

Correlation between Level of Interleukin-6, Interleukin-10 and Ratio of Interleukin with PELOD-2 Score as Predictor of Outcome in Children with Sepsis

Faiza Risty Aryanti Septarini, Muhamad Supriatna Toto Saputra, Wistiani, Omega Mellyana

Department of Child Health, Faculty of Medicine, Diponegoro University/Kariadi General Hospital, Semarang

Keyword:

Interleukin-6,
interleukin-10,
interleukin ratio,
sepsis,
prognosis

Abstract

Sepsis consist of inflammatory and anti-inflammatory processes. Biomarker as IL-6, IL-10 dan ratio of both interleukin has been associated with severity of sepsis which correlate with severity organ disfunction and usefull prognostic marker in patient with sepsis. One of sistem scoring that aimed to describe the severity of organ dysfunction is PELOD-2 score. Aim of this study is to prove that IL-6, IL-10 plasma concentrations and ratio IL-6/IL-10 can be used to predict outcome in children with sepsis by using PELOD-2 score. This is an analytic observational study in 31 children with sepsis in Dr. Kariadi general hospital, Semarang, from February to July 2019. The relationship between changes in IL-6, IL-10 and IL ratio with PELOD-2 scores were analyzed by the Pearson or Spearman test. Cut-off points for IL-6, IL-10 and IL ratio to predict the outcome of sepsis were determined using the ROC curve. The results were thirty one children with sepsis were included in the study. Nine children (31%) died during the study period. There were significant differences in IL-6 levels (D5-end observation), IL-10 levels (D0) and the IL-6 / IL-10 ratio (D0-end observation) between children death outcomes with life. The IL-6/IL-10 ratio with a cut-off point 1.35 (AUC 0.78 ± 0.1 ; $p = 0.019$) on D5, IL-6 level ≥ 17.15 pg/ml (AUC 0.85 ± 0.80 ; $p = 0.004$) and IL6 / IL-10 ratio ≥ 1.23 (AUC 0.89 ± 0.08 ; $p = 0.01$) at the end of monitoring can be used as an indicator of multiorgan damage. There was significant relationship between changes in IL-6 levels ≥ 1.25 pg/ml with increased multiorgan failure (RR 80; 95% CI 6.33 - 101.95; $p < 0.001$) at time interval D0-end observation, especially in the initial 5 days ≥ 1.05 pg/ml (RR 1.27; 95% CI 1.21 – 31.94; $p = 0.004$). Type of pathogen becomes a factor of the assault in this study. It was concluded that levels of IL-6 (D5 and end observation), IL-10 (D0) and the ratio of IL-6/IL-10 (D0, D5 and end observation) differed significantly between died with live outcomes. IL-6, IL-10 and IL-6 / IL-10 ratio can be used together in predicting multi-organ failures at D5 and end of monitoring.

Corresponding Author: faizaristy87@gmail.com (Faiza Risty Aryanti Septarini)

INTRODUCTION

Sepsis is a leading cause of death in children worldwide.^{1,2} A high incidence rate, complex management, high mortality and expensive cost, made sepsis to become a global problem in both developing and developed countries.³ In developing countries, sepsis mortality rate is up to 50% or more, especially when accompanied with shock and organ failure.² In pediatric intensive care unit at Dr. Kariadi Hospital Semarang, 44% of 120 pediatric patients treated since January 2013 – 31 August 2017 suffer from sepsis with mortality rate of 6,7%.⁴

Sepsis biomarker play an important role in identification of the presence of sepsis, to determine severity of sepsis, useful in prognostic area, guiding antimicrobial therapy, evaluation of therapeutic response, differentiating gram positive and negative infection, predicting sepsis complication and its development leading to organ dysfunction (heart, renal, liver or multiorgan dysfunction).^{5,6} There is a significant correlation between severity of sepsis with IL-6 and IL-10 concentration which may be useful in determining septic patient's prognosis. IL-6/IL-10 ratio have the highest AUC compared to IL-6 and IL-10 measurement themselves, IL-8, LDH and has strong association with severity of sepsis.^{8,9,10}

There are some scoring system used to investigate the severity of organ dysfunction in a critically ill child. One of those is PELOD-2 score. This scoring system showed that the greater accumulated effects of organ dysfunction and severity of sepsis, the higher the mortality risk also.¹⁰

Early diagnosis and stratification of severity of sepsis is critical, which may increased the possibility of specific therapy thus resulting in a better outcome. This research aims to prove a significant correlation between IL-6, IL-10 level and IL-6 to IL-10 ratio with PELOD 2 score in predicting the outcome of children with sepsis.

