID-19 severity (mild, moderate, critical) 5–6 months after the disease. Median and interquartile range (IRQ) are shown for 45 mild, 22 moderate and 8 critical cases. P values indicate statistical differences between groups. Data was analysed using ANOVA or Kruskal Wallis test with Post Hoc test and Bonferroni correction.

Decreased renal function has been associated with critical COVID-19 and poor prognosis before [27], and it has been shown that a significant proportion of critical patient can develop acute kidney failure (AKI), with lasting consequences beyond discharge from the hospital [28]. AKI patients have been associated with increased urea, creatinine, ferritin, CRP, LDH and D-dimer and decreased eGFR [29], many of which are also increased in our study, although no patient reported lasting renal problems. Also, Liu et al. showed that creatinine, urea and eGFR increase in the 3–12 months post infection compared to the values on discharge, but this increase was not dependent on disease severity [10]. To the best of our knowledge, no study so far observed Na and Cl levels months after disease resolution, however in acute disease, both hypernatremia and hyponatremia have been observed only in a fraction of critical patients that had worse outcomes (requirement for invasive ventilation, AKI) and longer hospital stay compared to patients with normal Na levels [30,31]. As the studies showed that hypernatremia occurred later in disease (normal Na levels were detected on admission) [32] and a study on cancer patients showed that pre-infection urea and creatinine levels do not correlate with COVID-19 mortality [33], we can assume that the observed markers of decreased renal function are a consequence of COVID-19.

Other significant differences in measured parameters were related to lifestyle. In agreement with higher BMI in critical cases

(Fig. 2), we also recorded higher total cholesterol and HbA1c levels (Table 3), an indicator of average long-term blood glucose concentration. Pre- and post-infection elevated HbA1c has been previously linked to higher probability of critical COVID-19 disease [34,35], which is in agreement with our data. As a previous study detected no difference in HbA1c levels between non-COVID-19 patients and COVID-19 patients 12 months after the infection [36], we can assume the differences in HbA1c detected between disease severity groups are the pre-infection baseline of these patients. On the other hand, low on-admission serum total cholesterol, HDL-cholesterol and LDL-cholesterol were associated with higher risk of critical disease and death [37,38]. Although studies report a transient decrease in serum lipid levels during acute COVID-19 disease compared to healthy controls [38–40], they return to normal levels in 2 months [38,41]. In our study, HDL-cholesterol measurements still show a similar, although non-statistically significant decrease with increasing disease severity even after 6 months. In contrast, statistically significant higher total cholesterol levels were detected in critical patients (Table 3, Fig. 6). This would suggest that the levels observed in our study are pre-infection and potentially reflect the BMI state of the patients.

The last statistically significant difference in biochemical parameters was increased serum ALP concentration in critical patients (Table S3, Fig. 7). This is consistent with a study that detected an increase in the incidence of elevated serum ALP concentrations 3 and 6 months after hospital discharge and a normalization of the incidence 12 months after hospital discharge [10]. This could be due to cholangiopathy, which is a normal occurrence during the acute course of the disease progressing to liver failure [42], but recently there have been publications reporting post-COVID-19 cholangiopathy that occurs weeks to months after the initial infection, more commonly noted among men, obese patients with metabolic syndromes and more severe COVID-19 disease [43].

3.2.5. Serum proteins

The levels of measured serum proteins and globulin fractions (proteinogram) (Table S5) were all within normal reference intervals. We detected higher values of alpha 2 (Fig. 7A) and beta 2 (Fig. 7B) globulin fractions in critically ill group compared to mild ($P = 0.002$ and 0.005, respectively) and moderate ($P = 0.007$ and 0.004, respectively) group. We have not determined the main proteins of the alpha 2 globulin fraction (alpha-2-macroglobulin, ceruloplasmin and haptoglobin), but we have measured two of the most abundant proteins in beta 2 globulin fraction (complement C3 component and IgA). Complement C3 component did not differ between disease severity groups, but IgA antibodies were increased in critical group with almost statistical significance (Table S4). As there was a significant correlation between beta 2 globulin fraction and IgA ($P = 2 \times 10^{-12}$, $r = 0.715$), we can reasonably conclude that the increase in beta 2 globulin fraction is due to increased IgA antibodies.

