New Microbiologica, 46, 2, 146-153, 2023, ISSN 1121-7138

FULL PAPER

Evaluation of laboratory parameters in 1587 COVID-19 patients admitted to metropolitan hospital area of Bologna, Italy

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SUMMARY

Since the outbreak of the 2019 pandemic coronavirus disease (COVID-19), great attention has been given to identifying the main clinical features of the disease. Identification of laboratory parameters able to classify patients based on their risk is mandatory to improve their clinical management. We retrospectively evaluated twenty-six laboratory tests measured in COVID-19 positive patients admitted to the hospital in March and April 2020 to find any correlation between their changes and the risk of death. We divided them into surviving and non-surviving patients. A total of 1587 patients were recruited, 854 males with median age of 71 (IQR 56-81) and 733 females with median age of 77 (IQR 61-87). On admission, death was found to be positively correlated with age (p=0.001), but not with sex (p=0.640) or with hospitalization in days (p=0.827). Brain natriuretic peptide (BNP), creatinine, C-reactive protein (CRP), INR, leukocyte count, lymphocyte count, neutrophil count, and procalcitonin (PCT) demonstrated a statistically significant difference between the two groups (p<0.001), suggesting their role as markers of disease severity; only lymphocyte count resulted as an independent risk factor for death.

Received September 20, 2022 *Accepted* February 18, 2023

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a systemic infection caused by a virus belonging to the Coronaviridae family (SARS-CoV-2) that mainly affects the respiratory apparatus. It first appeared in December 2019 in the city of Wuhan in China and rapidly spread around the world, evolving into a pandemic (Rota *et al.*, 2003). According to recent statistics from the World Health Organization (WHO) the disease affected all continents, with 624,748,971 confirmed cases and 6,560,620 confirmed deaths as of 26 October 2022 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports).

Although the clinical characteristics of the infection have been widely described and are therefore well defined and recognizable (Bohn *et al.*, 2020), it is important to find routinely analysed laboratory tests that, through their changes, may help to identify patients who will undergo rapid disease progression to severe complications and death and guarantee prompt treatment (Lippi *et al.*, 2020 b).

Inflammatory biomarkers

One of the main characteristics in critical COVID-19 patients is the development of clinical manifestations typical of septic shock (Ponti *et al.*, 2020; Li *et al.*, 2020), such as a huge increase in proinflammatory cytokines concentration, especially interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) (Henry *et al.*, 2020; Mehta *et al.*, 2020). Since a cytokine assay is not routinely required or is not always available in all diagnostic laboratories, some surrogate biochemical markers of inflammation, such as ferritin and C-reactive protein (CRP), may be helpful in assessing the severity of the disease (Qin *et al.*, 2020). Procalcitonin (PCT) can also be considered a useful inflammatory marker because its increase may indicate the onset of bacterial co- or super-infection in critically ill patients (Lippi *et al.*, 2020 c). In addition to the common inflammation biomarkers, it has also been suggested that lymphopenia and the high ratio between neutrophils and lymphocytes (NLR) (Shang *et al.*, 2020; Simadibrata *et al.*, 2021) have a potential prognostic value of poor prognosis. Likewise, an increase in D-dimer is constantly reported among the coagulation tests and is associated with worsening of the dis-

Key words:
COVID-19, laboratory biomarkers, prognosis.

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ease and with an increased risk of thromboembolic events development in COVID-19 patients (Li *et al.*, 2020; Terpos *et al.*, 2020; Henry *et al.*, 2020).

Cardiac biomarkers

Cardiovascular involvement in patients with severe COVID-19 infection is common due to profound systemic inflammation and/or microvascular dysfunction. Given that cardiac involvement has been associated with worse prognosis, ultrasound cardiac examinations and laboratory tests such as cardiac troponin and natriuretic peptides may be useful during the progression of disease to adequately stratify the risk of some patients (Lippi *et al.*, 2020 b; Mehta *et al.*, 2020; Lippi *et al.*, 2020 a; Huang *et al.*, 2020; Wang *et al.*, 2020; Guo *et al.*, 2020; Schilling *et al.*, 2020).

Hepatic biomarkers

Hepatic dysfunction is a common feature in most patients affected by a severe form of COVID-19, showing an increase in both liver and mixed type enzymes, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) (Huang *et al.*, 2020; Wang *et al.*, 2020; Guo *et al.*, 2020; Chen *et al.*, 2020; Guan *et al.*, 2020; Yang *et al.*, 2020; Fan *et al.*, 2020), probably due to immune-mediated damage due to severe inflammatory response following infection, direct cytotoxicity due to active viral replication in ACE2-expressing biliary epithelial cells, damage due to drug-induced liver disease (Sun *et al.*, 2020). Laboratory tests aimed at evaluating liver function should therefore be monitored routinely in COVID-19 patients, particularly those who receive antiviral treatment.

