Validity Test For C-Reactive Protein and Ferritin Level in Moderate and Severe Covid-19 Patients

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Abstract

Mild, moderate, severe, and critical COVID-19 are associated with hyperinflammation. The CRP and ferritin are acute phase proteins that marks incidence of inflammation and used as the paramaters of hyperinflammation. This study aimed to determine the validity of CRP and ferritin level examination in moderate and severe COVID-19 since the time of admission. This was a cross-sectional analytical retrospective study with on moderate and severe COVID-19 patients admitted to Dr. Hasan Sadikin General Hospital Bandung, Indonesia, during the period of March 2020 to December 2020. The CRP and ferritin levels were obtained since the beginning of admission to the fourth day since admission on patients without any history of anemia. Subjects in this study were divided into moderate and severe COVID-19 groups based on the 3rd edition of COVID-19 Prevention and Control Guideline issued by the Ministry of Health Republic of Indonesia. Each group consisted of 30 subjects. The cut-off value was 7.65 mg/dL (AUC 0.698) for CRP and 963.1 mcg/L (AUC 0.938) for ferritin. The validity of ferritin vs CRP were reflected respectively as follows: 93.3% vs 76.7% sensitivity; 80.0% vs 63.3% specificity; 82.4% vs 67.6% PPV; and 92.3% vs 73.1% NPV. The validity of ferritin was proven to be superior as it significantly increases since day one, persisted longer and reaches its peak on the16th day. Meanwhile, CRP increases within 6-8 hours and reaches its peak within 48 hours after inflammation, then declines soon afterwards.

Keywords: COVID-19, CRP, ferritin, validity

Introduction

Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) is a collective of clinical respiratory symptoms caused by coronavirus 2 genus β coronavirus which may also induce multi-organ disturbance and elicit clinical manifestation in various organs, including gastrointestinal, hepatic, cardiovascular and neural organs.[1] Based on World Health Organization (WHO) data in September 13th, 2021, the morbidity and mortality rate of Coronavirus Disease 2019 (COVID-19) cases reached 224,511,226 cases, especially on elderly patients with comorbidities, owing 4,627,540 cases.[2]

The incubation period for COVID-19 infection is around 5-6 days with various clinical symptoms, including mild symptoms, i.e., fever, anosmia, pharyngitis, cough, myalgia, cephalgia, gastrointestinal disturbance, to severe and critical degree symptoms, such as shortness of breath and sepsis which may end in death.[3]

The pathomechanism for appearance of various clinical symptoms in COVID-19, begins with innate and adaptive immune response, which will release pro-inflammatory cytokines, including TNF-α, IL-1, IL-6 and IL-8, also infection markers such as procalcitonin, CRP and ferritin. T-helper, T-suppressor and T-regulator cells are found reduced in COVID-19 patients, with T-helper and T-regulator cells significantly lower on severe cases.[4] There are various indicators to evaluate the severity and prognosis of COVID-19 based on the degree/clinical stage, which research is currently still ongoing.[5]

C-reactive protein is an acute phase protein synthesized by the liver as a response towards the increased IL-6 during systemic inflammation and severe infection, and is used as a marker to evaluate and monitor patients infected with SARS-CoV-2, especially those with severe cases.[6] On a study by Tan et al., showed that the increase of CRP is significant on the beginning of severe COVID-19 infection. C-reactive protein is also

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associated as prognostic marker on the initial phase of COVID-19 infection.[7,8] Based on a study by Huang et al., CRP level of ≥ 1 mg/dL is associated with poor COVID-19 clinical outcome and increased CRP is also associated with sepsis and mortality.[9]

Another marker, i.e., ferritin, is also an acute phase protein, which plays role as immune dysregulation mediator, and its incidence is associated with hyperferritinemia and cytokine storm as a result of overexpressed pro-inflammatory cytokine. Ferritin synthesis is mediated by interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF).[5] High ferritin level on patients with severe and critical disease showed systemic inflammation associated with the severity degree of COVID-19. A study by Gandini et al., showed that the mean level of ferritin on mild, moderate, severe and critical COVID-19 are 281 µg/L, 308 µg/L, 741 µg/L and 1640 µg/L, respectively.[10,11] Hence, examination of ferritin level during the beginning of the disease may identify the severity of the disease and the prognosis of patients infected with COVID-19.[12]

Considering that the increase of CRP and ferritin on COVID-19 patients during the initial phase of the disease may provide insight about the severity of the disease, the authors of this paper is intrigued to determine the validity of CRP and ferritin level for moderate and severe COVID-19 infection.

