

Correlation Between I/T Ratio, S100B, Interleukin-10 Serum, and NEWS2 Score in Sepsis

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Abstract- Immature/total neutrophils (I/T) ratio is a simple and inexpensive method used as an early sign of bacterial infection. Meanwhile, the S100B protein is found in glial cells and functions as a neuroprotection. Then, the interleukin-10 cytokine acts as an anti-inflammatory cytokine during infection. In addition, the NEWS2 score is used to see the early and fast changes in patients with sepsis. The purpose of the study was to prove the correlation between the I/T ratio, S100B, IL-10, and NEWS2 score in patients with sepsis. A cross-sectional study was conducted on 34 patients with sepsis from September 2020 to February 2021. I/T ratio was calculated in 100 leukocytes from microscopic blood smear preparation. Levels of S100B and IL-10 serum were measured using the ELISA method. Pearson correlation test was used for normally distributed data, and the Spearman correlation test was utilized for abnormally distributed data. Correlation test between I/T ratio, S100B, and IL-10 with NEWS2 score respectively showed values of $r=0.58$; $P=0.01$, $r=0.36$; $P=0.03$, and $r=0.39$; $P=0.02$, in which $P<0.05$. There was a moderate positive correlation between I/T ratio and NEWS2 score, a weak positive correlation between S100B and NEWS2 score, and a weak positive correlation between IL-10 and NEWS2 score.

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Keywords: I/T ratio; S100B; Interleukin-10; National early warning score (NEWS2); Sepsis

Introduction

Sepsis is the leading cause of death in intensive care units (ICU) overall and the second leading cause of death in non-coronary ICUs. The incidence and prevalence of sepsis worldwide have increased in the past few years. In addition, sepsis is defined as a life-threatening organ dysfunction due to impaired regulation of host response to infection and is recognized by the WHO as a global health priority (1-4). The study involving 16 countries in Asia by the Management of Severe Sepsis in Asia's Intensive Care Unit (MOSAIC) described an overall mortality rate in hospitals due to sepsis of 44.5%. Specifically, the mortality rate reported in Indonesia was 62.7% of the total number of patients treated with sepsis at National Central General Hospital Cipto Mangunkusumo between January 2014-July 2015, while the sepsis-related mortality rate at the ICUs of Central General Hospital Prof. Dr. R. D. Kandou Manado was

65.7% between December 2014-November 2015 (5,6).

While the widely used scores to help early detection of sepsis are systemic inflammatory response syndrome (SIRS) and quick sepsis-related organ failure assessment (qSOFA), recent studies found that National Early Warning Score (NEWS) is better than qSOFA. The score consists of steps necessary to assess the patient's physiological state, which comprises vital signs and direct awareness so that patients who experience clinical deterioration can be detected early, including patients with sepsis, to be treated promptly and transferred to the ICU. The seven physiological parameters in this score are not difficult to apply to patients with acute illness (7-9). Moreover, the gold standard of examination to diagnose sepsis is cultures as the marker of bacteremia, though it has limitations, including long preparation time. In this case, manual leukocyte count or immature to total neutrophil (I/T) ratio is a simple, fast, and inexpensive method that can assist in establishing the diagnosis and

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the management of acute infection or inflammation caused by bacteria. A left shift in the leukocyte count with an increase in the number of immature neutrophils is common in patients with acute bacterial infections (10,11). Sepsis-associated encephalopathy (SAE) is a term that describes sepsis-associated brain dysfunction (12). Meanwhile, the S100B protein is a calcium-bound protein produced by astrocytes in the central nervous system, mainly found in astroglia and Schwann cells. The protein has been reported as an indicator of damage in the process of circulatory failure, stroke, and head injury and is also thought to be associated with neuronal damage. Levels increase after brain ischemia occurs and can be used as a marker of brain damage due to ischemic hypoxia in sepsis-associated encephalopathy so that patients with neurological deficits can be detected early to produce a better prognosis (13-15). Besides, cytokines have pro- and anti-inflammatory functions that coordinate effectively with the body's defense mechanisms against pathogens. Concerning that, interleukin-10 (IL-10) is one of the most important anti-inflammatory cytokines produced during bacterial infection and is pleiotropic. Aside from fighting pathogens, it can also cause tissue damage if the levels are not balanced with pro-inflammatory cytokines. Interleukin-10 is produced by monocytes, macrophages, B lymphocytes, T lymphocytes, mast cells, dendritic cells, and non-hematopoietic cells, such as epithelial cells, microglia cells, and astrocytes (16). The production of IL-10 can mitigate damage to the host's cellular with clearance and neutralizing mechanism of immune response effectors (17). This parameter can be used as an anti-inflammatory marker as an immune response to sepsis and is produced by the body in large quantities, so it is easily detected in serum.

