

# Correlation of Immature Granulocytes and C-Reactive Protein with Blood Culture in Neonatal-SIRS at Gambiran Hospital

*by Sri Widyaningsih, Dosen Fik*

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## Correlation of Immature Granulocytes and C-Reactive Protein with Blood Culture in Neonatal-SIRS at Gambiran Hospital

Sri Widyaningsih, Muthia Maraya Hasna, M. Reza Utama, Gina Noor Djalilah

Faculty of Medicine, University of Muhammadiyah Surabaya, Indonesia. E-mail: [maraya.hasna@gmail.com](mailto:maraya.hasna@gmail.com)

### ABSTRACT

Sepsis can occur in patients with Systemic Inflammatory Response Syndrome (SIRS) symptoms, especially in neonates due to their underdeveloped immune systems. Blood culture as the gold standard test to diagnose sepsis in SIRS patients takes a long time to perform. The other rapid test parameters are needed to support the early diagnosis of infection (sepsis) in SIRS patients, including Immature Granulocytes (IG%) and C-Reactive Protein (CRP). This study aimed to determine the correlation between IG% with blood culture and CRP with blood culture in neonatal SIRS patients at Gambiran Hospital, Kediri. The research design used retrospectively using secondary data. A total of 63 samples were obtained using simple random sampling on the medical record of neonatal SIRS patients who met the inclusion criteria, without any exclusion criteria. There were significant results with a value of  $p=0.000$  ( $p < 0.05$ ) between IG% and blood culture using the Mann-Whitney test, as well as on CRP and blood culture using the independent samples T-test. The bivariate statistical test between IG% and CRP was carried out using the Spearman test and showed significant results with  $p=0.000$  ( $p < 0.05$ ) with a correlation coefficient ( $r_s$ ) of 0.740, which indicated a strong positive relationship. It was concluded that there was a correlation between IG% and CRP with blood culture in neonatal SIRS patients.

**Keywords:** Systemic inflammatory response syndrome, immature granulocytes, C-reactive protein, blood culture

### INTRODUCTION

Sepsis is one of the most common causes of mortality and morbidity in neonates due to different modes of spread of infectious agents, immature immunity, and diverse clinical manifestations.<sup>1</sup> Sepsis is defined as a systemic response of the body when a pathogen or toxin is in blood circulation.<sup>2</sup> The initial stage of sepsis can be Systemic Inflammatory Response Syndrome (SIRS), a non-specific inflammatory process that occurs in an infectious or non-infectious state. Sepsis occurs if there is an infection that triggers SIRS. Sepsis can then become severe with signs of organ dysfunction that increase the risk for septic shock.<sup>3</sup> Based on the World Health Organization (WHO), the global epidemiological burden of sepsis is difficult to ascertain; however, it is estimated that it occurs in 48.9 million people and has the potential to cause 11 million deaths, and an estimated 20 million children suffer from sepsis each year.<sup>4</sup> Neonatal mortality rate in Indonesia is estimated at 19 per 1000 live births, while the infant mortality rate is estimated at 34 per 1000 live births with 10.6% of death being related to neonatal sepsis.<sup>5</sup> The stillbirth rate in Kediri in 2017 was 2.8 per 1000 live births, while the infant mortality rate is 2 per 1000 live births.<sup>6</sup> Based on these data, the

most common causes of neonatal death are Low Birth Weight (LBW) (56.25%), asphyxia (37.5%), and infection (6.25%).<sup>6</sup>

Rapid and accurate diagnosis is very important to reducing mortality and morbidity of neonatal sepsis. Blood culture is the gold standard test in patients with sepsis; however, culture results can only be obtained after several days, while empirical antibiotics must be given immediately.<sup>7,2</sup> Currently, there are many tests to support neonatal sepsis such as hemato-infection parameters (WBC count, immature/total ratio (IT ratio), and neutrophil count), and C-Reactive Protein (CRP), procalcitonin (PCT), and Interleukin-6 (IL-6).<sup>7</sup> C-reactive protein is an acute-phase reactant synthesized by the liver in response to activation of proinflammatory cytokines within 4 to 6 hours of triggering conditions such as infection or tissue injury.<sup>8</sup> Several methods of WBC count are needed in the early diagnosis of sepsis.<sup>9</sup> According to a study by Nierhauss, the presence of Immature Granulocytes (IG) in peripheral blood indicates increased activity in bone marrow against bacteria; therefore, it can be used as an initial test in SIRS patients with suspected neonatal sepsis.<sup>10</sup>

