

Case Report**Relationship between CRP of COVID-19 Patients with Severity and Outcome***Suyoso¹, Farida Juliantina Rachmawaty², Bagastyo Afif Prabowo³***Abstract**

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Serum C-Reactive Protein (CRP) is an important marker that is significantly significant in the severity of COVID-19 patient outcomes. An increase in CRP indicates an increased risk of disease progression. IL-6 levels will also increase to become a strong predictor of the risk of damage to the respiratory system. This study aims to determine the relationship between CRP of COVID-19 patients with severity and outcome. This study is an observational study with a cross-sectional design. The subjects involved in the study were COVID-19 patients at the dr. Soedono Madiun in the period January 2020 - April 2021. The instrument used is secondary data in medical records from RSUD dr. Soedono Madiun. There was no significant difference in CRP levels in 114 patients hospitalized for <12 days and >12 days ($p=0.051$), then the duration of hospitalization was also not correlated with CRP levels ($p=0.70$, $r=-0.251$). In addition, there was a significant difference in CRP levels between patients with severe and non-severe severity ($p=0.0013$). The patient's severity was also positively correlated with CRP level ($p=0.012$, $r=0.233$). There was a significant difference in CRP levels in patients with recovered and dead outcomes and was positively correlated ($p=0.000$, $r=0.378$). CRP levels are related to the severity and outcome of COVID-19 patients. A high CRP indicates severity and risk of death.

Keywords: CRP, COVID-19, severity, outcome

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Introduction

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Common symptoms that can appear in patients with COVID-19 include fever, cough, shortness of breath, to anosmia. COVID-19 infection can progress to complications such as organ failure, septic shock, and venous thromboembolism. In some patients, the symptoms of COVID-19 can last for more than 12 weeks ¹. Coronavirus is a positive, encapsulated, unsegmented single-strain RNA virus. Coronaviruses have four main protein structures, namely: protein N (nucleocapsid), glycoprotein M (membrane), spike glycoprotein S (spike), and protein E (sheath). There are four genera

of coronaviruses, namely alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus ².

Researchers suspect the intermediate host in COVID-19 cases is pangolins because the coronavirus in pangolins has genome similarities to the coronavirus in bats and SARS-CoV-2 ³. SARS-CoV-2 is sensitive to ultraviolet light and high temperatures. SARS-CoV-2 has receptor binding on the spike protein with a three-dimensional structure, resulting in a strong affinity for angiotensin-converting enzyme 2 (ACE2). It can be concluded from these data that SARS-CoV-2 enters cells in the body through the ACE2 receptor ⁴.

The transmission of SARS-CoV-2 from human

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to human is the main factor in the spread of COVID-19 becoming more aggressive and faster. Transmission of COVID-19 occurs through droplets. These droplets can hit the oral and nasal mucosa and the eye's conjunctiva if a person is within proximity (1 meter) of an asymptomatic COVID-19 patient. SARS-CoV-2 has been reported to infect neonates, but there is no evidence that transmission occurs vertically. Virological examination of amniotic fluid, umbilical cord, and breast milk in mothers with COVID-19 showed negative results. SARS-CoV-2 can also infect the gastrointestinal tract. The virus can survive in the feces even though the examination of the respiratory tract sample has found the virus. From these data, it can be assumed that the transmission of SARS-CoV-2 can occur by fecal-oral ⁴.

SARS-CoV-2 carries out viral invasion of receptors on at-risk cells. SARS-CoV-2 has an S protein that plays an essential role for the virus in binding to cell receptors. Cells that are at risk of binding to SARS-CoV-2 are the presence of the ACE2 receptor. SARS-CoV-2 is transmitted through respiratory droplets and then directly infects nasal cilia and alveolar epithelial cells. In addition to the lungs, ACE2 receptors are also located in the small intestine, kidney, heart, thyroid, testes and fat tissue. When viremia occurs, the virus can infect cells in organ systems ⁵. Fusion between the virus and the cell membrane is also mediated by transmembrane serine protease 2 (TMPRSS2) to enter the cell. Viral and host factors play a role in SARS-CoV infection. Inadequate immune response leads to viral replication and tissue damage. On the other hand, an exaggerated immune response can cause tissue damage ⁵.

