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# Comparison of Changes in Inflammation Markers NLR, CRP, and LCR after Corticosteroid Therapy in Severe and Critical COVID-19 Patients

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**Abstract**

Corticosteroid, an immunomodulator agent for treating hyperinflammation, is widely used in severe and critical COVID-19 patients. This study is a retrospective observational cohort clinical study comparing changes in NLR, CRP, and LCR values with and without corticosteroid therapy in critically ill COVID-19 patients. Those samples were chosen due to widespread access and economically sound to be examined repetitively. Samples were collected by total sampling of medical records of patients admitted to the COVID-19 intensive care unit at RSUD Dr Soetomo between March 2020 to July 2021. Inclusion criteria are complete medical records of patients > 18 years with a confirmed COVID-19 diagnosis with severe and/or acute symptoms according to the WHO criteria and receiving corticosteroids (dexamethasone 6 mg/24 h or equivalent dose) for a minimum of 3 days. Patients not receiving corticosteroid treatment were included in the control group. NLR, CRP, and LCR were evaluated on day-0, day-3, day-6, and day-10 after initiation of corticosteroid therapy; in the control group, their hospital admission was designated as day-0. Comorbidities, complications, and other therapies that may affect NLR, CRP, and LCR values are noted. A total of 460 patients were included in the inclusion criteria. The control group had no significant median NLR, CRP, and LCR changes during observation ($p$-value > 0.05). In the therapy group, there was a significant increase in NLR and LCR and a decrease in CRP ($p$-value < 0.0001). When compared between control and therapy groups, the median changes in NLR and CRP differed significantly ($p$-value < 0.05), while significant differences in LCR occurred on days 6 and 10. Corticosteroid increases NLR and LCR and decreases CRP with significant differences between groups.

**Keywords:** Severe and critical COVID-19 patients, Corticosteroid, NLR, CRP, LCR, Inflammatory markers

## Introduction

The first report of the SARS-CoV-2 case was reported on 31st December 2019 in Wuhan, China. Since the World Health Organization (WHO) declared the pandemic situation in March 2020, 756 million people have been confirmed to be infected, and 6 million deaths have been caused by the coronavirus disease 2019 (COVID-19) until 15th February 2023 [1]. The National Institute of Health Research and Development, Ministry of Health of Indonesia, reported the first COVID-19 case in Indonesia on 1st March 2020 [2]. As of 16th February 2023, almost 7 million confirmed COVID-19 cases have been reported in Indonesia. With 160,000 deaths related to COVID-19, the mortality rate in Indonesia reached 2.39 % [3].

As the pandemic's source's epicentre, initial epidemiological reports from China provide a clinical picture that most COVID-19 sufferers (81 %) experience mild cases [4,5]. As many as 13.9 % of patients suffer from moderate or severe cases. In comparison, around 4.8 % of patients become critical due to acute respiratory distress syndrome (ARDS), sepsis and septic shock, encephalopathy, and multiorgan disorders that require treatment in an emergency unit - intensive care unit (ICU) [6]. The relatively low percentage of severe and critical COVID-19 cases compared to the total number of cases indicates that the severity of

the clinical condition does not depend on the pathogen's virulence but is influenced by the immune system response of each patient.

In severe or critical COVID-19 patients, there is an excessive and prolonged release of proinflammatory cytokines, which causes a cytokine storm or cytokine release syndrome. Cytokine storm causes vasodilation and coagulation disorders resulting in massive tissue and organ perfusion disorders, leading to multiorgan failure, including ARDS, liver failure, heart failure, and acute kidney failure [7,8]. This pathophysiology forms the basis for administering immunomodulatory drugs in severe and critical cases of COVID-19 to suppress the immune system [9]. The immune system status of COVID-19 patients needs to be evaluated periodically to 1) detect early patients who have the potential to become severe or critical and 2) evaluate the immunomodulation therapy that has been given.

While tests for quantifying levels of various proinflammatory cytokines are available, their routine implementation is hindered by cost constraints, limited laboratory facilities, and reagent availability. Several immuno-inflammatory parameters are indicators of immune system status in COVID-19 cases with statistically significant sensitivity, including the neutrophil-lymphocyte ratio (NLR) and C reactive protein (CRP). The lymphocyte-CRP ratio (lymphocyte-CRP ratio - LCR) is a parameter that has so far been more widely used as an indicator of immune status in cancer, but several studies have proven a correlation between LCR and morbidity and mortality in COVID-19 patients [10-12].

In severe and critical COVID-19 patients there is an increase in NLR and CRP values. The sustained neutrophilia and lymphopenia indicate ongoing infection with hindered adaptive immune response [13]. CRP is an active phase reactant; the upsurge in CRP levels is directly proportional to the degree of tissue damage and the concentration of inflammatory modulators. CRP's speed of production in response to inflammatory precipitating factors and short half-life describes the actual dynamics of inflammatory status [14]. LCR combines both absolute lymphocyte count and CRP; hence, LCR is inversely related to degree of inflammation and disease severity [12].

Corticosteroids, especially the glucocorticoid group, are one of the immunomodulatory modalities proven to provide therapeutic benefits in suppressing hyperinflammation. Since the Severe Acute Respiratory Syndrome (SARS) outbreak in 2003, corticosteroids have become the immunomodulator of choice; several studies have found that timely administration of corticosteroids in severe and critical cases leads to improvements in clinical symptoms, oxygenation, and radiological examination [9,15]. Corticosteroids possess anti-inflammatory and immunosuppression effects by altering the concentration, distribution, and effectiveness of leucocyte activity in periphery tissues [16].