METHODS

An analytic observational study was conducted on February 2019 to July 2019 in children with sepsis

aged 2-18 years old treated at Dr. Kariadi Hospital, Semarang. Subject of this study is 31 children who fulfill the research criteria with samples were obtained in a consecutive manner. The inclusion criteria for the study including children aged ≥ 2 years until ≤ 18 years old which suffer from sepsis and treated in Dr. Karadi Hospital Semarang for ≥ 5 days since diagnosed with sepsis. The diagnosis of sepsis was made according to sepsis criteria by American College of Chest Physicians (ACCP) and Society of Critical Care Medicine (SCCM) 2001. The exclusion criteria are including children whom was given long-term anti inflammation medication (corticosteroid, non-steroidal anti-inflammation drugs), has an underlying chronic systemic inflammation disease, has either a primary or acquired immunodeficiency disease, major congenital anomaly, asplenia, and parents that refuse to include their children in the study. Informed consent was obtained from the parents or family of subjects regarding the research. All subjects admitted to Dr. Kariadi Hospital in 24 hour period (H_0), day 5 of treatment (H_5) and when discharged from emergency treatment unit (H-end) either with improved or deteriorating condition underwent serum sample collection to measure the lactic acid, IL-10, and IL-6 level. Assessment with PELOD-2 score was also being done in the same day.

A total of 5 cc of venous blood were obtained as part of the study. It was then centrifuged at a speed of 3000 rpm for 15 minutes, then the serum formed was separated and stored in freezer at -20°C temperature. S100B test was measured with ELISA. Statistical analysis was used to compare IL-6, IL-10 level and IL-6/IL-10 ratio at H_0 , H_5 , and end-observation in children with sepsis with deceased or alive outcome. The difference was considered significant if $p < 0,05$. Analysis was done to investigate the correlation between IL-6, IL-10 level and IL-6/IL-10 ration with PELOD-2 score at H_0 , H_5 and end-observation. The correlation between the changes (delta) in IL-6 level, IL-10 level, and IL-6/IL-10 ratio with changes in PELOD-2 score which occurred in time range of H_0 - H_5 , H_5 – end-observation and H_0 – end-observation were also analyzed using Pearson test

if data distribution were normal or Spearman test if not. An ROC curve analysis was done to determine cut-off value of IL-6, IL-10 level, and IL-6/IL-10 ration which showed PELOD-2 score outcome ≥ 11 . Same thing also applied on the delta of interleukin level. The cut-off value of interleukin level were considered as a predictor if AUC ≥ 0.8 were obtained. Data analysis was done by using SPSS for Windows v.15.0 software. An ethical clearance was obtained from Medical Research Ethics Committee, Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital, Semarang.

RESULTS

This study was conducted at Dr. Kariadi Hospital Semarang, started from February 2019 to July 2019 on 36 subjects which met the inclusion criteria. Five subjects were excluded because of being hospitalized less than 5 days (2 subjects), diagnosed with SLE

and have given long-term corticosteroid (2 subjects), and diagnosed with malignancy (1 subjects), hence a total of 31 subjects participated in this study. The characteristic of research subject presented in table 1. PELOD-2 score outcome ≥ 11 or PELOD-2 score < 11 were not significantly different according to age, gender, nutritional status, and underlying disease, with p value $> 0,05$. There is, however, a significant difference regarding type of pathogen variable. Table 2 showed that the IL-6 level on H_5 , and H-end between decreased and living subjects were significantly different. The same result were also observed in IL-6/IL-10 ratio on H_5 , and end-observation between deceased and living subject. The interleukin level and ratio were significantly higher in the deceased subject compared to the living ones. IL-6 level between deceased and living subjects was insignificantly different on H_0 ($p < 0,05$) (Table 2).