Renal function markers

Literature data concerning the SARS (Severe Acute Respiratory Syndrome) epidemic, which occurred from 2002 to 2003, report that 6.7% of affected patients developed acute renal impairment and that the mortality of patients with acute kidney injury (AKI) was 91.7% (Chu *et al.*, 2005). Given the homology between the causative viruses, the evaluation of renal dysfunction in COVID-19 patients seems to be important. The data available today suggest that the prevalence of AKI is relatively low, but variable in COVID-19 patients, ranging from 0.5 to 19.1% (Huang et al., 2020; Wang *et al.*, 2020; Sun *et al.*, 2020; Chu *et al.*, 2005).

In a recent study by Cheng *et al.*, 2020, patients with elevated baseline serum creatinine had more likelihood of being admitted to the ICU and of undergoing mechanical ventilation, suggesting that early identification and treatment of kidney damage can be vital in the care of COVID-19 patients. The pathophysiological mechanisms underlying renal dysfunction of these patients are unknown and probably have multifactorial origin (i.e., intrarenal inflammation and damage due to inflammatory response to infection by cytokines, or direct cytopathic effects on renal tissue) (Ronco *et al.*, 2020).

This retrospective observational study was conducted on patients who were consecutively hospitalized with a confirmed diagnosis of SARS-CoV-2 virus infection at the departments of the Metropolitan Hospital Area of Bologna in March and April 2020. The inclusion criteria were having a SARS-CoV-2 RNA swab positive at Hospital access and an age ≥18 years.

Our aim was to evaluate the possible correlation between plasma levels of twenty-six inflammation and organ function laboratory biomarkers routinely tested and the patient's outcome (i.e., mortality, hospital stays), to contribute to the management and risk stratification of patients.

Ethical aspects

Being a retrospective study, it was difficult to obtain an informed consent from enrolled patients, but given the importance of this project for public health, this research was conducted in the context of the authorizations guaranteed by the 89th Article of the General Data Protection Regulation 2016/679 (GDPR), which is a regulation on data protection and privacy in the European Union (EU) and the European Economic Area (EEA). Data were collected anonymously on an encrypted server and analysed by the scientific coordinator of the project.

The study was performed according to Declaration of Helsinki guidelines and was approved by the local ethics committee (CE AVEC n. 821/2020/OSS/AUSLBO).

MATERIAL AND METHODS

Patients

All patients admitted to the Metropolitan Hospital Area of Bologna, Italy, with a PCR-positive nasopharyngeal swab test for SARS-CoV-2 infection in March and April 2020 were retrospectively included in the study. Blood samples were drawn at admittance and during the remaining days of hospitalization for purposes of routine monitoring and treatment. For all enrolled patients, we compared the concentration of twenty-six routine laboratory tests measured at admittance (T0) and after 7 (T1), 14 (T2) and 21 (T3) days from T0.

Recruited patients were grouped according to whether they were hospitalized in the intensive care unit (ICU) or not during the hospitalization period, and we considered if they were discharged from the hospital or if they died during the hospital stay.

Clinical and laboratory data were retrieved from electronic health records. In our study, we investigated the following biomarkers: albumin (LoD 0.07 g/L, CV% <5.0%), alkaline phosphatase (ALP) (LoD <1 U/L, CV% <6.0%), ALT (LoD <5 U/L, CV% <5.0%), AST (LoD <5 U/L, CV% <5.0%), creatine kinase (CK) (LoD 3 U/L, CV% <5.0%), creatinine (LoD: 0.05 mg/dL, CV% <5.0%), CRP (LoD: 0.20 mg/dL, CV% <5.0%),