World Health Organization<sup>4</sup> commends several laboratory tests to support the diagnosis of neonatal sepsis such as CRP, PCT, and IT ratio, which is useful

for detecting the presence of infection in the blood.<sup>11</sup> C-reactive protein and IT ratio are routine laboratory tests carried out in health services as predictors of sepsis while waiting for culture results. The IT ratio test was carried out using peripheral blood smears from neonates and is calculated manually.<sup>12</sup> Because the immature granulocytes parameters are already included in the complete blood count test using a hematology analyzer, the results could be obtained quickly and helpful for the treatment of neonatal sepsis.<sup>12</sup> Complete blood count tests and CRP measurements have been carried out routinely at Gambiran Hospital and are included in the Clinical Practice Guideline for neonatal sepsis. Based on this background, the authors tried to conduct research on the relationship between IG with blood culture and CRP with blood culture in neonatal SIRS patients.

## METHODS

The study was conducted from December 2020 to January 2021 using a retrospective research design. The data used in this study were medical records of neonates diagnosed with SIRS clinically at Gambiran Hospital Kediri from January 2017 to February 2020. The inclusion criteria were newborns (neonates), the presence of two or more SIRS criteria, as well as patients conducting complete blood count,<sup>42</sup> P, and blood culture at Gambiran Hospital, Kediri. Systemic inflammatory response syndrome criteria were defined as the presence of an abnormal body temperature ( $<36^{\circ}\text{C}$  or  $>38.5^{\circ}\text{C}$ ), an abnormal pulse rate, an increased respiratory rate ( $>60\text{x}/\text{min}$ ) or the use of mechanical ventilation, and an abnormal white blood cell count (based on age) or immature leukocytes  $>10\%$ .<sup>13</sup>

Laboratory tests were carried out at the Clinical Pathology Laboratory of Gambiran Hospital, Kediri.<sup>37</sup> Immature granulocytes% data were obtained from a complete blood count test using a hematology analyzer Sysmex XN 1000 with flow cytometry method, with a sensitivity of 97.3% and a specificity of 91.91% for IG marking.<sup>14</sup> Data of CRP levels were obtained from the Wondfo Finicare FIA Meter Plus using the fluorescence immunoassay method with an accuracy of 98.8%.<sup>15</sup> Blood cultures were identified using the Mindray TDR-300B with a semi-automatic method and a bottle sample according to the SOP in the NICU unit of the hospital.

The software used for data analysis in this study was SPSS version 25. Positive and negative culture results were reported as a nominal data scale, 1 for negative culture results and 2 for positive culture results,

whereas IG% and CRP values were included in the numerical data scale. Due to the different data scales, the correlation between IG% and blood culture results and CRP and blood culture results were analyzed using a comparative approach. This comparative approach aimed to compare the IG% and CRP values between two groups of patients (positive blood culture and negative blood culture) to determine whether high IG% and CRP values are associated with positive culture results or vice versa. The analytical technique used was the independent samples T-test parametric technique on the CRP comparison test and the non-parametric Mann-Whitney test on the IG% comparison test. The correlation between IG% and CRP was analyzed by bivariate correlation using the non-parametric spearman's rank test. The p-value  $<0.05$  indicated a significant correlation between both variables.<sup>16</sup>

## RESULTS AND DISCUSSIONS

This study found 63 patients who met inclusion criteria. Subjects involved in this study had several characteristics, which are fully presented in Table 1.

Based on Table 1, it was known that most of the neonates with SIRS symptoms were male (66.7%). This was in line with a study by Hakiem, which found that 18 (60%) of 30 neonates with sepsis were male.<sup>17</sup> Epidemiologically, a study by Izzati also found that 58.8% of the subjects of early-onset neonatal sepsis were male.<sup>18</sup> This risk was thought to be related to the body's defense factors in male infants with X-linked traits, that the number of X chromosomes for males (XY) is less than for females (XX). This has an impact on the body's immune system because the microRNA in the chromosome is needed to regulate proteins that are important for the immune system.<sup>18</sup>

More than half (52.4%) of neonates in this study were born in weeks 37 up to 42 weeks. These findings were in line with research by Ruan and Karad, which suggested that the risk of sepsis was mostly found in full-term neonates.<sup>19,20</sup> Premature infants can be a risk factor for neonatal sepsis due to premature immune system function.<sup>21</sup>

The number of LBW cases among subjects in this study was quite (41.27%); however, the majority of the baby's weight in this study was normal (58.73%). A similar number of normal birth weight babies with a risk of sepsis was also found in a study by Monga.<sup>22</sup> Neonates with low birth weight are estimated to have lower IgG levels than normal birth weights and are more susceptible to infection.<sup>22</sup> This makes low birth weight a risk factor for sepsis. Risk factors for

neonatal death from severe infection are often associated with prematurity, low birth weight, and low post-natal age.<sup>23</sup>

