RESEARCH ARTICLE

Laboratory parameters related to severe disease and death in SARS-CoV-2 pneumonia: Retrospective analysis

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Abstract

The clinical evolution of coronavirus disease 2019 (COVID-19) is highly variable and hospitalized patients can rapidly develop conditions requiring oxygen support, intensive care unit (ICU) or high dependency unit (HDU) care. Early identification of high-risk patients is mandatory. We retrospectively collected the medical history, symptoms, radiological, and laboratory findings of COVID-19 patients hospitalized between February and April 2020. Laboratory data were collected at the first, last, and middle times of hospitalization. We used arterial oxygen partial pressure and fractional inspired oxygen ratio (P/F) to evaluate respiratory status. Outcomes considered were death and ICU/HDU admission. We used the χ^2 or Fisher's exact test to examine differences between categorical variables. Continuous variables were analyzed using the Wilcoxon matched pairs signed-ranks test and Mann–Whitney test sample test. Of 71 patients admitted, 92% had interstitial pneumonia, and 17% an unfavorable outcome. Negative predictors were age, cerebrovascular disease, obesity, and chronic obstructive pulmonary disease. Baseline P/F was strongly associated with all outcomes. Markers linked to immunological dysregulation like elevated neutrophil-to-lymphocyte ratio exhibited prognostic significance over time. A validated prognostic score comprehensive of all these conditions for early staging and management of COVID-19 patients is urgently needed. Further studies are desirable to evaluate whether laboratory tests can target early treatment in high-risk patients.

KEYWORDS
laboratory parameters, neutrophil-to-lymphocyte ratio, prognostic factors, severe COVID-19

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) identified in patients presenting with viral pneumonia in Wuhan, China, in December 2019. Since then, COVID-19 has rapidly spread to the rest of the world causing a pandemic. To date, it has affected more than one hundred million patients globally, with over 2.5 million deaths.[1] The understanding of COVID-19 is evolving. It affects patients of all ages, causing mainly respiratory symptoms. These symptoms range from a mild flu-like syndrome to a clearly defined pneumonia with persistent fever and cough, up to a severe clinical condition of respiratory failure requiring oxygen support, invasive ventilation, and intensive care unit (ICU) care. Even patients with mild symptoms at presentation can experience a rapidly worsening of clinical conditions with respiratory failure, multiorgan, and systemic dysfunctions.

Given this variability, it is very important to focus on the clinical characteristics of patients at presentation to identify prognostic factors associated with poor outcomes.

Several authors have focused their attention on clinical findings in COVID-19. Many observational studies from different countries showed both an increased proportion of SARS-CoV-2 infection and a higher risk of severe disease, complications, and death from COVID-19 in several clinical categories. In particular, the most common conditions linked with worse prognosis were male sex, age over 65 years, hypertension, diabetes mellitus, obesity, cardiovascular disease, chronic obstructive pulmonary disease (COPD), malignancy.[2–6] Clinical features at presentation such as elevated body temperature and peripheral oxygen saturation <92% can also predict a poor prognosis.[7]

Many laboratory findings were investigated in COVID-19, including hematological, coagulative, inflammatory, and cardiovascular ones. Among these, low lymphocyte (LYM) count, high neutrophils-to-lymphocytes ratio (NLR), low platelets, elevated D-dimer, C-reactive protein (CRP), and interleukin-6 showed different degrees of prognostic value for poor outcome.[8–16] Furthermore, in some cases, these prognostic factors showed a cumulative impact.[7]

In this study, we retrospectively analyzed the characteristics of 71 COVID-19 hospitalized patients at presentation, to identify patients' features associated with death and severe disease. A specific analysis was addressed to laboratory data and their potential prognostic value over time.

2 | MATERIALS AND METHODS

2.1 | Data collection

This was a retrospective study on symptomatic patients with COVID-19 admitted to the Infectious disease ward from February, 27 to April, 23 2020, in San Salvatore Hospital of L'Aquila, Italy. Patients were consecutively enrolled. SARS-COV-2 was detected by a reverse-transcription polymerase chain reaction (RT-PCR) on a nasopharyngeal swab,

performed at the Clinical Analysis Laboratory of Santo Spirito Hospital in Pescara or the Experimental Zooprophylactic Institute of Abruzzo and Molise "G. Caporale", according to the epidemic period.

The following data were collected: demographic information, medical history, symptoms, radiological and laboratory findings, therapies, complications, and death.

From past medical history, we focused on major comorbidities such as hypertension, cardiovascular disease, diabetes, respiratory conditions such as chronic obstructive pulmonary disease, asthma, cerebrovascular disease, chronic kidney disease, liver disease, cancer or previous solid organ transplantation. All recent symptoms reported by the patients were reported. All patients underwent a chest CT scan or X-ray at presentation.