Dexamethasone is a synthetic glucocorticoid with immunosuppressive activity 5 times more potent than methylprednisolone and 30 times more potent than cortisol. The RECOVERY (Randomised Evaluation of COVID-19 Therapy) study by Horby *et al.* [17] is the first large-scale, randomised, open and controlled study to compare standard therapy with dexamethasone (6 mg per 24 h, equivalent to 32 mg methylprednisolone) for 10 days. The study found that administration of dexamethasone reduced day 28 mortality in COVID-19 patients who received oxygen therapy or mechanical ventilation at the start of therapy but not in patients who did not require oxygen therapy [17]. After the RECOVERY study results were published, administering low-dose dexamethasone became part of the protocol for treating severe and critical COVID-19 patients. However, until now, more clinical trials have examined clinical outcomes such as length of stay, probability of using a ventilator, and mortality.

No studies have compared changes in the values of the inflammatory parameters NLR, CRP, and LCR after dexamethasone administration in COVID-19 patients to monitor the effect of therapy. These 3 markers of inflammation can be done in clinical pathology laboratories in almost all hospitals, including remote areas. This study was made to see the effect of giving dexamethasone to COVID-19 severe and critical patients on changes in the parameters of inflammatory markers NLR, CRP, and LCR as indicators of therapy success.

## Materials and methods

### Study design and participants

We conducted a single-centre retrospective observational study on admission to the intensive care unit of Dr Soetomo General Hospital, Surabaya, East Java, Indonesia. The study was conducted between March 2020 and July 2021. The Ethics Committee of Airlangga University approved the study under 1207/LOE/301.4.2/I/2023.

The study sample was the medical records of all adult patients with confirmed severe/critical COVID-19 according to WHO standards which met the inclusion and exclusion criteria, who received corticosteroids (dexamethasone 6 mg/24 h or an equivalent dose orally or intravenously) for at least 3 days

while undergoing treatment RSUD Dr Soetomo within the study period. Patients with incomplete medical records did not meet the severe or critical degree, and patients who died or were discharged before completing the third day of treatment were excluded from this study.

**Data sources, variables, and measurements**

From the patients' electronic medical records, following the patient's consent to share the data and authorisation of the local research ethics committee. We collected the demographic data (age and gender), data associated with comorbidities, corticosteroid treatment (type, dose, and duration of administration), along with the length of stay in the ICU or days until death and outcome. Inflammatory marker data from this study consisted of NLR, CRP, and LCR values measured on day-0, 3, 6 and 10. To compare changes in inflammatory markers between patients who received and did not receive corticosteroids, study samples that met the inclusion criteria (n = 460) were then divided into 2 groups: The control group and the corticosteroid therapy group. The control group consisted of 122 (26.5 %) patients, whereas in the corticosteroid therapy group, there were 338 (73.5 %) patients. The discrepancy between both groups were due to time period of sampling; patients admitted after July 2020 were almost always given corticosteroids following the results of the RECOVERY trial, which is the reference for administering low-dose corticosteroids in severe and critical COVID-19 patients.

**Statistical analysis**

The obtained data were subjected to the normality distribution through the Saphiro-Wilk test. Categorical data are presented as counts and percentages in each category, while the continuous data are expressed as median ± interquartile range. Continuous data with no normal distribution were compared using the Mann-Whitney test (between 2 groups) or Kruskall-Wallis (more than 2 groups). Meanwhile, categorical data comparisons were completed using Fisher exact test. Data not normally distributed are presented as the median (interquartile), and the frequency distribution is expressed as a percentage (%).

Univariate and multivariate logistic regression models for mortality outcome were used to identify risk factors (age, sex, haematological parameters, systemic inflammation indexes, clinical comorbidity, treatment, and clinical complications). The odds ratio (OR), 95 % CI and *p*-values were also calculated. All tests were 2-tailed, and a $p$-value $< 0.05$ was considered statistically significant. GraphPad Prism version 9.5.1 for Windows, GraphPad Software, San Diego, California USA, was used to perform all statistical analyses.

## Results and discussion

**Demographic analysis**

From March 2020 to July 2021, a retrospective data search was carried out at the intensive care unit for COVID-19 patients in Dr Soetomo's general hospital and obtained 469 patients. A total of 9 patients were excluded because they underwent intensive care for less than 3 days or there was no data of repeated NLR, CRP, or LCR examinations. The demographic and clinical characteristic analysis of this study is presented in **Table 1**.

In this study, 61.74 % of the subjects were male and 38.26 % female. The mean age was 49.88 ± 13.06 years, while the median age category of patients was 51 years. The age category most infected with COVID-19 was the 50 - 60 years old group, with 139 (30.22 %) patients. A total of 251 (54.57 %) patients survived after undergoing treatment at ICU which was an Intensive Care Unit (ICU), while 209 patients (45.43 %) died. In the control group, 27.27 % of patients died, while in the corticosteroid therapy group, 74.10% of patients survived after treatment at ICU. The most common comorbidities were diabetes mellitus, hypertension and obesity, with percentages of 53.26, 48.91 and 26.74 %.