3.2.6. Trace elements

Trace element levels in blood and plasma were within normal reference intervals (Table S6). We did not observe differences in levels of both essential and non-essential elements and Cu/Zn plasma ratio between groups of patients with different disease severity. Only Cu in plasma in male patients almost reached statistical significance (Table S6) with higher detected levels in critical cases. Studies found higher Cu levels in survivors than in deceased patients [44], as well as higher levels in critical compared to mild group [45,46], which is in agreement with our results. Aryal et al. even found higher copper levels in acute and convalescent COVID-19 patients plasma samples compared to healthy controls, which is thought to be critical for the development of oxidative stress in infected patients [47]. This suggests that higher Cu levels might be a protective response to the disease rather than baseline difference that defines disease outcome.

3.2.7. Study limitations

This study has several limitations. A major limitation is that this is a one time-point follow-up (5–6 months after the infection) cross-sectional study and no other before- or during-infection data are available. This was a single centred study with no external validation control, which might have contributed to selection bias. The study was limited to healthy volunteers, excluding many severely ill patents with predispositions for worse COVID-19 outcomes, so the data do not represent a general population. The distribution of patients is highly uneven in disease severity and in gender, which was mostly attributed to initially low prevalence of COVID-19 in Slovenia and difficulty recruiting non-vaccinated volunteers (vaccination was freely available and highly promoted shortly after the beginning of our study and we had a high drop-out due to vaccination). Interpretation of our results is thus limited by the sample size and any correlations detected should be confirmed in an independent study on a larger population.

4. Conclusion

In this study we showed that although most parameters were within the normal reference intervals at 5–6 months after the infection, difference in distribution of the values of certain parameters were still observed, especially for the patients with critical COVID-19 disease. These differences are either baseline differences present already before the disease, which have promoted a different severity of the disease, or parameters that suggest prolonged consequences of the stress of the disease on organ systems and immune system. Especially noticeable were parameters related to reduced renal function (increased sodium, chloride, creatinine and urea levels), biliary tract dysfunction (ALP) and changes in globulin fractions (alpha2 and beta 2 globulin fractions, most probably connected to increased immune system related proteins), humoral immunity (B lymphocytes, NK cells) and inflammation (ferritin, cortisol). Our data also confirmed frequently detected risk parameters, showing that older age, higher BMI, higher cholesterol and higher HbA1c could lead to more severe disease progression.

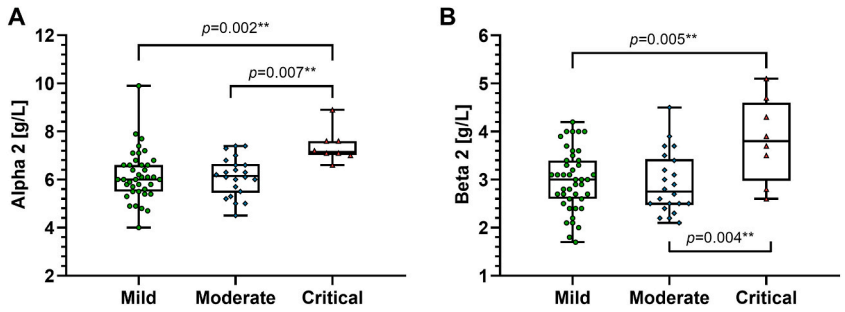

Fig. 7. Distribution of protein quantity in A) alpha 2 and B) beta 2 globulin fraction as obtained with serum protein electrophoresis, based on disease severity of COVID-19 patients (mild, moderate, critical) 5–6 months after the disease. Median and interquartile range (IRQ) are shown for 43 mild, 22 moderate and 8 critical cases. P values indicate statistical differences between groups. Data was analysed using ANOVA (Beta 2 globulin fraction) or Kruskal Wallis test (Alpha 2 globulin fraction) with Post Hoc test and Bonferroni correction.

