Predictive risk factors for worse outcomes in COVID-19 patients with different clinical features at baseline

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Abstract

Introduction: COVID-19 is characterized by a wide range of clinical expression and by possible progression to critical illness and death. Therefore it is essential to identify risk factors predicting progression towards serious and fatal diseases. The aim of our study was to identify laboratory predictive markers of clinical progression in patients with moderate/severe disease and in those with acute respiratory distress syndrome (ARDS).

Material and methods: Using electronic medical records for all demographic, clinical and laboratory data, a retrospective study on all consecutive patients with COVID-19 admitted to the Infectious Disease Clinic of Perugia was performed. The PaO_2/FiO_2 ratio (P/F) assessment cut-off of 200 mm Hg was used at baseline to categorize the patients into two clinical groups. The progression towards invasive ventilation and/or death was used to identify critical outcome. Statistical analysis was performed. Multivariate logistic regression analysis was adopted to identify risk factors of critical illness and mortality.

Results: In multivariate logistic regression analysis neutrophil/lymphocyte ratio (NLR) was the only significant predictive factor of progression to a critical outcome (p = 0.03) and of in-hospital mortality (p = 0.03). In ARDS patients no factors were associated with critical progression. Serum ferritin > 1006 ng/ml was the only predictive value of critical outcome in COVID-19 subjects with moderate/severe disease (p = 0.02).

Conclusions: Neutrophil/lymphocyte ratio and serum ferritin are the only biomarkers that can help to stratify the risk of severity and mortality in patients with COVID-19.

Key words: neutrophil/lymphocyte ratio, serum ferritin, COVID-19, predictive factors.

Introduction

Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, appears with a wide range of clinical expression: asymptomatic, mild, moderate, severe and critical features which can progress to death [1].

Most patients with SARS-CoV-2 have a mild disease and present common symptoms such as fever, cough, and fatigue [2]. Usually, they are not hospitalized. Other patients can show a moderate illness (fever and pneumonia), severe pictures (pneumonia with hypoxemia, SpO_2 < 92%), or critical forms with acute respiratory distress syndrome (ARDS), viral sepsis and so on.

It is worth noting that the clinical course is characterized by a first stage in which viral replication predominates, a second with pulmonary involvement, and a third one in which systemic hyper-inflammation prevails. Severe and fatal forms are the final stage of viral infection progression with which the host' s inflammatory and immunological response is associated [3]. However, only a small number of infected people progress to severe and critical forms with ARDS, multiple organ failure and death and, up to now, differences in pathogenesis between asymptomatic, mild and severe illnesses in COVID-19 patients are unknown, although age and comorbidities are factors associated with a more severe evolution [4–7]. Therefore, it is crucial to identify clinical and laboratory predictors of severe and fatal forms right away, in order to provide, apart from antiviral therapies, further interventional drugs aimed at preventing the possible hyper-inflammatory phase that leads to ARDS, cardiac failure, hypercoagulation, viral sepsis and death.

The aim of our study was to analyze clinical characteristics and laboratory biomarkers in patients with COVID-19 at hospital admission in order to define which parameters can discriminate between those who are at a higher risk of developing critical vs. non-critical forms of the disease, as well as those who are less likely to survive.

Material and methods

We performed a retrospective study on all consecutive patients admitted to the Infectious Disease Clinic of Perugia between March 16 and May 5, 2020 and tested positive for SARS-CoV-2.

Respiratory samples (nasopharyngeal swabs, sputum, tracheal aspirate, or bronchoalveolar lavage, BAL, fluid) were obtained from patients at admission and after 24 hours and they were tested for SARS-CoV-2 RNA using a commercial reverse transcriptase real-time PCR assay (RT-PCR assay, Allplex 2019-nCoV Assay, Seegene, Seoul) and/or with the Xpert Xpress SARS-CoV-2 (Cepheid).