Methods

This was an analytic observational study which data were obtained via retrospective cross-sectional method. Data was obtained using Laboratory Information System (*Sistem Informasi Laboratorium*) and medical records in March 2020 to December 2020 during the peak of Delta variant in Dr. Hasan Sadikin General Hospital Bandung. The subjects of this study were all moderate and severe COVID-19 patients admitted in isolation ward at Dr. Hasan Sadikin General Hospital Bandung. The inclusion criteria for the subjects in this study was adult patients (aged ≥ 18 years), admitted in isolation ward, COVID-19 diagnosis confirmed within ≤ 48 hours since admission based on nasopharyngeal swab examination with real-time PCR, included in moderate and severe COVID-19 cases based on 3rd Edition COVID-19 Prevention and Control Guideline Ministry of Health Republic of Indonesia (Year 2020) (*Pedoman Pencegahan dan Pengendalian* COVID-19 *Kementerian*

Kesehatan RI edisi 3 (*Tahun* 2020)), which stated that severe disease was marked by respiratory rate of >30 times/minute and/or SpO_2 <93% and/or presence of sepsis signs or respiratory distress, moderate disease was categorized on patients with pneumonia clinical signs (fever, cough, dyspnea) without signs and symptoms of severe disease, and mild disease was accompanied with symptoms but without evidence of viral pneumonia or without hypoxia. The categorization was conducted during initial assessment in the hospital, while CRP and ferritin level was examined during the initial time of admission, or 4 days the latest after isolation admission. The exclusion criteria of subjects in this study were COVID-19 patients with anemia and patients with incomplete laboratory results medical record data. The evaluated laboratory criteria for moderate and severe COVID-19 were ferritin and CRP levels. COVID-19 patients were divided into 2 groups, namely, moderate and severe degree groups.

Statistical analysis was conducted using unpaired t-test if the data were normally distributed and using non-parametric test (Mann-Whitney U test) if the data were not normally distributed. Analysis of receiver operator characteristic (ROC) curve was conducted to obtain cut-off value and area under curve (AUC) value, afterwards, validity test for CRP and ferritin was conducted for moderate and severe cases, remarking sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV). The study data were documented in Microsoft Excel® table and were processed using SPSS® 19.0 software. This study has been approved by Medical Research Ethical Committee, Dr. Hasan Sadikin Hospital Bandung (No.: LB.02.01/X.6.5/188/2021).

Results

Laboratory data and medical records data during March 2020 to December 2020 yielded data of 106 patients. The study subject characteristics data were presented on Table 1 below.

Table 1 showed that there was a significant association among gender, comorbidity, patient condition at discharge with disease severity degree, CRP and ferritin level (p<0.05%), and that there was no significant difference between age and disease severity degree (p>0.05). Females dominated moderate degree disease, while on severe disease was dominated by males (83.3%). On age group, it was known that the

Table 1 Characteristics Data of Study Subjects based on Degree of Severity

Patient Characteristics	Degree		p-value
	Moderate n=30	Severe n=30	
Gender:			
Male	13 (43.3)	25 (83.3)	0.001[a]*
Female	17 (56.7)	5 (16.7)	
Age (years)			
Mean ± SD	50 ± 18	57 ± 12	0.107[b]
Comorbidities			
With comorbidities	11 (36.7)	20 (66.7)	**0.020[a]***
Without comorbidity	19 (63.3)	10 (33.3)	
Discharge condition			
Improved	30 (100.0)	19 (63.3)	<0.001[a]*
Died	0 (0.0)	11 (36.7)	
CRP (mg/dL)			
Median (Min-Max)	6.74 (0.07 – 26.34)	10.24 (0.43 – 38.62)	0.008[c]*
Ferritin (mcg/L)			
Median (Min-Max)	618.2 (33.7 – 1636.7)	1741.8 (738.6 – 6766.2)	<0.001[c]*

Note: Analysis using [a]Chi Square test, [b]Unpaired t-test, [c]Mann-Whitney test, *significant if p<0.05

mean age was on the fifth decade. Severe cases were dominated by patients with comorbidities (66.7%) and those who were discharged with improvements (100%) were dominated by severe degree disease. Data analysis showed that the median of CRP value was (10.2 mg/dL) and for ferritin was (1741.8 mcg/L) which were higher on severe COVID-19 cases.