Materials and Methods

This research was an analytical observational study with a cross-sectional approach conducted at the Intensive Care Unit of Dr. Kariadi Hospital, Semarang, Indonesia. All subjects were asked for written informed consent, and their identities were kept confidential. Sampling was carried out from September 2020 to February 2021 by consecutive sampling. All prospective research subjects were given a complete explanation of research objectives, benefits, and procedures. The research data were taken from patients diagnosed with sepsis in the intensive care unit by an internist with a SOFA score of 2 points or more. The NEWS2 score and specimen collection were carried out within the first 24

hours of patients diagnosed with sepsis. Basic data were collected through history taking (history of pregnancy, drug use, central nervous system trauma, and chronic disease) and physical examination (consciousness, temperature, blood pressure, pulse, respiratory rate, and oxygen saturation). Inclusion criteria included age of 18 years, blood pressure of 120/80 mmHg, normal menstruation for women, free of radiation or chemotherapy, and negative SARS CoV-2 PCR test results. Meanwhile, exclusion criteria included patients with head trauma, autoimmune disease, HIV, and malignancy. In this study, 3 ml of venous blood was taken, then the I/T ratio was calculated in 100 leukocytes from microscopic blood smear preparation conducted by three readers who had been subjected to the Kappa test. The serum was separated from venous blood and stored at -80°C , used for biomarker assessment. Then, S100B and IL-10 were measured using the sandwich enzyme-linked immunoassay (ELISA) principle from Elabscience® USA. The research was conducted after obtaining ethical approval No.680/EC/KEPK-RSDK/2020, dated December 7, 2020, from the Ethics Commission of Dr. Kariadi Hospital, Semarang. Data were then analyzed by computer program. The normality test of the data was carried out with the Shapiro-Wilk test. In addition, the Pearson test was used to analyze the I/T ratio and IL-10 serum level. Spearman rank test was employed to analyze the S100B serum level. Moreover, $P<0.05$ was significant, with 95% confidence interval.

Results

Based on the data of 34 subjects who met the inclusion and exclusion criteria for patients with sepsis in the intensive care unit of Dr. Kariadi Hospital Semarang, 19 (55.8%) were male, and 15 (44.2%) were female. The overall patient age range was 19-82 years, with a mean age of 52.32 ± 15.14 . The median values of leukocyte, I/T ratio, and S100B were $13.05\times 10^3/\mu\text{L}$, 0.04%, and 430.25 pg/mL. The mean value of NEWS2 was 10.5, with the 5-16 range of 100% having a NEWS2 score of 5 points or more. The general characteristics of the research subjects are shown in Table 1.

Analysis of correlation test respectively showed values of $r=0.58$; $P=0.01$, $r=0.36$; $P=0.03$, and $r=0.39$; $P=0.02$, in which $P<0.05$. It can be concluded that there was correlation between I/T ratio, S100B, IL-10, and NEWS2 score (Table 2, Figure 1).

Table 1. General Characteristics of Research Subjects

Variables	Total (n=34)
Age (years) ^a	52.32 ± 15.14
Sex, n (%)	
Male	19 (55.8%)
Female	15 (44.2%)
Systolic blood pressure (mmHg) ^b	120 (53-185)
Respiratory rate (x/minute) ^b	20 (12-35)
Leukocytes (x10 ³ /uL) ^b	13.05 (2.7-37.9)
I/T ratio ^b	0.04 (0.01-0.12)
S100B (pg/mL) ^b	430.25 (26.7-1919.4)
Interleukin-10 (pg/mL) ^a	3.091 ± 1.19
National early warning score 2 ^a	10.59 ± 2.5

^aData is presented in mean (standard deviation). ^bData is presented in median (min-max). n: amount; I/T ratio: immature to total neutrophils ratio

Table 2. Correlation of I/T Ratio, S100B, and IL-10 Levels with NEWS2 Scores

Variables	National Early Warning Score 2	
	r	p
I/T ratio	0.58	0.01*
Protein S100B (pg/mL)	0.36	0.03*
Interleukin-10 (pg/mL)	0.39	0.02*

