



## ASSOCIATION BETWEEN INCREASED LEVELS OF C-REACTIVE PROTEIN (CRP) AND ABSOLUTE VALUES OF LYMPHOCYTES TO SEVERITY OF CHILDREN INFECTED WITH COVID-19

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**Abstract :** COVID-19 infection in children is often asymptomatic or mild. However, there is increased reported severe and critical COVID-19 cases in pediatric. A biomarker is needed to predict the severity progression of COVID-19 infection. This study aims to investigate the association between CRP and absolute lymphocytes to pediatric COVID-19 severity. This is an analytic observational study with cross-sectional approach including 271 confirmed by PCR COVID-19 pediatric cases from several hospital in Medan, Indonesia between July 2021 to September 2021. Data on children including demographic, clinical and laboratory findings, severity and clinical outcomes were collected and analyzed. Of 271 confirmed COVID-19 pediatric cases, mean age was  $7.56 \pm 5.75$  year old with age group of 1 month - < 1 year as the majority age group (49.4%), 57.9% male. 48% cases were mild, 25.5% cases intermediate, 12.2% severe and 14.4%. Fever (33.2%) and shortness of breath (30.6%) was the most common clinical symptoms. CRP was increased 22.9% cases. There was significant relationship found between increased CRP and COVID-19 case severity in pediatric patient (P value = < 0.001; PR = 3.569, 95% CI 2.457-5.184). There was no significant relationship found between absolute lymphocytes and COVID-19 case severity (p value > 0.05). There is significant relationship between increased CRP and COVID-19 severity in pediatric patients.

**Keywords - CRP, Lymphocytes, COVID-19**

### I. INTRODUCTION

In early December 2019, a number of cases of pneumonia of unknown origin appeared in Wuhan, Hubei province, China and on 11 February 2020, WHO named the disease associated with 2019-nCoV as coronavirus disease 2019 (COVID-19).<sup>1,2,3</sup> In a study conducted in China, researchers explored the epidemiological characteristics and transmission patterns of 2,193 pediatric patients with COVID-19. Cases are initially diagnosed based on clinical manifestations and history of exposure and the symptoms can range from asymptomatic to severe.<sup>4,5,6</sup>

The course of the disease in children is generally mild compared to adults. Nonetheless, the severe and fatal cases reported in children are expected to continue to increase as community transmission increases and the current prevalence of the disease. A recent report from China on 2,143 children with suspected COVID-19, described the severity of the disease in children, stratified by several age sub-categories. Specifically, the proportion of severe and critical cases by age group was 10.6% for <1 year, 7.3% for 1-5 years, 4.2% for 6 - 10 years, 4.1% for 11 - 15 years, and 3.0% at age  $\geq 16$  years. Previous research showed that there was a relationship between the CRP value and the severity of covid patients in adult samples with a sensitivity of 65%, a specificity of 83.7%, a positive predictive value (PPV) of 81.6%, and a negative predictive value (NPV) of 68.2%. Meanwhile for the absolute value of lymphocytes themselves in several studies showed a cut-off value ranging from 1100 /uL to 2000 /uL, with a sensitivity of 72% - 94%.<sup>7</sup>

Research conducted by Brandon et al showed an inconsistent pattern of changes in the leukocyte index in mild and severe cases of COVID-19 in children. In particular, changes in leukocyte count were observed only in 32% of cases of mild children. In mild disease, creatine kinase-MB (CK-MB) is often elevated. In severe disease, C-reactive protein (CRP), procalcitonin (PCT), and lactate dehydrogenase (LDH) are often elevated. Based on data obtained from the initial study of COVID-19, the leukocyte index in children appeared to be inconsistent, different from that reported in adults. An increase in CK-MB in mild cases of COVID-19 in children is an indication of possible heart damage. This shows the importance of monitoring cardiac biomarkers in hospitalized patients.<sup>7</sup>

Based on the description above, this study aims to assess the relationship between C-reactive protein (CRP) levels and lymphocyte absolute values on the degree of severity of children infected with COVID-19.