Demographic and medical history, the presence of ≥ 2 comorbidities (hypertension, coronary heart disease, diabetes, chronic obstructive lung disease, chronic kidney diseases, carcinoma and autoimmune diseases), clinical data (fever, cough, dyspnea) and laboratory and infection biomarkers, including blood routine, biochemistry, coagulation function, serum ferritin and C reactive protein (CRP), were obtained for each patient after admission. The presence of dyspnea (respiration rate ≥ 24 times/min), radiological characteristics of pneumonia, oxygen saturation (SpO_2) in resting state, arterial partial pressure of O_2 (PaO_2) and the fraction of inspired oxygen (PaO_2/FiO_2) ratio (P/F) were assessed in each patient, highlighting those who, at baseline, had a PaO_2/FiO_2 ratio < 200 mm Hg. Indeed, the P/F cut-off assessment of 200 mm Hg was used at baseline to distinguish two categories of patients: those with a P/F < 200 mm Hg indicating moderate/severe ARDS [8] and suggesting an advanced disease with an important inflammatory component and those with a P/F ≥ 200 mm Hg, indicating a less advanced illness.

The progression of the disease in our cohort was analyzed using two different outcomes: critical COVID-19 illness and in-hospital mortality. We defined critical COVID-19 illness as a composite of admission to the intensive care unit (ICU), invasive ventilation or death as adopted in previous studies [9, 10].

Furthermore, patients with P/F ≥ 200 mm Hg and with P/F < 200 mm Hg at hospital admission were analyzed separately to individuate predictors of critical COVID-19 in both groups.

The demographic and clinical information, laboratory results, and outcome data were extracted from electronic medical records, collected in an Excel file for processing and compared between critical and non-critical illness and survivors vs. non-survivors. Statistical analysis was performed.

The study was approved by our local ethics committee and was conducted according to the Declaration of Helsinki.

Statistical analyses

Continuous variables were summarized as median with the respective interquartile range (IQR) or mean with the respective standard deviation (SD). Categorial variables were represented as percentage of cases.

Differences between groups were determined using the Mann-Whitney test, Student's *t* test, or the χ^2 test as appropriate. Statistical significance was determined as $p < 0.05$.

Subsequently, multivariate logistic regression analysis was performed to identify risk factors of critical illness and mortality. Variables from the univariate analysis that showed a significant correlation with the outcome were included in the multivariate logistic regression analysis.

Continuous variables showing non-homogeneous data were transformed into categorical and then included in the multivariate logistic regression. To achieve this, performance of the single variable was evaluated by receiver operating characteristic (ROC) curve analysis. Subsequently, the most appropriate cut-off value was determined by calculating the Youden index. It corresponds to a point on the ROC curve with the highest vertical distance from the 45° diagonal line [11].

The goodness of fit of the model was assessed with the Hosmer-Lemeshow test (HL).

SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Seventy-nine adult patients with COVID-19 were admitted to the Infectious Disease Clinic of Perugia between March 16, and May 5, 2020. The median age was 65.5 years (IQR 32.2–87.6), most patients were male (83.5%) and 2 or more comorbidities were present in 22.7% of all patients. High blood pressure was the most frequent comorbidity (44.3%). Upon admission, fever, dyspnea and pneumonia were present in 93.6%, 51.9% and 97.5%, respectively (Table I).

At baseline 32/79 patients had a P/F < 200 mm Hg and 22 were admitted to the ICU during hospitalization, whereas 47/79 had a P/F ≥ 200 mm Hg and 21 needed invasive ventilation during hospitalization (p = 0.06, OR 2.7 (1.06–6.9)). The patients showed elevated median values of lactate dehydrogenase (LDH), serum ferritin, CRP, D-dimer and fibrinogen. Median values of the laboratory parameters are shown in Table I.

Considering the two groups of patients with P/F ≥ 200 mm Hg and < 200 mm Hg, upon admission dyspnea was observed in 49% and 59%, median NLR was 6.8 and 12.2, and median ferritin was 784 ng/ml and 990 ng/ml respectively.

Critical illness

Forty-three patients (54.4%) showed a critical course of COVID-19 illness with progression towards invasive ventilation. The comparison of characteristics, laboratory and infection biomarkers between critical and non-critical illness upon admission is shown in Table I.