A total of 66.7% of subjects in this study were neonates born with cloudy amniotic fluid. Green and smelly amniotic fluid is one of the risk factors for infection in neonates.<sup>18</sup> According to Kim, babies born with cloudy amniotic fluid have a 10 times risk of developing sepsis compared to babies born with clear amniotic fluid.<sup>24</sup> This is because a cloudy amniotic fluid especially those with a certain amount of meconium can be a growth medium for bacteria.<sup>24</sup>

The mean IG% in this study was 4.34%, higher than the normal value of 0.0–0.6%. The mean value of CRP in this study was 32.11 mg/L, higher than the normal value of 0–10 mg/L. The results of the blood culture test showed that positive results or bacteria were found in 55.6% of the patient samples, whereas negative results were reported in 44.4% of samples.

Positive blood cultures were obtained in 35 (55.6%) samples, suggesting that bacteria were the cause of the infection. Most of the infection-causing bacteria in this study were the Gram-positive group with a percentage of 57.14% (Table 2). The most common Gram-positive bacteria found in this study were *Staphylococcus saprophyticus* (20.1%), which is known as a common cause of Urinary Tract Infections (UTI) in sexually active young females.<sup>25</sup> The source of transmission of *Staphylococcus saprophyticus* in humans is still not fully known; however, research by Sousa revealed that this bacterium can be found in Minas cheese, polluted river water, and in the microbiota of pregnant females.<sup>25</sup> Based on this explanation, it can be suggested that the cause of *Staphylococcus saprophyticus* infection in neonates can originate from the patient's mother who was infected by this bacteria during pregnancy.

**Table 1.** Characteristics of research subjects

Variables	Mean±SD	n (%)
<b>Gender</b>		
Male		42 (66.7)
Female		21 (33.3)
<b>Gestational age:</b>		
37–42 weeks		33 (52.4)
32–37 weeks		29 (46)
28–32 weeks		1 (1.6)
<b>Newborn weight</b>		
Normal (2500–4000 gram)		37 (58.73)
Low (< 2500 gram)		26 (41.27)
<b>Mother's amniotic fluid</b>		
Clear		21 (33.3)
Cloudy		42 (66.7)
IG (%)	4.34±3.75	
CRP (mg/L)	32.11±13.27	
<b>Blood culture</b>		
Positive		35 (55.6)
Negative		28 (44.4)

**Table 2.** List of bacteria species based on blood culture results

Type of bacteria	Bacteria	n (%)
Gram (+)	<i>Staphylococcus saprophyticus</i>	9 (25.71)
	<i>Staphylococcus xylosum</i>	6 (17.14)
	<i>Staphylococcus aureus</i>	2 (5.71)
	<i>Staphylococcus lugdunensis</i>	2 (5.71)
	<i>Enterococcus faecalis</i>	1 (2.86)
Gram (-)	<i>Escherichia coli</i>	7 (20.0)
	<i>Burkholderia cepacia</i>	6 (17.14)
	<i>Pseudomonas diminuta</i>	1 (2.86)
	<i>Pseudomonas putida</i>	1 (2.86)

Other Gram-positive bacterial species identified in this study were *Staphylococcus xylosum*, *Staphylococcus aureus*, *Staphylococcus lugdunensis*, and *Enterococcus faecalis*. The most common species of Gram-negative bacteria found in this study were *Escherichia coli* (20%). *Escherichia coli* is mostly found in premature patients with LBW. Other Gram-negative bacterial species identified in this study were *Burkholderia cepacia*, *Pseudomonas diminuta*, and *Pseudomonas putida*.

This finding was in line with a study by Beltempo, which found Gram-positive bacteria in most blood culture samples.<sup>8</sup> Species of Gram-positive bacteria found were *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus agalactiae*, of which *Staphylococcus aureus* was the most abundant. The most common Gram-negative bacteria found were *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Candida lusitanae*. Several species found in this study were similar to the finding by Beltempo.<sup>8</sup> The differences in several types of bacteria found in each study are natural because the bacteria that cause sepsis differ among countries and hospitals.<sup>26</sup>

The bacteria found in the highest IG% value (15.2%) and the highest CRP value (67.5 mg/L) in this study were *Burkholderia cepacia* (CRP 41.7 mg/L) and *Pseudomonas putida* (IG% 3.5%). A study by Monga also found that Gram-positive bacteria were commonly found. In addition, a higher number of positive results of qualitative CRP was found in Gram-positive bacteria. Contrastingly, a higher number of positive results of quantitative CRP value was found in Gram-negative bacteria than in Gram-positive bacteria.<sup>22</sup> Similarly, this study also found that most of the Gram-negative bacteria had high CRP values.

