C-Reactive Protein as A Fungal Infection Marker in Acute Leukemia Patients

Brigitte Rina Aninda Sidharta, JB. Suparyatmo, Avanti Fitri Astuti

Department of Clinical Pathology, Faculty of Medicine, Sebelas Maret University/Dr. Moewardi Hospital, Surakarta, Indonesia. E-mail: rinaaninda@yahoo.com

ABSTRACT

Invasive Fungal Infections (IFIs) can cause serious problems in cancer patients and may result in high morbidity and mortality. C-reactive protein levels increase in response to injury, infection, and inflammation. C-reactive protein increases in bacterial infections (mean of 32 mg/L) and in fungal infections (mean of 9 mg/L). This study aimed to determine C-Reactive Protein (CRP) as a marker of fungal infections in patients with acute leukemia by establishing cut-off values of CRP. This study was an observational analytical study with a cross-sectional approach and was carried out at the Department of Clinical Pathology and Microbiology of Dr. Moewardi Hospital in Surakarta from May until August 2019. The inclusion criteria were patients with acute leukemia who were willing to participate in this study, while exclusion criteria were patients with liver disease. There were 61 samples consisting of 30 male and 31 female patients with ages ranging from 1 to 70 years. Fifty-four patients (88.5%) were diagnosed with Acute Lymphoblastic Leukemia (ALL) and 30 (49.18%) were in the maintenance phase. The risk factors found in those patients were neutropenia 50-1500 μL (23.8%), use of intravenous line (22%), and corticosteroid therapy for more than one week (20.9%). The median of CRP in the group of patients with positive culture results was 11.20 mg/L (11.20-26.23 mg/L) and negative culture results in 0.38 mg/L (0.01-18.63 mg/L). The cut-off value of CRP using the Receiver Operating Curve (ROC) was 9.54 mg/L (area under curve 0.996 and p. 0.026), with a sensitivity of 100%, specificity of 93.2%, Positive Predictive Value (PPV) of 33.3%, Negative Predictive Value (PPV) of 100%, Positive Likelihood Ratio (PLR) of 1.08, Negative Likelihood Ratio (NLR) of 0 and accuracy of 93.4%. C-reactive protein can be used as a screening marker for fungal infections in patients with acute leukemia.

Keywords: Leukemia, C-reactive protein

INTRODUCTION

Cancer remains a major cause of morbidity and mortality. There were 14 million new cases in 2012, and its mortality reached 8 million cases worldwide.[1,2] World Health Organization through the World Cancer Report 2014, estimate that there will be 25 million cancer case in 2025. In 2012 there were 352.000 new leukemia cases worldwide with 265.000 death at the same time.[3]

Epidemiology and laboratory data for Invasive Fungal Infections (IFIs) are important to improve antifungal therapy and reduced morbidity and mortality.[4] According to Gulhan *et al*. the frequency of probable IFI was 11.6% and possible IFI was 75% in children with hematology malignancy at Ankara Turkey Hospital.[5] A retrospective study by Yilmaz *et al*. on 240 patients with newly diagnosed acute leukemia showed that the probability of IFI in acute leukemia patients was 12.5%.[6] Aspergillosis and candidemia remain the most common causes of IFI in patients with acute leukemia. The most isolated

IFI-causing fungi were *Candida albicans*, non-*Candida albicans Candida* (NCAC), and *Aspergillus sp. Candida galbrata* is more frequent among NCAC causing IFI.[7] Tisi *et al*. demonstrated IFI incidence in his research was 3.2% (38 from 1191 patients) and invasive aspergillosis was the most frequent fungal infection. Pulmo was the most frequent organ involved in IFI.[8]

C-Reactive Protein (CRP) levels increase in response to injury, infection, and inflammation. C-reactive protein is a plasma protein originating from the liver and is part of the pentraxin family. C-reactive protein is the main mediator of inflammation synthesized in the liver in response to Interleukin-6 (IL-6) and is used as an early marker of opportunistic infection in patients.[9] The role of CRP in fungal infections remains to be elucidated. A study by Akin *et al*. showed that there were no significant differences in CRP levels in candidemia and bacteriemia patients and polymicrobial sepsis; however, there were significant differences in Procalcitonin (PCT) levels between candidemia and

bacteremia groups with p < 0.001.[10] C-reactive protein increased in bacterial infections (mean of 32 mg/L) and in fungal infections (mean of 9 mg/L).[11] Hazar and Tucker found that CRP level (60.5 mg/L) in fungal infection slightly lower than CRP level in Gram-negative (112 mg/L) and Gram-positive (184 mg/L) bacterial infection.[12]

This study aimed to determine CRP as a marker of fungal infections in patients with acute leukemia by establishing the cut-off value of CRP and determination of its sensitivity, specificity, PPV, NPV, Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), and accuracy.