In the univariate analysis the median values of white blood cell (WBC) count, NLR, LDH, serum ferritin, CRP, procalcitonin (PCT) and D-dimer were significantly different between critical vs. non-critical patients (p = 0.006; p < 0.0001; p = 0.001; p = 0.019; p = 0.009; p < 0.0001 and p = 0.017 respectively). For the multivariate analysis we included:

Table I. Demographic, clinic and laboratory characteristics of total patients with and without progression

Parameter	Total population	Critical illness		*P*-value
		Yes	No	
N (%)	79	43 (54.4)	36 (45.6)	
Age [years], mean (SD) [range]	65.5 (10.6) [32.2–87.6]	65.7 (9.2) [46.7–87.6]	65.3–12.1 [32.2–84.8]	0.72
Sex: male, *n* (%)	66/79 (83.5)	38/43 (88.4)	28/36 (77.8)	0.69
Comorbidities ≥ 2, *n* (%)	18/79 (22.8)	9/43 (20.9)	9/36 (25.0)	0.66
Fever, *n* (%)	75/79 (94.9)	42/42 (100)	32/36 (91.4)	0.052
Dyspnea, *n* (%)	42/78 (54.5)	25/42 (59.5)	16/36 (45.7)	0.22
Pneumonia, *n* (%)	77/79 (97.5)	43/43 (100)	33/35 (94.3)	0.11
pO_2/FiO_2 < 200 mm Hg at baseline No. (%)	32/79 (40.5)	22/43 (51.2)	10/36 (27.8)	0.035
ICU, *n* (%)	41/79 (51.9)	41/43 (95.3)	0/36 (0)	
White blood cells, median (IQR) [cells/mm^2]	7290.0 (5395.0–10590.0)	8620.0 (6680.0–11460.0)	6415.0 (4822.5–8010.0)	0.006
Neutrophil-lymphocyte ratio, median (IQR)	7.6 (3.9–12.8)	11.7 (7.6–16.2)	4.2 (2.6–7.6)	< 0.0001
Hb, median (IQR) [g/dl]	12.7 (11.3–14.0)	12.6 (11.1–13.9)	13.1 (11.8–14.3)	0.13
PLT, median (IQR), [× 1000/mm^2]	207.0 (158.5–321.5)	201.0 (257.0–302.0)	236.0 (159.8–339.8)	0.73
Albumin, median (IQR) [g/dl]	3.1 (2.7–3.4)	2.9 (2.7–3.3)	3.3 (3.1–3.6)	0.0017
Lactate dehydrogenase, median (IQR) [U/l]	331.0 (243.8– 437.3)	369.0 (297.0–455.0)	265.0 (210.5–342.5)	0.001
CPK median (IQR) [U/l]	77.0 (50.0–200.0)	91.0 (42.0–325.0)	74.0 (50.0–153.0)	0.46
Creatinine, median (IQR) [mg/dl]	1.0 (0.7–1.1)	0.9 (0.7–1.2)	1.0 (0.8–1.1)	0.38
Ferritin, median (IQR) [ng/ml]	892.4 (547.7–1342.5]	1101 (649.1–1381.5]	602.4 (435.5–1026.3]	0.019
CRP, mean (SD) [mg/dl]	13.6 (10.0)	16.2 (9.9)	10.3 (9.3)	0.009
PCT, median (IQR) [ng/ml]	0.3 (0.1–0.7)	0.5 (0.3–1.2]	0.2 (0.1–0.3)	< 0.0001
D-dimer, median (IQR) [ng/ml]	1547.0 (816.5–3215.5)	1727.0 (1139.8–4820.5)	845.0 (498.5–1877.5)	0.017
Fibrinogen, median (IQR) [mg/ml]	591.0 (523.5–651.5)	603.0 (539.5–663.5)	1177.0 (671.0–1879.0)	0.36

ICU – intensive care unit, Hb – hemoglobin, PLT – platelets, CPK – creatine phosphokinase, CRP – C-reactive protein, PCT – procalcitonin, IQR – interquartile range, SD – standard deviation

NLR, P/F < 200 mm Hg, LDH and serum ferritin, transforming the latter variable into categorical (the Youden index or optimal threshold value of serum ferritin was > 940 ng/ml). We found that NLR correlates with a severe disease outcome (p = 0.03). The model is representative of reality: the HL test was not significant, p = 0.1 (Table IV).

In-hospital mortality

Eighteen patients (22.7%) died during hospitalization (Table II). The median age was 69.3 years.

In univariate analysis the following differences between survivor and non-survivor patients were significant: dyspnea (p = 0.0083), admission to ICU (p = 0.0002), NLR (p = 0.0006) and hemoglobin (p = 0.02) (Table II). In multivariate analysis, transforming the serum ferritin into a categorical variable (> 1157 ng/ml), only NLR was associated with in-hospital mortality (p = 0.03). The model is representative of reality (HL, p = 0.3) (Table IV).

