Multiparametric correlation of laboratory biomarkers to multiorgan failure outcome in hospitalized COVID-19 patients: a retrospective observational study

A. BALLINI[1], S. CANTORE[2], E. SERRETIELLO[2], G. TROIANO[1], A. SMIMMO[2], M. DIOGUARDI[1], F. SPIRITO[1], F.C. SASSO[3], D. DE VITO[4], L. LO MUZIO[1], M. DI DOMENICO[2]

[1]Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy
[2]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy
[3]Department of Advanced Medical and Surgical Sciences, School of Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy
[4]Department of Basic Medical Sciences, Neurosciences, and Sense Organs, Medical School, University of Bari "Aldo Moro", Bari, Italy

Abstract. – OBJECTIVE: COVID-19 is an extremely contagious illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that will keep broadly circulating and evolving. Collected evidence revealed the clinical profile of COVID-19 patients as a potential predictor of their outcome. The aim of this study was to investigate the causal relationship between poor outcomes and laboratory parameters in hospitalized COVID-19 patients, in this sense observing how SARS-CoV-2 infection affects other organs.

PATIENTS AND METHODS: We retrospectively evaluated a cohort of 133 patients, positive for SARS-CoV-2, aged between 30 to 94 years, between January 12th and April 25th, 2021. Discharge from the hospital, transferral to the ordinary ward or nursing home, intensive care unit (ICU) admission, and in-hospital mortality were recorded, along with demographic, laboratory and clinical parameters. The whole sample was summarized by median (interquartile range) for quantitative data, and absolute and relative percentage frequencies for qualitative variables. Univariable logistic regression models were performed to assess the association between all the parameters of interest and COVID-19 adverse outcomes, single (in-hospital mortality) and composite (in-hospital mortality and ICU admission). Hence, a multivariable model was fitted to identify potential independent predictors of the composite outcome. The accuracy of the model was assessed through appropriate fitting indices, such as the C-statistic and Hosmer-Lemeshow test. Moreover, to detect multicollinearity, the variance inflation factor (VIF) was used.

RESULTS: Our study sample had a median age of 72 years old (59.0-83.0). The most common comorbidities were hypertension (63.7%), cardiovascular disease (41.9%), diabetes (33.6%), and cerebrovascular disease (21.5%); while as the most common symptoms, we observed dry cough (32.5%), dyspnoea (50.8%), and fatigue (29.8%). Totally, 18 patients died during hospitalization (13.5%), 10 required ICU admission (7.5%), 78 (58.6%) were discharged from the hospital, and 27 (20.3%) were transferred to either ordinary wards or nursing homes. We disclosed an association of older age with both composite [OR 1.06, 95% CI 1.02-1.09; *p*=0.003] and single outcome [OR 1.10, 95% CI 1.04-1.16; *p*=0.001]. A higher oxygen saturation (SpO_2) was associated with a better outcome [OR 0.75, 95% CI 0.60-0.93; *p*=0.009 and OR 0.76, 95% CI 0.61-0.95, *p*=0.009]. Among laboratory parameters, higher levels of neutrophils increased the risk of a poor outcome [OR 1.05, 95% CI 1.00-1.10; *p*=0.043]; while higher levels of lymphocytes seem associated with a better outcome [OR 0.94, 95% CI 0.88-0.99; *p*=0.043]. Higher levels of creatinine were associated with a higher risk of both adverse outcomes [OR 6.20, 95% CI 2.16-17.81; *p*<0.001 and OR 19.90, 95% CI 5.07-78.06; *p*<0.001, respectively]. Higher levels of sodium (Na) were associated with a higher risk of adverse events [OR 1.15, 95% CI 1.03-1.28; *p*=0.014 and OR 1.14, 95% CI 1.01-1.27]. Similar findings were also observed for C-reactive protein (CRP) levels [OR 1.01, 95% CI 1.00-1.02; *p*=0.010 and OR 1.01, 95% CI 1.00-1.02; *p*=0.024]. Conversely, being positive to IgM and IgG decreases the risk of adverse outcomes [IgM: OR 0.33, 95% CI 0.14-0.77; *p*=0.011 and OR 0.23, 95% CI 0.08-0.66; *p*=0.006. IgG: OR 0.30 95% CI 0.13-0.72; *p*=0.007 and OR 0.22 95% CI 0.07-0.66; *p*=0.007]. Hence, a multivariable model was fitted to identify potential independent laboratory predictors of the composite outcome, with laborato-

Corresponding Author: Stefania Cantore, MD; e-mail: stefaniacantore@pec.omceo.bari.it

ry parameters that showed an association with composite outcome. The model can be considered accurate according to Hosmer-Lemeshow test and C-statistic [*p*>0.83, C-stat=0.90].

CONCLUSIONS: **Our findings confirm that COVID-19 is a multiorgan disease. In fact, the analysis of laboratory parameters has revealed a strong relationship between poorer outcomes and multiple organ dysfunction, particularly established by higher levels of neutrophils, creatinine, sodium, and CRP. Alongside, cerebrovascular diseases, chronic kidney disease and older age supported this finding. Of note, higher levels of SpO_2, and lymphocytes, as well as positivity to IgM and IgG were associated with a lower risk of a poor outcome.**

Key Words:
COVID-19, SARS-CoV-2 pandemic, Hospitalization, Multivariable model multiorgan disease, Immunoglobulins M (IgM), Immunoglobulins G (IgG).

Introduction

On May 5, 2023, over 3 years after the spread of COronaVIrus Disease 2019 (COVID-19) pandemic, the World Health Organization (WHO) Emergency Committee established that COVID-19 currently represents an ongoing health issue that is no longer classifiable as a public health emergency of international concern (PHEIC)[1]. However, this does not mean that the pandemic itself has been defeated. In fact, COVID-19, an extremely contagious illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[2], will keep broadly circulating and evolving. Therefore, research should not stop. From the outbreak, globally, there have been over 7.6 hundred million confirmed cases of COVID-19, including 6.9 million, deaths reported by the WHO[3]. In March 2020, when the WHO declared a state of global emergency, the world was completely unprepared to challenge this "invisible" enemy. In the first wave of infection, the underestimated lethality and contagiousness of SARS-CoV-2 resulted in a large number of deaths[4,5]. At that time, the etiopathogenesis of the virus was completely unknown; consequently, there was no specific therapy[6,7]. Thanks to the great effort made by researchers, at the end of 2020, the first COVID-19 vaccine was approved[8]. Vaccines changed the course of the pandemic, reducing the rate of infection and mortality[9-11]. Three years after the outbreak of the pandemic, many breakthroughs were made. In particular, the major efforts were focused on the study of mechanisms of viruses such as Monkeypox and SARS-CoV-2 entry into host cells, the utility of vaccines and antibodies, and other potential therapeutics targeting SARS-CoV-2[12,13]. Furthermore, collected evidence[14-16] revealed that the clinical profile of COVID-19 patients can be a predictor of their outcome. Older individuals with multimorbidity are more susceptible to developing a more severe case of COVID-19 than either younger individuals or those without multimorbidity. In this sense, patients with chronic diseases, presenting a considerable proportion of hospitalized COVID-19 patients, need appropriate interventions to minimize the adverse events[17]. The strict correlation between multimorbidity and severe cases of COVID-19 seems to be related to the pathogenesis of the virus. Severe COVID-19 is characterized by the uncontrolled and high release of pro-inflammatory cytokines, leading to the so-called "cytokine storm". This storm increases levels of inflammatory mediators, endothelial dysfunction, coagulation abnormalities, and infiltration of inflammatory cells into the organs, contributing to the pathogenesis of COVID-19. One of the potential consequences of this process is the insurrection of edema, which may lead to death[18-20]. Since SARS-CoV-2 infection affects several organs, it is crucial to assess specific prognostic parameters for bad outcomes in vulnerable patients. Clinicians, seeing the "alarm" parameters in patients, could perform prompt interventions to improve patients' outcomes and decrease the COVID-19 general rate of mortality and morbidity. The aim of this study was to explore the cause-and-effect relationship between adverse outcomes and laboratory parameters in COVID-19 hospitalized patients. The organ-related laboratory parameters analyzed are summarized in Figure 1.