There is a positive correlation between IL-6 level ($r = 0.568$; $p = 0.01$), and IL-6/IL-10 ratio

Table 1. Characteristics of Research Subjects

Variables	PELOD-2 Score Outcome on First 24-Hour of Sepsis Diagnosis			p
	All subjects	PELOD-2 Score ≥ 11 (n=10)	PELOD-2 Score < 11 (n=21)	
Age (year),				
median (min-max)	4 (2-16)	3 (2 – 10)	4 (2 – 16)	0,175 [¥]
Gender, n (%)				
Male	13 (42)	3 (30)	10 (48)	0,297 [€]
Female	18 (58)	7 (70)	11 (52)	
Nutritional status, n (%)				
Malnutrition	10 (32)	2 (20)	8 (38)	0,281 [€]
Non Malnutrition	21 (68)	8 (80)	13 (62)	
Operative procedure, n (%)				
Surgical	6 (19)	2 (20)	4 (19)	0,650 [€]
Non surgical	25 (81)	8 (80)	17 (81)	
Type of Pathogen, n (%)				
Gram negative	18 (58)	9 (90)	9 (43)	0,020 ^{*€}
Gram positive	13 (42)	1 (10)	12 (57)	

[¥] Man Whitney U; [€] Fisher; ^{*} Significant

Table 2. The Difference in Interleukin Level with Outcome (Deceased/Lived)

Variables	Research subject final outcome		
	Deceased (n=9)	Lived (n=22)	<i>p</i>
IL-6 level (pg/ml)			
H ₀	16.00 (2.20 – 24.30)	20,65 (3.30 – 25,30)	0.184¥
H ₅	20.90 (1.40 – 26.00)	15.80 (1.90 – 27.00)	0.047*¥
H-end	20.57 (14.13 – 27.01)	10.51 (4.26 - 16.76)	<0.001* x
IL-10 level (pg/ml)			
H ₀	10.65 (5.32 – 15.98)	15.17 (10.15 – 20.19)	0.033* x
H ₅	15.10 (7.40 – 19.20)	15.90 (6.50 – 21.10)	0.489¥
H-end	14.20 (6.78 – 16.80)	14.20 (5.40 – 27.10)	0.499¥
IL-6/IL-10 ratio			
H ₀	1.84 (0.98 – 2.70)	1.29 (0.79 – 1.79)	<0.001* x
H ₅	1.55 (0.76 – 2.34)	1.03 (0.24 – 1.82)	0.014**
H-end	1.55 (0.46 – 2.77)	0.82 (0.21 – 1.56)	<0.001*¥
PELOD-2 Score,			
H ₀	9 (7-11)	10 (8-12)	0.127 x
H ₅	11 (7-13)	7 (6-11)	0.003**
H-end	17 (9-20)	2 (2-6)	<0.001*¥

($r = 0.528$; $p = 0.002$) with PELOD-2 score on H-end. The delta of IL-6 level and PELOD-2 score at the first 24-hour period to end observation were also positively correlated ($r = 0.627$; $p = < 0.001$).

ROC analysis of IL-6 level, IL-10 level and IL-6/IL-10 ratio as predictor of multiorgan failure assessed with PELOD-2 score in each measurement time (H₀, H₅, and H-end) on septic subjects are presented in table 3. The IL-6, IL-10 levels and IL-6/IL-10 ratio measurements on the first 24-hour of sepsis diagnosis could not be used as an indicator for multiorgan failure outcome using PELOD-2 score for children with sepsis. Analysis on H₅ showed IL-6/IL-10 ratio AUC with cut-off 1.35 was 0.78 ± 0.1 ($p = 0.019$) whereas IL-6 level AUC with cut-off 17.15 obtained at end observation was 0.85 ± 0.80 ($p = 0.004$) and IL-6/IL-10 ratio AUC with cut-off 1.23 was 0.89 ± 0.08 ($p = 0.01$).