This study's findings align with previous studies' findings since the start of the pandemic, in which the male gender was a risk factor for experiencing more severe complaints and symptoms of COVID-19. A meta-analysis conducted by Simadibrata *et al.* [18] found a more significant proportion of men in the group of patients with severe or dying degrees. This event arises from the genetic interplay, immunological, psychosocial, and behavioural health factors among individuals of different genders. Genetically, levels of the hormone ACE2 in plasma and expression of ACE2 receptors in various organ systems are higher in males than females [19]. Innate immune response in male shows greater strength against SARS-CoV-2 infection, as indicated by higher concentrations of IL-8 and IL-18 in plasma compared to females, along with increased monocytes induction. Instead, women's immune response is more adaptive to specific T cell activation. Sex hormones influence humoral and cellular immune responses that differ between the sexes; testosterone is generally immunosuppressive, whereas estrogen has a more potent immunomodulating

effect. These 2 factors contribute to males being more susceptible to severe or critical COVID-19 [20,21]. Regarding psychosocial and health behaviour, women tend to comply more with health protocol rules, such as washing hands, maintaining physical distance in social settings, wearing masks, avoiding smoking and excessive alcohol consumption, and actively seeking medical advice [19].

**Table 1** Demographic and clinical characteristics of the patients.

| Characteristic | Median | |
|---|---|---|
| | n | % |
| **Sex** | | |
| Women | 176 | 38.26 |
| Men | 284 | 61.74 |
| **Age group (mean 48.88 ± 13.06)** | | |
| < 40 | 116 | 25.22 |
| 40 - 49 | 89 | 19.35 |
| 50 - 60 | 139 | 30.22 |
| > 60 | 116 | 25.22 |
| **ICU survival** | | |
| Survive | 251 | 54.57 |
| Deceased | 209 | 45.43 |
| **Comorbidities** | | |
| Diabetes mellitus | 245 | 53.26 |
| Hypertension | 225 | 48.91 |
| Obesity | 123 | 26.74 |
| Cardiovascular disease (CVD) | 86 | 18.70 |
| Geriatric | 63 | 13.70 |
| **Time elapsed from symptom onset to ICU admission** | | |
| Median (days) | 8 | |
| IQR (days) | 6 - 12 | |
| **Length of stay (LOS)** | | |
| Median (days) | 16 | |
| IQR (days) | 11 - 23.75 | |

In this cohort, the average age of patients was 48.55 years, but 50 - 60 years was the age group with the most significant percentage. Compared to the demographics of COVID-19 patients who require intensive care in various countries, the mean and age range of the patients in this study is younger. The average age of severe and critical COVID-19 patients in ICU in China, America, and Europe is 63 - 65 years [22-24]. In the 3 epidemiological reports, most patients with severe or critical degrees of COVID-19 admitted to ICU were mainly from the age group above 65 years. A meta-analysis of reports on 611,583 patients found that the mortality rate was lower than 1.1 % in patients younger than 50 years; the increase in mortality increases exponentially above that age limit [25]. Ageing affects the immunological system, leading to immunosenescence. It impairs the adaptive and innate immune systems, disrupting the regulation of cytokine secretion and expression. Inflammation worsens this effect by intensifying the inflammatory effects of cytokines and chemokines [26].

During the study period, the survival rate of ICU patients was 54.57 %, which aligns with Mahase's reported ICU mortality ratio of 59.5 % in March 2020 and decreased to 41.6 % in May 2020 [27]. Along with the expansion of vaccine acceptance, infections dominated by new variants with lower pathogenicity,

and understanding of the pathophysiology and management of SARS-CoV-2 therapy, the mortality rate at ICU due to COVID-9 fell to 27.8 % by 2021 [28]. The success of treating patients is influenced by many things, including the patient's age and comorbidities, length of stay, available medical facilities and infrastructure. Therefore, the percentage of surviving COVID-19 patients varies between countries and ICUs within one country.

In our research, a total of 186 patients (74.1 %) obtained low-dose corticosteroid survival after intensive care. The magnitude of the survival rate is not much different from the results obtained from the RECOVERY study. In the study by Horby *et al.* [17], as many as 77.1 % of moderately severe COVID-19 patients who required oxygen therapy and received low-dose dexamethasone survived for 28 days. Corticosteroids work as immunomodulators by influencing cells of the immune system to produce pro- and anti-inflammatory cytokines, as well as preventing tissue damage triggered by excessive inflammation [29]. However, for inflammation caused by infection with a pathogen, administering corticosteroids must consider the patient's clinical criteria, time, dose, and administration duration to prevent immune suppression's side effects. In COVID-19, giving corticosteroids too early cause a delay in eliminating the virus from the body [30]. Doses that are too large or given for too long are a factor in causing secondary infections and prolonging the length of stay at the ICU or hospital [31]. In order to regulate the duration of corticosteroid administration, various inflammation markers, such as CRP, have been employed for monitoring purposes [32,33].

In this study, comorbid data were obtained from patients. The 3 most comorbidities are diabetes mellitus, hypertension, and obesity. Consistent with several previous studies, diabetes mellitus predicts severity and mortality in COVID-19 patients. Diabetes mellitus, especially those that are not well regulated, is related to the progression of COVID-19 because hyperglycemia conditions cause cellular and humoral immune dysfunction, which can exacerbate hyper-inflammatory conditions and facilitate secondary nosocomial infections [34].

Comorbid hypertension is a risk predictor of severe and critical COVID-19. The prevalence of COVID-19 patients with hypertension who needed treatment at the ICU compared to those who did not was 58.3 % vs. 21.6 % ($p$-value < 0.001). In Gao's [35] multivariate analysis, hypertension was independently associated with the risk of severe clinical manifestations (OR 1.562; $p$-value = 0.092) and death (OR 1.262; $p$-value = 0.458). This is because, in hypertensive patients, there is an increase in the expression of the ACE-2 enzyme, which facilitates SARS-CoV-2 infection, tissue damage, and organ failure [36].