Patients and Methods

Study Population

This is a retrospective cohort study including 133 patients who were hospitalized at General Hospital in Bari, Apulia, between January and April 2021, and who tested positive for Severe Acute Respiratory Syndrome related to coronavirus-2 (SARS-CoV-2) using real-time-polymerase chain reaction (RT-PCR) on a nasopharyngeal swab. The demographic, clinical and laboratory characteristics of the study population, were analyzed at the Labora-

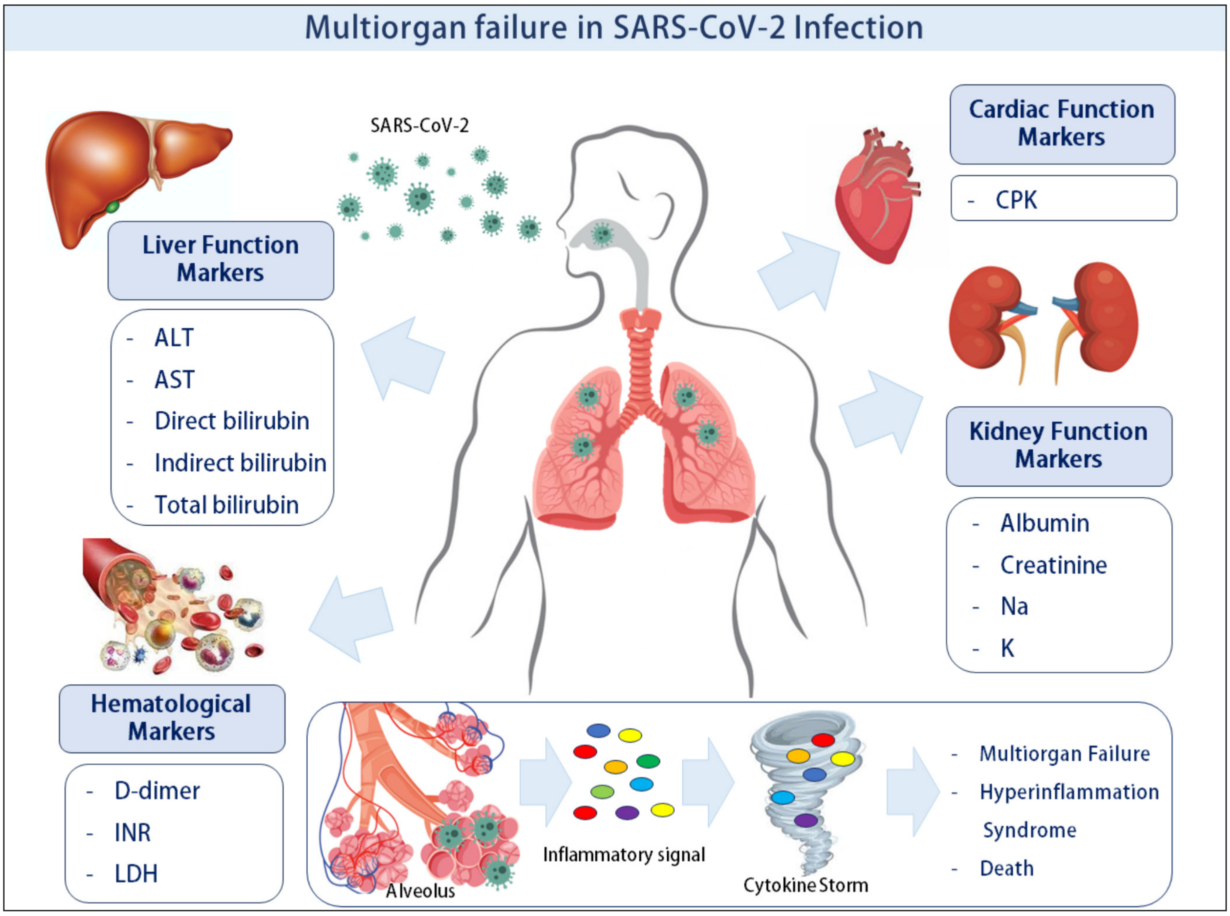

Figure 1. This representative image shows all the organ-related laboratory markers collected and how SARS-CoV-2 affects other organs. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Na, sodium; K, potassium; CPK, creatine phosphokinase; INR, international normalized ratio; LDH, lactate dehydrogenase; CRP, c-reactive protein.

tory Medicine Technical Sciences Unit, University of Campania Luigi Vanvitelli. The present study was conducted in accordance with the Declaration of Helsinki after obtaining the approval by 1-) Azienda Opedaliero-Universitaria Luigi Vanvitelli-AORN Ospedale dei Colli- Local Ethic Committees: Prot. N 0013965i/ (11 May 2023) "Analisi quantitativa e qualitativa delle Vescicole Extra-cellulari in soggetti ospedalizzati con infezione da SARS-CoV-2"; prot.: *ESOCOV*"; and 2-) Azienda Opedaliero-Universitaria "Consorziale Policlinico"- Independent Local Etic Committees approval: Prot. N 009921/21/12/20- Study number: 6665 (16 December 2020), "Studio sierologico ed immunologico di pazienti di età adulti affetti da COVID-19: la chiave per comprendere la suscettibilità al SARS-CoV-2".

Each patient underwent blood sampling on the day they were admitted to the Hospital. Additionally, a comprehensive clinical assessment was conducted, which included the examination of any existing comorbidities. Besides, none of the patients had received a COVID-19 vaccination prior to their admission.

Additionally, individuals with a history of receiving immunomodulating drugs or known blood diseases were also excluded as part of the study's exclusion criteria.

The study has a retrospective cohort design, so the data were obtained from the computer register of the laboratories. Epidemiological information such as age, sex and diagnosis were also assessed. Identification data of the patients, such as names, ID numbers, or contact information, were not collected for the present study. The requirement for informed consent was waived in view of the retrospective observational design of the research and the anonymized reporting of the confidential data obtained from the laboratory center database.

Outcomes

In-hospital mortality, intensive care unit (ICU) admission, discharges from the hospital and transferal to the ordinary ward or nursing homes, were recorded for our study population. The single outcome of the study included in-hospital mortality, whereas the composite outcome included in-hospital mortality and ICU admission.