CRediT authorship contribution statement

Taja Zore: Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Formal analysis, Data curation. **Jasna Lojk:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Conceptualization. **Katarina Reberšek:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Data curation. **Elizabeta Božnar Alič:** Writing – review & editing, Resources, Investigation. **Urška Čegovnik Primožič:** Writing – review & editing, Resources, Investigation. **Alenka France Štiglic:** Writing – review & editing, Writing – original draft, Resources, Investigation. **Aleš Jerin:** Writing – review & editing, Writing – original draft, Resources, Investigation. **Irena Prodan Žitnik:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Helena Podgornik:** Writing – review & editing, Resources, Investigation, Data curation, Conceptualization. **Nada Snoj:** Writing – review & editing, Resources, Investigation. **Barbara Ostanek:** Conceptualization. **Gabriele Turel:** Resources, Investigation. **Tatjana Lejko Zupanc:** Resources. **Janja Marc:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Darko Černe:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Data availability statement

Grouped data supporting this study conclusions are included within the article and/or supporting materials. The participants of this study did not give written consent for their raw data to be shared publicly, so due to the sensitive nature of the research supporting data is only available from the corresponding author, DČ, upon reasonable request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42535.

References

[1] R. Keni, A. Alexander, P.G. Nayak, J. Mudgal, K. Nandakumar, COVID-19: emergence, spread, possible treatments, and global burden, Front. Public Health 8 (2020). https://www.frontiersin.org/articles/10.3389/fpubh.2020.00216. (Accessed 18 July 2023).
[2] C.M. Roberts, M. Levi, M. McKee, R. Schilling, W.S. Lim, M.P.W. Grocott, COVID-19: a complex multisystem disorder, Br. J. Anaesth. 125 (2020) 238–242, https://doi.org/10.1016/j.bja.2020.06.013.
[3] G. Ponti, M. Maccaferri, C. Ruini, A. Tomasi, T. Ozben, Biomarkers associated with COVID-19 disease progression, Crit. Rev. Clin. Lab Sci. 57 (2020) 389–399, https://doi.org/10.1080/10408363.2020.1770685.

[4] B.M. Henry, M.H.S. de Oliveira, S. Benoit, M. Plebani, G. Lippi, Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis, Clin. Chem. Lab. Med. 58 (2020) 1021–1028, https://doi.org/10.1515/cclm-2020-0369.

[5] M. Sindelar, E. Stancliffe, M. Schwaiger-Haber, D.S. Anbukumar, K. Adkins-Travis, C.W. Goss, J.A. O'Halloran, P.A. Mudd, W.-C. Liu, R.A. Albrecht, A. García-Sastre, L.P. Shriver, G.J. Patti, Longitudinal metabolomics of human plasma reveals prognostic markers of COVID-19 disease severity, Cell Rep Med 2 (2021) 100369, https://doi.org/10.1016/j.xcrm.2021.100369.

[6] Y. Li, W. Luo, B. Liang, Circulating trace elements status in COVID-19 disease: a meta-analysis, Front. Nutr. 9 (2022) 982032, https://doi.org/10.3389/fnut.2022.982032.

[7] A.V. Skalny, T.R.R. Lima, T. Ke, J.-C. Zhou, J. Bornhorst, S.I. Alekseenko, J. Aaseth, O. Anesti, D.A. Sarigiannis, A. Tsatsakis, M. Aschner, A.A. Tinkov, Toxic metal exposure as a possible risk factor for COVID-19 and other respiratory infectious diseases, Food Chem. Toxicol. 146 (2020) 111809, https://doi.org/10.1016/j.fct.2020.111809.

[8] H.-L. Zeng, Q.-B. Lu, Q. Yang, X. Wang, D.-Y. Yue, L.-K. Zhang, H. Li, W. Liu, H.-J. Li, Longitudinal profile of laboratory parameters and their application in the prediction for fatal outcome among patients infected with SARS-CoV-2: a retrospective cohort study, Clin. Infect. Dis. (2020), https://doi.org/10.1093/cid/ciaa574 ciaa574.