GGT (LoD <1 U/L, CV% <5.0%), glucose (LoD 0.72 mg/dL, CV% <3.0%), lactate dehydrogenase (LDH) (LoD: 3 U/L, CV% <5.0%), total amylase (LoD <10 U/L, CV%, <5.0%), total bilirubin (LoD <0.01 mg/dl, CV% <5.0%) and total protein (LoD <1.0 g/L, CV% <4.0%), which were routinely determined on serum sample by enzymatic or colorimetric method on an AU5800 or AU680 system (Beckman Coulter Ireland Inc., USA).
Brain natriuretic peptide (BNP) (CLIA method, LoD: 1 pg/mL, CV% <7.0%), ferritin (LoD: <0.2 ng/mL, CV% <7.0%), PCT (LoD: 0.01 ng/mL, CV% <6.0%), were determined on serum sample by the immunoturbidimetric method on UniCel DxI (Beckman Coulter Ireland Inc., USA).
IL-6 (LoD 2.00 pg/mL, CV% <5.0%) was determined on serum sample by an IMMULITE® 2000 immunoassay system (Medical System S.p.A).
IL-8 (CV% <5.0%) and TNF-α (CV% <5.0%) were determined by cytofluorimetric method on a BD FACSCanto™ II Flow Cytometry System.
D-dimer (immunoturbidimetric method, CV% <8.0%), Fibrinogen (coagulative method, CV% <5.0%) and Prothrombin time (PT) expressed as international normalized ratio (INR) (coagulative method, CV <5%), were tested on a Sysmex-CS 5100 (Siemens).
Leukocyte count (WBC), (CV% <3.0%), Neutrophil count (CV% <8.0%) and Lymphocyte count (CV% <8.0%) were measured on blood samples with a K3EDTA by Sysmex XN haematology analyser. NLR was calculated from neutrophil and lymphocyte count.

Statistical analysis

All statistical analyses were performed using R statistical software (Version 4.1.2). A Kolmogorov-Smirnov test of normality was performed for all variables. Then, to analyse the differences between groups of interest, statistical significance was performed using the Chi-square (χ^2) test for dichotomous variables and Wilcoxon-Mann-Whitney test for continuous variables or with the independent sample *t*-test (parametric). The overall test used was the non-parametric Friedman test or ANOVA (parametric alternative) for repeated measures and, where significant, the comparison between two results was evaluated using paired t test (parametric) or Wilcoxon test (non-parametric). The *p*-values were finally adjusted according to the Bonferroni correction.
Categorical variables were described as frequency and percentages, and continuous variables were described using median and interquartile range (IQR) values, as appropriate. A *P*-value of <0.05 was considered significant.

RESULTS

From 1st of March to 30th of April 2020, a total of 1611 patients were consecutively admitted to Metropolitan Hospital Area of Bologna, Italy, with suspected COVID-19 infection and, according to the inclusion criteria mentioned above, 1587 of them were retrospectively recruited for our study: 854 males (53.8%) with median age of 71 (IQR 56-81) and 733 females (46.2%) with median age of 77 (IQR 61-87). During the hospitalization period, 401/1587 patients died (220 males and 181 females) and 1186/1587 survived until hospital discharge (634 males and 552 females). The main characteristics of enrolled patients are reported in *Table 1*. Admission to ICU or other Critical Care Department during the hospitalization period was significantly higher in patients who did not survive (75.1%) than in survivors (17.0%), p<0.001, and the difference in percentage of surviving patients admitted to ICU was not statistically significant between females and males (p=0.879). Surviving patients were more numerous and younger than those who did not survive, either among male or female (p<0.001), while even though the median hospitalization in days was statistically longer in surviving women than in those who did not survive (p<0.001), it was not the same for men (p=0.121). The original diagnosis that led to the patients' hospitalization and co-morbidities affecting them are listed in *Table 1*: in most cases, percentage differences of these characteristics were statistically significant (p<0.001) between the two groups. Median values of age, hospitalization in days and routine haematological tests concentration at patient admission are reported in *Table 2* for survivors and non-survivors: age was significantly different between the two groups, as was length of hospital stay (p<0.001), while, among the haematological findings, BNP, creatinine, CRP, INR, leukocyte count, lymphocyte count, neutrophil count, NLR, phosphatase alkaline and PCT demonstrated a statistically significant difference by univariate analysis. In fact, when compared to survivors, patients who did not survive presented, at hospital admission, a significantly higher percentage (p<0.05) of serum creatinine concentration >1.20 mg/dL (46% vs 13%), of INR >1.20 (38% *vs* 13%), of neutrophilia (neutrophil count >7.7 x 10^9/L, 61% *vs* 12%), of leukocyte count >10.50x10^9/L (29% *vs* 9%), of BNP >100 pg/mL (74% *vs* 1%), of CRP >0.5 mg/dL (98% vs 90%), of PCT >0.5 ng/mL (44% vs 11%), and lymphopenia (<1.1x10^9/L, 69% *vs* 52%). A multivariate analysis was then performed to find a possible correlation between risk of death and the biomarkers that resulted significant by univariate analysis, specifically: creatinine, fibrinogen, WBC, IL-6, INR, lymphocyte count, neutrophil count, NLR, CRP and PCT. By multivariate analysis, death was found to be positively correlated, on admission, with creatinine (OR 3.77, 95% CI 1.49 to 9.53, p=0.005), leukocyte count (OR 1.12, 95% CI 1.01 to 1.24, p=0.033) lymphocyte count (OR 0.10, 95% CI 0.10 to 0.60, p=0.027) and PCT, (OR 2.86, 95% CI 1.17 to 7.01, p=0.022) with multiple R-squared: 0.9846, adjusted R-squared: 0.9654, p-value: <0.001.