## II. METHODS

This is a analytic observational study with cross-sectional design including confirmed COVID-19 pediatric cases from several COVID-19 referral hospitals including Martha Friska Medan Hospital, Haji Mina Hospital, Haji Adam Malik Hospital, Bunda Thamrin hospital, Royal Prima hospital and Prof. dr. Chairuddin Panusunan Lubis hospital, during the period of July 2021 to September 2021. The study variables including patient characteristic, clinical and laboratory findings were obtained from medical records. The ethical clearance was obtained from ethical committee of related hospital to obtain access to patient medical records.

All COVID-19 cases confirmed from positive PCR test in patient aged 0-18 years old were included in the study. Exclusion criteria including missing or no CRP and absolute value of lymphocytes, lack of clinical and physical examination information from patient's medical record. Covid-19 case severity was classified base on clinical findings. Covid-19 case severity was divided into asymptomatic, mild, intermediate, severe and critically-ill.

Data was collected and analyzed with IBM SPSS version 20.0 software. Univariate analysis used to describe the patient characteristics. Categorical data was presented in frequency and percentage. Numerical data was presented in mean  $\pm$  SD for normal data distribution or median in abnormal data distribution. Chi-square was used to determine the relationship between elevated CRP and absolute value of lymphocyte with COVID-19 severity in pediatric cases with p value  $<$  0.05 and 95% confidence interval as statistically significant result. If the chi-square test doesn't meet the parametric requirement then fisher's exact test was used.

## III. RESULTS AND DISCUSSION

### 3.1 Results

A total of 271 confirmed pediatric COVID-19 cases were included from several COVID-19 referral hospitals including Martha Friska Medan Hospital, Haji Mina Hospital, Haji Adam Malik Hospital, Bunda Thamrin hospital, Royal Prima hospital and Prof. dr. Chairuddin Panusunan Lubis hospital, during the period of July 2021 to September 2021. The mean age was  $7.56 \pm 5.75$  year old with age group of 1 month -  $<$  1 year as the majority age group (49.4%) followed by 1 year -  $<$  5 year (33.2%) and  $<$  1 month (17.3%). Total of 157 children was male (57.9%) and 114 (42.1%) was female.

Base on COVID-19 severity, mild was the most prevalent in pediatric COVID-19 cases with 130 children (48%) followed by intermediate 69 children (25.5%), severe 33 children (12.2%) and critical 39 children (14.4%). There were no asymptomatic cases because all COVID-19 cases used in this study were inpatient cases. Fever was the most reported clinical symptom found in pediatric COVID-19 cases reported in 90 children (33.2%) followed by shortness of breath (30.6%), cough (20.7%), diarrhea (7.4%), abdominal discomfort (4.4%) and headache (3.7%). A total of 229 (84.5%) children had full recovery and 42 (15.5%) death (table 3.1).

Table 3.1: Patient Characteristics

Variables	n = 271
Sex, n (%)	
Male	157 (57.9)
Female	114 (42.1)
Age, years	
Mean (SD)	7.56 (5.75)
Median (min-max)	8 (0.08-17.58)
$<$ 1 month	47 (17.3)
1 month - $<$ 1 year	134 (49.4)
1 - $<$ 5 year	90 (33.2)
Severity, n (%)	
Mild	130 (48)
Intermediate	69 (25.5)
Severe	33 (12.2)
Critical	39 (14.4)
Clinical symptoms, n (%)	
Shortness of breath	83 (30.6)
Fever	90 (33.2)
Cough	56 (20.7)
Headache	10 (3.7)
Diarrhea	20 (7.4)
Abdominal discomfort	12 (4.4)
Outcome, n (%)	
Full recovery	229 (84.5)
Death	42 (15.5)