Fisher exact test analysis was being done to

determine the odd risk (OR) value. Odd risk (OR) of subject group with IL-6/IL-10 ratio for severe multiorgan failure (PELOD-2 score ≥ 11) was 6.86; CI 95% = 1,10-42,75 with p value = 0,037 on H% whereas on last day observation OR of subjects with IL-6/IL-10 ratio ≥ 1.23 for severe multiorgan failure was 33.25; CI 95% = 3,15 – 350,70 with $p=0,001$ and IL-6 ≥ 17.15 pg/mL has 8.5 risk; CI 95% = 1,34 – 54,13 with $p=0,022$ (Table 3, picture 1). However, based on logistic regression multivariate analysis, IL-6/IL-10 ratio ≥ 1.23 at end observation was the only independent variable that significantly correlated with the occurrence of multiorgan failure based on multivariate analysis multivariat (RR = 33.25; 95% CI 3.151 – 350.90; $p = 0.004$) whereas IL-6/IL10 ratio on treatment day-5 after sepsis diagnosis and IL-6 level ≥ 17.15 pg/ml at end observation were variables associated with multiorgan failure but not significantly correlated.

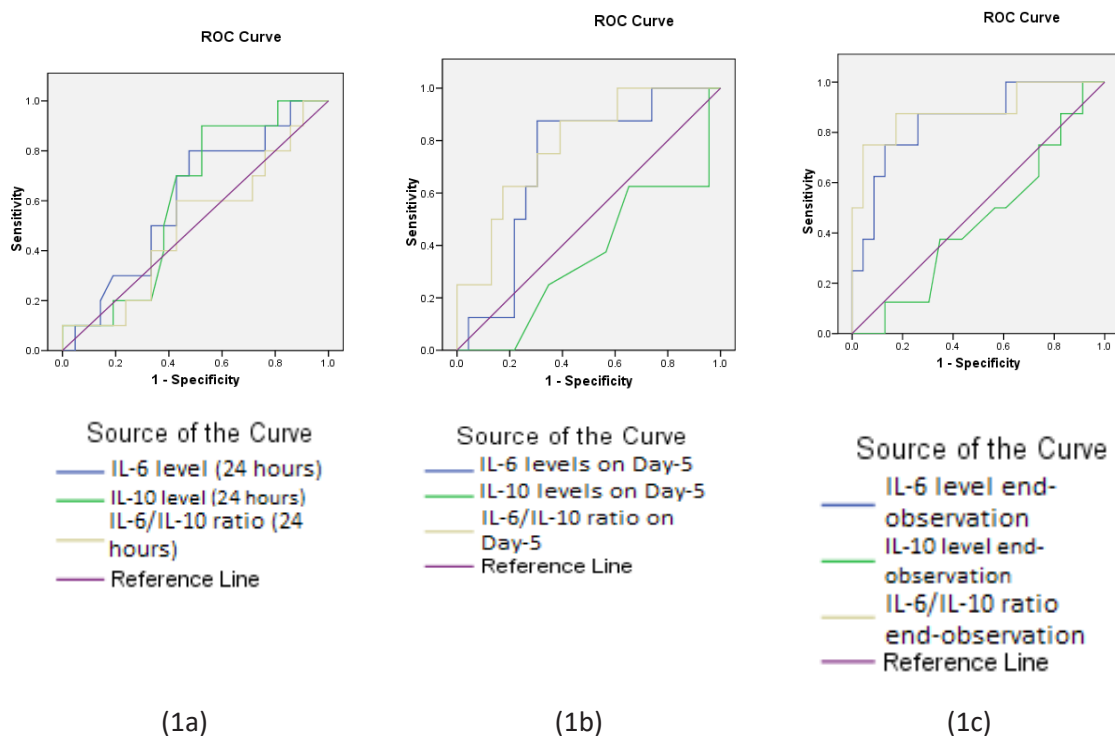
Table 3. Interleukin performance in predicting PELOD-2 score at certain times

Variable	Cut off Value	AUC	<i>p</i> value	Sensitivity (%)	Specificity (%)
First 24-hour measurement (H_0)					
IL-6	20.045	0.60 ± 0.11	0.364	60	57
IL-10	13.8	0.61 ± 0.10	0.353	70	57
IL6 / IL-10 ration	1.42	0.50 ± 0.11	1	60	57
Day 5 measurement (H_5)					
IL-6	18.9	0.71 ± 0.10	0.078	87.5	70
IL-10	15.5	0.36 ± 0.12	0.250	37.5	43.5
IL6 / IL-10 ratio	1.35	0.78 ± 0.10	0.019	75	70*
End observation measurement (H-end)					
IL-6	17.15	0.85 ± 0.80	0.004	75	74*
IL-10	13.9	0.45 ± 0.12	0.652	50	43.5
IL6 / IL-10 ration	1.23	0.89 ± 0.08	0.001	87.5	82*