[9] G. Lippi, M. Plebani, B.M. Henry, Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis, Clin. Chim. Acta 506 (2020) 145–148, https://doi.org/10.1016/j.cca.2020.03.022.

[10] T. Liu, D. Wu, W. Yan, X. Wang, X. Zhang, K. Ma, H. Chen, Z. Zeng, Y. Qin, H. Wang, M. Xing, D. Xu, W. Li, M. Ni, L. Zhu, L. Chen, G. Chen, W. Qi, T. Wu, H. Yu, J. Huang, M. Han, W. Zhu, W. Guo, X. Luo, T. Chen, Q. Ning, Twelve-month systemic consequences of coronavirus disease 2019 (COVID-19) in patients discharged from hospital: a prospective cohort study in wuhan, China, Clin. Infect. Dis. (2022) 1953–1965.

[11] COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health., (n.d.). https://www.covid19treatmentguidelines.nih.gov (accessed October 20, 2022).

[12] M. Gao, C. Piernas, N.M. Astbury, J. Hippisley-Cox, S. O'Rahilly, P. Aveyard, S.A. Jebb, Associations between body-mass index and COVID-19 severity in 6·9 million people in England: a prospective, community-based, cohort study, Lancet Diabetes Endocrinol. 9 (2021) 350–359, https://doi.org/10.1016/S2213-8587(21)00089-9.

[13] K.R. Starke, D. Reissig, G. Petereit-Haack, S. Schmauder, A. Nienhaus, A. Seidler, The isolated effect of age on the risk of COVID-19 severe outcomes: a systematic review with meta-analysis, BMJ Glob. Health 6 (2021) e006434, https://doi.org/10.1136/bmjgh-2021-006434.

[14] A. Rajamanickam, N.P. Kumar, A.N. Pandiarajan, N. Selvaraj, S. Munisankar, R.M. Renji, V. Venkatramani, M. Murhekar, J.W.V. Thangaraj, M.S. Kumar, C.P. G. Kumar, T. Bhatnagar, M. Ponnaiah, R. Sabarinathan, V. Saravanakumar, S. Babu, Dynamic alterations in monocyte numbers, subset frequencies and activation markers in acute and convalescent COVID-19 individuals, Sci. Rep. 11 (2021) 20254, https://doi.org/10.1038/s41598-021-99705-y.

[15] M.-L. Cheng, H.-Y. Liu, C. Zhou, R.-T. Li, J. Zheng, Y.-H. Qin, N. Yang, Y. Zhang, J.-J. Huang, Z. Zhu, Q.-Y. Meng, G.-Q. Wang, H. Zhao, Y. Chen, C.-Q. Bai, C.-F. Qin, F. Li, Longitudinal dynamics of cellular responses in recovered COVID-19 patients, Front. Immunol. 13 (2022). https://www.frontiersin.org/articles/10.3389/fimmu.2022.911859. (Accessed 26 May 2023).

[16] L. Cheng, H. Li, L. Li, C. Liu, S. Yan, H. Chen, Y. Li, Ferritin in the coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis, J. Clin. Lab. Anal. 34 (2020) e23618, https://doi.org/10.1002/jcla.23618.

[17] O. Para, L. Caruso, G. Pestelli, F. Tangianu, D. Carrara, L. Maddaluni, A. Tamburello, L. Castelnovo, G. Fedi, S. Guidi, C. Pestelli, B. Pennella, T. Ciarambino, C. Nozzoli, F. Dentali, Ferritin as prognostic marker in COVID-19: the FerVid study, Postgrad. Med. J. (n.d.) 1–6. https://doi.org/10.1080/00325481.2021.1990091.

[18] A. Eray, K. Murat, G. Abdullah, K. Ihsan, K. Royca, O. Didem, T.E. Ediz, Follow-up study of anti-SARS-CoV-2 IgG antibody response in COVID-19 patients up to 6 months after infection, Future Microbiol. 17 (2022) 1043–1049, https://doi.org/10.2217/fmb-2022-0006.

[19] K.F. Kernan, J.A. Carcillo, Hyperferritinemia and inflammation, Int. Immunol. 29 (2017) 401–409, https://doi.org/10.1093/intimm/dxx031.