Laboratory findings were collected at first, middle, and last times (T0, T1, and T2, respectively) of hospital stay with a focus on the complete blood count, levels of lactate dehydrogenase (LDH), ferritin, CRP, D-dimer, fibrinogen, international normalized ratio (INR), liver enzymes and blood gas analysis. We used the ratio between arterial oxygen partial pressure and fractional inspired oxygen (P/F) as oxygenation index to evaluate the levels of respiratory failure, considering that a proportion of patients were in oxygen support from the very beginning of observation. According to this, respiratory failure was categorized as follows: mild with P/F between 200 and 300, moderate with P/F between 100 and 200, severe with P/F < 100. Data about medical therapy were recorded including treatment with antiviral therapy, systemic corticosteroids, antibiotics, heparin and tocilizumab, duration of therapy, and adverse effects. Patients were classified according to World Health Organization (WHO) guidance and National Institute of Health (NIH) guidelines[17,18] as mild illness (mild clinical symptoms without pneumonia manifestations in imaging), moderate illness (having symptoms and pneumonia manifestation in imaging, with no requirement for supplemental oxygen), severe illness (having radiographic evidence of pneumonia or need for supplemental oxygen) and critical illness (with acute respiratory distress syndrome [ARDS], sepsis, or multiorgan failure).

2.2 | Statistical analysis

Descriptive statistics were calculated for all variables in the study, reporting mean and standard deviation (ds) or median and interquartile range (IQR) for numeric variables, as well as frequencies for categorical variables. We used the χ^2 or Fisher's exact test to examine the differences between categorical variables. Continuous variables were analyzed using the Mann–Whitney test for two independent groups. The Wilcoxon matched-pairs signed-ranks test was run to compare laboratory data at baseline and discharge.

All data regarding patients' characteristics at baseline were analysed by the following two outcomes: death and admission in a high dependency unit (HDU) or ICU. We performed a subanalysis of this last outcome considering separately patients in HDU and ICU for laboratory parameters.

All analyses were performed with STATA software setting $\alpha = 0.05$.

3 | RESULTS

Data from about 71 patients with symptomatic SARS-CoV-2 infection were collected and analysed. All patients were Caucasian, with ages ranging from 24 to 93 (median age 60 years), prevalently males (65%). Nine patients were health-care workers (13%) with a history of exposure to the virus in the work setting, and six patients (8%) were residential care hosts.

The most common comorbidities were hypertension (51%) and cardiovascular disease (24%). A third of the population was overweight or obese. Median time to admission after presentation was 8 days. The main symptoms reported at admission were fever (90%), cough (61%), and dyspnoea (39%). Respiratory failure (P/F < 300) at admission was observed in 48% of patients, with 20% and 6% overall in the moderate and severe degree group, respectively. Median SpO_2 at admission was 95% (range 85–100) and 21 (30%) were in oxygen therapy. Of 50 patients in an ambient room, 52% had a $SpO_2 ≤ 95\%$. A radiological diagnosis of pneumonia by Chest-CT or X-ray was confirmed in 65 patients (91.5%). Of 61 patients who underwent chest CT-Scan, 98.4% had bilateral and diffuse ground-glass opacities.

Table 1 reports all comorbidities, symptoms, and respiratory features at presentation and radiological patterns.

All pneumonia patients (91.5% overall) were treated with Protease inhibitors (PIs) therapy, while 87.3% overall with Hydrossychloroquine,

TABLE 1 Patient's comorbidities and characteristics at presentation

	n (%)
Comorbidities (n = 71)	
Hypertension	36 (51%)
-Therapy with ARBs	12 (17%)
-Therapy with ACE inhibitors	8 (11%)
Overweight or obese	23 (32%)
Cardiovascular disease	17 (24%)
Cerebrovascular disease	6 (8%)
Diabetes	10 (14%)
Smoke	16 (22%)
Asthma	2 (3%)
COPD	7 (10%)
Chronic liver disease	6 (8%)
Active/recent cancer	5 (7%)
Chronic kidney disease	3 (4%)
Symptoms (n = 71)	
Fever	64 (90%)
Cough	43 (61%)
Dyspnea	28 (39%)

TABLE 1 (Continued)

	n (%)
Gastroenteric	14 (20%)
Conjunctivits	11 (15%)
Anosmia	9 (13%)
Pharyngodynia	9 (13%)
Arthralgia and myalgia	8 (11%)
Rhinitis	3 (4%)
Syncope	3 (4%)
Respiratory and vital parameters (n = 71)	
$PaO_2/FiO_2 < 300$	34 (48%)
$PaO_2/FiO_2 < 200$	18 (25%)
$PaO_2/FiO_2 < 100$	4 (6%)
$200 < PaO_2/FiO_2 < 300$	16 (22%)
$100 < PaO_2/FiO_2 < 200$	14 (20%)
SpO_2	95% (85-100)
Oxygen support	21 (30%)
Rate of interstitial pneumonia (n = 71)	65 (91%)
Radiological features at CT (n = 61)	
Bilateral diffuse ground glass opacities	60 (98%)
Superimposed interlobular and intralobular septal thickening	34 (56%)
Pleural effusion	5 (8%)
Pericardial effusion	4 (7%)
Thoracic lymph node enlargement	27 (44%)
Lung consolidation	48 (79%)
Median time after onset (days)	8 [5–10]

Abbreviations: ARB, angiotensin II receptor blockers; ACE, angiotensin-converting enzyme; COPD, chronic obstructive pulmonary disease.