*bermakna

The delta of IL-6 level with cut-off value 1.05 pg/ml on H_0 - H_5 period, AUC= 0.72 ± 0.10 ($p = 0.05$),

and delta of IL-6 level with cut-off value 1.25 pg/ml (AUC = 0.97 ± 0.03 ; $p < 0.001$) on H_0 -end period



Picture 1. The receiver operating characteristic (ROC) difference for IL-6 level, IL-10 level and IL-6/IL-10 ratio at H_0 (1a), H_5 (1b) and end observation (1c)

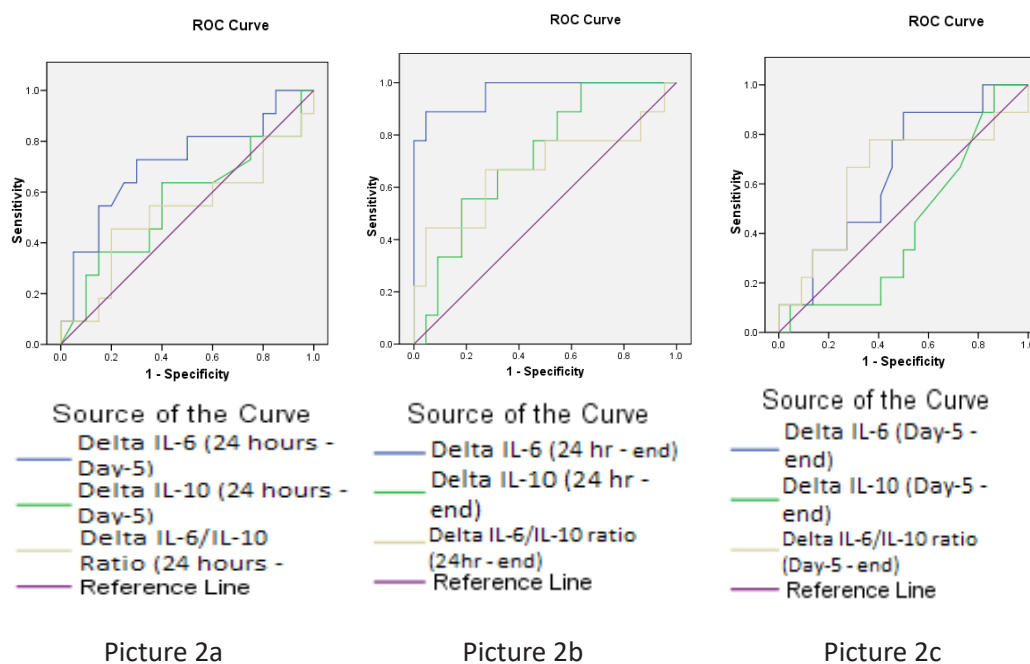
Table 4. The delta of interleukin level at measurement time period on predicting changes in PELOD-2 score

Variable	Cut off Value	AUC (mean $\pm$ SE)	P value	Sensitivity (%)	Specificity (%)
Changes in first 24-hour to day 5					
Delta IL-6	1.05	0.72 $\pm$ 0.10	0.05	72	70*
Delta IL-10	0.66	0.56 $\pm$ 0.11	0.591	63.6	60
Delta IL6 / IL-10 Ratio	- 0.47	0.52 $\pm$ 0.12	0.836	63.6	35
Changes in first 24-hor to end observation					
Delta IL-6	1.250	0.97 $\pm$ 0.03	0.000	89	90.9*
Delta IL-10	1.875	0.72 $\pm$ 0.10	0.061	66.7	68.2
Delta IL6 / IL-10 Ratio	- 0.274	0.67 $\pm$ 0.12	0.139	66.7	68.2
Changes from day 5 to end observation					
Delta IL-6	- 1.935	0.65 $\pm$ 0.10	0.199	55.5	59.1
Delta IL-10	- 1.610	0.42 $\pm$ 0.11	0.514	44.4	45.4
Delta IL6 / IL-10 Ratio	- 0.085	0.64 $\pm$ 0.12	0.240	66.7	68.2