[20] P.E. Taneri, S.A. Gómez-Ochoa, E. Llanaj, P.F. Raguindin, L.Z. Rojas, Z.M. Roa-Díaz, D. Salvador, D. Groothof, B. Minder, D. Kopp-Heim, W.E. Hautz, M. F. Eisenga, O.H. Franco, M. Glisic, T. Muka, Anemia and iron metabolism in COVID-19: a systematic review and meta-analysis, Eur. J. Epidemiol. 35 (2020) 763–773, https://doi.org/10.1007/s10654-020-00678-5.

[21] A. Khan, W.M. Khan, M. Ayub, M. Humayun, M. Haroon, Ferritin is a marker of inflammation rather than iron deficiency in overweight and obese people, J Obes 2016 (2016) 1937320, https://doi.org/10.1155/2016/1937320.

[22] R. Moll-Bernardes, A.S. de Sousa, A.V.S. Macedo, R.D. Lopes, N. Vera, L.C.R. Maia, A. Feldman, G.D.A.S. Arruda, M.J.C. Castro, P.M. Pimentel-Coelho, D.C. de Albuquerque, T.C. de Paula, T.A.B. Furquim, V.A. Loures, K.G.D. Giusti, N.M. de Oliveira, F.A. De Luca, M.D.M. Kotsugai, R.A.M. Domiciano, M.F. Santos, O. F. de Souza, F.A. Bozza, R.R. Luiz, E. Medei, IL-10 and IL-12 (P70) levels predict the risk of covid-19 progression in hypertensive patients: insights from the BRACE-CORONA trial, Front. Cardiovasc. Med. 8 (2021) 702507, https://doi.org/10.3389/fcvm.2021.702507.

[23] Y. Chen, J. Wang, C. Liu, L. Su, D. Zhang, J. Fan, Y. Yang, M. Xiao, J. Xie, Y. Xu, Y. Li, S. Zhang, IP-10 and MCP-1 as biomarkers associated with disease severity of COVID-19, Mol Med 26 (2020) 97, https://doi.org/10.1186/s10020-020-00230-x.

[24] S.L. Sommen, L.B. Havdal, J. Selvakumar, G. Einvik, T.M. Leegaard, F. Lund-Johansen, A.E. Michelsen, T.E. Mollnes, T. Stiansen-Sonerud, T. Tjade, V.B. B. Wyller, L.L. Berven, Inflammatory markers and pulmonary function in adolescents and young adults 6 months after mild COVID-19, Front. Immunol. 13 (2022) 1081718, https://doi.org/10.3389/fimmu.2022.1081718.

[25] W.F. Garcia-Beltran, E.C. Lam, M.G. Astudillo, D. Yang, T.E. Miller, J. Feldman, B.M. Hauser, T.M. Caradonna, K.L. Clayton, A.D. Nitido, M.R. Murali, G. Alter, R.C. Charles, A. Dighe, J.A. Branda, J.K. Lennerz, D. Lingwood, A.G. Schmidt, A.J. Iafrate, A.B. Balazs, COVID-19-neutralizing antibodies predict disease severity and survival, Cell 184 (2021) 476–488.e11, https://doi.org/10.1016/j.cell.2020.12.015.

[26] J.H. Park, M.J. Cha, H. Choi, M.-C. Kim, J.-W. Chung, K.-S. Lee, D.G. Jeong, M.S. Baek, W.-Y. Kim, Y. Lim, S.W. Yoon, S.-H. Choi, Relationship between SARS-CoV-2 antibody titer and the severity of COVID-19, J. Microbiol. Immunol. Infect. 55 (2022) 1094–1100, https://doi.org/10.1016/j.jmii.2022.04.005.

[27] E. Longhitano, C. Nardi, V. Calabrese, R. Messina, G. Mazzeo, E. Venanzi Rullo, M. Ceccarelli, A. Chatrenet, P. Saulnier, M. Torreggiani, G. Nunnari, G.B. Piccoli, D. Santoro, Hypernatraemia and low eGFR at hospitalization in COVID-19 patients: a deadly combination, Clinical Kidney Journal 14 (2021) 2227–2233, https://doi.org/10.1093/ckj/sfab122.