Statistical Analysis

Demographic, clinical and laboratory characteristics were summarized using median (interquartile range) and percentages for continuous and categorical variables, respectively. Therefore, the univariate logistic regression model was performed to evaluate the association between parameters of interest and COVID-19 adverse outcomes[21]. Hence, a multivariable model was fitted to identify potential independent predictors of the composite outcome. The accuracy of the model was assessed through appropriate fitting indices, such as the C-statistic and the Hosmer-Lemeshow test[22]. Furthermore, to detect multicollinearity, the variance inflation factor (VIF) was used[23]. All statistical analyses were performed with software R v 4.2.3 (R Core Team, 2022, Vienna, Austria). The *p*-values are derived from the multivariable logistic regression model. All *p*-values <0.05 are considered as considered as statistically significant.

Results

Descriptive Characteristics of the Study Population

Descriptive characteristics of the study population are summarized in Table I. The median age of the total population was 72 (59-83) years old and 53.6% of hospitalized patients were women.

Almost all patients hospitalized accessed by Emergency Room (ER) (96.4%), after a median time of 6.0 (1.0-9.5) days after the insurgence of symptoms. The median hospitalization time was 9 (5.0-15.0) days. IgG and IgM were dosed to understand the SARS-CoV-2 infection period: 63.9% of the study population tested positive for Immunoglobulins G (IgG) and 58.6% tested positive for Immunoglobulins M (IgM). Comorbidities of hospitalized COVID-19 patients are summarized in Figure 2 and Table II.

The most common pathologies were hypertension (63.7%), cardiovascular disease (41.9%), diabetes (33.6%) and cerebrovascular disease (21.5%).

Characteristics of the Study Population on Admission, During Hospitalization and Outcome After SARS-CoV-2 Infection

Patients' characteristics, markers of respiratory function, symptoms and outcomes of patients are demonstrated in Table III. On admission, patients had a median temperature of 36.5°C (36.2-36.7), cardiac frequency (Bpm) of 80.5 (74.0-93.0), fraction of inspired oxygen (FIO_2) of 40.0 (24.0-60.0), saturation percentage (SpO_2) of 97.0 (96.0-98.0) and median pressure of 77/130 (70/121-85/150 mm Hg). Most common symptoms were dry cough (32.5%), dyspnea (50.8%) and fatigue (29.8%), whereas the rarest symptoms were sore throat (1%), hemoptysis (0.8%), shivers (0.8%), ageusia (1.7%) and anosmia (0.8%).

During the hospitalization, 18 deaths (13.5%), 10 ICU admissions (7.5%), 78 (58.6%) discharges from the hospital and 27 (20.3%) transferals to ordinary departments or nursing homes were reported.

Table I. Descriptive characteristics of study population.

Variable	Overall* (n=133)
Demographic	
Age, yrs.	72.0 (59.0-83.0)
Gender	
M	71 (53.8%)
F	61 (46.2%)
*Accessed by *3 missing*	
ER	123 (96.4%)
Other hospital department	7 (3.7%)
Symptoms to hospitalization *10 missing	6.0 (1.0-9.5)
Hospitalization time *3 missing	9.0 (5.0-15.0)
COVID-19 Infection Parameters	
Positive IgM	85 (63.9%)
Positive IgG	78 (58.6%)

M: male; F: female; ER: Emergency Room; IgM: Immunoglobulins M; IgG: Immunoglobulins G. *Descriptive statistics are expressed as median (Interquartile Range) for quantitative variables, as absolute and percentage frequencies for qualitative variables.

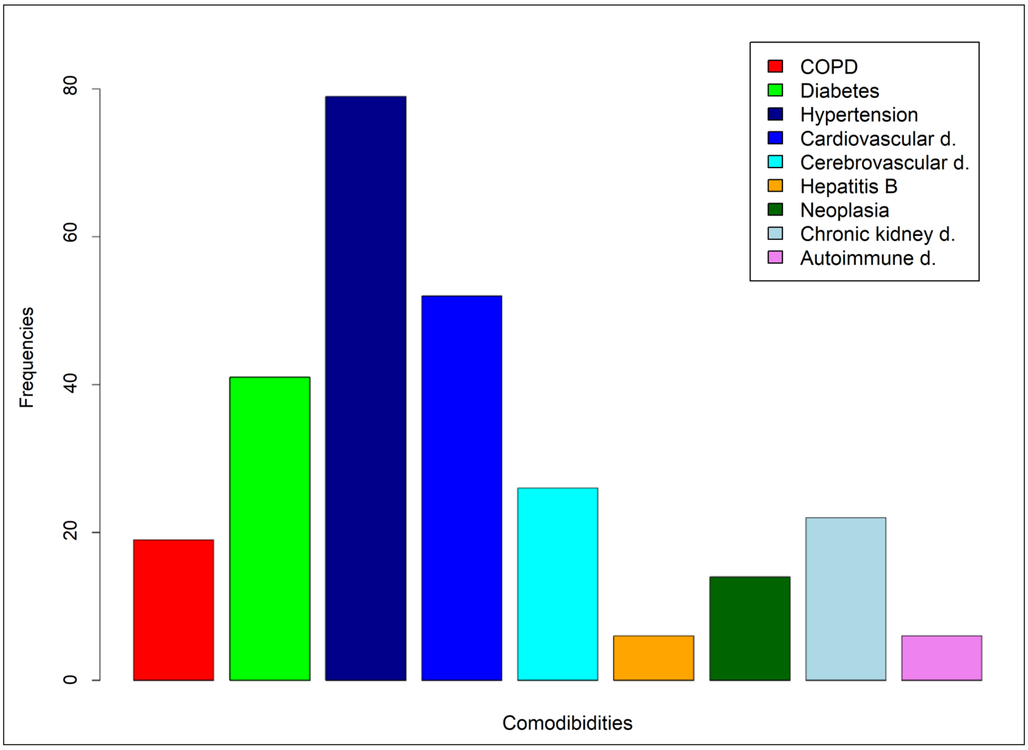

Figure 2. Barplot of comorbidities in our study population. Most frequent comorbidities were hypertension (79 out of 124), cardiovascular disease (52 out of 124) and diabetes (41 out of 122).

Table II. Comorbidities of study population.

Comorbidities	Overall* (n=133)
COPD **10 missing*	19 (15.4%)
Diabetes **11 missing*	41 (33.6%)
Hypertension **9 missing*	79 (63.7%)
Cardiovascular disease **9 missing*	52 (41.9%)
Cerebrovascular disease **12 missing*	26 (21.5%)
Hepatitis B **12 missing*	6 (5.0%)
Neoplasia **11 missing*	14 (11.5%)
Chronic kidney disease **11 missing*	22 (18.0%)
Autoimmune diseases **12 missing*	6 (5.0%)

COPD: chronic obstructive pulmonary disease. *Descriptive statistics are expressed as absolute and percentage frequencies for qualitative variables.

Laboratory parameters of the study population during hospitalization after SARS-CoV-2 infection

Patients' laboratory parameters were collected during the hospitalization to obtain a specific profile of each patient. All the variables are summarized in Table IV.

Neutrophils, lymphocytes, platelets, and hemoglobin, included in blood tests, have a median value of 78.3% (70.9-84.8), 14.3% (9.2-20.3), 211,000 U/mm^3 (154,000-280,000) and 125.0 g/l (111.0-137.0) respectively. As liver function markers, alanine aminotransferase (ALT), aspartate aminotransferase (AST), direct bilirubin, indirect bilirubin and total bilirubin were quantified, resulting respectively in median values of 27.0 U/l (20.0-47.0), 29.0 U/l (21.0-40.0), 0.010 mg/dl (0.008-0.014), 0.015 mg/dl (0.010-0.021) and 0.027 mg/dl (0.020-0.034). Albumin, creatinine, sodium (Na) and potassium (K), were dosed to test kidney function and have respectively a median value of 28.0 g/l (25.0-31.0), 0.88 mg/dl (0.70-1.17), 139.0

Table III. Clinical characteristics of study population.