avoided in four patients for glucose-6-phosphate dehydrogenase deficit or prolonged QT. Antibiotics were used in 50 patients (70.4%) with a median duration treatment of 7 days. The most used drugs were Azithromycin (28.2%) and Levofloxacin (32.4%). Seventy-two percent of patients received anticoagulants with low-molecular-weight Heparin, at therapeutic dosage (100 UI/Kg bis in die) for other indications in 8.45% and at prophylactic dosage (up to 100 UI/Kg die) in 63.4%. Median starting time since onset was 10 days. The median duration of therapy was 9.5 days. Thirty-eight percent of patients received glucocorticoid therapy; of these, 48.1% at low dosage (Methylprednisolone 40 mg/die) and 51.9% at high dosage (Methylprednisolone 1 mg/Kg/die), according to clinical severity. The median starting time since onset was 12 days and the median therapy duration was 10 days. Finally, 9.9% of patients received immunomodulant therapy with Tocilizumab (8 mg/kg [up to a maximum of 800 mg ev]); this drug was administered within the scope of a national multicentric study.[19]

3.1 | Laboratory features at presentation and overtime

Laboratory data were collected at the first, last, and middle times (T0, T1, and T2). Data at the middle time (T1) were not available for patients with short hospitalization time.

At presentation Neutrophil-to-lymphocyte ratio (NLR) values were over 3.13 in 42 patients (59.2%). Thrombocytopenia (under $142 \times 10^3/\mu l$) was observed in 19.7%, while thrombocytosis (above $424 \times 10^3/\mu l$) in 2,8%; anemia (hemoglobin levels <12 g/dl in female and <13 g/dl in male) in 26.8%. Altered values of LDH (above 220 UI/L) and ferritin (above 280 mg/dl) were observed in 79% and 76%, respectively. To notice, in 28% of patients, ferritin values were above 1000 mg/dl. Augmented values of D-dimer (above 0.5 µg/ml), fibrinogen (above 450 mg/dl), and INR (above 1.28) were observed in 65.7%, 67.1%, and 12.9%, respectively. Thirty-two patients (45.1%) had increased liver enzymes (ALT, alanine transaminase and AST, aspartate aminotransferase above 35 and 40 UI/L, respectively) and 59 (86.8%) had increased CRP (above 0.5 mg/dl). Only one patient, with a history of heart failure, had alteration of troponin value (normal value 0–34.2 pg/ml).

Laboratory data showed slight increases in lymphocyte count, haemoglobin levels, platelet count, and P/F ratio and decrease in NLR values during the hospital stay. Median laboratory values are reported in Table 2.

3.2 | Clinical course and outcome

According to WHO/NIH guidelines, patients were classified as follows: mild illness 7%, moderate illness 45%, severe illness 31%, and critical illness 17%. Of 71 patients, 5 (7%) were admitted to HDU, 4 (5.6%) were intubated in ICU, 4 (5.6%) died: 1 of these died after admission to ICU, and 3 in the Infectious Disease Yard because of contraindications for ICU treatment. The median time for moving to HDU or ICU was 2 days. Total patients with unfavorable clinical course were 12 (17%). The HDU/ICU outcome was reached by nine patients, while four patients died.

Fifty patients (70%) were discharged at home, 9 (13%) were moved to post-COVID Unit with sequelae or swab positivity persistence in clinical stability. Total patients with favorable clinical course were 59 (83%). The average length of hospitalization was 11 days for patients discharged or moved to the non-ICU.

The median overall duration of hospital stay in the Infectious Disease department was 10 days. The median time to defervescence (for 64 patients with fever at onset) was 12 days from symptoms onset.

The most common complication was respiratory failure (intended as P/F < 300) which occurred in 62% of patients; the median value of worse P/F was 231 (IQR: 146.8) and occurred after a median of 12 days from onset. A total of 41 patients (58%) needed oxygen support by nasal cannula or Venturi mask. As for other complications, 11 patients (16%) presented alteration in electrolyte equilibrium; microbiologically confirmed bacterial superinfections

occurred in 11 patients (16%), and arrhythmia in 2 patients (3%). Eleven patients developed hypomobility syndrome (16%).

Median time to viral healing (intended as two consecutive negative Rhyno-pharyngeal swabs) from the onset was 25 days, with four patients still positive at the time of data collection.

3.3 | Factors related to death and admission to ICU/HDU

Four patients died (5.6%). Being older age and having a cerebrovascular disease or COPD were conditions associated with death. Variables significantly related to death in univariate analysis are shown in Table 3. Nine patients were admitted in HDU or in ICU (12.7%). Obesity, COPD, and use of ARBs were significantly related to ICU or HDU admission, as well as dyspnoea at baseline. Significant comparisons of categorical and continuous variables between patients with HDU or ICU needing and those discharged are shown in Table 4. Among laboratory parameters, elevated NLR and D-dimer showed a correlation with mortality both at baseline and at overtime, while elevated NLR, PCR, and D-dimer at baseline showed a correlation with worse outcome, and both PCR and NLR preserved significance overtime. No clinical symptoms or radiologic features at presentation were significantly related to death. Results of analysis conducted for ICU and HDU subgroups are shown in Table 5.

4 | DISCUSSION

This was a retrospective study in 71 patients hospitalized for symptomatic SARS-CoV-2 infection whose data about comorbidities, clinical and laboratory findings were analysed by two outcomes: death and admission of HDU or ICU.