* Significant

may be used as indicator of progressing multiorgan failure (increased PELOD-2 score) of 3.25; CI 95% = 0,70 – 15,05 with $p=0,124$ on H_0-H_5 period whereas

OR of delta IL-6 ≥ 1.25 pg/ml group for progressing multiorgan failure was 80; CI 95% = 6,33 – 101,95 with $p<0,001$. The delta of IL-6 $\geq 1,05$ pg/ml was



Picture 2. ROC curve of delta IL-6 level, IL-10 level and IL-6/IL-10 ratio on range time of H0 - H5 (2a), H0 - H end (2b) dan H5 - H end (2c)

significant on multivariate analysis (RR 1.27; CI 95% 1.212 – 31.937; $p = 0.028$) (Table 4, picture 2).

There is a significant difference between IL-6 level ≥ 20.045 pg/ml with IL-6 level < 20.045 pg/ml in regards of type of infectious pathogen ($p < 0.05$) in first 24-hour. This indicated that type of pathogen affects IL-6 level outcome in the first 24-hour period. There was no significant difference between IL-6, IL-10 levels and interleukin ratio $\geq$ cut-off value with $<$ cut-off value on day-5 and end observation in regards to type of pathogen.

DISCUSSION

Sepsis is a complex syndrome as a result of simultaneous pro-inflammation and anti-inflammation cytokines expression. Sepsis immunopathogenesis begin with inflammation response, which triggered by massive release of pro-inflammation cytokines, including interferon (IFN)- γ , IL-6, IL-8 and IL-12. This event then followed by anti-inflammation response, by secreting immunosuppressing mediator, monocyte deactivation, anergy of T lymphocyte cells and immune cells apoptosis. Inflammation response which occurred lead to organ dysfunction and shock, whereas the excessive anti-inflammation process may increased the susceptibility to secondary infection.^{11,12} The undesirable effect of sepsis such as systemic inflammatory response syndrome (SIRS), disseminated intravascular coagulation (DIC), septic shock, syndrome caused by response to complement pathway activation and multiorgan failure is a condition associated with imbalance in pro-inflammatory cytokines mediator production, as well as excessive anti-inflammatory synthesis.¹²

Our study provides an overview that IL-6 and PELOD-2 score measurement in the first 24-hour of sepsis diagnosis was unable to predict the final outcome, whereas IL-6 measurement on day 5 could differentiate subject with possible poor outcome. These results were supported by the theory that IL-6 level could vary where its peak level may be obtained on the first 4 hour of sepsis and then fluctuates with various level. Persisten increased in IL-6 level was

associated with multiple organ system failure and mortality.^{13,14}

IL-10 measurement in our study showed a significant difference between the deceased and living subject ($p = 0.033$) on H0 period, however the difference of IL-10 level at H5 and end observation were not significant.

A study by Wu et al (2008) on 76 patients with severe sepsis whose IL-6, IL-10, IL-12 and TGF- β 1 level were measured on day 1 until day 7 showed similar result that IL-10 level in non-survivor was significantly higher on day 1 until day 3, but not on measurement day 4 until day 7.¹⁵

There were no significant correlation between IL-6 level at H0 ($r = 0.234$; $p = 0.204$) and H5 ($r = 0.030$; $p = 0.873$) for severe multiorgan failure which was assessed using PELOD-2 score and it could not be used as predictor for multiorgan failure. On the day 5, a decrease on IL-6 level occurred on average of our subject study, therefore IL-6 level on that measurement should not be a good predictor to represent progressing multiorgan failure. An IL-6 level ≥ 17.15 pg/ml may be used as multiorgan failure predictor at the end observation (AUC 0.85, $p = 0.004$), however this result was not significant on multivariate analysis. This result further emphasize the need of IL-6 and IL-10 measurement simultaneously and to calculate interleukin-6 to interleukin-10 ratio to detect severe multiorgan failure on day 5, where on our study IL-6/IL-10 ratio with cut-off value ≥ 1.35 on day 5 could represent multiorgan failure (AUC 0.78, $p = 0.019$).

