Presepsin Levels in Positive and Negative Blood Cultures of Febrile Neutropenic Pediatric Patients with Malignancies in Dr. Soetomo General Hospital, Surabaya, Indonesia

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ABSTRACT:
This was observational research with cross sectional type of study conducted from January to October 2020 at Dr. Soetomo General Hospital, Surabaya. The subjects of the research were 30 children's patients with febrile neutropenic episodes with malignancy based on the criteria of the American Society of Clinical Oncology and Infectious Disease Society of America, which were grouped into 2 groups based on blood culture results from secondary data. The control group was hemophilia patients without any complaint. The presepsin level was checked using ELISA. The differences in presepsin levels in positive and negative blood cultures and the control were analyzed using T2 free samples or Mann-Whitney U according to the data distribution. Median (min-max) presepsin levels in positive, negative and control cultures: 4.1(0.3 - 14) ng/mL, 2.95(0.2 - 13.8) ng/mL, 0.1(0.1 - 0.5)ng/mL. There is no significant difference in presepsin levels between positive and negative blood culture ($p = 0.606$). There is a significant difference between positive and negative culture presepsin levels towards controls ($p = 0.001$ and $p = <0.001$). There is an increase in the level of presepsin in febrile neutropenic pediatric patients with malignancy, but this examination cannot differentiate between bacteremia and non-bacteremia infections.

KEYWORDS: Presepsin, positive blood culture, negative blood culture.

INTRODUCTION:

Febrile neutropenia is the most common complication in children with malignancy that can be caused by chemotherapy treatment or due to an underlying disease[1]. The American Society of Clinical Oncology and the Infectious Disease Society of America (ASCO/IDSA) and the European Society for Medical Oncology (ESMO) define febrile neutropenia as a body temperature 38.3°C at one oral temperature measurement or a body temperature obtained 38.0°C

about one hour with absolute neutrophil count (ANC) < 500 cells/µL or a decrease in ANC to 500 cells/µL up to 48 hours[2,3].

The most important factor in the incidence of infection in malignancy is the state of neutropenia. The more severe and prolonged the state of neutropenia, the easier and more severe the infection that occurs. The state of neutropenia can increase the risk of infectious complications because the patient's body has a decreased ability to fight infection[3,4]. Febrile neutropenia has the potential to develop into a life-threatening condition with a mortality rate of 5% in patients with solid tumors to 10% in hematological malignancies[1].

Early diagnosis of microbial infection in a patient with febrile neutropenia is very difficult. Fever may be the only indicator of microbial infection in a neutropenic patient with malignancy. Patients with neutropenia following febrile neutropenia might be reviewed to be at high risk for severe infection, where almost all treatment guidelines recommend broad-spectrum antibiotics in this condition, but other causes of fever should be carefully evaluated

to avoid unnecessary use of antibiotics resulting in increased incidence of antibiotic resistance[2,4,5]. Although blood culture is the gold standard for the diagnosis of bacterial infection, the results are long and the incidence of bacteremia in febrile neutropenia is low, around 10–30%[1,6].

Since 2011, Soluble CD14 subtype (sCD14-ST) also known as presepsin has been reported as a new and promising biomarker for the diagnosis of bacteremia. Presepsin is a protein that is expressed on the surface of macrophages, dendritic cells, and neutrophils. CD14 is phagocytosed and cleaved together with pathogens during the infectious process. Presepsin levels increased as early as possible even earlier than procalcitonin (PCT) and C-reactive protein (CRP), ie within 2 hours of the onset of inflammation with peak concentrations after 3 hours. These characteristics make presepsin the fastest biomarker for sepsis in relation to PCT and CRP, which have activation times of 6-12 hours and 12-24 hours[7,8]. Several studies have reported elevated levels of presepsin in febrile neutropenia patients with bacterial infections. The aim of this research was to analyze differences in presepsin levels in positive and negative blood cultures of febrile neutropenic pediatric patients with malignancy.

MATERIALS AND METHODS:

This type of research is observational with a cross sectional conducted from January-October 2020 at Dr Soetomo Hospital Surabaya. This study used a population that was divided into two groups, the patient group and the control group. The patient group was a population of children with malignancy who had episodes of febrile neutropenia based on the criteria of the American Society of Clinical Oncology and the Infectious Disease Society of America. The inclusion criteria were patients with malignancy who met the criteria for a febrile neutropenia episode, aged 1-16 years. With the exclusion criteria: patients who were not examined for blood culture and insufficient samples collected for presepsin examination. There were 30 research subjects grouped into two groups based on the culture results from secondary data. While the control group is hemophilia patients without complaints.