A total of 26.74 % of patients in this study had comorbid obesity. In a population-based cohort study of 433,995 COVID-19 patients in Spain, it was concluded that obesity increases the risk of clinical deterioration (aRR 2.20) and hospitalisation (aRR 2.30); the correlation was even higher in patients in the age group under 50 years (aRR 13.80) compared to the age range 65 - 79 years (aRR 5.02) [37]. This clinical outcome is influenced by several pathophysiologies that contribute to increased secretion of inflammatory mediators, primarily IL-6, in the pulmonary tissue and the production of thrombosis. From the mechanics of the respiratory system, in obesity, there is a disturbance of chest wall elastance and decreased compliance. At the cellular level, there is an increase in ACE2 expression in adipocytes and pulmonary tissue lipofibroblast and an increase in insulin resistance so that the hyperinflammatory condition occurs chronically [35].

Inflammatory marker data from this study consisted of NLR, CRP, and LCR values measured on day -0, -3, -6 and -10. To compare changes in inflammatory markers between patients who received and did not receive corticosteroids, study samples that met the inclusion criteria (n = 460). The control group consisted of 122 (26.5 %) patients, whereas in the corticosteroid therapy group, there were 338 (73.5 %) patients.

**Median inflammatory markers on day-0, -3, -6, and -10**

**Table 2** shows data on inflammatory markers at initial hospitalisation (day-0) in all study subjects. The mean number of neutrophils of all patients was 8,450/µL. There was a statistically significant difference between the median of absolute neutrophils in the corticosteroid therapy group (7.920 (IQR 5.120 - 11.120)/µL) and the control group (9.770 (6.360-13.2240)/µL; $p$-value < 0.0005). The absolute number of lymphocytes between the 2 groups was not significantly different; the median lymphocyte for the treatment group was 960/µL, and for the control group, 1,010/µL with a $p$-value of 0.458.

The median NLR value for all patients was 8.3 (5.0 - 15). There was a statistically significant difference between the median NLR values of the corticosteroid therapy group and the control group (7.6 (4.8 - 14) vs. 9.5 (5.6 - 17)), $p$-value = 0.0162. CRP analysis yielded a median CRP of 11 mg/dL (IQR 4.8 - 18) in all study subjects. In the steroid group, the median CRP value was 11 (4.9 - 20) mg/dL, while in the control group, it was 10 (4.3 - 15) mg/dL. The 2 comparative tests gave $p$ = 0.066, indicating that there

was no significant difference between the CRP of the 2 groups at the start of treatment. The median LCR of all study subjects was 88 (45 - 253), in the therapy group 85 (41 - 253), and 96 (55 - 275) in the control group. From the Mann-Whitney comparison test, *p*-value = 0.1415 was obtained, indicating no significant difference in the median initial LCR between the 2 groups.

**Table 2** Median inflammatory markers on day 0.

| Inflammatory markers | Total patients (n = 460) | Group | | *p*-value |
|---|---|---|---|---|
| | | Steroid (n = 338) | Control (n = 122) | |
| Neutrophil absolute (/µL) | 8,450 (5,410 - 11,690) | 7,920 (5,120 - 11,120) | 9,770 (6,360 - 13,240) | **0.0005*** |
| Lymphocyte absolute (/µL) | 990 (690 - 1.350) | 960 (650 - 1.340) | 1,010 (720 - 1,350) | 0.458 |
| NLR | 8.3 (5.0 - 15) | 7.6 (4.8 - 14) | 9.5 (5.6 - 17) | **0.016*** |
| *C-reactive protein* (mg/dL) | 11 (4.8 - 18) | 11 (4.9 - 20) | 10 (4.3 - 15) | 0.066 |
| LCR CRP mg/dL | 88 (45 - 253) | 85 (41 - 253) | 96 (55 - 275) | 0.142 |

Data presented as median (interquartile range).
*Mann-Whitney test between steroid and control groups, significantly different if *p*-value < 0.05.

The median of each inflammatory marker was calculated and analysed based on the measurement day between the 2 groups. Comparisons were calculated and analysed between 1) the median of day-0 and 10 for each marker by treatment group, 2) the median for each day of measurement between the 2 groups, and 3) the median between the 0 - 3 day, 3 - 6 day, and 6 - 10 day in the therapy group. In the therapy group, day-0 of treatment was counted from the first day of receiving corticosteroids, while in the control group, day-0 was counted from the day of admission to the hospital.

In the control group, the median neutrophils and lymphocytes significantly increased during the first 10 days of treatment. The NLR median increased on the 3$^{rd}$ and 6$^{th}$ days but then decreased on the tenth. A comparison of the median NLR at the beginning and the end of the observation period showed no significant differences (*p*-value = 0.225). The CRP median peaked on day 3 and gradually decreased on days 6 and 10 without statistical significance (*p*-value = 0.548). Median LCR in the control group showed biphasic changes, with increases on days 3 and 10 and decreased on days 6. Overall, the median LCR of the control group experienced no significant change during the first 10 days of treatment, as shown in **Table 3**.