A study by Bozza et al (2007) which stated IL-6 concentration besides IL-8 and G-SF have positive correlation with organ dysfunction which measured using SOFA score system and could predict organ dysfunction that get worse on day 3. In the study it was also stated that there are not a single biomarker that could 100% accurately predict the outcome of septic patient, thus using a combination of biomarker and scoring system was suggested to obtain a better assessment result.⁵

IL-10 level measurement alone could not represent a significant correlation between multiorgan

failure which assessed using PELOD-2 score on each measurement time H0, H5 and end observation. This study results further support the hypothesis that there is a role of cytokine autoregulation which induced by the same inflammation stimulus. IL-10 as anti-inflammatory mediator is produced by a few types of immune cells including T-helper cell (T2), B cells, macrophage, monocytes and keratinocytes to suppress production of pro-inflammatory mediator and down-regulate co-stimulator molecules in T cells activation. The IL-10 response were at the same level as IL-6 on the first day of sepsis then IL-10 response is not too apparent on the next day on septic patients. A supra-physiologic dose of IL-10 played a role as protection to mortality and production of pro-inflammatory cytokines. However, over-production of IL-10 could resulted in immune system failure (immunoparalysis) causing inability to eradicate infection thus causing progression of severe sepsis disease. This hypothesis also supported by other studies which showed that an anti-inflammation strategy could further worsen the outcome.^{15,16,17}

The difference in IL-6/IL-10 ratio at 24-hour, day 5, and end observation measurement between the deceased subject and the living ones were statistically significant. When correlated with multiorgan failure assessed using PELOD-2 score, the IL-6/IL-10 ratio showed a positive correlation that statistically significant on measurement day 5 with cut-off value 1,35 (AUC 0,78; $p = 0.019$), and on end observation with cut off value 1,23 (AUC 0,89; $p = 0.001$), making it useful as multiorgan failure indicator which assessed by PELOD-2 score, rather than IL-6 and IL-10 measurement that being done separately. The positive correlation indicated that with higher IL-6/IL-10 ratio, along with higher PELOD-2 score thus showed a more progressing multiorgan failure caused by sepsis. The risk of developing multiorgan failure was 6,86 times higher in subject with IL-6/IL-10 ratio value $\geq 1,35$ on day 5 ($p = 0.037$). however this result were not statistically significant on multivariate analysis ($p = 0.379$). on end observation, the risk for developing severe multiorgan failure was 33,25 times higher in subject with IL-6/IL-10 ratio $\geq 1,23$

($p = 0,001$), with significant multivariate analysis ($p = 0.001$).

A similar study conducted by Simpson et al (2000) founded that log IL-6/IL-10 transformation was significantly correlated with mortality in septic patients, where a higher IL-6/IL-10 ratio was significantly correlated on univariate analysis and increase risk of mortality by 3,46 times (95% CI 1.73 – 6.93; $p < 0.001$), whereas on multivariate analysis it was not significant as an independent factor risk. Simpson also stated that IL-6/IL-10 ratio may represent mortality risk better than measuring variables in separate manner. This study by Simpson was different from our study in regards to study subject which were adult subjects with sepsis caused by acute myeloidosis.¹⁸

In this study we found that IL-6 delta ≥ 1.05 pg/ml in H0-H5 time period may be used as indicator of progressing multiorgan failure (increasing PELOD-2 score) (AUC 0.72, $p = 0.05$) as well as IL-6 delta ≥ 1.25 pg/ml on H0 until end observation time period also may be used as indicator of progressing severe multiorgan failure (AUC 0.97, $p < 0.01$) (Table 15 and table 16). The risk of developing severe multiorgan failure was 1,27 times higher in subject with IL-6 level delta $\geq 1,05$ on H0-H5 time period H5 (95% CI 1.21 – 31.94.75; $p = 0.028$), and 80 times higher in subject with IL-6 level delta $\geq 1,25$ on H0 until end observation time period. We could also state that an IL-6 level that remain high on day 5 after diagnosis of sepsis have poorer outcome prognosis for progressing severe multiorgan failure, which could be seen with PELOD-2 score outcome.¹⁹