Variable	Overall* (n=133)
On admission	
Fever at hospitalization (°C) **14 missing*	36.5 (36.2-36.7)
Cardiac Frequency (Bpm)	80.5 (74.0-93.0)
SpO_2 (%) **15 missing*	97.0 (96.0-98.0)
FIO_2 (%) **12 missing*	40.0 (24.0-60.0)
Systolic blood pressure (mmHg)	130.0 (120.8-150.0)
Diastolic blood pressure (mmHg)	77.0 (70.0-85.0)
Symptoms	
Arrhythmia **29 missing*	15 (14.4%)
Nasal congestion **19 missing*	4 (3.5%)
Conjunctival congestion **15 missing*	0 (0.0%)
Headache **15 missing*	13 (11.0%)
Dry cough **13 missing*	39 (32.5%)
Sore Throat **15 missing*	7 (5.9%)
Cough up *14 missing	2 (1.7%)
Hemoptysis **15 missing*	1 (0.8%)
Dyspnoea **9 missing*	63 (50.8%)
Fatigue **12 missing*	36 (29.8%)
Nausea **15 missing*	8 (6.8%)
Vomit **15 missing*	6 (5.1%)
Arthralgias **15 missing*	3 (2.5%)
Myalgia **14 missing*	3 (2.5%)
Shivers **14 missing*	1 (0.8%)
Ageusia **15 missing*	2 (1.7%)
Anosmia **15 missing*	1 (0.8%)
Tonsillar hypertrophy **15 missing*	0 (0.0%)
Lymphadenopathies **15 missing*	0 (0.0%)
Rash **15 missing*	0 (0.0%)
Obnubilation **15 missing*	10 (8.5%)
Outcome	
In-hospital mortality	18 (13.5%)
ICU admission	10 (7.5%)
Discharged from hospital	78 (58.6%)
Transferal to ordinary ward or nursing home	27 (20.3%)

ICU, intensive care unit; FIO_2, Fraction of inspired oxygen; SpO_2, oxygen saturation percentage. *Descriptive statistics are expressed as median (Interquartile Range) for quantitative variables, as absolute and percentage frequencies for qualitative variables.

mmol/l (137.0-141.0) and 4.0 mmol/l (3.8-4.5). The cardiac function marker, creatine phosphokinase (CPK), showed a median value of 77.0 U/l (44.0-149.0). Whereas hematological marker, D-dimer, international normalized ratio (INR) and lactate dehydrogenase (LDH) had a median value of 0.88 µg/ml (0.53-2.03), 1.05 (1.00-1.11) and 265.0 U/l (210.5-320.2), respectively. C-reactive protein (CRP) was dosed as an inflammation marker, having a median value of 47.0 mg/dl (18.1-97.8).

Association of Clinical Characteristic and Laboratory Markers with Adverse Outcomes in COVID-19 Patients

During the observation time, eighteen deaths and ten ICU admissions were reported (Table II). We evaluate the association of demographic, clinical and laboratory variables with adverse outcomes in COVID-19 patients. We identify two adverse outcomes: in-hospital mortality (single outcome) and the combination of in-hospital mortality and ICU admission (composite outcome). Univariable logistic regression models disclosed an association of older age with both composite [OR 1.06, 95% CI 1.02-1.09; p=0.003] and single outcome [OR 1.10, 95% CI 1.04-1.16; p=0.001]. A higher SpO_2 was associated with a better outcome [OR 0.75, 95% CI 0.60-0.93; p=0.009 and OR 0.76, 95% CI 0.61-0.95, p=0.009]. Cerebrovascular diseases determined three times the risk of a poor outcome [OR 3.07, 95% CI 1.10-8.61; p=0.003]. Also, chronic kidney disease (CKD) was associated with a higher risk of an adverse outcome in COVID-19 patients [OR 4.19, 95% CI 1.46-12.07; p=0.008 and OR 3.78, 95% CI 2.26-11.28; p=0.017, respectively]. Among laboratory para-

Table IV. Laboratory characteristics of study population.

Laboratory variables	Overall* (n=133)
Blood tests	
Neutrophils (%)	78.3 (70.9-84.8)
Lymphocytes (%)	14.3 (9.2-20.3)
Platelets (U/mm3)	211,000 (154,000-280,000)
Haemoglobin (g/l)	125.0 (111.0-137.0)
Liver Function Markers	
ALT (U/l) **1 missing*	27.0 (20.0-47.0)
AST (U/l)	29.0 (21.0-40.0)
Direct bilirubin (µmol/l)	0.010 (0.008-0.014)
Indirect bilirubin (µmol/l)	0.015 (0.010-0.021)
Total bilirubin (µmol/l)	0.027 (0.020-0.034)
Kidney Function Markers	
Albumin (g/l)	28.0 (25.0-31.0)
Creatinine (µmol/l)	0.88 (0.70-1.17)
Na (mmol/l)	139.0 (137.0-141.0)
K (mmol/l) **1 missing*	4.0 (3.8-4.5)
Cardiac Function Markers	
CPK (U/l) *4 missing	77.0 (44.0-149.0)
Hematologic Markers	
D-dimer (µg/ml) **1 missing*	0.88 (0.53-2.03)
INR	1.05 (1.00-1.11)
LDH (U/ml) **3 missing*	265.0 (210.5-320.2)
Inflammation Markers	
CRP (mg/dl) **2 missing*	47.0 (18.1-97.8)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Na, sodium; K, potassium; CPK, creatine phosphokinase; INR, international normalized ratio; LDH, lactate dehydrogenase; CRP, c-reactive protein. *Descriptive statistics are expressed as median (Interquartile Range) for quantitative variables.

meters, higher levels of neutrophils increased the risk of a poor outcome [OR 1.05, 95% CI 1.00-1.10; *p*=0.043]; while higher levels of lymphocytes seemed to be associated with a better outcome [OR 0.94, 95% CI 0.88-0.99; *p*=0.043]. Consistently with CKD, higher levels of creatinine were associated with a higher risk of both adverse events [OR 6.20, 95% CI 2.16-17.81; *p*<0.001 and OR 19.90, 95% CI 5.07-78.06; *p*<0.001, respectively]. Higher levels of sodium (Na) were associated with a higher risk of adverse events [OR 1.15, 95% CI 1.03-1.28; *p*=0.014 and OR 1.14, 95% CI 1.01-1.27]. Similar findings were also observed for C-reactive protein (CRP) levels [OR 1.01, 95% CI 1.00-1.02; *p*=0.010 and OR 1.01, 95% CI 1.00-1.02; *p*=0.024] (Table V).

Conversely, being positive to IgM and IgG decreased the risk of adverse outcomes [IgM: OR 0.33, 95% CI 0.14-0.77; *p*=0.011 and OR 0.23, 95% CI 0.08-0.66; *p*=0.006. IgG: OR 0.30 95% CI 0.13-0.72; *p*=0.007 and OR 0.22 95% CI 0.07-0.66; *p*=0.007] (Figure 3).