The median age of this male-dominated population was 60 years. A third of the population was overweight or obese. Hypertension was the most common comorbidity (51%), and high levels of cardiovascular and respiratory disease were also observed. The majority of patients were hospitalized with fever and respiratory symptoms, and with a high prevalence of interstitial pneumonia. This is congruent with the median time to admission after presentation of 8 days: it corresponds to the "pulmonary phase" of COVID-19.[20] It has to be noticed that only 61% of patients presented with cough, besides 91% with radiologically confirmed pneumonia. This inconsistency between the severity of radiological pattern and mild clinical manifestation has largely been observed before.[21] Moreover, 48% had a degree of hypoxemia, 39% of patients complained of shortness of breath at baseline and 30% were already in oxygen support from Emergency Department, because of low peripheral oxygen saturation values. Those discrepancies probably reflect the contrast between preserved oxygen saturation and arterial hypoxemia ("ventilation-perfusion mismatch") previously described in SARS-CoV-2 pneumonia patients.[22]

During the hospital stay, defervescence was observed in a median of 12 days after the onset. A high percentage (62%) of patients developed

TABLE 2 Laboratory features over time

	Median values (n)			T0 vs. T2 p[a]
	T0	T1	T2	
NEU, x1000 cell/μl	3.56 (n = 71)	3.70 (n = 44)	3.74 (n = 63)	0.5676
LYM, x1000 cell/μl	1.02 (n = 71)	1.19 (n = 44)	1.42 (n = 63)	0.0078
NLR	3.68 (n = 71)	3.19 (n = 44)	2.52 (n = 63)	0.0285
Hb, g/dl	13.9 (n = 71)	13.0 (n = 44)	13.0 (n = 63)	<0.0001
PLT, x1000 cell/μl	196 (n = 71)	239 (n = 44)	229 (n = 63)	<0.0001
LDH UI/L	277 (n = 71)	244 (n = 39)	225 (n = 52)	<0.0001
Ferritin mg/dl	503 (n = 58)	490 (n = 33)	499 (n = 46)	0.0635
D-Dimer μg/ml	0.62 (n = 70)	0.53 (n = 34)	0.53 (n = 44)	0.0729
Fibrinogen mg/dl	515 (n = 70)	534 (n = 31)	492 (n = 42)	0.0034
INR	1.1 (n = 70)	1.08 (n = 27)	1.1 (n = 45)	0.1775
AST UI/L	29.5 (n = 70)	19.1 (n = 24)	21.0 (n = 32)	0.0002
ALT UI/L	24.5 (n = 70)	32.0 (n = 24)	33.0 (n = 31)	0.6310
GGT UI/L	40 (n = 67)	37 (n = 23)	41.5 (n = 28)	0.2793
CRP mg/dl	2.99 (n = 68)	3.20 (n = 42)	0.74 (n = 57)	0.0005
PaO2/FiO2	300 (n = 69)	267.5 (40)	324.0 (n = 54)	0.0056

Abbreviations: AST, aspartate aminotransferase; ALT, alanine transaminase; CRP, C-reactive protein; GGT, gamma glutamyl transferase; LDH, lactate dehydrogenase; NLR, Neutrophil-to-lymphocyte ratio; NEU, neutrophil count; LYM, lymphocyte count.

[a]Wilcoxon matched-pairs signed-ranks test.

TABLE 3 Factors related to death, univariate analysis

	Death		
	Yes (n = 4), n (%)	No (n = 67), n (%)	p^a
Cerebrovascular disease (yes)	2 (33%)	4 (67%)	0.033
COPD (yes)	2 (29%)	5 (71%)	0.046
PaO_2/FiO_2 < 200 (yes)	3 (17%)	15 (83%)	0.048
PaO_2/FiO_2 < 100 (yes)	2 (50%)	2 (50%)	0.014
Mid-flow oxygen support (yes)	3 (21%)	11 (79%)	0.022
	Median (IQR)	Median (IQR)	p^b
Age	78.0 (10.5)	59.0 (21)	0.024
NLR (t0)	11.8 (18.7)	3.34 (3.04)	0.051
D-Dimer (t0)	2.53 (3.13)	0.61 (0.58)	0.023
LYM (t0)	0.67 (0.38)	1.08 (0.65)	0.005
PaO2/FiO2 (t0)	142 (160.6)	310 (123.8)	0.025
LYM (t1)	0.54 (0.45)	1.22 (0.86)	0.018
LDH (t1)	438 (127)	242 (73)	0.033
NLR (t2)	9.60 (6.51)	3.67 (2.83)	0.014
LYM (t2)	0.38 (0.3)	1.44 (0.86)	0.006
NLR (t2)	31.7 (31.3)	2.43 (2.75)	0.007
D-Dimer (t2)	4.0 (0.0)	0.49 (0.6)	0.033
AST (t2)	52.0 (28)	19.5 (12)	0.047

Abbreviations: ARB, angiotensin II receptor blockers; COPD, chronic obstructive pulmonary disease; LDH, lactate dehydrogenase; LYM, lymphocyte count; NLR, neutrophil-to-lymphocyte ratio.

[a]p: Fisher exact test

[b]p: Mann–Whitney test

respiratory failure and a worse respiratory performance was again observed at a median time from onset of 12 days. Overall, 9 patients (12.6%) were admitted to ICU or HDU with a median time of 2 days after hospitalization and 10 days after onset, reflecting the timing of respiratory dysfunction observed before in SARS-CoV-2-infection.[21] Comprehensively, the timeline observed seems to confirm the 10th–12th day of disease as the "key moment" in COVID-19 evolution.