[28] A. Hadadi, H. Farrokhpour, S. Rashedi, S. Kafan, M. Sotoudehnia, H. Rahimzadeh, S. Tabatabaei, E. Razeghi, Z. Aghsaeifard, Long-term impact of the COVID-19 associated AKI: the relationship between kidney recovery and mortality in a 10-month follow-up cohort study, Kidney Blood Press. Res. (2022) 1–6, https://doi.org/10.1159/000524451.

[29] M. Taha, D. Sano, S. Hanoudi, Z. Esber, M. Elahi, A. Gabali, T. Chopra, S. Draghici, L. Samavati, Platelets and renal failure in the SARS-CoV-2 syndrome, Platelets 32 (2021) 130–137, https://doi.org/10.1080/09537104.2020.1817361.

[30] M.A. Zimmer, A.K. Zink, C.W. Weißer, U. Vogt, A. Michelsen, H.-J. Priebe, G. Mols, Hypernatremia—a manifestation of COVID-19: a case series, A A, In Pract. 14 (2020) e01295, https://doi.org/10.1213/XAA.0000000000001295.

[31] M.S. Asghar, S.J. Haider Kazmi, N.A. Khan, M. Akram, R. Jawed, W. Rafaey, M. Hassan, U. Rasheed, M. Khan, A.R. Khan, Role of Biochemical Markers in Invasive Ventilation of Coronavirus Disease 2019 Patients: Multinomial Regression and Survival Analysis, Cureus 12 (n.d.) e10054. https://doi.org/10.7759/cureus.10054.

[32] S. Redant, J. Vanderhulst, E. Maillart, R. Attou, A. Gallerani, P.M. Honoré, D.D. Bels, Significance of hypernatremia due to SARS-CoV-2 associated ARDS in critically ill patients, J Transl Int Med 8 (2020) 255–260, https://doi.org/10.2478/jtim-2020-0038.

[33] A. Kiani, R. Roesch, C.M. Wendtner, F. Kullmann, T. Kubin, T. Südhoff, M. Augustin, M. Schaich, C. Müller-Naendrup, G. Illerhaus, F. Hartmann, H. Hebart, R. Seggewiss-Bernhardt, M. Bentz, E. Späth-Schwalbe, P. Reimer, U. Kaiser, M. Kapp, U. Graeven, J.-M. Chemnitz, J. Baesecke, H. Lambertz, R. Naumann, Preinfection laboratory parameters may predict COVID-19 severity in tumor patients, Cancer Med. 10 (2021) 4424–4436, https://doi.org/10.1002/cam4.4023.

[34] F. Prattichizzo, P. de Candia, A. Nicolucci, A. Ceriello, Elevated HbA1c levels in pre-Covid-19 infection increases the risk of mortality: a sistematic review and meta-analysis, Diabetes Metab Res Rev 38 (2022) e3476, https://doi.org/10.1002/dmrr.3476.