The immune response to SARS-CoV2 is not fully known but can be studied from the immune response to SARS-CoV and MERS-CoV. The viral antigen will be presented to the antigen presentation cells (APC) when it enters the cell. Viral antigen presentation depends on major histocompatibility complex (MHC) class I. In addition, there is also a role for MHC class II. Furthermore, the body's humoral and cellular immune responses will be stimulation mediated by T cells and B cells. The humoral immune response will form IgM and IgG against SARS-CoV. SARS-CoV IgM disappears by the end of the 12th week, and SARS-CoV IgG can persist for a long time ⁶. A study explained that patients who recovered from SARS-CoV after four years could have CD4+ and CD8+ memory T cells specific to SARS-CoV, which decreased gradually. SARS-CoV can evade immune responses by producing double-membrane vesicles that do not have pattern recognition receptors (PRRs) and replicate within these vesicles so that the host

cannot recognize them. SARS-CoV and MERS-CoV also inhibit the IFN-I pathway. In addition to MERS-CoV, antigen presentation is also inhibited in MERS-CoV infection ⁴.

Research conducted in China explained the difference in the immune response between COVID-19 with mild symptoms and COVID-19 with severe symptoms⁷. There will be a lower lymphocyte count, a higher leukocyte and neutrophil-lymphocyte ratio, and a lower percentage of monocytes, eosinophils, and basophils in severe cases. In addition, in severe cases, proinflammatory cytokines (TNF- α , IL-1, IL-6, IL-8) were also found and higher infection markers such as procalcitonin, ferritin and C-reactive protein. In COVID-19 patients, a decrease in T helper, T suppressor, and regulatory T cells was found with a lower decrease in T helper and T regulator levels in severe cases.

The leading cause of death in COVID-19 is Acute Respiratory Distress Syndrome (ARDS). A cytokine storm causes the occurrence of ARDS in COVID-19 patients, a systemic inflammatory response that cannot be controlled due to the release of large amounts of proinflammatory cytokines (IFN- α , IFN- γ , IL-1 β , IL-2, IL-6, IL-7, IL-10 IL-12, IL-18, IL-33, TNF- α , and TGF β) and large amounts of chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, and CXCL10).

There was also an increase in Granulocyte-colony stimulating factor, interferon- γ -inducible protein 10, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1 alpha. As a result of this excessive immune response, it can cause lung damage and fibrosis that causes functional disability ⁸. In addition, COVID-19 patients with ARDS showed decreased CD4 and CD8 T lymphocytes. CD4 and CD8 T lymphocytes were in a hyperactivated state characterized by a high proportion of the HLA-DR+CD38+ fraction. CD8 T lymphocytes contain high concentrations of cytotoxic granules (31.6% positive for perforin, 64.2% positive for granulysin, and 30.5% positive for granulysin and perforin). An increase in proinflammatory Th17 CCR6+ was also found ⁴.

Clinical manifestations of COVID-19 patients occur gradually from asymptomatic or asymptomatic, mild symptoms, pneumonia, severe pneumonia, ARDS, sepsis, to septic shock. About 80% of them are mild or moderate cases, 13.8% are severe cases, and 6.1% are in critical condition. The proportion of asymptomatic infections is not known. According to data from countries affected by the pandemic, 40% of cases are mild, 40% will experience moderate illness including pneumonia, 15% will experience severe illness, and 5% will

experience a critical condition ⁴.

COVID-19 cases can be divided into asymptomatic, mild, moderate, severe and critical ⁹ :

1. Asymptomatic

In this condition, there are no symptoms in the patient. This condition is the mildest.

2. Mild

The symptoms are fever, cough, fatigue, anorexia, shortness of breath, and myalgia in this condition. Other symptoms such as sore throat, nasal congestion, headache, diarrhea, nausea and vomiting, anosmia or ageusia may also occur.

3. Moderate

Symptoms that appear in adolescent or adult patients are symptoms of pneumonia (fever, cough, shortness of breath, fast breathing). However, there are no signs of severe pneumonia, including SpO₂ > 93% with room air. In pediatric patients, the symptoms are mild pneumonia (cough or difficulty breathing + rapid breathing and chest indrawing) and no signs of severe pneumonia.