METHODS

This study was an observational analytical study with a cross-sectional approach. The research was carried out at the Department of Clinical Pathology and Microbiology of the Dr. Moewardi Hospital in Surakarta. The study was performed from May 2019 to August 2019 in acute leukemia patients (adults and children) who fulfilled the inclusion and exclusion criteria and signed informed consent. Acute leukemia was diagnosed based on the results of routine blood tests, peripheral blood smears, and bone marrow smears. The exclusion criteria were history of liver disease according to anamnesis or medical records [characterized by an increase in alanine aminotransferase/ALT levels 3 times above the upper reference value (♂ > 135 IU/L, ♀ > 102 IU/L)].

A total of 11 mL venous blood was collected at the Department of Clinical Pathology of the Dr. Moewardi Hospital. Total 5 mL of blood was used for fungal culture examination, 3 mL of Ethylene Diamine Tetraacetic Acid (EDTA) blood was used for complete blood count and peripheral blood smear, while the remaining 3 mL of blood was used without anticoagulant for CRP test. A bone marrow sample was used to make a bone marrow smear. Variables in this study were acute leukemia patients, CRP, and fungal culture. C-reactive protein levels were measured using the immunoturbidimetric method using an Advia 1800 chemistry analyzer. Fungal culture from blood samples was carried out with a BacT alert analyzer and identified using Vitek mass spectrometry.

The characteristics of the research subjects were presented in a table, which contained mean and Standard Deviation (SD) if applicable. Normality test was performed using Kolmogorov-Smirnov test (p-value > 0.05). The cut-off was determined using Area Under Curve (AUC) derived from Receiving Operating Curve (ROC) and sensitivity, specificity,

PPV, NPV, accuracy, PLR, and NLR were then calculated. Statistical analysis was performed using SPSS version 22, p < 0.05.

This study had received approval from the Research Ethics Committee of Dr. Moewardi Hospital with number 810/VI/HREC/2019. Informed consent was obtained from the study subjects and patient identity was kept confidential.

RESULTS AND DISCUSSION

A total of 61 patients with acute leukemia participated in this study. Kolmogorov-Smirnov test was used to calculate normality and it was found that the data distribution for age parameters was not normal (median; minimum and maximum were used), while data distribution for the hemoglobin, leukocytes, platelets, absolute neutrophils, and absolute lymphocytes parameters were normal (mean±SD was used). The research subjects consisted of 49.2% males and 50.8% females with an age range of 1-70 years. Of 61 patients in this study, 44% were diagnosed with Acute Lymphoblastic Leukemia (ALL)-L2, while the most risk factors for fungal infections were the condition of neutropenia 500-1500 µL (23.8%), use of intravenous line (22%) and corticosteroid therapy for more than one week (20.9%). The most therapeutic phase was the maintenance phase.

The maintenance phase is the last phase of chemotherapy in ALL patients, in which the majority of drugs are given orally in this phase and lasts for 84 days. Hemoglobin and platelets of patients in this phase were normal, despite some low leukocyte yields (Table 1).

The cut-off value obtained for CRP in this study was 9.54 mg/L with AUC 0.966 (p=0.026; CI 0.0905-1,000) with a sensitivity of 100%, specificity of 93.2%, PPV of 33.3%, NPV of 100%, PLR of 1.08, NLR of 0 and accuracy of 93.4%.

A total of 61 leukemia patients of the Dr. Moewardi Hospital participated in this study, consisting of 49.2% male and 50.8% female patients. These results were different from a study by Li *et al.*, which showed a higher number of male patients (55.7%) compared to female patients (44.3%).[13] Based on GLOBOCAN data in 2018, the incidence rate of leukemia males was higher (4.0-7.5/100.000 with a mortality rate of 3.2-4.5/100,000) than in females (3.0-5.3/100.000 with a mortality rate of 2.4-2.9/100,000).[14]

The most common type of leukemia in this study was ALL (88.5%) with an age range of 18 years (90.7%). There were only 7 cases of AML leukemia in

this study with a mean age > 18 years (57.1%). The result was in accordance with a study by Hutter, which showed that 80% of leukemia patients in his research were children with ALL.[2] Li *et al*. mentioned in their study that the age of leukemia patients varied from 15.3 years in ALL to 77.3 years in Chronic Myelomonocytic Leukemia (CMML) patients.[13] Acute lymphoblastic leukemia is cancer with a high incidence in children, with 5.2 cases per 100,000 found per year in children aged 0-4 years, and 1.9 cases found in children aged 10-14 years. Acute myeloblastic leukemia was more common in adults with an incidence rate of 3.4 per 100.000 per year and two-thirds of cases occurred at age of 60 or older.[3]

The highest risk factors for fungal infections in leukemia patients were the duration of neutropenia of 10 days (69%) compared with neutropenia < 10 days (31%) and higher in patients with severe neutropenia (95%) compared with mild neutropenia (5%).[15] According to the research of Papachristou *et al*. patients with AML leukemia were more affected by IFI (3.7-28%) compared to relapse ALL (4-9%) and acute ALL (0.6-2%) due to a relative decrease in absolute neutrophil counts at the start of treatment. Other risk factors were the cytotoxic effects of chemotherapy drugs, the use of broad-spectrum antibiotics, and neutropenia.[16]

The normality test of CRP levels in this study showed a p-value of 0.00003, indicating an abnormal distribution. Two of 61 patients were positive for *Candida tropicalis*.