**Table 3** Median inflammatory markers of the control group on treatment day-0,-3, -6, and -10.

| Inflammatory markers | | Day-0 | Day-3 | Day-6 | Day-10 | *p*-value |
|---|---|---|---|---|---|---|
| Neutrophils (/µL) | Median | 9,774 | 11,084 | 12,276 | 13,603 | **0.0004*** |
| | IQR | (6,248 - 13,239) | (8,501 - 14,012) | (8,644 - 16,362) | (8,714 -19,822) | |
| Lymphocytes (/µL) | Median | 1,014 | 1,002 | 1,112 | 1,384 | **0.0007*** |
| | IQR | (723 - 1,350) | (743 - 1,496) | (705 - 1,465) | (926 - 1,384) | |
| NLR | Median | 9.5 | 10.61 | 12.25 | 10.52 | 0.225 |
| | IQR | (5.6 - 17) | (6.6 - 16) | (7.1 - 20) | (5.1 - 18) | |
| CRP (mg/dL) | Median | 10 | 11,45 | 10.7 | 10.4 | 0.548 |
| | IQR | (4.3 - 15) | (7.1 - 18) | (4.3 - 22) | (3.5 - 20) | |
| LCR | Median | 96 | 115.9 | 80.8 | 120.3 | 0.728 |
| | IQR | (55 - 275) | (45 - 220) | (43 - 246) | (63 - 435) | |

*Kruskal-Wallis test, significant if *p*-value < 0.05

The treatment group showed a different result, as shown in **Table 4**. There was an increase in the median NLR and LCR and a decrease in CRP values in the 3 repeat examinations in the exposure group. The comparative test between the median before and after corticosteroid treatment was significantly different for the 3 ($p$-value < 0.0001). When broken down per day of examination, the increase in NLR and LCR and decrease in CRP were significantly different between day-0 and -3 and day-3 and -6; the median change of the 3 parameters was insignificant between day-6 and day-10.

**Table 4** Median inflammatory markers of the therapy group on treatment day-0, -3, -6, and -10.

| Inflammatory markers | | Day-0 | Day-3 | Day-6 | Day-10 | $p$-value |
|---|---|---|---|---|---|---|
| Neutrophils (/μL) | Median | 9,605 | 9,955 | 13,035 | 15,932 | **< 0.0001*** |
| | IQR | (6,890 - 14,161) | (7,265 -14,195) | (9.038 - 18,623) | (11,310 - 21,766) | |
| | $p$-value | Day-0 v. Day-3 = 0.310 | | Day-6 v. Day-10 **< 0.0001**** | | |
| | | - | Day-3 v. Day-6 **< 0.0001**** | | - | |
| Lymphocyte (/μL) | Median | 827 | 773 | 850 | 1,000 | **0.0073*** |
| | IQR | (580 - 1,245) | (550 - 1.183) | (524 - 1,397) | (570 - 1,000) | |
| | $p$-value | Day-0 v. Day-3 = 0,276 | | Day-6 v. Day-10 = 0.060 | | |
| | | - | Day-3 v. Day-6 = 0,1412 | | - | |
| NLR | Median | 7.61 | 12.06 | 15.22 | 16.62 | **< 0.0001*** |
| | IQR | (4.8 - 14) | (7.5 - 20) | (8.6 - 25) | (8.1 - 29) | |
| | $p$-value | Day-0 v. Day-3 **< 0.0001**** | | Day-6 v. Day-10 = 0.265 | | |
| | | - | Day-3 v. Day-6 **0.0133**** | | - | |
| CRP (mg/dL) | Median | 11.1 | 6.65 | 3.5 | 3.9 | **< 0.0001*** |
| | IQR | (4.9 - 20) | (2.2 - 13) | (1.2 - 99) | (0.6 - 12) | |
| | $p$-value | Day-0 v. Day-3 **< 0.0001**** | | Day-6 v. Day-10 = 0.638 | | |
| | | - | Day-3 v. Day-6 **0.0002**** | | - | |
| LCR | Median | 85.32 | 122 | 266.7 | 306.5 | **< 0.0001*** |
| | IQR | (40.8 - 253) | (63 - 381) | (74 - 924) | (65 - 1888) | |
| | $p$-value | Day-0 v. Day-3 **0.0010**** | | Day-6 v. Day-10 = 0.320 | | |
| | | - | Day-3 v. Day-6 **< 0.0001**** | | - | |

*Kruskal-Wallis test, significant if $p$-value < 0.05
**Mann-Whitney test, significant if $p$-value < 0.05

The median NLR at admission for all patients was 8.3 (IQR 5.0 - 15); a score below 9 indicates mild physiological stress and is not in a critical clinical condition (Farkas, 2019). When divided into treatment groups, patients receiving steroids had a lower median NLR than the control group (7.6 (IQR 4.8 - 14) vs 9.5 (5.6 - 17) with $p$-value = 0.0162). This result is probably because the control group mainly came from the early COVID-19 pandemic (before July 2020), when there was no standardised corticosteroid therapy protocol for severe and critical patients requiring oxygen therapy and observation at ICU.

The comparison of NLR, CRP, and LCR values between the control and treatment groups on days 3, 6, and 10 is shown in **Figure 1**. On day-3, the median NLR and CRP between the control and treatment groups showed a significant difference, while the LCR values did not differ significantly between the 2 groups. Comparison tests of the median NLR, CRP, and LCR of the 2 treatment groups on day-6 and 10 resulted in a statistically significant difference ($p$-value < 0.05) between the 2 groups.