Five patients (7%) were admitted to HDU and survived. Patients admitted to ICU were 4 (5.6%) and one of these died. The prevalence of mortality was 5.6% that is quite lower than that observed in SARS-CoV-2 hospitalized patients in the same epidemic period.[23] Three of these four dead patients were not moved to ICU because of contraindications (age and cancer). A total of 59 patients (83%) had a favorable clinical course and, at a median time of 11 days since hospitalization and 19 days after onset, were discharged at home or moved to the post-COVID department, mainly for hypomobility syndrome and/or swab positivity persistence. This favorable outcome, together with the observation of mild illness in 45% of patients, seems to be more frequent than expected also considering high levels of comorbidities and the median age of our population. A possible explanation could be the early timing of hospital admission of these patients: in fact, it has been speculated that a delay in

hospitalization may correspond to worse respiratory conditions at admission and worse prognosis.[24] Regarding the therapeutic approach, it has to be noticed that clear indications about COVID-19 treatment were lacking at the time of hospitalization and guidelines were consequently unclear. So, the majority of patients received antiviral therapy with PI (91.5%) and/or hydroxychloroquine (HCQ) (87.3%), according to the Italian Society of Infectious Disease (SIMIT) indications at that time. Steroidal therapy was used in 38% of our patients, according to the severity of hypoxemia. Use of this therapy, later associated with a clear benefit in COVID-19 prognosis,[25] had been particularly controversial given the initial contraindication provided by WHO.[26] To note, steroidal use was addressed to patients with worse respiratory performance (worse P/F 163, IQR: 112.1 vs. 291, IQR: 125 in nontreated patients, p = p = 0.0003, Mann–Whitney test; data not shown), and this could partly explain the data of favorable outcome. Anticoagulant therapy was given to 72% of patients. Despite this uncertainty in the therapeutic approach, mortality remained still low. Antibiotics were used in a higher proportion of patients (70,4%) than that with confirmed bacterial superinfection (16%): this is mainly due to an initially supposed efficacy of azithromycin in combination with HCQ against SARS-CoV-2,[27] later not confirmed.[28] It is also likely that clinicians considered some CT-scan

TABLE 4 Factors related to HDU/ICU, univariate analysis

| | HDU/ICU | | |
	Yes: $n = 9$, n (%)	No: $n = 62$, n (%)	P^a
Obesity (yes)	6 (26%)	17 (74%)	0.050
ARBs (yes)	5 (42%)	7 (58%)	0.005
COPD (yes)	3 (43%)	4 (57%)	0.039
DYSPNOEA (yes)	7 (25%)	21 (75%)	0.024
PaO$_2$/FiO2 < 300 (yes)	8 (24%)	26 (76%)	0.011
PaO$_2$/FiO2 < 200 (yes)	7 (29%)	11 (61%)	<0.001
PaO$_2$/FiO2 < 100 (yes)	3 (75%)	1 (25%)	0.005
	Median (IQR)	Median (IQR)	P^b
NLR	5.74 (4.95)	3.31 (2.9)	0.055
D-dimer (t0)	1.06 (5.91)	0.58 (0.42)	0.033
CRP (t0)	10.80 (5.91)	2.28 (5.7)	0.002
PLT (t1)	134.50 (9)	249 (127)	0.030
CRP (t1)	16.24 (8.8)	2.60 (5.4)	0.042
NEU (t2)	5.85 (5.13)	3.40 (3.0)	0.026
LYM (t2)	0.67 (0.45)	1.46 (0.8)	0.004
NLR (t2)	13.09 (10.2)	13.09 (10.2)	<0.001

Abbreviations: AST, aspartate aminotransferase; COPD, chronic obstructive pulmonary disease; HDU, high dependency unit; ICU, intensive care unit; LDH, lactate dehydrogenase; LYM, lymphocyte count; NLR, neutrophil-to-lymphocyte ratio.

$^a p$: Fisher exact test.

$^b p$: Mann–Whitney test.

features (e.g., interlobular and intralobular septal thickening, lung consolidation) or laboratory parameters (e.g. altered procalcitonin [PCT], very elevated CRP) as indicators of bacterial superinfection also in absence of microbiological confirmation.

Viral healing, intended as two consecutive negative Rhynopharyngeal swabs, was observed after a median of 25 days from onset.

Despite the small number of patients in "death" and "HDU/ICU" groups, statistical analysis showed a significant relationship between comorbidities, and death or ICU/HDU admission. In particular, cerebrovascular disease was significantly related to death ($p = 0.033$), as well as age, showing a median of 78 years old in the death group versus 59 years old in the survivors ($p = 0.024$). The presence of COPD was significantly related both to death ($p = 0.046$) and to ICU/HDU admission ($p = 0.039$), in agreement with global data recently reviewed.[29] Obesity resulted as a significant risk factor for disease severity rather than mortality; this is also well-known[29] and not unexpected, considering the repercussions of obesity on pulmonary function.

