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**Original Research Article**

# Inflammation Associated with Disease Severity and Fatality of COVID-19

Boumraya Sara[1*], Hajar Skali[1], Fatima Zahrae Lazrak[1], Aboulmakarim Siham[1], Chellak Saliha[2]

[1]Department of biochemistry, E-rrazi Hospital, Mohamed VI Medical Center, Faculty of Medicine and Pharmacy of Marrakech, Cadi Ayyad University, Marrakech, Morocco
[2]Biochemistry Department, Avicenne Military Hospital, Marrakech, Morocco

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***Corresponding author:** Boumraya Sara

## Abstract

COVID-19 has become one of the worst infectious disease outbreaks of recent times, with over 6.3 million cases and 367000 deaths. Our study aims to analyze characteristics, biomarkers changes of patients with COVID-19 and their associations with severity and outcomes of the disease. Laboratory parameters included inflammatory markers such as procalcitonin (PCT), serum ferritin, C-reactive protein (CRP), lactate dehydrogenase (LDH) and interleukin-6 (IL-6). Fifty-two consecutive hospitalized patients in intensive care unit with confirmed COVID-19, in the university Hospital of Mohammed 6 of Marrakech were enrolled in this retrospective study between March 20, 2020, and May20, 2020. Demographics, underlying diseases and laboratory data were collected. 39 (75%) were man and 13 (25%) were woman; the mean age was 58 years, 76, 1% were up to 50 years old. Among all non-survivors, 81% were male. The median course of the disease was 9 days. The most common co morbidity was hypertension (13/52, 25 %) and diabetes (13/52, 25%). Respirator was used in 23 patients (23/52, 44.2%). 78.59% patients had received Hydroxychloroquine+Azythromycine and 15.8% patients had received antiviral medications: lopinavir/ritonavir. The fatality rate was 71.2 % in this population considered critical. On admission, regardless of the severity, majority of patients present an identical level of inflammation marker levels. Levels of serum ferritin (median 845 [IQR 102.5–4275]), CRP (median 174.15 [IQR 5.19–508.39]), PCT (median 0.68 [IQR 0.04– 45]), LDH (median 519.5 [90–1238]), IL6 concerning 7/52 (median 101[IQR 17– 201]. Dynamic changes of biochemical and immunologic biomarkers during progression of COVID-19 across on admission, mid-hospitalization and end-hospitalization. Measurement of inflammatory markers especially CRP, IL-6 and serum ferritin might assist clinicians to evaluate the severity of COVID-19 because the variations of these biomarkers could serve to predict recovery of fatal outcome.

**Keywords**: COVID-19; SARS-CoV-2; inflammatory markers; severity.



## INTRODUCTION

On December 2019, a severe pneumonia emerged in Wuhan, China, caused by a novel coronavirus named 2019-nCoV. The terminology was later updated, the disease is named COVID-19 and it is caused by the virus SARS-CoV-2 [1, 2]. Severe acute respiratory syndrome coronavirus (SARS-CoV) is likely originated from bat [3], spread from person to person [4], cause not specific symptoms including fever, myalgia, fatigue, respiratory symptoms, pneumonia and severe respiratory syndrome with a typic ground glass on imaging[5].

As of March, 21, 2020, a total of 266, 073 confirmed cases from 150 countries and territories were reported, including 11,183 deaths (WHO, 2020b). On 01 june, 2020, the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) OMS reported that 6, 3 million infected with COVID-19 worldwide, with 376895 cases dead with a case fatality rate of 5.9 %.

Evidence has suggested that inflammatory responses play a critical role in the progression of Covid 19 [6]. Althought most of COVID-19 patients are mild, patients with severe type may quickly progress to acute respiratory distress syndrome (ARDS), ending up with multiple organ failure (MOF) and even death [7].

Therefore, exploring severe form of COVID-19 shares several clinical and laboratory features related to ARDS and MOF. This study aims to evaluate the characteristics and prognostic factors of disease severity in patients with COVID-19 in Marrakech investigating the correlation between abnormalities on laboratory

parameters including inflammatory markers such as procalcitonin (PCT), serum ferritin, C-reactive protein (CRP), lactate dehydrogenase (LDH) and interleukin-6 (IL-6) and the severity or mortality of the disease.