This study results also showed that interleukin 10 measurement could not be used as predictor for multiorgan failure severity. A high IL-10 level in the first 240-hour, however, could differentiate between patients with fatal and living outcome which relevant with the theory that excessive anti-inflammatory or cytokins pro-inflammation response could impair the host, which then developed into respiratory distress syndrome, shock, multiorgan failure, or death. This setting prevented the clearance of infectious agents, known as *compensatory anti-inflammatory response*

syndrome (CARS).^{17,20,21}

The correlation between IL-6/IL-10 delta level with increasing severity of multiorgan failure which measured with PELOD-2 score delta was not obtained in this study. Measurements on H_0-H_5 , H_0-H_{end} , and H_5-H_{end} were not significantly correlated.

The type of pathogen causing sepsis (gram negative or gram positive bacteria) is a confounding factor in our study. Gram negative bacteria have lipopolysaccharides (LPS), whereas gram positive bacteria have lipoteichoic acid (LTA) as an antigen which induce inflammation in infection. Gram negative bacteria cell wall contained endotoxin which is not present on almost all Gram positive bacteria. Endotoxin could stimulate IL-6 release and induced a faster increase of CRP and procalcitonin. The nature of infecting pathogen influenced the host reaction mechanism and the response to therapy. Patients with Gram negative bacteria infection are more likely to develop septic shock than patients with Gram positive bacteria infection and have a higher mortality risk. This makes early identification of causative organism a critical matter as a consideration for a more effective and narrow spectrum of antibiotic usage.^{21,22,23}

There are studies which suggested that the TNF- α level was two-times higher on a group with gram negative bacteria infection, and 1.7 times higher on mixed infection compared to the ones infected by gram positive bacteria. IFN- γ level was 13-times higher in gram negative bacteria-infected group and 8,6 times higher in mixed infection, IL-1 α level was 2,1 times higher in mixed infection, IL-10 level was 1.83 times higher compared to gram negative bacteria infection whereas in mixed infection was 1,75 times higher than in gram positive bacteria infection. In our study, a gram negative bacterial infection showed the risk of having IL-6 level outcome ≥ 20.045 pg/ml was 42-times higher compared to gram positive bacterial infection on the first 24-hour.^{20,21}

The limitation of this study was that the results of blood, sputum, urine or CSF culture as a reference for sepsis diagnosis were not all obtained on the first 24-hour. There were 6 subjects with culture results obtained more than first 24-hours after sepsis

diagnosis. Despite the author efforts to obtain a blood culture sample in 24-hours period, some showed a sterile results. Furthermore, end observation point in this study was different for each subject which could be affected with possible nosocomial infection.

From this study we can concluded that IL-6 level at H_5 , and end observation, IL-10 at H_0 , and IL-6/IL-10 ratio at H_0 , H_5 and end observation could differentiate the outcome between deceased subjects with the living ones. IL-10 level should not be used as multiorgan failure predictor marked by PELOD-2 score increase ≥ 11 at H_0 , H_5 or H_{end} observation. And IL-6/IL-10 ratio $\geq 1,35$ at H_5 could be used as multiorgan failure predictor assessed using PELOD-2 score, but not statistically significant whereas IL-6/IL-10 ratio at end observation was statistically significant. The risk for developing severe multiorgan failure in PELOD-2 score ≥ 11 was 1,27 times higher in IL-6 level $\geq 17,15$ pg/ml and 33,25 times higher in IL-6/IL-10 ratio $\geq 1,23$ at end observation time.

This study suggested (1) the need for measuring IL-6/IL-10 ratio on treatment day-5 as an outcome predictor in children with sepsis, (2) antibiotic treatment on Gram negative bacterial infection should be continuous and more aggressive, using a narrow spectrum based on culture results as Gram negative bacterial infection have a higher risk for increased IL-6, IL-10 levels as well as increased PELOD-2 score ≥ 11 higher than Gram positive bacterial infection and (3) further studies comparing IL-6, IL-10 levels and IL-6/IL-10 ratio with clinical manifestation in septic patients are warranted to improve clinical skill in regards to future patients prognosis.

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