The ROC curve of CRP towards severity degree of COVID-19 patients was presented in Figure 1, while the ROC curve of ferritin towards severity degree of COVID-19 patients was presented in Figure 2.

Table 2 showed that combined CRP and ferritin level has lower validity compared to CRP alone and ferritin alone. Ferritin has a higher

validity with optimum cut-off value of 963.1 mcg/L (p=<0.001) with sensitivity of 93.3% and specificity of 80.0% in predicting the severity degree of COVID-19 patients at the beginning of admission.

Discussion

This study utilizes data of 60 COVID-19 patients, in which moderate degree cases were dominated by female, while on severe degree were dominated by male. This result was in accordance with the study result by Gandini et al., which showed that there were 54.3% female subjects and 45.7% male subjects on mild-moderate degree, while on

Table 2 Validity Test of CRP and Ferritin towards Severity Degree of COVID-19

Cut-off Value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CRP (mg/dL)				
7.65	76.7	63.3	67.6	73.1
Ferritin (mcg/L)				
963.1	93.3	80.0	82.4	92.3
CRP 7.65 mg/dL and Ferritin 963.1 mcg/L	73.3	70.0	71.0	72.4

Note: PPV: positive predictive value, NPV: negative predictive value
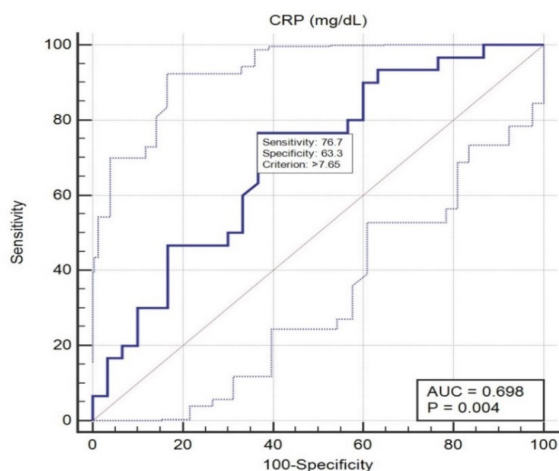
Figure 1 ROC Curve of CRP towards Severity Degree of COVID-19 Patients
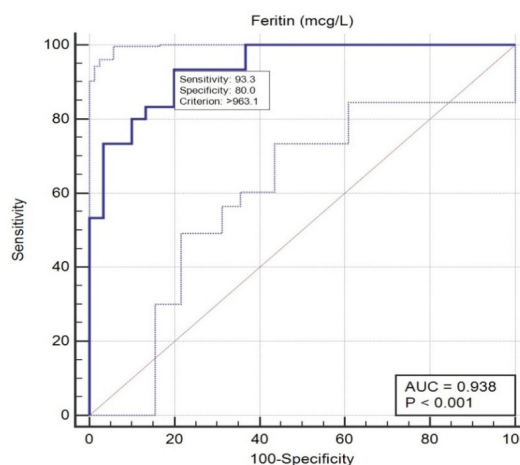
Figure 2 ROC Curve of Ferritin towards Severity Degree of COVID-19 Patients

the severe degree cases there were 26.7% female subjects and 73.35% male subjects. Because the X-chromosome encodes several genes associated with immune responses, women are likely to have lower inflammation responses than men. Moreover, men have higher susceptibility to infections because testosterone's role that inhibit immune processes.[13] Based on that study, the incidence of acute respiratory distress syndrome (ARDS) and systemic inflammation was associated with history of comorbidities such as cardiovascular disease, diabetes mellitus, hypertension, chronic obstructive pulmonary disease, lung cancer on male subjects.[11] In this study, male subjects have comorbidities, which was mostly diabetes mellitus, hypertension and cardiovascular disease.