Table 1 - *Characteristics of enrolled patients.*

Characteristics	*Surviving patients*	*Non-surviving patients*	*p-value*[a]
n (%):	1186 (74.7%)	401 (25.3%)	**<0.001**
– Women (%)	552 (46.6%)	181 (45.1%)	
– Men (%)	634 (53.4%)	220 (54.9%)	0.667
– Women age, years (median, IQR)	71 (58, 84)	88 (80, 92)	**<0.001**
– Male age, years (median, IQR)	65 (52, 76)	81 (75, 88)	**<0.001**
– Hospitalization time, in days	11 (6-22)	8 (4-16)	**<0.001**
– Hospitalization time, in days, for women (median, IQR)	11 (6, 24)	8 (3, 15)	**<0.001**
– Hospitalization time, in days, for men (median, IQR)	10 (6, 22)	10 (4, 18)	0.121
Number of patients admitted to ICU during the hospitalization period (%):	212 (17.0%)	302 (75.1%)	**<0.001**
– Women (%)	68 (32.1%)	100 (33.1%)	
– Men (%)	144 (67.9%)	202 (66.9%)	0.879
– Women age, years (median, IQR)	68 (61, 73)	69 (62, 77)	0.251
– Male age, years (median, IQR)	64 (54, 73)	67 (56, 76)	**0.031**
– Hospitalization time, in days, for women (median, IQR)	8 (3, 15)	11 (6, 24)	0.104
– Hospitalization time, in days, for men (median, IQR)	15 (8, 31)	16 (10, 26)	0.046
Original diagnosis that led to hospitalization (%):			
– Simple pneumonia (radiographically confirmed) and pleurisy with complications	530 (44.7%)	175 (43.6%)	**<0.001**
– Pulmonary edema and respiratory failure	136 (11.5%)	90 (22.4%)	**<0.001**
– Simple pneumonia and pleurisy without complications	346 (29.3%)	36 (9.0%)	**<0.001**
– Tracheotomy with mechanical ventilation	26 (2.2%)	36 (9.0%)	**<0.001**
– Assisted breathing	30 (2.5%)	26 (6.5%)	**<0.001**
others:			
Non-SARS-CoV-2 viral infections	45 (3.8%)	12 (3.0%)	**<0.001**
Cardiovascular diseases	13 (1.1%)	6 (1.5%)	**<0.001**
Malignancy	2 (0.2%)	2 (0.5%)	**<0.001**
Haematological disease	3 (0.2%)	-	-
Cerebrovascular disease	3 (0.2%)	2 (0.5%)	**<0.001**
Kidney disease	10 (0.8%)	3 (0.7%)	**<0.001**
Gastrointestinal disease	4 (0.3%)	1 (0.3%)	**<0.001**
Respiratory disease	8 (0.7%)	–	
Infection	3 (0.2%)	1 (0.3%)	**<0.001**
Surgical intervention	20 (1.7%)	3 (0.8%)	**<0.001**
Childbirth	3 (0.2%)	–	–
Complications after surgical intervention	2 (0.2%)	–	–
Sepsis	2 (0.2%)	7 (1.7%)	**<0.001**
Mental disease	–	1 (0.2%)	–

Data are reported as median (interquartile range, IQR) and percentages. ICU, intensive care unit. [a]*p*-value comparing survived and not-survived patients result from χ^2 test, or Wilcoxon-Mann-Whitney U test. $p<0.05$ was considered statistically significant.