[35] A. Valle, J. Rodriguez, F. Camiña, M.A. Martínez-Olmos, J.B. Ortola, S. Rodriguez-Segade, At-admission HbA1c levels in hospitalized COVID-19 participants with and without known diabetes, Clin. Chim. Acta 532 (2022) 188–192, https://doi.org/10.1016/j.cca.2022.05.027.
[36] L. Huang, Q. Yao, X. Gu, Q. Wang, L. Ren, Y. Wang, P. Hu, L. Guo, M. Liu, J. Xu, X. Zhang, Y. Qu, Y. Fan, X. Li, C. Li, T. Yu, J. Xia, M. Wei, L. Chen, Y. Li, F. Xiao, D. Liu, J. Wang, X. Wang, B. Cao, 1-year outcomes in hospital survivors with COVID-19: a longitudinal cohort study, Lancet 398 (2021) 747–758, https://doi.org/10.1016/S0140-6736(21)01755-4.
[37] V. Chidambaram, H. Shanmugavel Geetha, A. Kumar, M.G. Majella, R.K. Sivakumar, D. Voruganti, J.L. Mehta, P.C. Karakousis, Association of lipid levels with COVID-19 infection, disease severity and mortality: a systematic review and meta-analysis, Front. Cardiovasc. Med. 9 (2022). https://www.frontiersin.org/articles/10.3389/fcvm.2022.862999. (Accessed 25 November 2022).
[38] X. Hu, D. Chen, L. Wu, G. He, W. Ye, Declined serum high density lipoprotein cholesterol is associated with the severity of COVID-19 infection, Clin. Chim. Acta 510 (2020) 105–110, https://doi.org/10.1016/j.cca.2020.07.015.
[39] K.R. Feingold, The bidirectional link between HDL and COVID-19 infections, J. Lipid Res. 62 (2021) 100067, https://doi.org/10.1016/j.jlr.2021.100067.
[40] Z. Lv, W. Wang, B. Qiao, X. Cui, Y. Feng, L. Chen, Q. Ma, X. Liu, The prognostic value of general laboratory testing in patients with COVID-19, J. Clin. Lab. Anal. 35 (2021), https://doi.org/10.1002/jcla.23668.
[41] V. Chidambaram, A. Kumar, M.G. Majella, B. Seth, R.K. Sivakumar, D. Voruganti, M. Bavineni, A. Baghal, K. Gates, A. Kumari, S.J. Al'Aref, P. Galiatsatos, P. C. Karakousis, J.L. Mehta, HDL cholesterol levels and susceptibility to COVID-19, EBioMedicine 82 (2022), https://doi.org/10.1016/j.ebiom.2022.104166.
[42] L. Hartl, K. Haslinger, M. Angerer, G. Semmler, M. Schneeweiss-Gleixner, M. Jachs, B. Simbrunner, D.J.M. Bauer, E. Eigenbauer, R. Strassl, M. Breuer, O. Kimberger, D. Laxar, K. Lampichler, E. Halilbasic, A.F. Stättermayer, A. Ba-Ssalamah, M. Mandorfer, B. Scheiner, T. Reiberger, M. Trauner, Progressive cholestasis and associated sclerosing cholangitis are frequent complications of COVID-19 in patients with chronic liver disease, Hepatology 76 (2022) 1563–1575, https://doi.org/10.1002/hep.32582.
[43] S. Yadlapati, S.A. Jarrett, D. Baik, A. Chaaya, COVID-19 related biliary injury: a review of recent literature, World J. Gastroenterol. 29 (2023) 2127–2133, https://doi.org/10.3748/wjg.v29.i14.2127.
[44] J. Hackler, R.A. Heller, Q. Sun, M. Schwarzer, J. Diegmann, M. Bachmann, A. Moghaddam, L. Schomburg, Relation of serum copper status to survival in COVID-19, Nutrients 13 (2021) 1898, https://doi.org/10.3390/nu13061898.
[45] H.-L. Zeng, Q. Yang, P. Yuan, X. Wang, L. Cheng, Associations of essential and toxic metals/metalloids in whole blood with both disease severity and mortality in patients with COVID-19, FASEB J. 35 (2021) e21392, https://doi.org/10.1096/fj.202002346RR.
[46] S. Zhou, F. Zhang, F. Chen, P. Li, Y. He, J. Wu, L. Dong, C. Wang, X. Wang, W. Zhang, W. Sun, L. Yin, R. Zhang, J. Zhao, B. Sun, Micronutrient level is negatively correlated with the neutrophil-lymphocyte ratio in patients with severe COVID-19, Int. J. Clin. Pract. (2022) 6498794, https://doi.org/10.1155/2022/6498794 (2022).
[47] B. Aryal, J. Tillotson, K. Ok, A.T. Stoltzfus, S.L.J. Michel, V.A. Rao, Metal-induced oxidative stress and human plasma protein oxidation after SARS-CoV-2 infection, Sci. Rep. 13 (2023) 2441, https://doi.org/10.1038/s41598-023-29119-5.