Hence, a multivariable model was fitted to identify potential independent laboratory predictors of the composite outcome, with age, SpO_2, lymphocytes, neutrophils, creatinine, Na and CRP; laboratory parameters that showed an association with composite outcome. Among these selected parameters, the VIF method showed that lymphocytes and neutrophils were highly correlated with other variables. Since the focus of the study was on multiorgan dysfunction, only creatinine, sodium and CRP along to age and SpO_2 were included in model. Age, creatinine, and Na were confirmed as independent risk factors for the composite outcome, while SpO_2 revealed an independent predictor of a lower risk of adverse events (Table VI). Sodium's confidence interval is too wide, meaning that data could be influenced by anomalous values. Therefore, this independent variable was removed from the model. The final model confirms previous findings, and it can be considered still accurate (C-statistic=0.88; HL-test: *p*-value=0.73) (Table VII and Figure 4).

Association of Vitamin D with Adverse Events on Subpopulation of COVID-19 Patients

Among our study population, vitamin D was tested for 79 hospitalized COVID-19 patients. The median value of D25-OH and D1-25-OH dosage in this subpopulation were 15.0 (8.5-23.0)

Table V. Association of risk factors and laboratory markers with outcomes in COVID-19 patients.

	ICU admission + In-hospital mortality		In-hospital mortality	
Variable	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Demographic				
Age	1.06 (1.02-1.09)	0.003	1.10 (1.04-1.16)	0.001
Sex (male)	0.44 (1.01-2.34)	0.979	0.92 (0.34-2.50)	0.871
Respiratory Parameters				
SpO_2 (%)	0.75 (0.60-0.93)	0.009	0.76 (0.61-0.95)	0.014
FIO_2 (%)	1.01 (0.99-1.03)	0.252	1.01 (0.99-1.02)	0.516
Symptoms				
Dry cough	0.80 (0.29-2.25)	0.673	0.55 (0.17-1.79)	0.318
Arrhythmia	1.23 (0.31-4.91)	0.766	1.46 (0.36-5.90)	0.594
Headache	0.88 (0.18-4.31)	0.873	0.43 (0.05-3.54)	0.434
Dyspnea	1.50 (0.59-3.82)	0.393	0.96 (0.36-2.62)	0.941
Fatigue	1.58 (0.59-4.23)	0.360	0.89 (0.29-2.72)	0.843
Obnubilation	0.52 (0.06-4.36)	0.547	0.60 (0.07 -5.0)	0.633
Comorbidities				
COPD	2.54 (0.84-7.66)	0.098	1.11 (0.29-4.29)	0.877
Diabetes	2.60 (1.0-6.78)	0.050	1.72 (0.62-4.76)	0.295
Hypertension	0.50 (0.20-1.27)	0.145	0.39 (0.14-1.09)	0.072
Cardiovascular disease	2.33 (0.91-5.97)	0.077	1.91 (0.70-5.22)	0.210
Cerebrovascular disease	3.07 (1.10-8.61)	0.033	2.81 (0.97-8.21)	0.058
Neoplasia	1.46 (0.37-5.80)	0.590	1.69 (0.42-6.78)	0.458
Chronic kidney disease	4.19 (1.46-12.07)	0.008	3.78 (1.26-11.28)	0.017
Blood Tests				
Neutrophils	1.05 (1.00-1.10)	0.043	1.05 (0.99-1.11)	0.116
Lymphocytes	0.94 (0.88-0.99)	0.043	0.95 (0.88-1.02)	0.134
Platelets	1.00 (1.00-1.00)	0.133	1.00 (1.00-1.00)	0.185
Hemoglobin	1.00 (1.00-1.01)	0.359	1.00 (1.00-1.01)	0.349
Liver Function Markers				
ALT	1.00 (0.99-1.01)	0.747	0.99 (0.96-1.01)	0.270
AST	1.01 (1.00-1.02)	0.141	1.01 (1.00-1.02)	0.124
Direct bilirubin	0.001 (2e-10-4e+06)	0.792	2.3e-09 (4.1e-30-1.3e+12)	0.414
Indirect bilirubin	4.1e-08 (1.2e-23-1.4e+08)	0.351	6.0e-01 (9.0e-09- 4.1e+07)	0.956
Total bilirubin	7.0e-03 (2.8e-08-1.8e+04)	0.510	1.9e-02 (2.1e-09-1.8e+05)	0.629
Kidney Function Markers				
Albumin	0.95 (0.87-1.03)	0.218	0.96 (0.87-1.06)	0.417
Creatinine	6.20 (2.16-17.81)	<0.001	19.90 (5.07-78.06)	<0.001
Na	1.15 (1.03-1.28)	0.014	1.14 (1.01-1.27)	0.02
K	0.54 (0.23-1.25)	0.152	0.58 (0.21-1.50)	0.248
Cardiac Function Markers				
CPK	1.00 (1.00-1.00)	0.537	1.00 (1.00-1.00)	0.307
Hematological Markers				
D-dimer	1.00 (0.97-1.02)	0.726	0.99 (0.94-1.05)	0.748
INR	2.36 (0.52-10.64)	0.263	3.33 (0.68-16.32)	0.138
LDH	1.00 (1.00-1.00)	0.197	1.00 (1.00-1.00)	0.498
Inflammation Markers				
CRP	1.01 (1.00-1.02)	0.010	1.01 (1.00-1.02)	0.024

OR, odds ratio. The *p*-values are derived from the multivariable logistic regression model. All *p*-values <0.05 are considered as considered as statistically significant.

and 87.3 (58.6-122.2). Furthermore, we observed that higher levels of vitamin D25-OH and D1-25-OH, respectively, reduced the risk of in-hospital mortality [OR 0.63 95% CI 0.44-0.92; p=0.002 and OR 0.95 95% CI 0.92-0.99; p=0.008].

Discussion

Severe COVID-19 is characterized by an uncontrolled and high release of pro-inflammatory cytokines, that increases the levels of inflamma-