## MATERIAL AND METHODS

**Study design**

The university Hospital of Mohammed 6 was designated to treat COVID-19 patients. This study was a retrospective observational study respecting patient anonimity. A total of 52 hospitalized patients in intensive care unit, confirmed with COVID-19 in Errazi hospital between March 20, 2020, and May20, 2020 were enrolled. All patients were confirmed positively by real-time reverse transcription polymerase chain reaction (SARS-CoV-2 nucleic acid RT-PCR), using specimens derived from oropharyngeal swabs or sputum. Severe Covid-19 was defined based on the guidelines of the National Health Commission of Morocco, collaborating with the Moroccan Society of Anesthesia, Analgesia and Critical care (SMAAR) and the Moroccan Society of Emergency medicine (SMMU). Venous blood samples were collected every 48 h for monitoring pace. Biochemical indicators were measured by cobas 6000 (Roche, switzerland) immediately after sampling like Inflammatory markers such as Procalcitonin (PCT), serum ferritin, lactate déshydrogénase (LDH),C-reactive protein (CRP) and Serum cytokine levels like interleukin-6 (IL-6).

## DATA COLLECTION

Demographic characteristics, comorbidities, hospitalization time in ICU, and laboratory tests were collected from electronic medical records.

## STATISTICAL ANALYSIS

The descriptive statistics are median and interquartile range for continuous data. The statistics for categorical variables are counts and percentages. Student's t test and Mann-Whitney U test were performed respectively for normally and non-normally distributed quantitative data. A P value of less than 0 .05 was regarded as statistically significant. All statistical analyses were performed using IBM SPSS statistics version 16.0 for Windows.

## RESULTS

**1. Demographic and clinical characteristics:**

A total of 52 hospitalized patients with SARS-CoV-2 infection were included in this study, among them 39 (75%) were man and 13 (25%) were woman, the mean age was 58 years, 76,1% were up to 50 years old. with significantly older age for non-survivors than survivors (66.9 vs. 50.5, $p<0.05$). Among all non-survivors, 81% were male. The median course of the disease was 9 days, range from 1 to 31 days.

Underlying diseases, the most common was hypertension and diabetes with 25 % respectively, the most common co-existing comorbidities, followed by heart diseases (5 %), kidney diseases (5%), which was significantly higher in non-survivors than survivors (all $p<0.05$).

Respirator was used in 23 patients (44.2 %). A total of 30/38 patients (78.59 %) had received Hydroxychloroquine+Azythromycine. Only 6/38 patients (15.8%) had received antiviral medications: lopinavir/ritonavir. The fatality rate was 71.2 % in this population considered critical.

**2. Laboratory findings**

The routine blood test, procalcitonin (PCT), c-reactive protein (CRP) and ferritin , Lactate dehydrogenase (LDH), IL6 were used to reflect changes of inflammatory response in COVID-19. All measures were recorded on the day of admission for all patients, then every 48 hours.

Laboratory tests on admission showed the level of serum ferritin (median 845 [IQR 102.5–4275]; normal range30–400 ng/ml), CRP (median 174.15 [IQR 5.19–508.39] normal range 0–5 mg/l), PCT (median 0.68 [IQR 0.04– 45]; normal range 0 – 0.05 ng/ml), LDH (median 519.5 [90–1238]; normal range 240–480 UI/L), IL6 concerning 7/52 (median 101[IQR 17–201]; normal range >7 pg/ml, AST (median 47.5 [IQR 11–399]; normal range 10—50UI/L), TG (median 1.36 [IQR 1.19– 1.5]; normal range 0.35—1.5g/l).

**Table-1: Demographic characteristics and laboratory findings on admission of patients with COVID19**

| ICU patients (n=52) | | | Non-survivors | survivors |
|---|---|---|---|---|
| | Median (IQR) | No. (%) | Median (IQR) | Median (IQR) |
| **DEMOGRAPHIC CHARACTERISTICS** | | | | |

**Laboratory biochemical findings at admission**

| **Age** | | **58 (29-85)** | **——** | **>55 y : 26 ≤55 Y : 11** | **>55 y : 6 ≤55 Y : 9** |
|---|---|---|---|---|---|
| **Gender** | **Male** | 39 | 74 | 28 | 11 |
| | **Female** | 13 | 26 | 9 | 4 |
| **Comoribities** | **Diabetes** | 13 | 25 | 10 | 3 |
| | **HTA** | 13 | 25 | 10 | 3 |
| | **D+HTA** | 22 | 52 | 4 | 1 |
| | **heart diseases** | 4 | 5 | 2 | 2 |
| | **kidney diseases** | 4 | 5 | 3 | 1 |
| | **Tabac** | 4 | 7,7 | 3 | 1 |
| **Patient under respirator** | | 23 | 44,8 | 21 | 2 |
| **TTT Antiviral** | **HYDROXYCHLOROQUIN+AZYTHRO** | 30 | 78,9 | 20 | 10 |
| | **Lopinavir+Ritonavir** | 6 | 15,8 | 5 | 1 |