The mean hemoglobin value was  $10.84 \pm 1.74$  g/dL, leukocyte  $9.73 \pm 11.2$   $10^3/\mu\text{L}$  and thrombocyte  $284.96 \pm 130.88$   $10^3/\mu\text{L}$ . The mean of absolute lymphocyte was  $3.51 \pm 2.91$   $10^9/\text{L}$  and CRP  $4.98 \pm 11.67$  mg/dL. Absolute lymphocyte was found normal in 172 children (63.5%), increased in 59 children (21.8%) and decreased in 40 children (14.8%). CRP was found increased in 62 children (22.9%) and normal in 209 children (77.1%) (table 3.2). The statistical analysis with Chi-Square found no significant relationship between absolute lymphocyte and COVID-19 case severity in pediatric patient (P value = 0.164). There was significant relationship found between increased CRP and COVID-19 case severity in pediatric patient (P value =  $<$  0.001; PR = 3.569, 95% CI 2.457-5.184) (table 3.3).

Table 3.2: Laboratory Characteristics

Variables	Mean (SD)	Median (Min-Max)
Hemoglobin, g/dL	10.84 (1.74)	11 (3.9 – 16.8)
Hematocrit, %	34.95 (8.02)	36.7 (10.4 – 56.1)
Leukocyte, 10 <sup>3</sup> /μL	9.73 (11.2)	8.15 (1.53 – 171.05)
Thrombocyte, 10 <sup>3</sup> /μL	284.96 (130.88)	276 (2 – 721)
Eosinophil, %	0.79 (0.94)	0.4 (0 – 6)
Basophil, %	0.8 (0.92)	0.5 (0 – 6)
Neutrophil, %	47.78 (17.73)	44.6 (10 – 95.4)
Lymphocyte, %	51.44 (18.73)	48.8 (9 – 93.2)
Monocyte, %	9.33 (2.78)	9.2 (1.8 – 19.2)
Absolute Lymphocyte, 10 <sup>9</sup> /L	3.51 (2.91)	2.5 (0.3 – 23.9)
Normal, n (%)	172 (63.5)	
Increased, n (%)	59 (21.8)	
Decreased, n (%)	40 (14.8)	
CRP, mg/L	4.98 (11.67)	2.8 (0 – 120)
Normal, n (%)	209 (77.1%)	
Increased, n (%)	62 (22.9%)	

Table 3.3: Association of Absolute Lymphocyte and CRP with COVID-19 Cases Severity in Pediatric Patient

Variables	Severity		P value	Prevalence Risk CI 95%
	Severe-Critical n = 70	Mild-Moderate n=201		
Absolute Lymphocyte, 10 <sup>9</sup> /L				
Normal, n (%)	38 (22.1)	134 (77.9)	0.164 <sup>a</sup>	-
Increased, n (%)	20 (33.9)	39 (66.1)		
Decreased, n (%)	12 (30)	28 (70)		
CRP, mg/L				
Increased, n (%)	36 (58.1)	26 (41.9)	<0.001 <sup>a</sup>	3.569 2.457-5.184
Normal, n (%)	34 (16.3)	175 (83.7)		
<sup>a</sup> Chi-Square				

### 3.2 Discussion

In December 2019 a new type of Corona virus was discovered called Severe Acute Respiratory Syndrome – Coronavirus 2 (SARS-CoV-2) in Wuhan, China. The disease caused by this new virus is called Coronavirus Disease 19 (COVID-19). Since first reported, the number of this novel virus infection continues to increase throughout the world and in 12 march 2020, WHO declared COVID-19 a global pandemic.<sup>8,9</sup> Although often asymptomatic or with mild symptoms, pediatric patient also prone to SAR-CoV-2 infection. Hospitalization and mortality risk reported to be lower in COVID-19 cases in pediatric.<sup>10-14</sup> A systematic review show that severe infection of COVID-19 in children are rarer and 27.7% cases are asymptomatic.<sup>15</sup>

Clinical spectrum of COVID-19 in pediatric reported from CDC database in China shown from 2143 children, 4.4% are asymptomatic, 50.9% mild, 38.8% intermediate and 5.8% with severe or critical symptoms.<sup>16</sup> Other study found that fever (46%), dry cough (37%), diarrhea (19%) and sore throat (13%) are symptoms often reported from pediatric COVID-19 cases.<sup>17</sup> The mean age in this study is 7.5 age year old and age group of 1 month - < 1 year is the most prevalent. All 271 pediatric cases were confirmed using PCR. The most prevalent symptom was fever (33.2%), shortness of breath (30.6%) and cough (20.7%). Based on COVID-19 severity, mild-intermediate severity was the most prevalent with 74.2%. However, in this study there were no asymptomatic patient reported because all samples used was inpatient cases.