Table 2 - *Characteristics and haematological findings of patients with COVID-19 at admission divided into surviving and non-surviving.*

		Surviving patients, n= 1186	*Non-surviving patients, n=401*	*p-value*[a]
Age, years		67 (55-80)	73 (59-84)	**<0.001**
Duration of hospitalization, days		11 (6-22)	8 (4-16)	**<0.001**
Biomarker (unit)	*Reference range*	*Median (IQR)*	*Median (IQR)*	
Albumin (g/L)	35-52	33.7 (27.9, 37.4)	32.9 (29.4, 35.9)	0.639
ALT (U/L)	Female: < 35 Male: < 50	25 (16, 39)	20 (15, 36)	0.271
AST (U/L)	Female: < 35 Male: < 50	32 (22,45)	28 (23,51)	0.764
BNP (pg/mL)	<100	81 (35,149)	188 (138,378)	**0.037**
CRP (mg/dL)	<0.50	5.31 (1.95, 11.20)	9.88 (6.60, 14.80)	**0.006**
Creatinine (mg/dL)	0.50-1.20	0.88 (0.72, 1.03)	1.01 (0.77, 1.29)	**0.030**
D-dimer (mg/L FEU)	<0.55	0.84 (0.44, 1.34)	0.53 (0.41, 1.00)	0.294
Ferritin (ng/mL)	Female: 11-306 Male: 24-336	353 (159, 728)	309 (197, 1054)	0.518

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		Surviving patients, n= 1186	*Non-surviving patients, n=401*	*p-value*[a]
Fibrinogen (mg/dL)	150-400	473 (364, 583)	444 (356, 559)	0.637
GGT (U/L)	Female: < 38 Male: < 55	44 (33, 74)	48 (39, 67)	0.689
Glucose (mg/dL)	60-110	107 (93, 133)	128 (96, 166)	0.149
IL-6 (pg/mL)	<5.9	26.7(12.9, 52.9)	51.4 (24.0, 115.0)	0.158
IL-8 (pg/mL)	<70	19 (10,35)	87 (48, 161)	0.248
INR (pg/mL)	<1.20	1.10 (1.03, 1.14)	1.11 (1.04, 1.24)	**0.035**
LDH (U/L)	<248	290 (230, 379)	291 (228, 513)	0.259
Lymphocyte (x10^9/L)	1.10-4.00	1.10 (0.86, 1.48)	0.83 (0.58,1.26)	**0.005**
Neutrophil count (x10^9/L)	1.50-7.70	3.90 (3.16, 5.61)	5.22 (3.65, 8.03)	**0.014**
NLR	na	3.50 (2.22, 5.89)	7.72 (4.15, 13.32)	**<0.001**
PCT (ng/mL)	low risk of sepsis: <0.5 moderate risk of sepsis: 0.5-<2.0 high risk of sepsis: 2.0-<10 likely severe sepsis: 10.0	0.1 (0.1,0.2)	0.2 (0.1, 0.6)	**<0.001**
Phosphatase alkaline (U/L)	30-120	76 (68, 80)	62 (51, 66)	**0.046**
TNF-α (pg/mL)	<8.1	9 (1, 77)	11 (5, 95)	0.619
Total amylase (U/L)	28-100	56 (43, 80)	69 (42, 97)	0.499
Total bilirubin (mg/dL)	<1.20	0.50 (0.47, 0.80)	0.62 (0.48, 0.94)	0.192
Total protein (g/dL)	6.6-8.3	6.2 (5.7 ,6.6)	6.1 (5.8, 6.4)	0.768
WBC (x10^9/L)	3.60-10.50	5.61 (4.59, 7.64)	6.82 (5.51, 9.92)	**0.023**

Data are reported as median (interquartile range, IQR) and percentages. [a]p-value comparing survived and not-survived patients result from χ^2 test, Fisher's exact test or Mann-Whitney U test. $p<0.05$ was considered statistically significant. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BNP, Brain natriuretic peptide; CK, creatine kinase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; IL, interleukin; INR, prothrombin time expressed as international normalized ratio; LDH, Lactate dehydrogenase; na, not applicable; NLR, neutrophils to lymphocytes ratio; PCT, Procalcitonin; TNF-α, tumour necrosis factor-α; na, not available; WBC, leukocyte count.

When major confounding factors such as age and gender were introduced in a multivariable adjusted analysis, death was found to be positively correlated, on admission, with age (OR 1.07, 95% CI 1.02 to 1.12, p=0.005), but not with sex (p=0.640) and with hospitalization in days (p=0.827), while, among all laboratory biomarkers tested, only lymphocyte count resulted as an independent risk factor for death (OR 0.15, 95% CI 0.03 to 0.69, p=0.015), with multiple R-squared: 0.8079, adjusted R-squared: 0.5773, p-value: 0.0279. Lymphopenia (<1.1 x10^9/L) at admission was associated with death compared with a lymphocyte count in the reference range (1.1-4.0×10^9/L) (OR 2.04, 95% CI 0.27 to 15.3, p 0.013).