*p<0.05: significant value

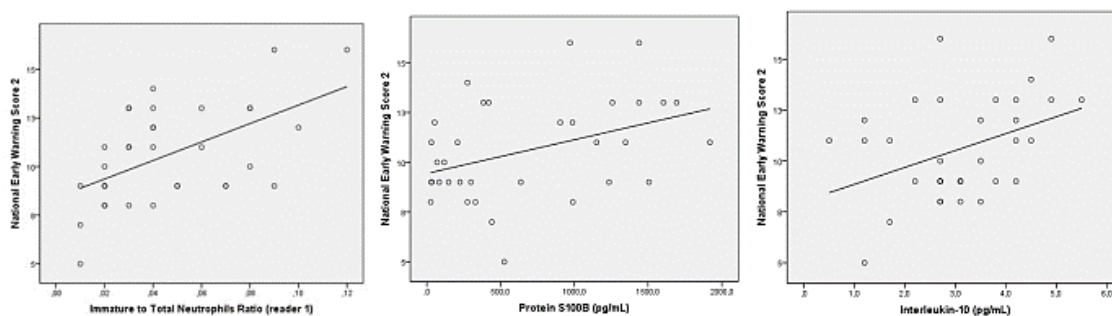


Figure 1. Correlation of I/T Ratio, S100B, and IL-10 Levels with NEWS2 Scores

Discussion

The mean age of the research subjects was 56 (19-82) years, and this is in accordance with several previous studies, such as the MOSAIC study in 16 countries in Asia, with a mean age of patients with septic admitted to the ICU of 59 years (5). According to a study by Zhou *et al.*, the mean age of patients with sepsis was 66 years, with an age range of 51 years to 77 years (18). In a study by Kotfis *et al.*, 32.8% of the incidence of sepsis occurred at the age of 61 years to 70 years (19). The increased incidence of sepsis in elderly patients with infection is

related to the effects of aging and the presence of comorbidities or chronic disease (20). In addition, the effect of age involves a decline in the function of the innate (neutrophils, macrophages, dendritic cells, and NK cells) and adaptive (T cells and B cells) immune systems (21,22). The prevalence of sepsis in this study was lower in female than in male patients due to the protective effect of estrogen, which has been reported in several previous studies (5,18,23,24).

Most of the patients in this study had systolic blood pressure ≥100 mmHg (28 patients). It was because the patient had received vasopressor therapy. The difference

with a study by Novita *et al.*, is that most patients had systolic blood pressure ≤ 100 mmHg (25). In this regard, pro-inflammatory cytokines activate endothelial cells by increasing the expression of adhesion receptors, and it causes endothelial cell damage by inducing the adhesion of neutrophils, monocytes, macrophages, and platelets to endothelial cells. In addition, several mediators, such as protease, prostaglandin, and leukotriene, are also released, which will damage the endothelium, resulting in increased permeability and vasodilation. It will decrease cardiac output so that systolic blood pressure will decrease (26).

Furthermore, patients with leukocytes $>12,000/\mu\text{L}$ in this study were 70.6%, showing that the function of phagocytosis as the body's immune response to extracellular bacterial would cause an increase in the number of leukocytes, especially neutrophils, seen in the presence of immature granulocytes in the patients with peripheral blood septic (27).

The overall NEWS2 scores were in the range of 5-16, with a median value of 10.5. Thus, this study showed that 100% of the subjects had a NEWS2 score ≥ 5 . Related to this, a study by Melhammar *et al.*, demonstrated that patients with septic who had a NEWS2 score ≥ 5 (65.2%) and had multiorgan disorders were admitted to the ICU and died in the first 72 hours after treatment, with an AUC of 0.80 (8). In addition, Liu *et al.*, stated that a NEWS score ≥ 6 had a sensitivity of 87-89% with an AUC of 0.87 (95% CI:0.87-0.87) compared to other scoring systems in predicting death and patient transfer to the ICU (28). Previous studies align with this study that patients with a NEWS2 score ≥ 5 tended to deteriorate clinically and had a greater risk of sepsis, even death; thus, further biomarker examination is necessary to confirm the diagnosis. Further, several parameters on the NEWS2, such as respiratory rate, oxygen saturation, and oxygen supplementation, are affected by stress, pain, metabolic acidosis, acute respiratory distress syndrome (ARDS), asthma, and chronic obstructive pulmonary disease (COPD). Moreover, systolic blood pressure and pulse are influenced by a history of hypertension, heart disease, hyperthyroidism, obesity, and the administration of beta-blockers. Meanwhile, consciousness can be affected by sedation and head trauma (29). Spinal injuries (tetraplegia or high paraplegia) can also affect the patient's autonomic nervous system regulations, such as pulse, temperature, and blood pressure, influencing NEWS2 (30). However, this study did not consider these, which is a point to consider in the future.

In addition, neutrophils play an important role in the innate immune response in eliminating pathogens.