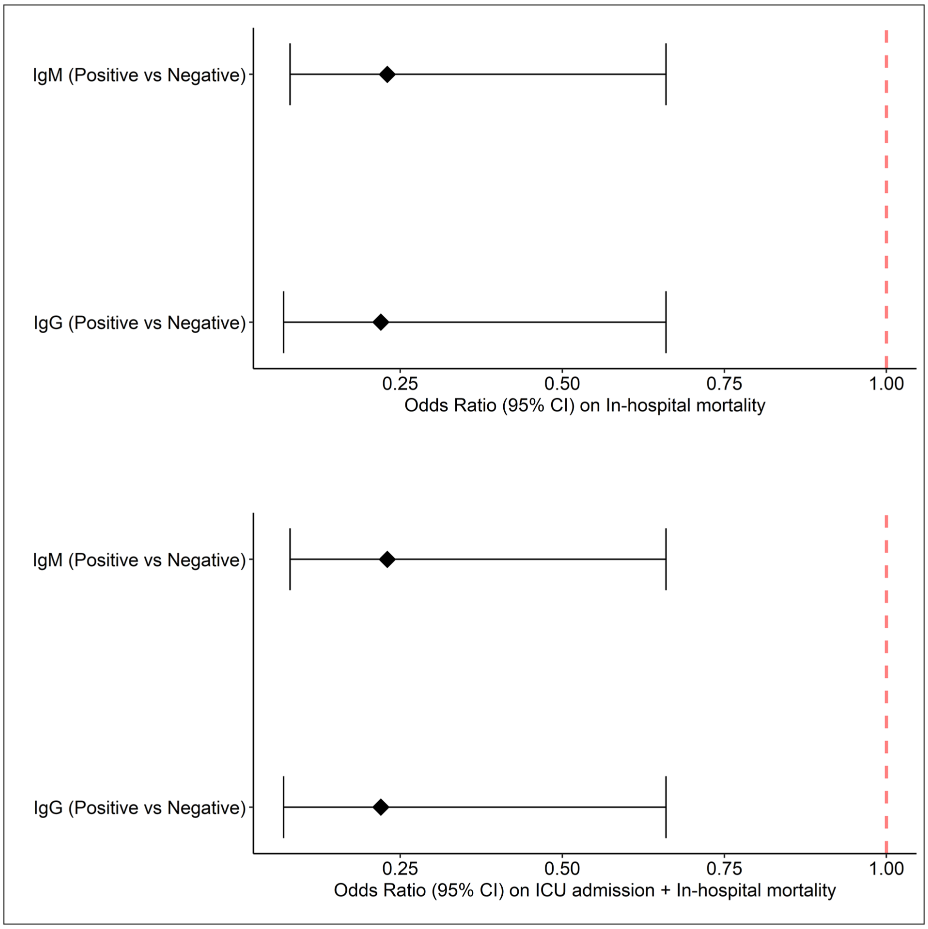

Figure 3. ORs of IgM and IgG on poor outcome and in-hospital mortality are presented. Red dashed line marks non-significance threshold. Values before the threshold means that the considered level factor decreases the risk of verify the event compared to the reference level. Therefore, being positive to IgM and IgG are protection factor for both outcomes. All ORs come from univariate logistic regression.

tory mediators, endothelial dysfunction, and coagulation abnormalities[24,25]. Indeed, the infiltration of inflammatory cells into the organs contributes to COVID-19 pathogenesis[26]. Older individuals with multimorbidity are more susceptible to developing a severe form of COVID-19 than either younger individuals or those without multimorbidity[27,28].

The mortality rate of COVID-19 is influenced by the involvement of different organs. Yildiz et al[29] conducted a study with 360 cases, revealing that non-survivors had significantly higher occurrences of chronic obstructive pulmonary disease (COPD). Similarly, in an article by Mayet et al[30], it was found that acute respiratory failure (ARF) contributed to increased mortality in ICU patients experiencing respiratory distress and shock. Another meta-analysis by Hansrivijit et al[31], involving 26 studies and 5,497 patients, indicated a 13-fold elevated risk of mortality associated with ARF. Thromboembolism and renal microangiopathy were considered potential mechanisms leading to ARF in COVID-19 patients[32,33].

Table VI. Multivariable regression model with previous statistically significant identified laboratory markers on composite outcome.

	ICU admission + In-hospital mortality	
Variable	**OR (95% CI)**	***p*-value**
Age	1.08 (1.02-1.41)	0.013
SpO_2	0.70 (0.49-0.94)	0.024
Creatinine	5.70 (1.67-32.7)	0.025
Na	1.19 (1.02-1.41)	0.032
CRP	1.01 (0.99-1.02)	0.183

OR, odds ratio. The *p*-values are derived from multivariable logistic regression model. All *p*-values <0.05 are considered as considered as statistically significant.

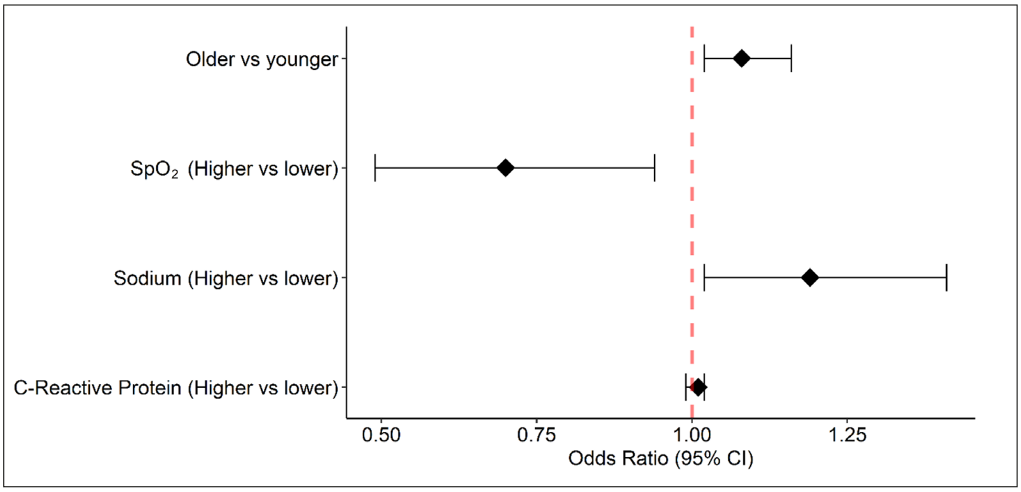

Figure 4. ORs from multivariate logistic regression on poor outcome are presented. Red dashed line marks non-significance threshold. Values before the threshold means that the considered level factor decreases the risk of verify the event compared to the reference level, whereas values after the threshold increase the risk of verify the event compared to the reference level. Therefore, older age, high levels of sodium can be considered as risk factor to poor outcome, whereas higher levels of SpO_2 can be considered as protection factor.

In COVID-19, the inflammatory response has been linked to several biomarkers, that play important roles in understanding the inflammatory processes and potential outcomes in COVID-19 patients. Monitoring and interpreting these markers can aid in the assessment and management of the disease; in fact, although good knowledge has been gained on clinical features of SARS-CoV-2 infection, less clear information has been provided on laboratory abnormalities.

In this study, our primary objective was to investigate any potential correlation between laboratory biomarkers and the severity of the disease. Additionally, we aimed to identify any specific biomarkers that could serve as potential risk factors for predicting disease progression and severity. By examining a range of laboratory parameters and their associations with disease outcomes, we sought to enhance our understanding of how these biomarkers could aid in early prediction and better management of disease progression in affected individuals.

For this study, a multivariable model was fitted to identify potential independent laboratory predictors of the composite outcome, with age, SpO_2, creatinine, Na and CRP; laboratory parameters that showed an association with the composite outcome. Age, creatinine, and Na were confirmed as independent risk factors for the composite outcome, while SpO_2 revealed an independent predictor of a lower risk of adverse events. The model can be considered accurate according to Hosmer-Lemeshow test and C-statistic [$p>0.73$, C-stat=0.88].

The findings of this investigation validate the unfavorable predictive significance of advanced age in individuals hospitalized due to COVID-19. Moreover, the study also revealed that the levels of nucleocapsid protein-targeted IgG against SARS-CoV-2 detected upon admission offer protection against mortality, regardless of the patient's age.

Positivity for both IgM and IgG antibodies against the virus appears to decrease the risk of adverse outcomes. The presence of these antibodies indicates that the individual's immune system has mounted a response to the infection, which could potentially offer pro-

Table VII. Multivariable regression model with previous statistically significant identified laboratory markers on composite outcome.

