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Diagnostic Performance of Serum (1,3) β-D Glucan to Detect Fungal Infection in Acute Leukemia Patients with Chemotherapy

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ABSTRACT

Chemotherapy is a predisposing factor for infection in patients with malignancy, while culture, as the gold standard, limits the diagnosis of fungal infections. (1,3) β-D glucans, the most abundant polysaccharide component of the fungal wall, are increased in patients with Invasive Fungal Infections (IFI). This research was an analytical observational study with a cross-sectional approach involving 60 acute leukemia patients who received chemotherapy with suspicion of fungal infection at the General Hospital of Dr. Moewardi, Surakarta, from September to October 2019. Fungal blood cultures and serum (1,3) β-D glucan levels by the enzyme-linked immunoassay method were examined. Diagnostic tests were performed to determine sensitivity, specificity, Positive Predict Value (PPV), Negative Predict Value (NPV), Positive Likehood Ratio (PLR), Negative Likehood Ratio (NLR), and the serum's accuracy value (1,3) β-D glucan levels to fungal culture. Most (88.3%) of patients were diagnosed with Acute Lymphocytic Leukemia (ALL), maintenance chemotherapy phase (51.3%), risk factors for neutropenia (50%), and intravenous (IV) line use (56.7%). Serum (1,3) β-D glucan levels in patients with positive fungal cultures (4) in blood samples had a median of 482.87 (476.13-640.56) pg/mL, while patients with negative fungal cultures (56) had a mean±SD 298,68±114,39 pg/mL. Diagnostic test with a cut-off of 471,717 pg/mL showed sensitivity of 100.0%, specificity of 96.4%, NPV of 100%, PLR of 28.00, and NLR of 0.00 with an Area Under Curve (AUC) value of 0.982 and Coefficient Interval (CI) 95% (0.950-1.014). The measurement of serum (1,3) β-D glucan at a cut-off value of 471,717 pg/mL showed good performance as a biomarker for diagnosing and screening IFIs.

Keyword: (1,3) β-D-glucan, invasive fungal infection, acute leukemia, chemotherapy

INTRODUCTION

Invasive Fungal Infections (IFIs) are life-threatening complications in leukemic patients with neutropenia. The prevalence of IFI in acute leukemia cases is 38.8%.[1] Research by Patricia showed that the prevalence of IFI in malignancy cases at the Dr. Moewardi Hospital, Surakarta was 38.2%.[2] Chemotherapy is a predisposing factor for infection in malignant patients.[3]

A fungal culture is a gold standard in the diagnosis of IFI. The diagnosis of IFI is frequently delayed because it is often difficult to get positive culture results from blood or tissue in a critical patient, and it takes a long time.

Glucan is the central, most influential, and abundant component of the fungal polysaccharide.[4,5] (1-3)-β-D-glucans test in high-risk patients has become a crucial non-cultural method for diagnosis of IFI. Serum (1-3)-β-D-glucans levels can be measured by a kinetic method based on the Limulus Amebocyte Lysate (LAL) pathway. This method has been widely used in research despite its complicated process and limited availability.[6,7] Another way is Enzyme-Linked Immunosorbent Assay (ELISA), which needs further development and research.[6] This research aimed to determine the diagnostic performance of serum (1-3) β-D-glucans using the ELISA method to detect fungal infections in patients with acute leukemia on chemotherapy as a predisposing factor to help clinicians establish early and accurate diagnosis and management of patients.

METHODS

This research was an analytical observational study with a cross-sectional approach, which involved 60 patients with acute leukemia on chemotherapy with IFI suspicion between September-October 2019 at Dr. Moewardi Hospital, Surakarta. Inclusion criteria included all patients with acute leukemia who had been diagnosed based on the results of routine blood tests, peripheral blood smear, bone marrow smear, had received chemotherapy, and patients who had one or more

risk factors for fungal infections (fever > three days, neutropenia (neutrophils < 500 cells/μL), lymphopenia (lymphocytes < 500 cells/μL), patients with urinary catheters, patients with intravenous lines, corticosteroid therapy > 1 week, antibiotic therapy > 1 week); and patients who approved and signed informed consent. Exclusion criteria were patients who had received antifungal therapy based on anamnesis and medical records.

Subject's data that met the inclusion criteria during the specified time period were then collected through medical records, anamnesis, and physical examination to complete the patient characteristic data until the number of samples was fulfilled (consecutive random sampling). Blood samples were withdrawn from 3-5 mL to be examined by Matrix-Assisted Laser Desorption Ionization-Mass Spectrometry (MALDI-TOF-MS) followed by fungal culture and measurement of serum (1,3) β-D-glucan levels (BDG ELISA kit, MyBioSource). This research has been approved by the Dr. Moewardi Hospital Ethics Committee, with number 1.096/IX/HREC/2019.