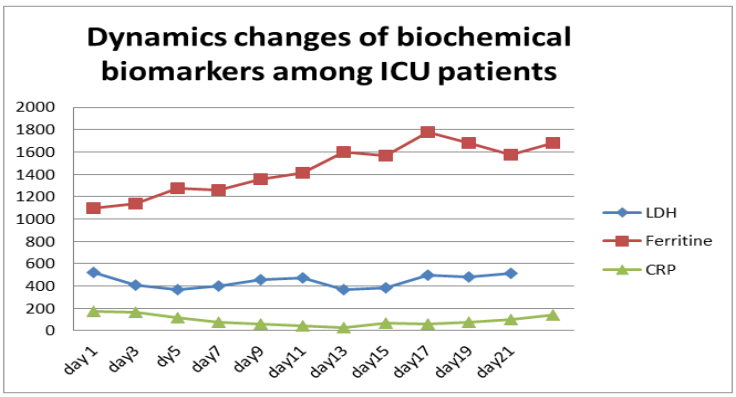
**Laboratory biochemical findings at admission**

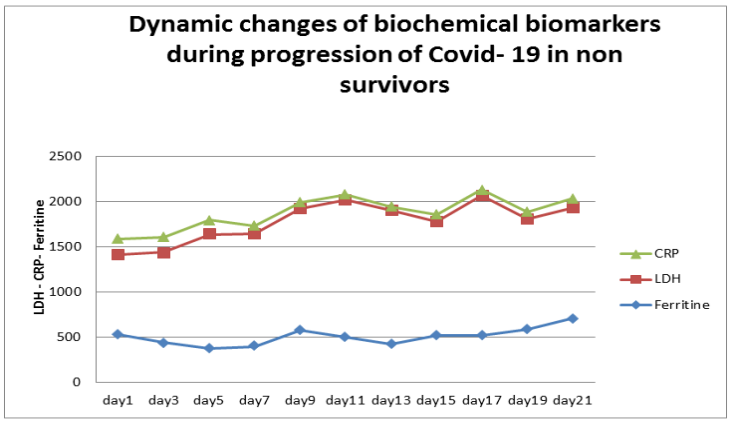
| **Ferritin (Moy)** | 84 (201, 17) | 845 | 1142 |
|---|---|---|---|
| **CRP** | 174,15 (508, 5) | 172 | 182 |
| **LDH** | 519,5 (1238, 90) | 519 | 533 |
| **PCT** | 0.68 (45,0.04) | 0.70 | --- |
| **IL6** | 101 (201,17) | --- | --- |
| **ASAT** | 47 (399,11) | --- | 59.15 |
| **TG** | 1.36 (1.5,1.19) | --- | --- |

On admission, regardless of the severity, majority of patients present an identical level of inflammation marker levels.

Compared with survivors, non-survivors showed significantly increased levels of IL-6, CRP, PCT, ferritin and LDH (all p<0.05). CRP, PCT and ferritin also showed a significant increasing trend with severer condition of the disease.

Dynamic changes of biochemical and immunologic biomarkers during progression of COVID-19 across on admission, mid-hospitalization and end-hospitalization



During hospitalization, survivors showed an increasing trend for both CRP (mid-term, the end of hospitalization, $p<0.05$) and serum level of ferritin (mid-term, and at the end of hospitalization, $p<0.05$), however, non-survivors remained at high-level without a significant increase.

For LDH, survivors showed a significantly higher level on admission and an increasing trend during hospitalization (mid-term and the end of hospitalization, $p<0.05$) whereas non-survivors started with a very high-level.

Biomarkers including IL-6 and PCT were maintained at lower levels or showed a slightly downward trend in survivors. In contrast, those biomarkers maintained at higher levels in non-survivors since admission, during hospitalization and of course at end of hospitalization. Significantly higher levels were found in non-survivors for IL-6, in critical patients, on admission.

## DISCUSSION

COVID-19 has been declared a Public Health Emergency of International Concern by WHOM, putting a huge amount of pressure on medical systems in a short time. Prognostic indicators are of great significance in conserving medical resources, guiding treatment and reducing mortality.

The inflammatory response plays a critical role in COVID-19, and inflammatory cytokine storm increases the severity of COVID-19[8].

For the main results, we found that the cases reported to date suggest that most are older adults more poor prognostic is observed and there is no difference in susceptibility between male and female. The most prevalent comorbidities are hypertension and diabetes which are associated with the rapid development of severe illness.

Xun et al. reported 25 death cases of with COVID-19, the clinical characters of these patients indicated that the age and underlying diseases were the most important risk factors for death [9].

In a retrospective study of 1591 consecutive hospitalized cases in intensive care in Lombardy (Italy), 82% were male. Grasselli et al., have found that 49% of patients had chronic hypertension, 21% were followed for cardiovascular disease, 17% for diabetes, and only 8% for neoplasia, 4% for chronic obstructive pulmonary disease, 3% for chronic renal failure [10].