NLR has long been used as a parameter of inflammation in cases of bacterial infection, which can be used as a predictor of clinical correlation and mortality. De Jager's prospective cohort study concluded that

in patients with bacterial community-acquired pneumonia (CAP), NLR values at baseline were more accurately predictive of clinical deterioration and mortality than conventional parameters of inflammation, such as CRP, leukocyte count, and absolute count neutrophils [38]. NLR also has a positive correlation with scores or clinical severity indexes of bacterial pneumonia, such as CURB-65 (Confusion, Uremia, Respiratory rate, Blood pressure, age > 65) or PSI (Pneumonia Severity Index) [38,39]. However, the COVID-19 pandemic is the first time NLR has been recognised as a predictor of inflammatory status, clinical correlates, and mortality for viral CAP. In SARS-CoV-1 and MERS infections, neutropenia occurs, so the NLR value does not increase [40]. The NLR value in this study is similar to a study conducted in Mexico involving 242 participants from March to June 2020. In that retrospective study, Albarrán-Sanchez and colleagues found that in severe COVID-19 patients who survived the average NLR upon admission was significantly higher than non-survivor patients (8.31 (IQR 4.22 - 13.05) and 17.66 (9.99 - 26.25), respectively; $p$-value < 0.001) [41].

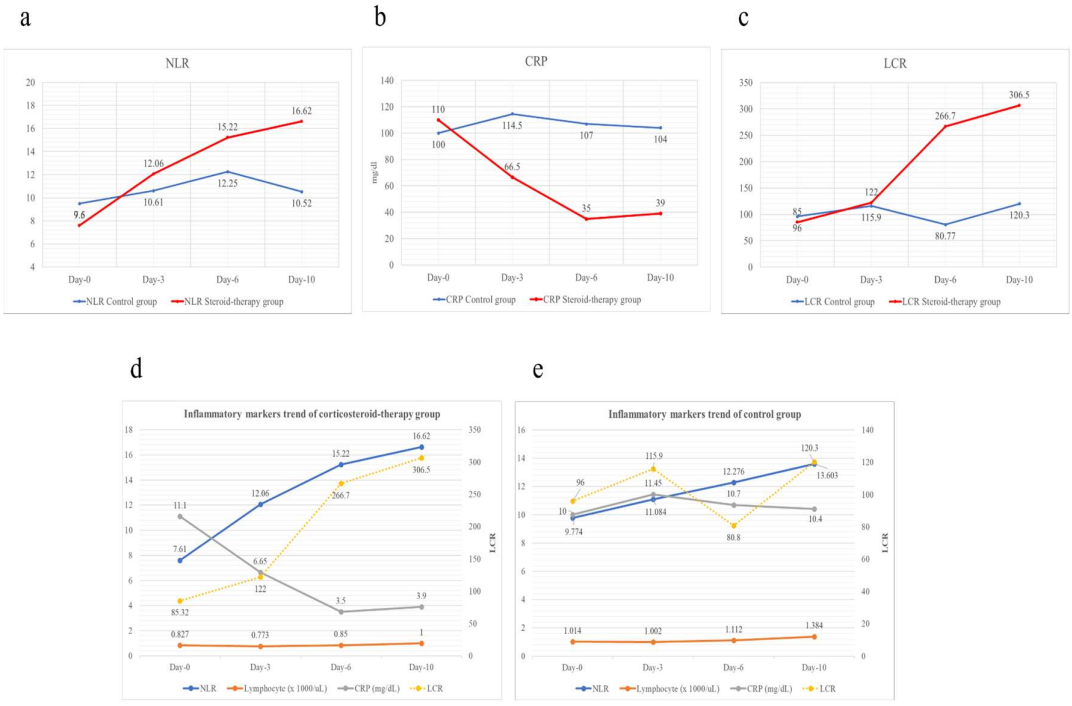


**Figure 1** Examination of variations in median a) NLR, b) CRP and c) LCR between corticosteroid-exposed groups and controls on day-0, -3, -6, and -10 and changes in median of NLR, absolute lymphocytes, CRP, and LCR for both the d) corticosteroid-treated group and e) control group on the specified days.

The median value of initial CRP during hospitalisation for all patients in the study was 11 mg/dL (IQR 4.8 - 18) or the equivalent of 110 mg/L. When grouped based on the received corticosteroid therapy, there was no significant difference between the median CRP and corticosteroid treatment group (110 (49 - 200) mg/L compared to control group 100 (43 - 150) mg/L, $p$-value = 0.066). CRP has been one of the most widely used biological markers and an indicator of inflammation since the pandemic's start, especially since it was discovered that IL-6 is one of the most elevated proinflammatory cytokines in acute phase of hyperinflammation. According to the pathophysiology in which CRP production is influenced by changes in levels of IL-1 and IL-6 in the systemic circulation, it is not uncommon for CRP to be used as a surrogate for IL-6 examination, especially in healthcare centres with limited funds and examination laboratory facilities.

Increased CRP values up to 100 mg/L can occur in acute uncomplicated viral infections caused by adenovirus, influenza, and cytomegalovirus. Acute bacterial infection generally causes an increase in higher CRP, up to 15 - 350 mg/L [42]. It can be concluded that the median CRP value at the beginning of hospital admission in this study reflects an acute viral infection that is likely to present with complications. This observation aligns with the clinical condition of patients who suffered from severe or critical COVID-19

pneumonia but without any occurrence of secondary bacterial infections or complications related to organ failure.