After that, we used Friedman test for repeated measures with Bonferroni correction, and for each group of patients we compared serum biomarkers concentration measured at admission (T0) with those measured after 7 (T1), 14 (T2) and 21 (T3) days (data are reported in *Table 3*) to understand the dynamic profile of routine laboratory findings. In this way, we found that in surviving patients several parameters, specifically ALT, AST, CK, CRP, D-dimer, ferritin, fibrinogen, neutrophil count, NLR, total protein and WBC had statistically significant changes in their concentration during follow-up, while, in those who did not survive, only albumin, ALT, glucose, IL-6, PCT and WBC.

DISCUSSION

Since the beginning of the pandemic, several studies have been carried out to describe the main epidemiological, clinical, laboratory and radiological conditions that could be characteristics of COVID-19 patients, most of which concerned the Chinese population and with a limited number of subjects (Simadibrata *et al*., 2021; Huang *et al*., 2020; Wang *et al*., 2020; Chen *et al*., 2020; Guan *et al*., 2020; Ding *et al*., 2020; Ghahramani *et al*., 2020; Salinas *et al*., 2021). In our study we evaluated the prognostic value of twenty-six routine laboratory analyses measured at hospital admission on 1587 COVID-19-positive patients and explored their dynamic profile during hospitalization, in order to find any correlation between their changes and the patients' mortality. In our study non-surviving patients were significantly older than surviving patients, while hospitalization in days was longer in surviving individuals, even if this difference disappeared in males when patients were grouped by gender (*Table 1*). When compared to survivors, patients who did not survive presented, at hospital admission, a significantly higher percentage ($p<0.05$) of out of range parameters, such as serum creatinine concentration >1.20 mg/dL, INR >1.20, neutrophilia (neutrophil count >7.7x10^9/L), leukocyte count >10.50x10^9/L, BNP >100 pg/mL, CRP >0.5 mg/dL, PCT >0.5 ng/mL and lymphopenia (<1.1 x10^9/L),

in line with what is reported in other scientific papers (Ding *et al*., 2020; Ghahramani *et al*., 2020). During follow-up, the median value of lymphocyte count remained below the normal lower cut-off (<1.1x10^9/L) in non-surviving patients, while it reached normal values after 14 days in those who survived (*Table 3*).

The difference of ALP concentration between survivors and non-surviving patients was slightly statistically significant at hospital admission (p=0.046): 15% of patients who did not survive had pathological values of ALP (>120 U/L), while none showed alteration of ALP concentration among survivors.

Neutrophil count showed normal median values in surviving patients during follow-up, while neutrophilia was present from T2 to T3 in patients who did not survive. Finally, the median value of NLR was also statistically different between survivors and non-surviving individuals at T0, although no consensus cut-off has been established, to date, to determine normal and elevated NLR values, especially for COVID-19. A meta-analysis reported a range from 3.3 and 5.9 to predict severity and from 7.9 to 11.8 to predict mortality, but obviously it may vary from one population to another (Shang *et al*., 2020). Our study confirmed