Zhe Zhu et al., found higher age, and proportion of hypertension in the severe group [11]. In Wuhan, the patients aged 65 years or older in severe cases .Slightly more than half of all patients were male, and the proportion of males in severe cases was higher than in non severe cases. This difference is possibly explained by the frequency of risk factors for disease severity in the male population.

Our study confirmed again that higher age (>50 years old) was observed in 68% of death, 75% were man and 85% of our patients have underlying diseases.

Inflammation-related marker levels (high sensitivity C-reactive protein, lactate déshydrogénase (LDH) and ferritin) were significantly higher in severe cases, suggesting that there is a severe inflammatory cascade in patients with COVID-19 and used as indicators of disease progression [9].

CRP is a gauge of inflammation; it plays an important role in host defense against invading pathogens [12]. In the present study, CRP was elevated since admission, before death in 65% of patients, and then increased as compared to the first test, suggesting that the rising of CRP levels can be used as indicators of disease progression, what must be confirmed by bigger sample size study.

An early clinical study in Wuhan reported that 86, 3% of patients with COVID-19 had elevated hs-CRP levels [13]. Increased hs-CRP levels were likely due to COVID-19 related acute inflammatory pathogenesis during which multiple cytokines were released and their amount was associated with disease severity [5].

In addition, PCT is a useful marker and a sensitive indicator of bacterial infection who may play an important role in promoting the death of patients [14].

In line with prior studies, we also showed that 96.3% of our patients had elevated levels of PCT in admission, and 98.4% in the last test among dead patients.

Previous studies have found that the levels of procalcitonin, LDH were elevated in 9.5%, 73.6% of all patients, respectively. As well as PCT levels were elevated in 90.5% of severe patients [14, 15].

Severe patients had significantly high markers of inflammation (CRP, ferritin, Interleukin 6) [16]. Elevated CRP, up to 150mg/L, hyperferritinemia, elevated LDH in approximately 40% of patients associated with an increase in D-dimers, stigmas of coagulopathy associated with severe and predictive forms of mortality [17, 18].

Other conventional markers related to inflammatory damage, such as Ferritin also belong to acute phase proteins. Serum ferritin levels can be affected by iron status and may indicate a hyper immune state. So, hyperferritinemia can be used as

prognostic markers for tissue injury or acute infections. Also, it is a marker for hemophagocytic lymphohistiocytosis, which is a known complication of viral infections [19].

In accord with previous studies, we confirmed the observation of the presence of hyperferritinemia in patients with covid-19 since admission with a dynamic increasing during the hospitalization. Another study on COVID-19, found out a dynamic increasing of ferritin in non-survivors since on admission [16].

Also we have found that LDH levels were much higher in the dead patients, correlated with the severity of Covid-19. In the study of Wu et al., he indicated that a high LDH level is a risk factor for Covid-19 severity [18].

The cytokine storm caused by an excessive immune response against the virus may cause deleterious consequence during SARS-COV-2 infection. Especially, IL-6 plays a key role in cytokine storm, it is more widely used and more meaningful clinically, compared with other cytokine [17].

In our study, IL-6 is not a routine laboratory test, which limits their usage, and it was higher in patients (n=10). In constent, it was found that IL-6 was correlated with other inflammation parameters, and a most close relationship was observed between IL-6 and severity [17].

Similary, the elderly (≥55 years) and a high level of IL-6 (≥20 pg/mL) were markers associated with the progression to severe and critical illness among COVID-19 patients [20].

Yang et al. pointed out those peripheral blood IL-6 levels could be used as an independent factor to predict the progression of Journal COVID-19, which is consistent with the results of this study [8].

The concept of "cytokine storm" proposed by Professor Li Lanjuan, demonstrated that inflammatory factors played a crucial role in the progression from mild to severe disease. As one of the most distinctive acute phase reactants, CRP increased rapidly after the onset of inflammation [20].

In clinical practice, the curve of inflammatory indicators should be drawn dynamically. In our study, the serum levels of IL-6 and CRP presented with a zigzag curve in critically ill patients, we think that this obvious change may be caused by medical intervention. We conclude that refractor inflammatory reaction can predict a poor outcome of patients.

This study indicated preadmission medication for patients with severe COVID-19. Only 2 cases of young patients without comorbidities used AINS.

In our study, 79% of our severe patients take Hydroxychloroquine + Azythromycine treatment and 15.8% take lopinavir/ritonavir treatment. The last one offered no significant benefit over standard care for patients with COVID-19. However, our study was an observational study; thus, the benefit of treatments for patients with severe COVID-19 needs to be further confirmed.

Patients with older age, hypertension and diabete, and high level of inflammatory markers need careful observation in ICU. Measurement of inflammatory markers especially CRP, IL-6 and serum ferritin might assist clinicians to evaluate the severity of COVID-19 because the variations of these biomarkers could serve to predict recovery of fatal outcome.

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