Immature neutrophils are less potent in their immune defense function than mature forms due to reduced receptor expression, chemotaxis, phagocytosis, ROS production, and cytokine production (31). Saboohi *et al.*, demonstrated a significant correlation between the I/T ratio and a positive culture result ($r=0.721$; $P<0.001$), with equally high specificity and NPV (83.82% and 93.44%, respectively) in neonatal sepsis (32). A study by Nierhaus *et al.*, also found that immature neutrophils had a high AUC score in the first 48 hours after the sign of SIRS, with an AUC of 0.861 ($P<0.0001$), and the mean value of immature neutrophils in patients with infection was higher than in non-infected individuals ($P<0.0001$). Concerning this, immature neutrophils are absent in healthy people's peripheral circulation, and the presence of immature neutrophils in the peripheral blood indicates an increase in bone marrow activity as in sepsis (27). In addition, neutrophils have other effects that can cause tissue damage and organ failure (33). It signifies that the I/T ratio can be used as an indicator of the occurrence of sepsis in predicting early sepsis. The results of this study indicated a moderately positive correlation between the I/T ratio and NEWS2 score, which showed that the I/T ratio increased along with decreasing clinical conditions in patients with sepsis. Nevertheless, the I/T ratio might be influenced by comorbid diseases suffered by patients, which had not been considered in this study.

In this study, the median value of S100B was 430.25 (26.7-1919.4). The correlation test results between S100B levels and the NEWS2 in this study showed a weak positive correlation ($r=0.36$; $P=0.03$), meaning that S100B levels would increase in patients with sepsis in proportion to the increased risk of a decline in the clinical condition of the patients assessed by the NEWS2. A study by Pfister *et al.*, using the APACHE II score, obtained a relatively high median with 22.5% but not statistically significant ($P=0.09$) in patients with sepsis with delirium (34). In contrast, Nguyen *et al.*, found a significant result ($P=0.03$) between elevated S100B levels and APACHE III scores in patients with sepsis and septic shock (35). Their study described elevated S100B levels in patients with sepsis with brain dysfunction ($P=0.028$), and comatose patients had higher S100B levels than patients with delirium ($P=0.007$) (36). Here, elevated levels of S100B indicate a worsening of brain dysfunction, one of the multiorgan failures caused by sepsis.

A study by Wu *et al.*, described that peak levels of S100B in sepsis were found at 48 hours of treatment compared to 24 hours of treatment because, in sepsis, the brain damage that occurred would increase S100B levels continuously, especially in SAE (37). In this study, the

S100B examination was carried out 24 hours after the patient was diagnosed with sepsis in intensive care, and no re-examination was performed, and it was very likely that the S100B levels were not yet maximum. For critically ill patients in the ICU, it is very difficult to diagnose the presence of impaired brain function since the patient has been given sedation and pain medication at the time of admission. In this study, nearly 50% of patients admitted to intensive care were unconscious patients who had undergone surgery and had been given sedation. Those unconscious patients might not have elevated S100B levels, affecting the assessment of consciousness on the NEWS2 score. It is consistent with the previous studies by Routsis *et al.*, and Duda *et al.*, (38,39). Increased S100B in sepsis is due to a response from pro-inflammatory cytokines in the brain, but this increase may be due to catecholamine stimulation of muscle, cartilage, and adipocytes damaged by sepsis (40). Elevated levels of S100B can also be affected by obesity because this protein is not only produced by glial cells but also comes from adipose tissue (41).

Moreover, the results of this study uncovered a weak positive relationship between IL-10 and NEWS2 scores in patients with sepsis. The higher IL-10 levels indicated a patient's declining clinical condition, which was proportional to the increase in the NEWS2 score. In a study by Gogos *et al.*, patients with sepsis in ICUs using SAPS II scores demonstrated a significant correlation based on the severity of the disease with IL-10 levels at 48 hours of treatment ($r=0.677$) while TNF- α levels had a relationship with the beginning of treatment ($r=0.724$). Significantly high SAPS II scores were found in non-survival patients ($P<0.05$). A decrease in IL-10 levels was found in surviving patients 48 hours after treatment, while no changes in IL-10 levels were found in non-survival patients (42). A study by Matsumoto *et al.*, revealed a significant correlation between APACHE II scores and increased IL-10 levels in critically ill patients in intensive care versus non-critically ill patients in the acute phase on days 1, 2, and 4 (43). In addition, Surbatovic *et al.*, stated that IL-10 levels were higher in patients with gram-negative bacterial infections than in gram-positive and polymicrobial bacteria in abdominal sepsis; also, IL-10 levels were lower in survivors than in non-survivors (44). Decreased levels of IL-10 can be found in obesity and diabetes mellitus, as in the study by Chang *et al.*, and Barry *et al.*, (45,46). Besides, anti-inflammatory cytokine response and overproduction of pro-inflammatory cytokines will occur. The degree of damage inflicted by sepsis depends on the timing of cytokine release and the balance between pro-and anti-

inflammatory cytokines.

The study results revealed a correlation between markers of infection, neuroprotection, and anti-inflammatory and the NEWS2 score in patients with sepsis. It is expected to help in the early detection of sepsis. Still, further studies are needed to pay attention to comorbidities and levels of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 and the sampling time of S100B protein due to its short half-life.

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