Patients with positive blood culture results had a higher IG% value than patients with negative blood culture results. The statistical test showed that there was a significant difference in IG% ( $p=0.000$ ) between the IG% variable and blood culture.<sup>16</sup> Thus, it can be concluded that there was a significant relationship between IG% and blood culture results in neonates diagnosed with SIRS. The IG% value tends to be higher in the group with positive blood

culture results, and vice versa. This was in line with the study by Cimenti, which showed that there was a relationship between IG% and neonatal sepsis.<sup>27</sup> The percentage of IG% increased significantly in the group of neonates with sepsis compared to healthy neonates.<sup>27</sup>

According to research by Ayres, there was a statistically significant relationship ( $p=0.009$ ) between blood culture results and IG% analyzed with the Pearson Chi-Square test.<sup>28</sup> In addition, a significant relationship was also found by comparing blood culture and clinical confirmation results with IG% above and below 2%.<sup>28</sup> Through the Pearson Chi-Square test, a significant result ( $p < 0.001$ ) was obtained for this comparison where patients with negative blood cultures and clinical confirmations tended to have an IG% value  $< 2\%$ .<sup>28</sup> Studies by Ayres were carried out on different ages of subjects, whereas this study used a sample with an age  $> 18$  years.<sup>28</sup>

Statistical tests showed a significant difference in CRP ( $p=0.000$ ) between the CRP variables and blood cultures, indicating a significant relationship between high CRP values and positive culture results, and low CRP values are often associated with negative culture results (Table 3). These findings support a finding in a study by Chacha that the CRP test can support the diagnosis of neonatal sepsis in SIRS patients.<sup>29</sup> Higher CRP values were reported in neonates with culture-confirmed sepsis compared with negative culture results.<sup>29</sup> There was a significant correlation between CRP and blood culture with a  $p$ -value  $< 0.05$ .<sup>29</sup> It was also found that neonates with Gram-negative bacterial infection had a high mean CRP value.<sup>29</sup>

According to a study by Madavi, which studied CRP as an early indicator of neonatal sepsis, it was found that there was a relationship between positive CRP values and positive blood culture results, in which positive CRP values were expressed as  $CRP > 0.6$  mg/dL.<sup>30</sup> A total of 92.52% of positive blood cultures had a positive CRP value. Statistical analysis showed that this relationship was significant with  $p$ -value=0.000.<sup>30</sup> Another study on the relationship between CRP and blood culture conducted by Monga, which used quantitative and

**Table 3.** Comparison test results between IG and CRP based on blood culture

Variable	Blood Culture Results		P
	Positive	Negative	
IG (%)	4.8 (0.6–15.2)	1.65 (0.5–13.0)	0.000
CRP (mg/L)	39.90±10.44	22.38±9.51	0.000

qualitative CRP results, there was a relationship between positive blood culture results and positive CRP.<sup>22</sup> Positive CRP results were found in 85.1% of positive blood culture samples with a mean CRP

**Table 4.** Bivariate correlation test results between IG dan CRP

$r_s$	P
0.760	0.000

Spearman's rank correlation test showed a correlation coefficient ( $r_s$ ) of 0.740, indicating a strong relationship in a positive direction (directly proportional) between IG and CRP (Table 4). It was concluded that there was a significant correlation ( $p=0.000$ ) between IG% and CRP in neonates diagnosed with SIRS. These findings empirically indicated that the IG% and CRP parameters had the same pattern of changes when infection occurs. This was in accordance with a study by Park, which suggested that both IG% and CRP tests could distinguish between patients at risk for sepsis and non-sepsis with local infection among 6 studied biomarkers.<sup>31</sup>

## CONCLUSIONS AND SUGGESTIONS

This study found a significant correlation ( $p=0.000$ ) between IG% and blood culture and between CRP and blood culture. In addition, there was a significant correlation between IG% and CRP ( $p=0.000$ ) with a strong correlation strength ( $r=0.760$ ). Based on these results, it was concluded that there was a relationship between IG% and CRP with blood culture in neonatal SIRS patients. Examination of IG% and CRP can be used as an initial test to identify infection that can cause sepsis in neonates diagnosed with SIRS. Both markers can be obtained quickly they can be used together in practice to support early diagnosis and treatment. However, this study did not analyze the cut-off IG% and CRP as markers of infection in SIRS patients, making further research an important need to determine the cut-off of IG% and CRP as a marker of infection.

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