Characteristics data (Table 1) regarding age distribution showed that the mean age for moderate degree was from 56-65 years age group and for severe degree was from >65 years age group. This result was consistent with a study by Tural et al., which stated that the mean age of study subjects was from 62 years age group and with presence of comorbidities, namely cardiovascular disease, diabetes mellitus, hypertension. This study result showed that severe degree group has more comorbidities compared with moderate degree group. COVID-19 patients were mostly from the age group of >60 years and has poorer prognosis considering the declined immune system and specific immunoregulation in the elderly. Elderly patients had the tendency to develop multisystem organ dysfunction with age, and it

has also been known that elderly patients had a decreased natural innate and adaptive immune system, causing a continuous production of inflammatory mediators and cytokines, known as chronic systemic inflammation which occurs physiologically with aging and might potentially induce cytokine storm.[14,15]

This study showed that there was still several complaints or symptoms at discharge on 36.7% subjects on severe degree group (Table 1), which was consistent with the study findings of Mahmud et al., stating that severe COVID-19 patients were still symptomatic at discharge probably associated with severity of the infection, length of stay, risk factor and history of comorbidities in the patient.[16]

This study showed that there was a difference of CRP and ferritin level between moderate and severe cases, in which the median CRP and ferritin on severe cases were higher than the moderate cases. This result was reciprocal with study result by Smilowitz et al., which stated that the high CRP along with increased ferritin level was associated with the severity of COVID-19 infection in addition of pre-existing comorbidities such as hypertension, cardiovascular disease, diabetes mellitus and kidney failure. The median CRP on the study was 136.0 mg/L while the median of the ferritin was 811 mcg/L.[8] Ferritin and CRP were acute phase proteins used as inflammation marker, and the increase of ferritin level correlated with severity degree of COVID-19 which was associated with cytokine storm and immune response.[10] Suzuki et al., showed that ferritin and CRP levels were

lower in the Omicron group than in the Delta group that correlated with the milder symptoms in Omicron phase.[17] Wang also found that ferritin and CRP levels had the highest value in the Delta group compared to B.1.338 group and Omicron group.[18]

This study result showed that the best cut-off value for ferritin level was 963.1 mcg/L which was considered very excellent for severe disease. This result was in accordance with study result by Gandini et al., who published ferritin cut-off value of 500 mcg/L for severe COVID-19 patients group.[11] Based on a study by Zhou et al., the ferritin level in severe COVID-19 significantly increased on 4th day and reached its peak in 16th day.[10] Increased ferritin level was presumably associated with incidence of cytokine storm categorized in hyperferritinemia syndrome, and was associated with inflammation in COVID-19 infection; thus, ferritin might be utilized to predict the severity degree of the disease and the extent of cytokine storm.[19] Ferritin occurs as a cytosolic protein in most tissues, although a mitochondrial form also exists and nuclear localization has been proposed. Even though widely recognized as a representative of total body iron stores, its prognostic utility is linked with acute and chronic inflammatory processes and is nonspecifically raised in a variety of such disorders, including chronic kidney disease, rheumatoid arthritis, and autoimmune disorders, etc.[12]

Several studies have varied cut-off value for CRP level ranged from 1-10 mg/dL on severe COVID-19 group which was correlated with severity degree in the patients.[11,12,16,20] This study result showed that the optimum cut-off value for CRP was 7.65 mg/dL in order to predict severe degree of COVID-19 infection. C-reactive protein increased rapidly during acute inflammation within the first 608 hours and significantly peaked after 48 hours, declining afterwards as the inflammatory response resided.[3,6] Moreover, the timing of CRP increase should be taken into account since the time was shorter compared with ferritin and that there were other factors which affected CRP levels, including liver damage, history of administered treatments and timing of CRP level examination.[9]

Ferritin has sensitivity of 93.3% and specificity of 80.0%, thus, could be categorized as good validity, while CRP has sensitivity of 76.7% and specificity of 63.3%, thus, could be categorized as low validity. The limitation on this study was that on the moderate and severe cases, the comorbidities were not equally proportioned, which potentially may confound the study result.

In conclusion, validity of ferritin level was more superior than CRP and was presumably associated with incidence of cytokine storm. This study could be further advanced by accounting the proportion of comorbidities in moderate and severe COVID-19 groups.

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