Table 3 - *Comparison of laboratory findings during hospitalization period.*

Laboratory test (Unit)	*Surviving patients*				*p-value*	*Non surviving patients*				*p-value*
	T0 median (IQR) N=1186	*T1 median (IQR) N=309*	*T2 median (IQR) N=139*	*T3 median (IQR) N=77*		*T0 median (IQR) N= 401*	*T1 median (IQR) N=93*	*T2 Median (IQR) N=46*	*T3 median (IQR) N=27*	
Albumin (g/L)	33.7 (27.9, 37.4)	22.0 (11.0,39.0)	28.6 (25.8,31.1)	28.7 (27.5,32.1)	0.179	32.9 (29.4, 35.9)	24.1 (22.7,27.1)	25.2 (23.6,26.8)	25.5 (22.7,28.3)	**0.004**
ALT (U/L)	25 (16, 39)	51 (28,90)	54 (31,93)	42 (23,74)	**<0.001**	20 (15, 36)	38 (23, 73)	48 (30,76)	48 (32,118)	**0.013**
AST (U/L)	32 (22, 45)	50 (32,70)	34 (27,52)	na	**0.043**	28 (23, 51)	51 (34, 75)	42 (33,61)	na	0.149
BNP (pg/mL)	81 (35, 149)	na	na	na	–	188 (138, 378)	na	na	na	–
CK (U/L)	114 (51, 204)	50 (28, 127)	35 (19, 88)	39 (24, 61)	**<0.001**	54 (38, 114)	53 (31, 176)	93 (47, 189)	27 (18, 65)	0.112
Creatinine (mg/dL)	0.88 (0.72, 1.03)	0.76 (0.64, 0.97)	0.76 (0.62, 1.01)	0.84 (0.61, 1.07)	0.236	1.01 (0.77,1.29)	1.02 (0.72,1.46)	0.89 (0.66, 1.40)	0.80 (0.57, 1.20)	0.073
CRP (mg/dL)	5.31 (1.95, 11.20)	2.32 (0.83, 6.86)	0.93 (0.22, 3.31)	4.99 (0.27, 5.26)	**<0.001**	9.88 (6.60,14.80)	11.83 (2.85, 14.68)	12.23 (1.09, 13.32)	6.58 (1.69, 13.05)	0.172
D-dimer (FEU)	0.84 (0.44, 1.34)	1.29 (0.71, 2.95)	1.78 (0.79, 2.98)	1.47 (1.01, 2.26)	**0.008**	0.53 (0.41, 1.00)	2.67 (1.04, 6.85)	3.77 (2.30, 6.60)	2.47 (1.08, 3.26)	0.308
Ferritin (ng/mL)	353 (159, 728)	496 (306, 879)	346 (174, 550)	NA	**0.004**	309 (197, 1054)	342 (195, 964)	673 (543,822)	na	0.861
Fibrinogen (mg/dL)	473 (364, 583)	450 (360, 574)	269 (231, 368)	348 (297, 451)	**<0.001**	444 (356, 559)	409 (273, 531)	360 (238, 581)	360 (258, 556)	0.615
GGT (U/L)	44 (33, 74)	68 (28,164)	na	na	0.668	48 (39, 67)	73 (62, 145)	na	na	0.275
Glucose (mg/dL)	107 (93, 133)	108 (88, 135)	92 (83, 118)	105 (84, 130)	0.064	128 (96, 166)	148 (110, 186)	126 (108, 163)	131 (105, 152)	**0.003**
IL-6 (pg/mL)	26.7 (12.9, 52.9)	319.5 (170.3, 911.8)	221.0 (14.5, 246.5)	na	0.264	51.4 (24.0, 115.0)	817.5 (384.3, 1136.0)	26.0 (18.0, 41.0)	na	0.015
IL-8 (pg/mL)	19 (10, 35)	na	na	na	–	87 (48,161)	na	na	na	–
INR	1.10 (1.03,1.14)	1.10 (1.06, 1.20)	1.07 (1.03, 1.18)	1.09 (1.05, 1.17)	0.143	1.11 (1.04,1.24)	1.17 (1.09, 1.28)	1.10 (1.06, 1.19)	1.07 (1.04, 1.17)	0.074
LDH (U/L)	290 (230, 379)	292 (233, 367)	270 (219, 327)	269 (218, 914)	0.447	291 (228, 513)	397 (293, 525)	360 (287, 465)	393 (349, 518)	0.878
Lymphocites (x10^9/L)	1.10 (0.86, 1.48)	1.08 (0.73, 1.52)	1.32 (0.91, 1.88)	1.21 (0.91, 1.95)	0.434	0.83 (0.58,1.26)	0.67 (0.43, 0.96)	0.80 (0.49, 1.14)	0.94 (0.69, 1.45)	0.358
Neutrophilis (x10^9/L)	3.90 (3.16, 5.61)	4.77 (7.62, 3.14)	5.43 (8.37, 3.68)	5.46 (3.65, 7.65)	**<0.001**	5.22 (3.65, 8.03)	7.71 (5.57, 10.44)	11.12 (7.15, 14.96)	8.11 (5.24, 12.91)	0.072
NLR	3.50 (2.22, 5.89)	4.13 (2.39-8.79)	4.09 (2.46-8.66)	3.76 (2.52, 8.69)	**0.015**	7.72 (4.15, 13.32)	12.84 (7.09-19.05)	15.94 (6.83-28.74)	9.17 (4.24-17.17)	0.543
PCT (ng/mL)	0.1 (0.1,0.2)	0.1 (0.1,0.3)	0.1 (0.1,0.3)	na	1	0.2 (0.1, 0.6)	0.3 (0.1, 0.6)	0.4 (0.1, 0.5)	na	**0.023**