Critical progression for patients with $PaO_2/FiO_2 \geq 200$ mm Hg and $PaO_2/FiO_2 < 200$ mm Hg

In Table III data on critical progression of patients with P/F ≥ 200 mm Hg are summarized.

Forty-seven out of 79 patients had a P/F ≥ 200 mm Hg at baseline and 21 of them developed a critical illness during hospitalization (44.7%). The in-hospital mortality rate in this group was 19.1% (9/47).

We evaluated which variables were correlated with critical COVID-19 in this subgroup of 47 patients. In univariate analysis, WBC count (p = 0.019), NLR (p <0.0001), LDH (p = 0.017), serum ferritin (p = 0.011) and CRP (p = 0.029) and PCT (p = 0.0013) correlated significantly with critical COVID-19 in patients with P/F ≥ 200 mm Hg at hospital admission.

Multiple logistic regression was performed including the following variables: white blood

Table II. Demographic, clinic and laboratory characteristics of patients referred to the "in-hospital mortality" outcome

Parameter	Total population	In-hospital mortality		*P*
		Yes	No	
N (%)	79	18	59	
Age [years], mean (SD) [range]	65.5 (10.6) [32.2–87.6]	69.3 (8.6) [51.6–87.6]	64.5 (11.1) [32.2–84.8]	0.12
Sex: male, *n* (%)	66/79 (83.5)	17/18 (94.4)	47/59 (79.7)	0.14
Comorbidities ≥ 2, *n* (%)	18/79 (22.8)	6/18 (33.3)	11/58 (19.0)	0.19
Fever, *n* (%)	75/79 (94.9)	18/18 (100.0)	55/58 (94.8)	0.20
Dyspnea, *n* (%)	42/78 (54.5)	12/18 (66.7)	30/58 (51.7)	0.0083
Pneumonia, *n* (%)	77/79 (97.5)	18/18 (100.0)	57/59 (96.6)	0.42
pO_2/FiO_2 < 200 mm Hg at baseline, *n* (%)	32/79 (40.5)	9/18 (50.0)	22/59 (37.3)	0.34
ICU, *n* (%)	41/79 (51.9)	16/18 (88.9)	23/59 (39.0)	0.0002
White blood cells, median (IQR) [cells/mm^2]	7290.0 [5395.0–10590.0]	8845.0 [6675.0–11222.5]	7100.0 [5225.0–10480.0]	0.16
Neutrophil-lymphocyte ratio, median (IQR)	7.6 [3.9–12.8]	12.3 [8.3–21.5]	6.0 [3.6–12.1]	0.0006
Hb, median (IQR) [g/dl]	12.7 [11.3–14.0]	12.0 [9.9–13.0]	12.8 [11.6–14.2]	0.02
PLT, median (IQR), [× 1000/mm^2]	207.0 [158.5–321.5]	195.0 [154.5–302.0]	223.0 [161.5–341.5]	0.77
Albumin, median (IQR) [g/dl]	3.1 [2.7–3.4]	2.9 [2.6–3.2]	3.1 [2.8–3.4]	0.09
Lactate dehydrogenase, median (IQR) [U/l]	331.0 [243.8– 437.3]	380.0 [299.5–455.8]	303.0 [243.8–415.0]	0.11
CPK median (IQR) [U/l]	77.0 [50.0–200.0]	70.0 [34.0–148.5]	83.5 [51.3–223.0]	0.49
Creatinine, median (IQR) [mg/dl]	1.0 [0.7–1.1]	1.0 [0.7–1.6]	0.9 [0.7–1.1]	0.50
Ferritin, median (IQR) [ng/ml]	892.4 [547.7–1342.5]	1246.0 [691.3–1388.3]	776.6 [547.7–1323.5]	0.25
RCP, mean (SD) [mg/dl]	13.6 (10.0)	14.5 (10.2)	13.3 (10.0)	0.63
PCT, median (IQR) [ng/ml]	0.3 [0.1–0.7]	0.5 [0.3–1.2]	0.3 [0.1–0.6]	0.10
D-dimer, median (IQR) [ng/ml]	1547.0 [816.5–3215.5]	1998.0 [1080.0–6388.0]	1488.5 [774.8–2310.8]	0.20
Fibrinogen, median (IQR) [mg/ml]	591.0 [523.5–651.5]	597.5 [521.3–669.0]	593.0 [530.0–648.0]	0.98

ICU – intensive care unit, Hb – hemoglobin, PLT – platelets, CPK – creatine phosphokinase, CRP – C-reactive protein, PCT – procalcitonin, IQR – interquartile range, SD – standard deviation

cells, neutrophils/lymphocyte ratio, ferritin as a categorical variable (> 1006 ng/ml), C-reactive protein, and serum lactate dehydrogenase. As shown in Table IV, the only variable significantly associated with critical illness in this sub group was ferritin values ≥ 1006 ng/ml (p = 0.02). The model is representative of reality (HL, p = 0.3).