LCR is the ratio of lymphocytes to CRP; Median LCR at initial hospitalisation for all patients was 88 (IQR 45 - 253), 85 (41 - 253) in the therapy group and 96 (55 - 275) in the control group. Compared to NLR and CRP, LCR is rarely used as a biological marker in inflammation elicited by an infectious process. During the COVID-19 pandemic, LCR is starting to be looked at as one of the parameters of inflammation because it has several advantages: 1) compared to NLR, LCR value is not affected by neutrophilia caused by immune system dysfunction, physiological stress, or post steroid therapy; 2) sensitive to changes in the immune response because it uses the absolute number of lymphocytes (reflecting the status of the immune response adaptive of cellular components) and CRP (reflects the concentration of the phase reactant acute and as a substitute for IL-6); and 3) cost-effective for monitoring disease progression and treatment effectiveness [12].

The LCR value in this study population is lower when compared with results obtained in Harbin, China (n = 184 between January 2020 - March 2021). Zhang *et al.* [43] reported a significant difference between initial LCR hospitalisation of mild/moderate patients with severe/critical (2,407.47 (IQR 640 - 8,755) vs 140.42 (71.32 - 402.27; *p*-value = 0.000)). They excluded patients who may have chronically elevated CRP so that the median CRP is reported lower. However, lymphopenia in patients with severe degrees is very low (median 650 (420 - 990)/μL), so the range of LCR values for this group is lower than in this study. Seen from the demographic data provided by the research team, the median age of the patients was high (68 (58.25 - 75.75) years) could be the cause of lymphopenia [43].

Research in Mexico resulted in lower LCR values than in Surabaya and China; of 242 patients treated between March - June 2020, the average initial LCR of patients with severe degrees of COVID-19 who survive is significantly lower than non-survivors (6 (IQR 0.3 - 13) vs 0.3 (0.2- 0.7); *p*-value < 0.001). This very low LCR is due to the high average CRP in the study population (111 (43 - 216) mg/L vs 217 (150 - 284) mg/L), which is likely to be influenced by the study sample which is dominated by male patients (67 %) and the higher incidence of patients who have more than one comorbid in between hypertension, diabetes, and heart disease [41].

**Comparison of median changes in inflammatory markers before and after initiation of corticosteroid therapy**

Inflammatory marker data collected from the therapy group in this study were analysed following the intention-to-treat principle (ITT) modified; of all patients receiving corticosteroids with dose and duration according to the operational definition and are not included in the criteria exclusion, then the data from the examination of inflammatory markers is carried out were included in the statistical calculations even though complete data were not available until day 10 after therapy. Incomplete data were filled using statistical interpolation [44].

Since the beginning of the COVID-19 pandemic, NLR has become one of the tests widely used to predict clinical deterioration and morbidity. However, not many clinical trials have reported using NLRs as an indicator of the efficacy of corticosteroid therapy in COVID-19 patients; generally, these studies use qualitative output results such as the reduced risk of clinical deterioration, ventilator use, and mortality.

The comparative test of changes in median NLR in the corticosteroid treatment group on day-3, -6, and -10 supported the study's hypotheses. After administering corticosteroids, there was a significant median increase in NLR compared to pre-therapy levels. The median NLR increase was statistically significant on day-3 (*p*-value < 0.0001) and day-6 (*p*-value < 0.0133). On day-10, there was still an increase in the median NLR, but it was not statistically significant (*p*-value = 0.2645).

The findings of this study are consistent with Hyun and colleagues' prospective study, which also examined NLR serially after steroid therapy. The clinical trials compared changes in inflammatory parameters, the absolute number of neutrophils, lymphocytes, and CRP in the group of severe COVID-19 patients receiving steroid therapy early (less than 10 days after a confirmed diagnosis of COVID-19) and late (after 10 days). Although the ratio and statistical analysis were not calculated, there was an increase in neutrophil values on the 3$^{rd}$, 7$^{th}$, and 14$^{th}$ days of observation and lymphopenia on day 3 in both groups. Changes in the 2 components cause an increase in NLR after steroid therapy until day-14 [45].

Causes of increased NLR after steroid therapy in severe COVID-19 patients can be caused by 2 factors: 1) the patient is still in the neutrophilia phase and lymphopenia caused by SARS-CoV-2 infections, and 2) neutrophilia and systemic lymphopenia as a direct result of steroids on the immune system. The first factor is in line with the study reported by Lucas *et al.* [46] on the production and circulation patterns of the Lucas humoral and cellular immune systems conducted longitudinal observation for 60 days after confirmation of the diagnosis in 113 patients in Connecticut, United States between March - May 2020,

and found that in patients with severe COVID-19, it still occurs neutrophilia up to day 20 and lymphopenia (mainly $CD4^+$ T cells and $CD8^+$) until day 25.

A study by LaSalle (n = 306, conducted between March and May 2020) provided a more comprehensive insight into the impact of neutrophilia on patients with severe COVID-19. The study extensively examined the types of neutrophils present in systemic circulation, the subtypes of genes expressed by these neutrophils, and their correlation with the humoral component of the immune system. This prospective clinical trial was conducted before the RECOVERY research results were released, resulting in changes. The events that occur are not affected by the immunosuppressive effects of steroids. On the 7th day of symptoms onset (DfSO), in patients with severe COVID-19 mature neutrophils dominate the circulating neutrophil types. Furthermore, on days 10 to 14, neutrophil hyperactivation triggers emergency myelopoiesis and the release of immature neutrophils into systemic circulation. Between day-7 and day-14, gene expression increased that regulates neutrophil degranulation, TNF-α signalling through NF-κB, processes ROS metabolism, and neutrophil migration [47].