In this analysis, therapy with Angiotensin II receptor blockers (ARBs) showed a significant association with ICU/HDU admission ($p = 0.005$). The relationship between this therapy and severe COVID-19 was largely discussed considering the possible role of these drugs in the SARS-CoV-2 life cycle but this correlation was not confirmed in large studies.[30]

Statistical analysis showed that the presence of hypoxemia at presentation is significantly related to both outcomes. In particular, having a P/F lower than 200 and 100 resulted in an increased risk of mortality ($p = 0.048$ and $p = 0.014$, respectively) and ICU/HDU treatment (P/F < 200 with OR 16.2, $p < 0.001$, P/F < 100 with $p < 0.001$). In the last group, also having a P/F < 300 was significantly related to ICU/HDU admission ($p = 0.011$). Overall, to have a low P/F index at baseline was significantly related with death ($p = 0.025$), equally to be in oxygen support ($p = 0.022$). These results are controversial: while it is evident that high levels of hypoxemia are one of the strongest indications for ICU or HDU admission, it is also evident that a more compromised respiratory situation at the time of hospitalization implies a more severe clinical course, a raised probability of high intensity of care and, at least, a higher prevalence of death. For this reason, it is important to emphasize the use of basal hypoxemia as a guiding parameter for the clinician approaching patient management. This is important regardless of respiratory symptoms, which as we said before, are not always congruent with the laboratory data. Anyway, it has to be noted that the presence of dyspnoea at hospitalization was predictive of HDU/ICU admission

TABLE 5 Factors related to HDU and ICU, univariate analysis

	HDU		
	Yes (n = 5)n (%) or median [IQR]	No (n = 62)n (%) or median [IQR]	P^a
PaO$_2$/FiO$_2$ < 200 (yes)	4 (22%)	14 (78%)	<0.001
100 < PaO$_2$/FiO$_2$ < 200 (yes)	3 (21%)	11 (79%)	0.047
NEU (t0)	5.34 (1.0)	3.52 (2.9)	0.058
LYM (t0)	0.68 (0.4)	1.09 (0.6)	0.026
NLR (t0)	8.85 (15.4)	3.31 (2.9)	0.007
Ferritin (t0)	1750 (1920.1)	479.6 (706)	0.053
ALT (t0)	82 (79)	23.5 (27)	0.038
GGT (t0)	152 (158)	39.0 (38)	0.004
FiO2 (t0)	0.60 (0.2)	0.21 (0.1)	0.009
CRP (t0)	10.3 (5.7)	2.28(5.8)	0.018
PaO$_2$/FiO$_2$ (t0)	120 (14.2)	314 (92.8)	0.019
LYM (t2)	0.67 (1.0)	1.45 (0.8)	0.002
NLR (t2)	13.1 (6.7)	2.07 (2.2)	0.001
Fibrinogen (t2)	603 (105.5)	474.5 (235)	0.021
INR (t2)	1.16 (0)	1.08 (0.1)	0.022
GGT (t2)	261 (480)	38.0 (29)	0.034
	ICU		
	Yes (n = 4), n (%) or median [IQR]	No (n = 62), n (%) or median [IQR]	
Oxygen support (yes)	3 (30%)	7 (70%)	0.010
PaO$_2$/FiO$_2$ < 100 (yes)	2 (67%)	1 (33%)	0.008
CRP (t0)	11.91 (5.5)	2.28 (5.8)	0.028
FiO$_2$ (t2)	0. 44 (0.2)	0.21 (0.1)	0.049
pO$_2$ (t0)	52.00 (9.0)	74.00 (18.0)	0.002
PaO$_2$/FiO$_2$ (t0)	127.30 (107.3)	314.30 (92.8)	0.006

Abbreviations: AST, aspartate aminotransferase; COPD, chronic obstructive pulmonary disease; HDU, high dependency unit; IQR, interquartile range; ICU, intensive care unit; LDH, lactate dehydrogenase; LYM, lymphocyte count; NLR, neutrophil-to-lymphocyte ratio.
^{a}χ^2 or Fisher exact test, Mann–Whitney test.

(p = 0.024). It is important to highlight also that the number of patients who reached the two outcomes is very low.

At admission, the majority of patients had alteration of laboratory parameters. During the first spread of SARS-CoV-2, it was clear that the majority of patients showed alteration in neutrophil and lymphocyte count resulting in a high NLR, and a rise in the level of CPR, D-dimer, fibrinogen, ferritin, and LDH.

The most common alteration found is increased CRP (86.8%). The majority of patients (79% and 76%, respectively) showed high levels of LDH and ferritin and a very high prevalence of altered values of D-Dimer (66%) and fibrinogen (67%) were observed. We also observed in 59% of patients an NLR over 3.13, which is the value identified, in some studies[31] as the cut-off for poor prognosis in COVID-19 patients. These altered parameters showed in many cases a significant slight improvement over time

(lymphocyte count, haemoglobin levels, platelet count PCR, LDH, fibrinogen, and D-Dimer).

Statistical analysis showed a significant relationship between many of these alterations at baseline with death and/or HDU/ICU admission.