Thirty-two patients had a P/F_2 < 200 mm Hg at baseline and 22 needed invasive ventilation during hospitalization (68.7%). The hospital mortality rate in this group was 28% (9/32).

In univariate analysis, NLR, LDH and PCT values were significantly associated with progression (p = 0.03; p = 0.036; p = 0.02 respectively). None of these variables were associated with critical COVID-19 in multivariate logistic regression (Table IV).

Discussion

COVID-19 infection is characterized by a wide range of clinical expression, ranging from asymptomatic to mild forms with involvement of the upper airways, to moderate or severe features of pneumonia, to critical forms with ARDS, derangement of hemostasis, and viral sepsis [1]. However, usually, the disease does not appear at the onset in its full expression but it progresses over two to three weeks, during which the maximum viral replication of the first week is replaced by a condition of a severe inflammatory state which conditions the disease severity, even leading to death. Several studies have reported risk factors associated with the development of critical illness: Wu *et al*. reported that risk factors associated with development of ARDS and death included older age, neutrophilia, organ dysfunction, coagulopathy and elevated D-dimer levels [12]. Wenhua *et al*. developed a clinical risk score and validated a web-based risk calculator based on 10 variables commonly measured on admission to the hospital, to predict the development of critical illness among hospitalized COVID-19 infected patients [13]. In hospitalized patients with respiratory distress, Brandon *et al.* recommend that clinicians closely monitor WBC count, lymphocyte count, platelet count, IL-6 and serum ferritin as markers for potential progression to critical illness [14].

Table III. Demographic, clinic and laboratory characteristics of patients with PO2/FiO2 >= 200 mmHg at admission with and without progression

Parameter	Total of patients with $PO_2/FiO_2 \geq 200$ at admission	Critical		*P*
		Yes	No	
N (%)	47	21	26	
Age [years], mean (SD) [range]	64.9 (10.9) [32.2–84.8]	66.2 (9.3) [46.7–77.5]	63.8 (12.1) [32.2–84.8]	0.45
Sex: male, *n* (%)	39/47 (83.0)	19/21 (90.5)	20/26 (76.9)	0.21
Comorbidities ≥ 2, *n* (%)	10/47 (21.3)	3/21 (14.3)	7/26 (26.9)	0.29
Fever, *n* (%)	45/47 (95.7)	21/21 (100)	24/26 (92.3)	0.19
Dyspnea, *n* (%)	23/47 (48.9)	12/21 (57.1)	11/26 (42.3)	0.31
Pneumonia, *n* (%)	45/47 (95.7)	21/21 (100)	24/26 (92.3)	0.19
White blood cells, median (IQR), [cells/mm^2]	6760.0 [5135.0–9670.0]	9180.0 [5400.0–11520.0]	5685.0 [4667.5–7552.5]	0.019
Neutrophil-lymphocyte ratio, median (IQR)	6.8 [4–12.5]	11.7 [7.6–13.8]	4.5 [2.5–7.0]	<0.0001
Hb, mean (SD) [g/dl]	12.8 (1.9)	12.6 (2.2)	13.0 (1.6)	0.48
PLT, median (IQR) [× 1000/mm^2]	223.0 [150.0–317.5]	227.0 [148.0–323.0]	217.5 [157.3–314.0]	
Lactate dehydrogenase, median (IQR) [U/l]	325.0 [231.0–402.0]	379.5 [326.5–471.5]	251.0 [207.0–345.0]	0.017
CPK median (IQR) [U/l]	90.0 [54.8–200.3]	93.0 [63.5–362.8]	83.0 [50.8–158.5]	0.47
Creatinine, median (IQR) [mg/dl]	0.9 [0.8–1.1]	0.8 [0.7–1.1]	1.1 [0.8–1.1]	
Ferritin, median (IQR) [ng/ml]	784.1 [539.1–1375.0]	1217.0 [748.8–1444.0]	592.3 [415.4–921.4]	0.011
RCP, median (IQR) [mg/dl]	10.6 [4.4–19.1]	16.5 [6.9–20.9]	6.3 [2.8–15.4]	0.029
PCT, median (IQR) [ng/ml]	0.3 [0.2–0.7]	0.5 [0.4–1.0]	0.2 [0.1–0.3]	0.0013
D-dimer, median (IQR) [ng/ml]	1419.5 [701.0–2980.5]	1524.0 [673.8–5137.8]	1163.5 [667.0–1817.5]	
Fibrinogen, median (IQR) [mg/ml]	591.0 [528.0–623.0]	603.0 [569.5–629.5]	591.0 [523.8–623.0]	