The specimens of this study were peripheral blood samples of pediatric patients with malignancy who had episodes of febrile neutropenia in Ethylenediamine Tetraacetic Acid (EDTA) tubes for presepsin examination using the Presepsin ELISA Reagent Kit from MyBioSource. This tool uses the principle of Sandwich Enzyme Linked Immune-sorbent Assay (ELISA). Anti-presepsin antibodies were previously attached to the solid phase. Furthermore, antipresensin which has been conjugated with biotin is added to detect antibodies. The standard or sample to be examined and antipresensin which has been conjugated with biotin were added to the well and washed with wash buffer. HRP-streptavidin was then added to the well and the unbound conjugate was washed with wash buffer. Tetramethylbenzidine (TMB) substrate was used to see the enzymatic reaction of HRP. TMB catalyzed by HRP produces a blue color which turns yellow after adding an acidic stop solution. The density of the yellow color is proportional to the amount of presepsin captured in the well. Further reading of O.D. absorbance at a wavelength of 450 nm in a microplate reader to see the concentration of presepsin.

Blood culture was not done specifically in this study considering that the culture examination was carried out on all febrile neutropenia patients according to the Standard Operating Procedure applicable in the Pediatric Inpatient Dr. Soetomo Hospital Surabaya, so that the culture results were obtained from secondary data. The same procedure was carried out in the control group except for examination of blood cultures and complete blood counts. Statistical analysis for differences in presepsin levels in positive and negative blood cultures as well as against controls were analyzed by free sample T2 or Mann-Whitney U according to data distribution.

RESULTS AND DISCUSSION:

The patient group consisted of 30 episodes of febrile neutropenia with both positive and negative culture results, consisting of 12 male patients and 18 female patients with various malignancies. The control group consisted of 10 people consisting of 10 boys who were pediatric hemophilia patients without complaints. The diagnosis of malignancy in the patient group was dominated by hematologic malignancies as many as 29 (96.66%) and the remaining 1 (3.34%) non-hematological. Hematological malignancies were generally 21 (72.4%) with ALL and 8 (27.6%) AML, while the non-haematological diagnosis was neuroblastoma. The results of blood culture examination of all episodes of febrile neutropenia were taken from secondary data and obtained 8 positive blood cultures and 22 negative blood cultures. The data characteristic of the study sample including gender, diagnosis of malignancy, age, neutrophil count (ANC), temperature of the positive, negative and control blood culture groups are shown in Table 1.

The number of positive blood cultures obtained from 30 samples of febrile neutropenia episodes as many as 8 (26.66%) samples, with the number of Gram-positive bacteria as many as 3(37.5%) samples and Gram-negative bacteria as many as 5(62.5%) samples and the number of negative blood cultures were 22(73.33%) samples. Data on the types of bacteria found in positive blood culture samples can be seen in Table 2.

Table 1. Characteristics of Subjects.

Characteristics	Group		
	Positive Culture (n = 8)	**Negative Culture (n = 22)**	**Control (n = 10)**
Age (year)			
Mean ± Standard Deviation	7.5±3.928	7.41±3.899	11.20±3.084
Median (min – max)	6 (4–16)	6.5 (1–14)	11 (7–16)
Gender [n(%)]			
Man	3 (37.5%)	9 (40.9%)	10 (100%)
Woman	5 (62.5%)	13 (59.1%)	0 (0%)
Diagnosis [n(%)]			
ALL	6 (75%)	15 (68.2%)	0 (0%)
AML	2 (25%)	6 (27.3%)	0 (0%)
Neuroblastoma	0 (0%)	1 (4.5%)	0 (0%)
Hemophilia	0 (0%)	0 (0%)	10 (100%)
ANC (cell/µL)			
Mean ± Standard Deviation	157.5±132,315	240.91±119.957	
Median (min – max)	120 (10–400)	220 (90–480)	
Temp (°C)			
Mean ± Standard Deviation	38.325±0.403	38.26±0.353	36.19±0.238
Median (min – max)	38.15 (38–39)	38.3 (38–39.5)	36.1 (36–36.7)

Table 2. Positive Blood Culture Results in 8 Samples of Febrile Neutropenia Episodes.

Gram (+)	Number (%)	Gram (-)	Number (%)
Staphylococcus hominis	2 (25%)	*Enterobacter cloacae*	1 (12.5%)
Streptococcus agalactiae	1 (12.5%)	*Salmonella species*	1 (12.5%)
		*Escherichia coli**	1 (12.5%)
		Klebsiella pneumonia	1 (12.5%)
		Morganella morganii	1 (12.5%)

The results of the Mann-Whitney U test showed that there was no significant difference in presepsin levels in the group of febrile neutropenia episodes with positive and negative blood cultures ($p > 0.05$). The median values are 4.1 ng/mL and 2.95 ng/mL, respectively. The data is shown with the median, because the data are not normally distributed (Table 3).