There were elevated levels of baseline CRP in patients admitted to HDU/ICU (median 10.8 vs. 2.28; p = 0.002), and the significance was partially preserved over time, while no relation was found with death. Also considering ICU and HDU separately, PCR levels were still significantly higher in these groups than in other patients. Correlation between CRP levels and disease severity has already been noticed[32] and the strength of this evidence seems to be high in the meta-analysis study.[29]

Elevation of D-dimer was significantly related to both outcomes, and this relation is preserved over time for the death group. The prognostic role of D-dimer, also in his evolution over time, has largely

been observed before,[12–14] and D-dimer and seems to be the cardiovascular parameter with the best correlation with ICU treatment and death.[33] This probably reflects the role of coagulation in the physiopathology of severe COVID-19 and supports the use of low-molecular-weight heparin in these cases.[14]

Elevated NLR was significantly related to both death and severity of disease. This relation is preserved over time and with increasing significance, specifically in the HDU group, showing a possible role of this index not only at hospital admission but also during clinical course, and suggesting a link with disease evolution. In this perspective, it is interesting to notice that NLR seems to increase its significance over time and that lymphopenia considered in itself has the same trend. In contrast, Neutrophilia in itself showed inconstant correlations with disease severity and mortality.

Lymphopenia is common in SARS-CoV-2 infection and many studies evidenced its correlation with mortality[8] and severe disease,[9] may be related to an insufficient specific immune response to the virus.[34] Interestingly, a geographic variability in this parameter was observed, with a much higher percentage in patients from Italy if compared with those from some Far East countries.[35]

NLR is an index used as a surrogate to assess the extent of systemic inflammation that may have a correlation with severe disease and death.[10,36] In fact, the "cytokine storm" associated with the dysregulation of inflammatory response seems to play a crucial role in COVID-19 evolution, as observed in other Coronaviruses infections.[37] The NLR cut-off with the best predictive value has not been identified.[38] It is important to highlight that this parameter could be influenced over time by steroidal use. For this reason, also domiciliary usage of steroids should be taken into account. Anyway, none of these patients reported a history of steroidal use. Considering that the beneficial role of steroidal therapy in COVID-19 could be explained by its effects on inflammation, it is also possible to speculate that the use of this drug could be guided by NLR values in an early phase of disease before respiratory failure develops.

Isolated significance was found for high levels of LDH at middle hospitalization and for AST at the end of it for the death group ($p = 0.033$ and 0.047 respectively), while having low platelets at middle-time of hospitalization was significantly related with ICU/HDU admission ($p = .030$). A correlation of these alterations with severity of disease and with death has been described in some studies, but their predictive role is controversial[29] so they are probably less specific.

5 | CONCLUSIONS

This observational retrospective study conducted during the first epidemic period of the SARS-CoV-2 pandemic highlights the importance of many factors that need to be examined in-depth for future epidemic waves.

Comorbidities, and in particular cerebrovascular and respiratory ones, affect prognosis together with obesity and age. Patients with more than one of these conditions need to be considered as very high-risk patients since the onset of COVID-19 symptoms. Regarding the clinical

manifestations, the absence of an evident "shortness of breath" and low levels of peripheral oxygen saturation is probably not accurate enough to define a patient as at low risk for severe-COVID-19. Assessing and quantifying hypoxemia is essential for this purpose. The clinical course of these patients underlines the importance of the 10th–12th day since onset as the key moment in COVID-19 evolution toward a severe or mild disease. For this reason, all SARS-CoV-2 symptomatic patients should be monitored accurately at least until this time.

Data about our population confirmed the importance of some laboratory findings, especially those related to hyperinflammation, as predictors of worse outcomes, both at baseline and over time. Worldwide the role of those alterations was largely investigated as the prognostic score, combined with high-risk conditions such as comorbidities, age, and sex. It is mandatory to assess in larger studies the weight of these alterations in early staging of COVID-19 patients, creating a validated prognostic score to address those patients with a worse prognostic score with hospitalization and intensive clinical observation. This is essential considering the large numbers of patients simultaneously observed in emergency rooms during the epidemic peaks, and the above-mentioned cruciality of early hospital admission for supportive therapy, to decrease mortality of COVID-19. Lacking a specific antiviral therapy, the potential benefit of an early medical treatment able to modulate inflammatory response in high-risk patients needs to be further investigated. The timing of steroidal therapy remains anyway controversial considering its effect on viral clearance.[26]

This study has many limitations. The population size is small and the number of events for all three outcomes is scarce; for this reason, no multivariable model was performed, reducing the prognostic value of all parameters detected. Data were collected retrospectively in a context of uncertainty about the therapeutic approach and this reflects the variability in clinical choices. Further and larger studies are needed to confirm these observational findings.

CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS
Giovanna Picchi and Monica Di Norcia conceived the presented idea, wrote the manuscript with support from Gaia Sinatti, and Vincenza Cofini. Vincenza Cofini and Stefano Necozione developed the statistical model and performed all statistical analysis; Vincenza Cofini also encouraged Giovanna Picchi and Monica Di Norcia to focus on some specific data, wrote all tables, and supervised all data exposition in the manuscript. Giovanna Picchi, Monica Di Norcia Gaia Sinatti, Benedetta Cosimini, Paola Vertolli, Franco Tonello, and Anna

Cecilia Carucci managed all patients and collected all data. Clara Balsano and Alessandro Grimaldi supervised the project. All authors discussed the results and contributed to the final manuscript.

REFERENCES

1. World Health Organization. *Report on COVID-19*; 2021. https://www.who.int/publications/m/item/weekly-epidemiological-update—16-march-2021

2. Centers for Disease Control and Prevention. *National Diabetes Statistics Report, 2020*. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services; 2020.