	ICU admission + In-hospital mortality	
Variable	**OR (95% CI)**	***p*-value**
Age	1.09 (1.04-1.16)	0.002
SpO*2*	0.77 (0.58-0.99)	0.048
Na	1.24 (1.08-1.45)	0.004
CRP	1.01 (1.00-1.02)	0.006

OR, odds ratio. The *p*-values are derived from multivariable logistic regression model. All *p*-values <0.05 are considered as considered as statistically significant.

tection against severe disease outcomes. This suggests that individuals who have developed a detectable immune response through IgM and IgG antibodies may have a better chance of experiencing milder symptoms and a lower risk of severe complications or adverse outcomes compared to those who do not show such antibody positivity.

Limitations

This study has several limitations that should be acknowledged. Firstly, its retrospective design may introduce biases due to the exclusion of patients with missing values, potentially affecting the study's results. Secondly, since the data were collected from a single center, the generalizability of the findings to other populations or settings may be limited. Thirdly, the lack of information about the patients' medication history, such as prior antibiotic use that could influence some laboratory biomarkers levels, might impact the interpretation of the results. Fourthly, there was inadequate data on the prevalence of bacterial co-infection in each group, which could have an impact on the study's conclusions. Lastly, the absence of regular monitoring and comparison of biomarker levels during the disease limits the understanding of how some biochemical values change over time in relation to clinical outcomes.

Conclusions

Our findings confirm that COVID-19 is a multiorgan disease. In fact, the analysis of laboratory parameters has revealed a strong relationship between poorer outcomes and multiple organ dysfunction, particularly established by higher levels of neutrophils, creatinine, sodium, and CRP. Alongside, cerebrovascular diseases, chronic kidney disease, and older age supported this finding. Of note, instead, higher levels of SpO_2, and lymphocytes, as well as positivity to IgM and IgG, were associated with a lower risk of a poor outcome. Taken together, in this study were presented laboratory data helpful to provide a valuable set of tools indispensable for better understanding this specific viral infection that is fundamentally different, and markedly more complex, than all the infectious diseases that we have studied to date, and which may also cause other organ involvement in the long term.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Conceptualization, A.B., S.C., and M.D.D.; methodology, E.S., G.T., A.S, D.D.V. and A.B.; software, A.S., F.S. and A.B.; validation, A.S., E.S., and A.B.; formal analysis, A.B., E.S., S.C. and A.S.; investigation, A.B., S.C. and D.D.V.; data curation, A.S., and G.T.; bibliographic research, A.S., A.B., F.S. and M.D.; writing-original draft preparation, A.B., A.S., and S.C.; writing-review and editing, A.B., S.C. and E.S.; visualization, F.C.S., M.D. and L.L.M.; statistical analysis: A.S.; critical revision of the manuscript for important intellectual content, M.D.D.; G.T., S.C., and A.B.; supervision, M.D.D., L.L.M., S.C., and A.B.; project administration M.D.D., E.S., and S.C. Finally, Andrea Ballini and Stefania Cantore equally contributed as co-first authors. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by Regione Campania - POR FESR CAMPANIA 2014-2020 - OS 1.3 - AZIONE 1.3.1 AVVISO PUBBLICO DI CUI AL DD n.19/2022 DG 50.04 – PROGETTO: "Analisi molecolare e tipizzazione linfocitaria finalizzata al monitoraggio della memoria immunitaria nell'infezione da SARS-CoV-2", UNICAMPANIA - CUP B63C22001210007 - SURF 21058BP000000020 - P.I: Prof. Marina Di Domenico.

Ethics Approval

1-) Azienda Opedaliero-Universitaria "Luigi Vanvitelli"-A-ORN "Ospedale dei Colli"- Local Ethic Committees approval: Prot. N 0013965i/ (11 May 2023), "Analisi quantitativa e qualitativa delle Vescicole Extra-cellulari in soggetti ospedalizzati con infezione da SARS-CoV-2"; PROT.: ESOCOV"; 2-) Azienda Opedaliero-Universitaria "Consorziale Policlinico"- Independent Local Etic Committees approval: Prot. N 009921/21/12/20 Study number: 6665 (16 December 2020), "Studio sierologico ed immunologico di pazienti di età adulti affetti da COVID-19: la chiave per comprendere la suscettibilità al SARS-CoV-2".

Informed Consent

The study has a retrospective cohort character, so the patient's written consent was not required, as the data were obtained from the computer register of the laboratories. Epidemiological information such as age, sex and diagnosis were also assessed. Identification data of the patients, such as names, ID numbers or contact information, were not collected for the present study. The study was conducted in accordance with the Declaration of Helsinki.

Data Availability

All data were contained within the manuscript.

ORCID ID

Andrea Ballini: 0000-0001-8758-1415
Stefania Cantore: 0000-0001-9521-2521
Giuseppe Troiano: 0000-0001-5647-4414
Mario Dioguardi: 0000-0003-0949-3457
Francesca Spirito: 0000-0002-8364-2425
Lorenzo Lo Muzio: 0000-0003-4633-4893
Marina Di Domenico: 0000-0002-6201-4200
Annafrancesca Smimmo: 0009-0002-3877-8398
Ferdinando Sasso: 0000-0002-9142-7848
Danila De Vito: 0000-0002-4816-4456
Enrica Serretiello: 0000-0001-6556-0760