Table 3. Different Presepsin Levels in Positive and Negative Blood Culture Groups.

Group	n	Median (min – max) (ng/mL)	p value
Positive Culture	8	4.1 (0.3 – 14)	0.606
Negative Culture	22	2.95 (0.2 – 13.8)	

The results of the Mann-Whitney U test showed that there was a significant difference in presepsin levels in the positive and negative blood culture group against the control group ($p < 0.05$). The medians are 4.1ng/mL, 2.95ng/mL and 0.1ng/mL, respectively. The data is shown with the median, because the data are not normally distributed (Table 4).

Tabel 4. Results of Different Presepsin Levels in Positive and Negative Blood Culture Groups against Control.

Group	n	Median (min – max) (ng/mL)	Nilai p
Positive Culture	8	4.1 (0.3–14)	0.001
Control	10	0.1 (0.1–0.5)	
Kultur Negatif	22	2.95 (0.2–13.8)	<0.001
Control	10	0.1 (0.1–0.5)	

This study obtained a diagnosis of malignancy in the group of patients dominated by hematological malignancies as many as 29(96.66%). Hematologic malignancies generally constituted ALL as many as 21 (72.4%). The results of this study are identical to those of Baraka and Zakaria., 2018 where in this study the majority of patients diagnosed with febrile neutropenia were diagnosed with ALL[1]. ALL is the most common type of hematological malignancy of the leukemia group in children, accounting for about 25% of all childhood cancers and 80% of all leukemias, followed by AML and acute monocytic leukemia (AMoL)[9].

A number of studies have shown that only 10-30% of patients have proven incidence of bacteremia[1,6]. This study also found a low number of positive blood cultures, as many as 8 samples (26.66%) out of a total of 30 episodes of febrile neutropenia. This result is because many samples in this study have received prophylactic antibiotic therapy. The results of blood cultures during antibiotic therapy were associated with a significant loss of pathogen detection, so it is highly suggested to obtain blood cultures before administration of antibiotics[10], but in fact around 28-63% of patients have received therapy. antibiotics when blood cultures are performed, especially for those with severe clinical conditions[11].

In this study, there was no significant difference in presepsin levels in the positive and negative group of patients with episodes of febrile neutropenia with a p value of 0.606 ($p>0.05$), although a higher median value of presepsin was found in the positive blood culture group of 4.1 (0.3–14) ng/mL than negative blood culture median value 2.95(0.2–13.8)ng/mL. An increase in presepsin levels in positive blood cultures (1.579ng/mL (0.243–20ng/mL) higher than in negative blood cultures (1.168ng/mL (0.187–16.764ng/mL), but there was no difference in presepsin levels which was significant in both groups ($p >0.05$)[12]. Febrile neutropenia is the most common complication found in children with malignancy that can be caused by chemotherapy treatment because this treatment will cause myelosuppression. Meanwhile, presepsin is the most common found on the surface of human monocytes.Low levels of leukocytes, including monocytes, can cause low levels of presepsin due to the low source of presepsin production itself[13].

The results of this study are different from the results of the study by Baraka and Zakaria, 2018 which found a significant difference ($p<0.05$) in presepsin levels in positive blood cultures with a mean value of 2.7208± 1.1514ng/mL and in negative blood cultures with a mean 1.411±0.646ng/mL, so it can be used as a differentiator for the cause of fever in hematological malignancies due to infectious agents or not due to infection[1]. The difference in the results of this study may be due to the high number of cases of local infection in the negative culture group in this study. In this study 14 patients (63%) with episodes of febrile neutropenia in the culture negative group had local infection. The most common local infections in this study were mucositis in 5 patients (27%), pneumonia in 4 patients (21%), unspecified hepatitis in 3 patients (16%), cellulitis in 2 patients (11%), stomatitis in 1 patient (5%), candidiasis 1 patient (5%), urinary tract infection 1 patient (5%), cholecystitis 1 patient (5%), sinusitis 1 patient (5%).

This study has several limitations, including: Uniformity of length of illness before patients come to RSUD Dr. Soetomo, Surabaya varies, patients often receive prophylactic antibiotics, no evaluation of other types of infection other than bacteria is carried out. The limitations of this study may allow for considerable bias[14,15,16,17,18,19,20,21].

CONCLUSION:

In summary, there is an increase in the level of presepsin in febrile neutropenic pediatric patients with malignancy, but this examination cannot differentiate between bacteremia and non-bacteremia infections.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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