3. Guan WJ, Ni ZY, Hu Y. China medical treatment expert group for covid-19. clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020; 382(18):1708-1720.

4. Onder G, Rezza G, Brusaferro S. Case-fatality rate and characteristics of patients dying in relation to COVID-19 in Italy. *JAMA*. 2020; 323(18):1775-1776.

5. Yang J, Zheng Y, Gou X, et al. Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. *Int J Infect Dis*. 2020;94:91-95.

6. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. 2020; 8(5):475-481.

7. Jang JG, Hur J, Choi EY, et al. Prognostic factors for severe coronavirus disease 2019 in Daegu, Korea. *J Korean Med Sci*. 2020; 35(23):209.

8. Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. *Clin Chem Lab Med*. 2020; 58(7):1131-1134.

9. Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID-19 infection. *Am J Hematol*. 2020;95(6):E131-E134.

10. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis*. 2020; 71(15):762-768.

11. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a meta-analysis. *Clin Chim Acta*. 2020;506:145-148.

12. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA J Am Med Assoc*. 2020;323:1061.

13. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395:1054-1062.

14. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost*. 2020;18:844-847.

15. Liu F, Li L, Xu M, et al. Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. *J Clin Virol*. 2020; 127:104370.

16. Sun H, Ning R, Tao Y, et al. Risk factors for mortality in 244 older adults with COVID-19 in Wuhan, China: a retrospective study. *J Am Geriatr Soc*. 2020; 68(6):E19-E23.

17. World Health Organization. *Clinical managment of COVID-19*; 2020

18. COVID-19 Treatment Guidelines Panel. *Coronavirus disease 2019 (COVID-19) treatment guidelines*. National Institutes of Health; 2020. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed April 15, 2021.

19. Piccirillo MC. TOCIVID-19 - A multicenter study on the efficacy and tolerability of tocilizumab in the treatment of patients with COVID-19 pneumonia. Study protocol. Contemp Clin Trials. 2020; 98:106165.

20. Siddiqi HK, Mehra MR. COVID-19 illness in native and immunosuppressed states: a clinical–therapeutic staging proposal. *J Heart Lung Transplant*. 2020; 39(5):405-407.

21. Li Xu, Ma X. Acute respiratory failure in COVID-19: is it 'typical' ARDS? *Crit Care*. 2020; 24(1):198.

22. Dhont S, Derom E, Van Braeckel E, Depuydt P, Lambrecht BN. The pathophysiology of 'happy' hypoxemia in COVID-19. *Respir Res*. 2020;21(1):198.

23. International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC). *COVID-19 Report*; 2020. https://isaric.org/research/covid-19-clinical-research-resources/evidence-reports/

24. de Simone G, Mancusi C. COVID-19: timing is important. *Eur J Intern Med*. 2020;77:134-135.

25. Horby P, Lim WS, Emberson JR, et al. Dexamethasone in hospitalized patients with Covid-19—preliminary report. *N Engl J Med*. 2020;384(8):693-704.

26. World Health Organization. *Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected: interim guidance*; 2020. https://apps.who.int/iris/handle/10665/330893. Accessed April, 23, 2020.

27. Gautret P, Lagier J-C, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *Int J Antimicrob Agents*. 2020;56(1):105949.

28. Arshad S, Kilgore P, Chaudhry ZS, et al. Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. *Int J Infect Dis*. 2020;97:396-403.

29. Izcovich A. Prognostic factors for severity and mortality in patients infected with COVID-19: a systematic review. *PLOS One*. 2020; 15(11):e0241955.

30. Iaccarino G, Grassi G, Borghi C, et al. Age and multimorbidity predict death among COVID-19 patients. Results of the SARS-RAS study of the italian society of hypertension. *Hypertension*. 2020;76:366-372.

31. Liu J, Liu Y, Xiang P, et al. Neutrophil-to-lymphocyte ratio predicts severe illness patients with 2019 novel coronavirus in the early stage. *J Transl Med*. 2020;18:206. https://doi.org/10.1186/s12967-020-02374-0

32. Zhang J, Dong X, Cao Y, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy Jul*. 2020;75(7):1730-1741.

33. Aboughdir M, Kirwin T, Abdul Khader A, Wang B. Prognostic value of vardiovascular biomarkers in COVID-19: a review. *Viruses*. 2020; 12(5):527.

34. Lippi G, Plebani M. The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks. *Clin Chem Lab Med*. 2020; 58(7):1063-1069.

35. Frater JL, Zini G, d'Onofrio G, Rogers HJ. COVID-19 and the clinical hematology laboratory. *Int Journal Lab Hemat Rev*. 2020; 42(Suppl 1):11-18.

36. Tatum D, Taghavi S, Houghton A, Stover J, Toraih E, Duchesne J. Neutrophil-to-lymphocyte ratio and outcomes in Louisiana Covid-19 patients. *Shock*. 2020; 54(5):652-658.

37. Ryabkovaa VA, Churilova LP, Shoenfeldab Y. Influenza infection, SARS, MERS and COVID-19: cytokine storm–the common denominator and the lessons to be learned. *Clin Immunol*. 2021; 223:108652.

38. Li X, Liu C, Mao Z, et al. Predictive values of neutrophil-to-lymphocyte ratio on disease severity and mortality in COVID-19 patients: a systematic review and meta-analysis. *Crit Care*. 647 2020;24:647.