The NLR measurements cannot be used to indicate changes in post-therapy inflammatory status corticosteroids if the observation period is still included in the immunopathological process due to COVID-19 or in a period of neutrophilia due to steroid administration. If NLR is to be used in conditions other than COVID-19, it is ideally observed over a longer period (> 14 days) or in combination with other markers of inflammation that are more specific, including the type or subset count of lymphocytes.

Median change in CRP on day 3, -6, and -10 in the exposure group corticosteroids proved the second hypothesis but disproved the hypothesis of these studies. After administration of corticosteroids, there was a decrease in CRP significantly compared to before therapy ($p$-value < 0.0001). The decrease in CRP on day-3 and day-6 was significantly different compared to the previous period examination ($p$-value < 0.0001 and $p$-value = 0.0002). Median CRP on day 10 increased compared to the sixth day, but the increase was insignificant.

This study's results align with a retrospective study by Cui *et al.* [48] in the United States and a prospective study by Hyun *et al.* [45] in South Korea. Both studies performed repeat monitoring of CRP up to a week after steroid therapy. Cui *et al.* [48] (N = 324 between 10th March - 2nd May 2020) reported the CRP decreased to less than 50 % of the initial value on the third-day post steroid therapy (prednisone 0.5 mg/kg/day; equivalent to dexamethasone 0.075 mg/kg/day). Statistically, the decrease is significantly different from the value $p$-value < 0.001. The average CRP decrease continues gradually from day-12 post-treatment observations [48].

CRP increased on day-10 in this study, and day-14 in Hyun *et al.* [45] indicates a new active pathological process; some possible causes in patients receiving steroid therapy is infection secondary to bacterial or organ failure due to worsening clinical condition. That matter must be correlated with other supporting tests such as procalcitonin, culture, and examination of organ function such as kidney, liver, lung, or heart [42]. Corticosteroids cause a decrease in CRP through suppression of the genes that regulate neutrophil degranulation, TNF-α signalling through NF-κB processes ROS metabolism, and increased synthesis of anti-inflammatory mediators [47].

The observed changes in the median LCR within the corticosteroid treatment group on day-3, -6, and -10 support the initial hypothesis. Following corticosteroid administration, there was a significant and distinct increase in LCR compared to the previous LCR therapy ($p$-value < 0.0001). The increase in LCR on the 3rd and 6th day was significantly different compared to the previous inspection period ($p$-value = 0.0010 and $p$-value < 0.0001). The median LCR on the 10th day increased compared to the 6th day, but the increase is statistically insignificant. This event is caused by increased CRP between the 6th and 10th day, so the difference in LCR between the 2 measurement periods decreased.

Clinical trials examining changes in LCR on corticosteroid administration are scarce; In general, what is studied is clinical outcomes such as decreased risk of mechanical ventilation or mortality. Calcaianu clinical trials in France (n = 181 between 14th March - 5th June 2020) compared changes in absolute lymphocyte count and LCR in severe COVID-19 patients receiving methylprednisolone 1 - 2 mg/kg/day (dexamethasone equivalent 0.1875 - 0.375 mg/kg/day) for 7 days, followed by prednisolone at the same dose down gradually over 4 - 6 weeks. in patients who experience improvement lymphopenia within 1 - 7 days and an increase in LCR on the 7th day after initiation steroids had a significant reduction in the risk of mortality (OR 0.17; 95 % CI 0.05 - 0.61; $p$-value = 0.006) [49].

This study is consistent with Calcaianu's study [46]. After corticosteroid administration, the absolute number of lymphocytes decreases from the first day and then increases. Therefore, an increase in the absolute number of lymphocytes coupled with a decrease in CRP concentration causes an increase in post-LCR steroid therapy.

**Comparison of changes in NLR, CRP, and LCR between groups of corticosteroid treatment and control**

Delta analysis of inflammatory markers between control groups was compared with the corticosteroid exposure group, which yielded different findings significant for NLR and CRP on days-3, -6, and -10. Median LCR on the 3rd day of observation did not differ significantly between the 2 groups, but there was a difference between the 2 groups that became significant on day-6 and -10. The decreased mean CRP in the corticosteroid treatment group was caused by the immunosuppressive effects of steroids through various genomic mechanisms as well as non-genomic, such as repression of proinflammatory cytokine production, activation of anti-inflammatory synthesis, and modification of various signalling cascades regulate humoral and cellular immune system responses [50]. The median LCR between the control and therapy groups began to show a significant difference from day 6. This is due to the decline in the absolute number of lymphocytes in the corticosteroid exposure group in the examination on the third day. Hence, the median LCR increases in that measurement are not significant.

## Conclusions

NLR measurements may not be suitable as indicators of post-therapy inflammatory status when the observation period overlaps with the ongoing immunopathological process of COVID-19 or a period of increased neutrophil count due to steroid administration. If NLR is used in other contexts beyond COVID-19, it is recommended to monitor it over an extended period (> 14 days) or in combination with more specific inflammation markers, such as counts of lymphocyte types or subsets. On the other hand, the study findings suggest that CRP measurements are a reliable indicator of changes in inflammatory status for up to 10 days after corticosteroid therapy in severe and critical COVID-19 patients. The LCR measurements indicate changes in inflammatory status up to the 10th day after corticosteroid therapy in severe COVID-19 patients.

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