ICU – intensive care unit, Hb – hemoglobin, PLT – platelets, CPK – creatine phosphokinase, CRP – C-reactive protein, PCT – procalcitonin, IQR – interquartile range, SD – standard deviation

Table IV. Multivariate logistic regression analyses

Outcome: critical COVID-19	Multivariate logistic regression
Neutrophil–lymphocyte ratio, OR (95% CI, *p*)	1.1 (1.0–1.2, 0.03)
PO_2/FiO_2 < 200 mm Hg at baseline, OR (95% CI, *p*)	2.4 (0.8–7.4, 0.11)
Ferritin > 940.1, OR (95% CI, *p*)	3.0 (0.9–9.6, 0.06)
Lactate dehydrogenase, OR (95% CI, *p*)	1.0 (0.99–1.0, 0.6)
AUROC (STD, 95% CI)	0.8 (0.05; 0.7–0.9, < 0.0001)
HL (*p*)	13.4 (0.1)
Outcome: in-hospital mortality for COVID-19	
Dyspnea, OR (95% CI, *p*)	1.4 (0.4–5.0, 0.5)
Neutrophil–lymphocyte ratio, OR (95% CI, *p*)	1.1 (1.0–1.2, 0.03)
Hemoglobin, OR (95% CI, *p*)	0.7 (0.5–1, 0.07)
Ferritin > 1157.0, OR (95% CI, *p*)	2.3 (0.7–8.5, 0.19)
AUROC (STD, 95% CI, *p*)	0.8 (0.07, 0.7–0.9, 0.0002)
HL (*p*)	7.6 (0.5)
Outcome: critical COVID-19 in patients with pO_2/FiO_2 > 200 mm Hg at hospital admission	
White blood cells, OR (95% CI, *p*)	1.1 (0.9–1.5, 0.2)
Neutrophil–lymphocyte ratio, OR (95% CI, *p*)	1.1 (1.0–1.2, 0.4)
Ferritin > 1006, OR (95% CI, *p*)	6.0 (1.3–30.6, 0.02)
CRP, OR (95% CI, *p*)	1.0 (1.0–1.1, 0.4)
Lactate dehydrogenase, OR (95% CI, *p*)	1.0 (0.99–1.00, 0.9)
AUROC (STD, 95% CI, *p*)	0.9 (0.06, 0.8–1.0, < 0.0001)
HL (*p*)	9.7 (0.3)
Outcome: critical COVID-19 in patients with pO_2/FiO_2 < 200 mm Hg at hospital admission	
Neutrophil–lymphocyte ratio, OR (95% CI, *p*)	1.1 (1.0–1.3, 0.09)
Lactate dehydrogenase, OR (95% CI, *p*)	1.0 (0.99–1.02, 0.12)
AUROC (STD, 95% CI, *p*)	0.8 (0.09, 0.6–1, 0.008)
HL (*p*)	12.0 (0.2)

OR – odds ratio, CI – confidence interval, PO_2/FiO_2 – partial pressure of oxygen/fraction of inspired oxygen, AUROC – area under the receiver operating characteristics, HL – Hosmer-Lemeshow test, CRP – C reactive protein.

In our study we evaluated standard validated biomarkers at the time of admission in COVID-19 positive patients, but we used clinical criteria in dividing patients into two groups both at baseline (± ARDS moderate/severe) and for clinical progression (± ICU/death). Our population, of not advanced average age, was made up of 40% of subjects with a severe picture (therefore suggestive of the disease having already progressed towards an inflammatory phase) and 60% with a milder or more recent form. Overall, over 54% of the patients required intensive ventilation, 69% of those with ARDS at onset, 44% of those with a milder form at the time of hospitalization; in addition, 28% of patients with baseline ARDS and 19% of the others died. We found that patients with a critical course of COVID-19 illness showed median values of WBC count, NLR, LDH, serum ferritin, CRP, PCT and D-dimer higher than those who had a non-critical course and only NLR correlated with both the progression towards invasive ventilation and mortality.