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Laboratory test (Unit)	*Surviving patients*				*p-value*	*Non surviving patients*				*p-value*
	T0 median (IQR) N=1186	*T1 median (IQR) N=309*	*T2 median (IQR) N=139*	*T3 median (IQR) N=77*		*T0 median (IQR) N= 401*	*T1 median (IQR) N=93*	*T2 Median (IQR) N=46*	*T3 median (IQR) N=27*	
Phosphatase alkaline (U/L)	76 (68, 80)	19 (11,33)	na	na	0.668	62 (51, 66)	79 (66,99)	na	na	0.371
TNF-α (pg/mL)	9 (1, 77)	na	na	na	–	11 (5, 95)	na	na	na	–
Total Amylase (pg/mL)	56 (43,80)	na	na	na	–	69 (42,97)	na	na	na	–
Total bilirubine (mg/dL)	0.50 (0.47,0.80)	0.58 (0.46, 0.84)	0.65 (0.52, 0.86)	0.76 (0.54, 0.88)	0.118	0.62 (0.48, 0.94)	0.60 (0.47, 1.01)	0.62 (0.51, 0.91)	0.62 (0.47, 1.34)	0.433
Total protein (g/L)	6.2 (5.7 ,6.6)	6.0 (5.4, 6.5)	5.7 (5.2, 6.2)	5.6 (5.3, 6.1)	**<0.001**	6.1 (5.8, 6.4)	5.4 (4.9, 5.8)	5.0 (4.7, 5.6)	4.9 (4.6, 5.7)	0.065
WBC ($x10^9$/L)	5.61 (4.59,7.64)	6.50 (4.74, 9.53)	7.56 (5.77, 10.79)	7.97 (5.79, 9.56)	**<0.001**	6.82 (5.51, 9.92)	9.16 (6.04, 12.51)	12.17 (8.59, 17.61)	11.52 (7.35, 16.01)	**0.008**

Data are reported as median (interquartile range, IQR) and percentages. [a]p-value result from the Friedman test for repeated measures with Bonferroni correction. $p<0.05$ was considered statistically significant. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BNP, Brain natriuretic peptide; CK, creatine kinase; CRP, C-reactive protein; GGT, gamma-glutamyl transferase; IL, interleukin; INR, prothrombin time expressed as international normalized ratio; LDH, Lactate dehydrogenase; NLR, neutrophils to lymphocytes ratio; PCT, Procalcitonin; TNF-α, tumour necrosis factor-α, WBC, leukocyte count.

previous results, given that the median values of NLR were between a lower median value of 3.50 (IQR, 2.22-5.89) and a maximum of 4.13 (IQR, 2.39-8.79) in survivors, and were significantly higher in patients who died, with a median value ranging from 7.72 (IQR 4.15-13.32) at admission to a maximum of 15.94 (7.09-19.05) after 14 days.

No statistically significant difference between the two groups was found at admission in median concentration of other analytes. Typical inflammatory markers were above normality cut-off in both survivors and non-surviving individuals either at admission or during follow-up (i.e., LDH, IL-6, IL-8, Ferritin); nevertheless, the median values of these biomarkers were always higher in patients who died. Likewise, the median albumin concentration was below the lower cut-off of normality (35g/L) at admission and during follow-up in both groups of patients, but its decrease was significantly higher from T0 to T1 in non-surviving individuals, remaining stable until T4, while in surviving patients the albumin concentration started to increase between T1 and T2 (*Table 3*). Although IL-6 and ferritin are considered the most involved biomarkers in the "COVID-19 cytokines storm" (Mehta *et al.*, 2020; Qin *et al.*, 2020), which is why they are still used as outcome indicators in hospitalized SARS-CoV-2 positive patients, it is very interesting to note that, in our study, there was no significant increase for these inflammatory indexes at T0 in non-surviving patients when compared to those who survived (p>0.05). On the other hand, our results suggest that a more useful prognostic value, either at admission and/or during follow-up, can be given by PCT, despite the fact that it is an analyte with very narrow reference ranges and that its elevation could be very small.

CONCLUSION

This retrospective study, involving 1587 patients with COVID-19, confirmed the fundamental role of several haematological parameters routinely tested during hospitalization, such as creatinine, BNP, INR, leukocyte count, neutrophil count, NLR, CRP and PCT, as markers of disease severity, that could help clinicians stratify patients according to their risk of death. Particularly, at hospital admission, death correlated positively with lymphopenia and with age but not with sex or with days of hospitalization, indicating that elderly SARS-CoV-2 infected people have a greater risk of death and for this reason need to be protected through preventive actions such as anti-COVID-19 vaccination. Although our study analysed the association of several laboratory markers with symptomatic SARS-CoV-2 infection at the beginning of the pandemic, when there was no vaccine immunization, it could still be of use to understand the severity of the infection at the systemic level in immunocompromised individuals who may develop the disease in a severe form.

A limitation of our study was the lack of patients' symptomatic features; nevertheless, it was one of the studies that collected the largest number of individuals.

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