References

1) WHO. Available at: www.who.int/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic (last accessed 10 June 2023).
2) Çil E, Sezgin Sayiner H. Length of hospital stay and risk factors associated with prognosis in COVID-19 patients: surprising results. Eur Rev Med Pharmacol Sci 2022; 26: 5268-5277.
3) WHO Coronavirus (COVID-19) Dashboard. Available at: https://covid19.who.int/ (last accessed 13 July 2023).
4) Moreno-Torres V, Muñoz-Serrano A, Calderón-Parra J, Mills-Sánchez P, Pintos-Pascual I, Rodríguez-Olleros C, Ibánez-Estéllez F, Tung-Chen Y, Ramos-Martínez A, Vargas-Núñez JA, Cuervas-Mons PV, de Mendoza C. Mortality by COVID-19 Before Vaccination - One Year Experience of Hospitalized Patients in Madrid. Int J Infect Dis 2022; 116: 339-343.
5) Corapli G, Çil E, Tutak AŞ, Corapli M. Effect of first application laboratory values on the prognosis of COVID-19 patients hospitalized in the intensive care unit. Eur Rev Med Pharmacol Sci 2022; 26: 3361-3366.
6) COVID-19 Excess Mortality Collaborators. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020-21. Lancet 2022; 399: 1513-1536.
7) Hayat AA, Meny A, Hamdan QU, Sami W, Albadrani G. Seeing the unforeseen: an insight into kingdom-wide psychological impact of COVID-19 pandemic. Eur Rev Med Pharmacol Sci 2022; 26: 5956-5962.
8) Coronavirus disease (COVID-19): Vaccines and vaccine safety. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/question-and-answers-hub/q-a-detail/coronavirus-disease-(covid-19)-vaccines?adgroupsurvey={adgroupsurvey}&gclid=CjwKCAjwp6CkBhB_EiwAlQVyxZSnSzdUDFei-ICBRYUW8xqriee4rRIMC0NXSM6WZ18kWNzNqjE32hoCouMQAvD_BwE (last accessed 16 July 2023).
9) Fodor E, Olmos Calvo I, Kuten-Pella O, Hamar E, Bukva M, Madár Á, Hornyák I, Hinsenkamp A, Hetényi R, Földes F, Brigitta Z, Jakab F, Kemenesi G, Lacza Z. Comparison of immune activation of the COVID vaccines: ChAdOx1, BNT162b2, mRNA-1273, BBIBP-CorV, and Gam-COVID-Vac from serological human samples in Hungary showed higher protection after mRNA-based immunization. Eur Rev Med Pharmacol Sci 2022; 26: 5297-5306.
10) Fisher KA, Nguyen N, Fouayzi H, Crawford S, Singh S, Dong M, Wittenberg R, Mazor KM. From COVID-19 Vaccine Hesitancy to Vaccine Acceptance: Results of a Longitudinal Survey. Public Health Rep 2023; 138: 681-690.
11) Dellino M, Lamanna B, Vinciguerra M, Tafuri S, Stefanizzi P, Malvasi A, Di Vagno G, Cormio G, Loizzi V, Cazzato G, Tinelli R, Cicinelli E, Pinto V, Daniele A, Maiorano E, Resta L, De Vito D, Scacco S, Cascardi E. SARS-CoV-2 Vaccines and Adverse Effects in Gynecology and Obstetrics: The First Italian Retrospective Study. Int J Environ Res Public Health 2022; 19: 13167.
12) Spirito F, Guida A, Caponio VCA, Lo Muzio L. Monkeypox: A New Challenge for Global Health System? Life (Basel) 2023; 13: 1250.
13) Charitos IA, Ballini A, Lovero R, Castellaneta F, Colella M, Scacco S, Cantore S, Arrigoni R, Mastrangelo F, Dioguardi M. Update on COVID-19 and Effectiveness of a Vaccination Campaign in a Global Context. Int J Environ Res Public Health 2022; 19: 10712.
14) Lleal M, Corral-Vazquez C, Baré M, Comet R, Herranz S, Baigorri F, Gimeno-Miguel A, Raurich M, Fortià C, Navarro M, Poblador-Plou B, Baré M. Multimorbidity patterns in COVID-19 patients and their relationship with infection severity: MRisk-COVID study. PLoS One 2023; 18: e0290969.
15) Delerue Matos A, Fonseca de Paiva A, Cunha C, Voss G. Precautionary behaviours of individuals with multimorbidity during the COVID-19 pandemic. Eur J Ageing 2022; 19: 827-835.
16) Rezoagli E, McNicholas BA, Madotto F, Pham T, Bellani G, Laffey JG; LUNG SAFE Investigators, the ESICM Trials Group. Presence of comorbidities alters management and worsens outcome of patients with acute respiratory distress syndrome: insights from the LUNG SAFE study. Ann Intensive Care 2022; 12: 42.
17) Fekadu G, Bekele F, Tolossa T, Fetensa G, Turi E, Getachew M, Abdisa E, Assefa L, Afeta M, Demisew W, Dugassa D, Diriba DC, Labata BG. Impact of COVID-19 pandemic on chronic diseases care follow-up and current perspectives in low resource settings: a narrative review. Int J Physiol Pathophysiol Pharmacol 2021; 13: 86-93.

18) Ramos-Casals M, Brito-Zerón P, Mariette X. Systemic and organ-specific immune-related manifestations of COVID-19. Nat Rev Rheumatol 2021; 17: 315-332.
19) Mokhtari T, Hassani F, Ghaffari N, Ebrahimi B, Yarahmadi A, Hassanzadeh G. COVID-19 and multiorgan failure: A narrative review on potential mechanisms. J Mol Histol 2020; 51: 613-628.
20) Paidas MJ, Sampath N, Schindler EA, Cosio DS, Ndubizu CO, Shamaladevi N, Kwal J, Rodriguez S, Ahmad A, Kenyon NS, Jayakumar AR. Mechanism of Multi-Organ Injury in Experimental COVID-19 and Its Inhibition by a Small Molecule Peptide. Front Pharmacol 2022; 13: 864798.
21) Tripepi G, Jager KJ, Dekker FW, Zoccali C. Linear and logistic regression analysis. Kidney Int 2008; 73: 806-810.
22) Fagerland MW, Hosmer DW. A Generalized Hosmer–Lemeshow Goodness-of-Fit Test for Multinomial Logistic Regression Models. Stata J 2012; 12: 447-453.
23) Miles J. Tolerance and variance inflation factor: Wiley Stats Ref: Statistics Reference Online; 2014. Available at: https://doi.org/10.1002/9781118445112.stat06593 (last access 16 July 2023).
24) Cazzolla AP, Lovero R, Spirito F, Di Cosola M, Santacroce L, Lo Muzio E, Ciavarella D, Dioguardi M, Crincoli V, Pepe M, Varraso L, Contino R, Di Serio F, Lo Muzio L. Evaluation of qualitative and quantitative taste alterations in COVID-19. Biomol Biomed 2023; 23: 344-350.
25) Zhurakivska K, Troiano G, Pannone G, Caponio VCA, Lo Muzio L. An Overview of the Temporal Shedding of SARS-CoV-2 RNA in Clinical Specimens. Front Public Health 2020; 8: 487.
26) Spirito F, Amato A, Scelza G, Pisano M, Caponio VCA, Martina S. Education during the COVID-19 pandemic: the perception of Italian dental and medical students. Minerva Dent Oral Sci 2022; 71: 277-286.
27) Pannone G, Caponio VCA, De Stefano IS, De Stefano IS, Ramunno MA, Meccariello M, Agostinone A, Pedicillo MC, Troiano G, Zhurakivska K, Cassano T, Bizzoca ME, Papagerakis S, Buonaguro FM, Advani S, Muzio LL. Lung histopathological findings in COVID-19 disease - a systematic review. Infect Agent Cancer 2021; 16: 34.
28) Caponio VCA, Lipsi MR, Fortunato F, Arena F, Lo Muzio L. Symptomatic SARS-CoV-2 Infection with Ageusia after Two mRNA Vaccine Doses. Int J Environ Res Public Health 2022; 19: 886.
29) Yıldız M, Uzel Şener M, Kavurgacı S, Özturk A, Öztürk FE, Çiçek T. The role of Neutrophil/Lymphocyte Ratio in Determining COVID-19 Mortality. J Izmir Chest Hospital 2021; 36: 7-13.
30) Mayet V, Mousseaux C, Petit-Hoang C, Ouali N, Rafat C, Mesnard L, Luque Y, Rondeau E, Buob D, François H. COVID-19-associated acute kidney injury: after the tubule and the glomerulus, now the vessel? Clin Kidney J 2020; 13: 1105-1106.
31) Ercan Z, Evren Öztop K, Pınar M, Varim C, Dheir H, Karacaer C, Yaylaci S, Bilal Genç A, Çekiç D, Nalbant A, Cihad Genç A. The aggregate index of systemic inflammation may predict mortality in COVID-19 patients with chronic renal failure. Eur Rev Med Pharmacol Sci 2023; 27: 3747-3752.
32) Renberg M, Jonmarker O, Kilhamn N, Rimes-Stigare C, Bell M, Hertzberg D. Renal resistive index is associated with acute kidney injury in COVID-19 patients treated in the intensive care unit. Ultrasound J 2021; 13: 3.
33) Kutlusoy S, Koca E. Routine laboratory parameters in estimating mortality and morbidity in COVID-19 diagnosed cases followed in the intensive care unit. Eur Rev Med Pharmacol Sci 2023; 27: 5885-5892.