4. Severe

In adult patients, symptoms of pneumonia (fever, cough, shortness of breath, rapid breathing) plus one of the following: respiratory rate > 30 breaths/minute, severe respiratory distress, or SpO₂ < 93% on room air. Meanwhile, in pediatric patients, symptoms that appear are pneumonia (cough or difficulty breathing), plus at least one of the following signs: (1) central cyanosis or SpO₂ < 93%; (2) severe respiratory distress (eg rapid breathing, grunting, very heavy chest indrawing); (3) general danger signs: inability to suckle or drink, lethargy or loss of consciousness, or seizures; (4) rapid breathing/chest indrawing/tachypnea: age < 2 months, 60x/minute; age 2–11 months, 50x/minute; 1–5 years old, 40x/minute; > 5 years old, 30x/minute.

5. Critical

In this condition, the patient has ARDS, sepsis and septic shock.

Viruses can damage several vital organs, such as the heart, liver and kidneys. Hence, analyzing biochemical factors is the right way for doctors to evaluate the functional activity of these organs ¹⁰. Routine laboratory tests performed on COVID-19 patients are complete blood count, coagulation tests (PT, aPTT and D-dimer) and inflammatory parameters (ESR, CRP, ferritin and procalcitonin). During the incubation period, which lasts from 1 to 14 days and during the early phase of the disease, leukocytes and lymphocytes are generally normal

or slightly reduced. After viremia, SARS-CoV-2 affects lung tissue, heart, and digestive system to express large amounts of ACE2. Within 7-14 days of the onset of initial symptoms, there is a spike in symptoms of the disease due to an increase in inflammatory mediators and cytokines known as a cytokine storm. In this phase, lymphopenia becomes more pronounced ¹⁰

Lymphopenia was the most striking finding in the predominance of SARS-CoV-2 patients. Several studies have also reported an increase in neutrophils. Overall, decreased neutrophil count accompanied by thrombocytopenia is one of the most common findings in complete blood counts of COVID-19 patients. In COVID-19 patients, an increase in prothrombin time (PT) was also accompanied by a prolongation of the activated partial thromboplastin time (aPTT). An increase in D-dimer was also found that supported the occurrence of coagulopathy. In the acute phase, inflammation parameters increased: erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and procalcitonin. Research on 2020 showed an increase in ferritin from average values, and ferritin will decrease as CRP decreases ^{6,10}.

C-Reactive Protein (CRP) is a protein produced by the liver as a marker of infection and inflammation. As a clinical parameter, serum CRP is an important marker that is significantly significant in the severity of COVID-19 patient outcomes. CRP binds strongly to phosphocholine that appears on the surface of damaged cells. This binding forms the classic pathway of the immune system and modulates phagocytic activity to rid microbes and damaged cells of damage. The average blood concentration of CRP is 10 mg/L but increases rapidly within 6-8 hours, reaching a peak 48 hours after disease onset. The half-life of CRP is approximately 19 hours. When inflammation or tissue damage improves, the CRP concentration will decrease so that the CRP concentration can be a helpful marker to see the severity of the disease ¹¹.

CRP concentrations are often used as markers of inflammation in other diseases. In dengue infection, CRP concentration is used as a prognostic marker of disease. An increase in CRP indicates an increased risk of disease progression. This is worth considering because the dengue virus and SARS-CoV-2 are RNA viruses with a similar infection course. Clinically, elevated CRP levels can be an early indication of nosocomial infection in COVID-19 patients experiencing slow recovery and help health workers provide appropriate antibiotic therapy to prevent worsening of the disease condition ². Increased levels of CRP are

also followed by increased levels of IL-6 so that it becomes a strong predictor of the risk of damage to the respiratory system and requires adaptive treatment with the patient's condition ⁽¹²⁾. This study aims to determine the relationship between CRP of COVID-19 patients with severity and outcome.

Research Method

Subjects

This study is an observational study with a cross-sectional design. The subjects involved in the study were COVID-19 patients at the dr. Soedono Madiun in the period January 2020 - April 2021. The number of samples is estimated using the Slovin formula. The results of the calculation of the Slovin formula show that 152 samples are needed to represent the population. The subject inclusion criteria were patients diagnosed with COVID-19 who received a CRP examination at the dr. Soedono Madiun for the period January 2020 - April 2021. The exclusion criteria for this study were patients diagnosed with COVID-19 with incomplete medical records and who did not receive a CRP examination.