However, in patients with a not particularly severe form (P/F ≥ 200 mm Hg) only serum ferritin was a significant predictive value of disease progression.

Therefore, our study reveals two main laboratory characteristics as predictive risk factors of critical illness and death: NLR and serum ferritin. Both are expressions of a systemic inflammatory response that tends to escalate toward a cytokine storm.

Neutrophil/lymphocyte ratio is reported as having great value indicating a patient's overall inflammatory status [15] and it is considered a risk factor of mortality for many diseases other than infections [16, 17]. Ferritin is a blood protein that contains iron and is used as a marker of iron deficiency. However, it arises from damaged cells [18] and its synthesis is induced in animal models by inflammation [19]. Therefore, serum ferritin is a well-known acute-phase reactant, with levels that mirror the degree of acute and chronic inflammation

in infectious, rheumatologic, hematologic and malignant disease. In adult onset Still's disease high serum ferritin levels are an important diagnostic tool [20]. Unlike many bacterial infections, viral infections are commonly characterized by elevated levels of the pro-inflammatory cytokine IL-18 and increased circulating ferritin concentrations [21].

In our whole population NLR is proposed as an independent predictive factor for the risk of progression to invasive ventilation and death, confirming other experiences with COVID-19 infection [22–24]. However, it does not achieve statistical significance if the analysis is carried out on all the population at an earlier or the milder phase.

A high NLR has already been reported in SARS-CoV [25, 26], in MERS [27], and in severe RSV infections [28]; lymphopenia and neutrophilia have also been reported in Ebola [29]. Viral infections such as SARS, RSV and Ebola are characterized by a great inflammatory response but are also debilitating diseases, and inevitably lead to activation of the hypothalamic–pituitary–adrenal (HPA) axis and excess cortisol secretion [30].

The massive phlogistic response with TNF, IL-1β, IL-6, IL-18 and GMCSF that stimulates neutrophils and macrophages tends to be counter-regulated by IL-4, IL-10, IL-1RA and by cortisol production [29–33].

This leads to an increase of neutrophils, a decrease of lymphocytes, and an increase in NLR. Lymphopenia and neutrophilia in SARS were related to the prevailing serum cortisol [34], which probably reflected the integrity of the HPA axis and a stress response [33–35].

In addition, cortisol seems, on the one hand, to promote the apoptosis of lymphocytes, and on the other to counter-regulate the apoptosis of neutrophils [36].

In our study serum ferritin levels were not in the normal range (30–400 ng/ml) in the whole COVID-19 population, both in non-severe and severe disease, although they were more elevated in patients with ARDS. These data are in accordance with those reported in international literature and prompt the suggestion that COVID-19 may be the fifth member of hyper-ferritinemic syndromes so far comprising four clinical entities: septic shock, macrophage activation syndrome (MAS), Still's disease in adults (AOSD) and catastrophic antiphospholipid syndrome (CAPS). All these diseases are characterized by both an extremely elevated serum ferritin level and partly life-threatening hyperinflammation [37].

In our population, the evaluation of ferritin as a predictive factor of progression towards invasive ventilation and death has given contradictory answers. Only univariate analysis demonstrated serum ferritin as a predictor of disease progression, not of death. Nevertheless, in the patient group with P/F ≥ 200 it was a strong predictor of progression (median levels of 1217 ng/ml vs. 592 ng/ml in critical vs. non-critical course) both in univariate and multivariate logistic regression analysis considering levels > 940 ng/ml. However, focusing attention only on patients with P/F ≥ 200 at hospital admission, ferritin levels > 1006 ng/ml were the only predictive factor of progression also for multivariate logistic regression.

In conclusion, the study of a small cohort does not allow definitive conclusions to be drawn and needs validation in a much wider case study. While on the one hand the insufficiency of ventilation on admission already places the medical staff on clinical alert, in less severe patients the dosage of ferritin, also accompanied by NLR, can be a reason to activate strategies focused on the control of systemic inflammation.

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