Increased CRP can be detected at 6-8 hours after inflammatory stimulation and its level reaches a peak at 24-48 hours, and with a half-life around 19 hours.

Table 1. Characteristics of research subjects

Variable	n (%)	Median (min-max)	Mean±SD
Age (year)		9 (1-70)	
Gender			
Male	30 (49.2%)		
Female	31 (50.8%)		
Type of leukemia			
ALL-L1	10 (16.4%)		
ALL-L2	44 (72.1%)		
AML-M2	1 (1.6%)		
AML-M3	2 (3.3%)		
AML-M4	4 (6.6%)		
Risk factor for fungal infection			
Fever > 3 days	13 (12.4%)		
Neutropenia			
< 500 µL	8 (7.6%)		
500-1500 µL	25 (23.8%)		
Urine catheter	-		
Intravenous line	23 (22.0%)		
Corticosteroid therapy > 1 week	22 (20.9%)		
Antibiotic therapy > 1 week	14 (13.3%)		
Therapeutic phase			
Induction	17 (27.87%)		
Consolidation	5 (8.20%)		
Reinduction	2 (3.28%)		
Maintenance	30 (49.18%)		
AML therapy	7 (11.47%)		
Complete blood count results			
Hemoglobin (g/dL)			10.89±1.81
Thrombocytes (/µL)			217,57±122,42
Leukocytes (/µL)			3970,49±1704,54
Absolute Neutrophils (cells/µL)			1442,13±806,54
Absolute Lymphocytes (cells/µL)			1930±1122,58

n: number of samples; min: minimum; max: maximum; ALL: Acute Lymphoblastic Leukemia; AML: Acute Myelocytic Leukemia; µL: microliter; SD: Standard Deviation; g: gram; dL: deciliter

Tabel 2. Characteristic of research parameters

Variable	Unit	Median	Culture
CRP	mg/dL	11.20 (11.20–26.23)	Positive (2 samples/3.28%)
		0.38 (0.01–18.63)	Negative (59 samples/96.72%)

CRP: C-Reactive Protein; mg: milligram; dL: deciliter

Table 3. CRP levels and blood culture results

CRP Levels	Fungal Culture		
	Positive	Negative	Total
> 9.54 mg/L	2	4	6
≤ 9.54 mg/L	0	55	55
Total	2	59	61

CRP: C-reactive protein; mg: milligram; L: liter
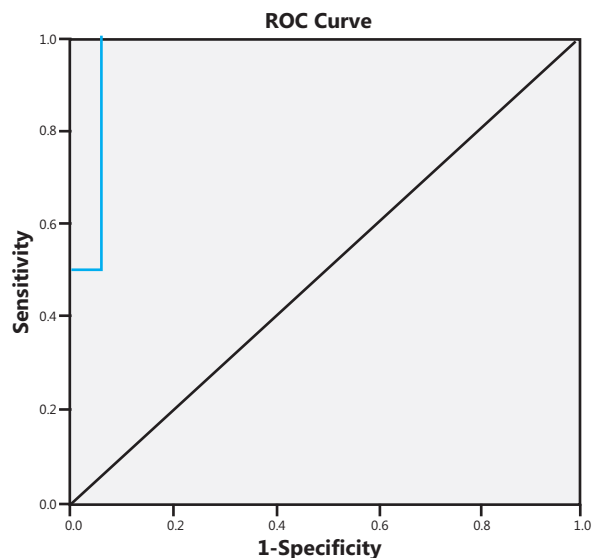
Figure 1. ROC curve of CRP and blood culture results

The main synthesis site for CRP was in the liver, but its mRNA was also found on extrahepatic (adipose tissue, lung, epithelial cell, lymphocyte, and atherosclerotic lesion).[9]

Holder *et al.* stated that CRP cut-off 160 mg/dL at 48 hours has sensitivity 100%, specificity 48%, PPV 33.3%, NPV 100% for diagnosing fever caused by fungal infection. Abas in his research conducted a diagnostic Polymerase Chain Reaction (PCR) test for the detection of fungi in blood samples with a gold standard of culture and obtained a sensitivity of 50%, specificity of 75.4%, PPV of 6.3%, NPV of 97.9%, PLR of 2.0, NLR of 0.7 and accuracy of 74.6%.[17]

CONCLUSION AND SUGGESTION

C-reactive protein could be used as a screening marker for fungal infections in patients with acute leukemia. Cut-off value of CRP was 9.54 mg/L with AUC of 0.966 (p=0.026; CI: 0.0905-1,000), while it had a sensitivity of 100%, specificity of 93.2%, PPV of 33.3%, NPV of 100%, PLR of 1.08, NLR of 0 and accuracy of 93.4%.

A fungal culture test can be performed in patients at risk of fungal infections before administration of antibiotic therapy. Measurement of CRP levels and a fungal culture can be routinely performed in relapsed ALL patients, AML patients, and ALL with neutropenia after chemotherapy.

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