Presentation of COVID-19 disease varies widely from asymptomatic, mild, intermediate, severe and critical. Therefore, an indicator is necessary as predictor for severe or critical disease progression in SARS-CoV-2 infection. Infection of SARS-CoV-2 was known to trigger strong immune reaction known as cytokine storm. Several subsets of COVID-19 patient shown organ dysfunction such as hepatic, heart, coagulopathy and renal. This has resulted in an increase in markers that can be used to predict the outcome of the course of COVID-19 case.<sup>18</sup> Previous studies shown several biomarkers can be used to predict the outcome of COVID-19. These biomarkers include CRP, lymphocyte count, D-dimer, pro-calcitonin, neutrophil count, neutrophil-lymphocyte ratio (NLR), ferritin, hemoglobin, white blood cell (WBC), platelet, prothrombin time (PT), activated partial thromboplastin time (aPTT), lactate dehydrogenase (LDH), hepatic enzymes, creatinine, blood urea nitrogen (BUN).<sup>13,15,18,19,20,21</sup> Abnormal laboratory value typical in COVID-19 infection often found was lymphopenia (31%), leukopenia (19%) and increase creatine kinase-MB (31%) and procalcitonin (17%). In other study, CRP was elevated in 45% COVID-19 cases in pediatric.<sup>15</sup>

Early response from host to viral or bacterial infection is activation of acute phase reactants like CRP, ferritin, proinflammation cytokine and blood sedimentation rate. CRP, part of the pentraxins group is a non-specific conventional biomarker acting as a marker of inflammatory reactions secreted by the liver. CRP was known to interact with complement factor C1 on complement activation cascade.<sup>18,19</sup> Elevated CRP had positive correlation to increase of inflammation process and disease severity.<sup>21</sup> A comparative study between COVID-19 patients shown that acute protein phase such as CRP, ferritin, procalcitonin and IL-6 significantly higher in patients with death outcome.<sup>19</sup>

In this study the laboratory test parameter such as CRP, leucocyte count and absolute lymphocyte was collected from patient medical record. A total of 62 children (22.9%) with COVID-19 had increased CRP. Of which, 36 children (58.1%) had severe-critical COVID-19 infection and 26 children (41.9%) children with mild-intermediate severity. Increased CRP was statistically

significant associated with more severe infection (PR 3.569;  $p < 0.001$ ). This result was similar with meta-analysis from Figliozzi et al which shown increased CRP in COVID-19 cases with odd ratio of 3.87 chance of disease worsening ( $p < 0.001$ ). However, in this study CRP doesn't shown significant relationship in predicting COVID-19 mortality (OR 5.82; CI 0.60-56.88;  $p = 0.05$ ).<sup>22</sup> Aside from being a marker for the progression of SARS-CoV-2 disease, increased CRP levels also act as a risk factor for death in cases of severe COVID-19.<sup>23</sup> A comparative study shown a trend of increased acute phase reactants like CRP, ferritin, procalcitonin and IL-6 in patients who do not survive COVID-19 disease.<sup>18</sup> Patients with death outcome in one study shown very high CRP level reaching 10 times from patients with full-recovery outcome.<sup>10</sup>

### III. CONCLUSION

The majority of COVID-19 pediatric patients admitted to hospitalization were male (57.9%), age group 1 month - < 1 year (49.4%) with mild severity (48%). Mean CRP value was 4.98 mg/L and increased in 22.9%. There is significant relationship between increased CRP and severity of COVID-19 cases in pediatric. Patient with higher CRP value are more likely to had more severe COVID-19 infection

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