Data collection and research instrument

Data collection was obtained through secondary data, namely patient medical records. The instrument used is secondary data in medical records from RSUD dr. Soedono Madiun. The research instruments used in this study were writing instruments and worksheets.

Data analysis

Data were analyzed descriptively for all variables. The relationship between two variables using Chi-square. Multivariate analysis to examine the relationship between various variables using logistic regression.

Results

Collections were made on 155 patients. However, 41 patients did not have CRP data, so there were only 114 patient data that could be analyzed. The study sample was divided into 63 male patients and 51 female patients. Furthermore, the data were analyzed using statistical tests with SPSS software. Based on the normality test conducted by looking at the results of Kolmogorov Smirnov, it can be seen that the significance values of CRP, NER, and length of stay were not normally distributed ($p < 0.05$).

The results were analyzed to determine the relationship between CRP values with the length of stay, the severity of patients' clinical symptoms, and outcomes (recovered or died). The difference

power test uses Mann Whitney because the data is not normally distributed, while the correlation test uses the Spearman test. The test results are summarized in Table 1.

Table 1. Results of the analysis with the Mann Whitney test and the Spearman Correlation

	Dependent variable	Mann Whitney	Spearman Correlation	Interpretation
CRP	Length of stay 1 = <12 (48) 2 = > 12 (66)	p = 0.051		p>0.05
	Length of stay		p = 0.070 r = -0.251	p<0.05
	Severity 1 = Non critical (60) 2 = Critical (54)	p = 0.013	p = 0.012 r = 0.233	p<0.05
	Outcome 1 = Recovered (81) 2 = Death (33)	p = 0.000	p = 0.000 r = 0.378	p<0.05

The length of stay for patients who were less than 12 days was 42.11%, while patients who were treated for more than 12 days were 57.89%. Patients with moderate clinical conditions were 52.63%, while patients with severe conditions were 47.37%—patients with recovered conditions recovered as much as 71.05% and 28.95% who died.

Discussion

The length of hospitalization showed no significant difference, $p > 0.05$ (more or less), with an inverse and weak correlation. This can occur because of patients with high CRP levels but short hospitalizations because some of these patients died, so they seem to be hospitalized for a short time.

Patients with CRP levels associated with the severity of clinical symptoms showed significantly different results ($p < 0.05$). This is in accordance with Shilpa et al. (2021) research that COVID-19 patients with CRP levels > 100 mg/dL had more severe clinical manifestations of COVID-19, especially respiratory distress those below 100 mg/dL. Meanwhile, according to Herold et al. (2020), patients with CRP levels >97 mg/dL have a poorer clinical prognosis and risk of respiratory

failure. The correlation test results showed positive results, but the relationship was weak ($r=0.333$). It is possible that only two categories are used, namely not heavy (medium) and heavy. Patients with mild clinical conditions were not hospitalized and were not tested for CRP, so data were not available. In addition, not all patients had CRP data, so these patients could not be included in the study data.

CRP levels associated with the outcome of recovering or dying showed significant results ($p < 0.05$). However, the correlation between the two is weak ($r = 0.378$). The increase in CRP levels in Covid-19 patients can be understood because CRP is a protein produced by the liver in response to inflammation. Healthy people generally have low CRP levels. On the other hand, high CRP levels are a sign of inflammation or infection.

Conclusion

CRP levels are related to the severity and outcome of Covid-19 patients. A high CRP indicates severity

and risk of death. However, the relationship in this study is still weak. Researchers have not measured CRP levels in COVID-19 patients with mild symptoms. So it is necessary to measure CRP levels in patients with mild COVID symptoms in future studies, in order to show results that are in accordance with actual conditions.

Conflict of interest

All authors declare that they have no conflicts of interest.

Ethical clearance

The research has been declared ethically feasible by the Head of the Health Research Ethics Committee at RSUD dr. Soedono Madiun with code number 070/23741/303/2021.

Authors' contribution

All authors were involved equally in patient management, data collection, literature review, analysis, manuscript writing, revision and finalizing.

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