The characteristics of the research subjects were presented in descriptive statistics. Serum (1,3) β-D glucan levels were presented into two different groups, positive and negative fungal blood culture. The normality test of the Kolmogorov-Smirnov test was used in patients with negative fungal cultures. The Shapiro-Wilk test was used in the group of patients with positive fungal cultures group. Data were then presented in mean±SD or median (25^{th}-75^{th} percentile) according to the data distribution with a significance value of $p > 0.05$. Mann-Whitney test/T-test was then performed to determine the difference according to the data distribution and type of data with a significance value of $p < 0.05$.

Statistical analysis was processed using the SPSS program to determine the cut-off value of serum (1,3) β-D glucan using a Receiver Operating Characteristic (ROC) curve. A diagnostic test was performed to determine sensitivity, specificity, NPV, PPV, accuracy, NLR, and PLR.

RESULTS AND DISCUSSIONS

Table 1. Basic characteristics of research subjects

Characteristic	F (%)	Mean±SD	Median (percentile 25-75)
Age			9.50 (6.25-14.75)
Gender			
Male	28 (46.7%)		
Female	32 (53.3%)		
Diagnosis			
ALL	53 (88.3%)		
AML	7 (11.7%)		
Phase of chemotherapy			
Induction phase	16 (26.7%)		
Consolidation phase	4 (6.7%)		
Maintenance phase	31 (51.7%)		
Reinduction phase	2 (3.3%)		
AML regiment	7 (11.7%)		
Risk factor			
Fever > 3 days	12 (2.0%)		
Neutropenia	30 (50.0%)		
Use of urine catheter	0 (0.0%)		
Use of IV line	34 (56.7%)		
Antibiotic therapy > 1 week	17 (28.3%)		
Corticosteroid therapy > 1 week	18 (30.0%)		
Hemoglobin[a] (g/dL)		10.83±1.85	
Leukocytes (cells/μL)		3906,67±1645,11	
Platelets ($X10^3$/μL)			215,00 (78.25-296,00)
Absolute neutrophils (cells/μL)		1439,70±809,18	
Absolute lymphocytes (cells/μL)		1890,52±1143,87	

Note: a. Data distribution was normal (meana±SD); b. Data distribution was abnormal (median (25^{th}-75^{th} percentile); SD= Standard Deviation, ALL= Acute Lymphoblastic Leukemia, AML= Acute Myeloblastic Leukemia, F=frequency, IV=intravenous, g/dL= gram/desilitre, μL= microlitre, %=percent

Table 2. Characteristics of research variable: (1,3) β-D glucan and fungal culture

Research Variable	Blood Culture		P
	Positive (n=4)	Negative (n=56)	
(1,3) β-D-glucan (pg/mL)	482,87 (476,13-640,56)	298,68±114,39	0.0001

Note: data were presented as median (25th – 75th percentile) due to abnormal distribution; the difference was determined using Mann-Whitney test. Significant if p < 0.05; pg/mL= picogram/mililitre, n=number of samples, p=significance value

Based on the inclusion and exclusion criteria, a total of 60 subjects was involved, consisting of 88.3% patients with a clinical diagnosis of ALL and 51.7% patients with the maintenance phase of chemotherapy. Complete data of the basic characteristics of subjects can be seen in Table 1.

Based on Table 2, the median of serum (1,3) β-D-glucan levels in patients with positive fungal blood cultures was 482,87 (476,13-640,56) pg/mL, while the mean±SD of serum (1,3) β-D-glucan levels in patients with negative fungal blood cultures was 298,68±114,39 pg/mL. Mann-Whitney test results showed a significantly different result of serum (1-3) β-D-glucan levels between patients with positive and negative fungal blood cultures in this study with p-values < 0.05.

The cut-off value was determined using the ROC curve (Figure 1). The cut-off value of serum (1,3) β-D glucan level obtained from the ROC curve was 471.72 pg/mL with an AUC value of 0.982 and Confident Interval (CI) of 95% (0.950-1.014). The diagnostic performance of (1,3) β-D serum glucan levels was compared to the gold standard method of fungal culture.

Based on the 2x2 table (Table 3), it was found that four patients with positive fungal blood cultures had serum (1-3) -β-D-glucan levels > 471,72 pg/mL, while most (54) of 56 patients with negative fungal blood cultures had serum (1-3) -β-D-glucan levels < 471.72 pg/mL. Besides, compared to fungal blood culture as a gold standard, serum (1-3) -β-D-glucan levels had sensitivity, specificity, NDN, PLR, and NLR of 100.0%, 96.4%, 100%, 28.00, 0.00 with an AUC value of 0.982 CI 95% (0.950-1.014), respectively in detecting fungal infections in patients with acute leukemia on chemotherapy.

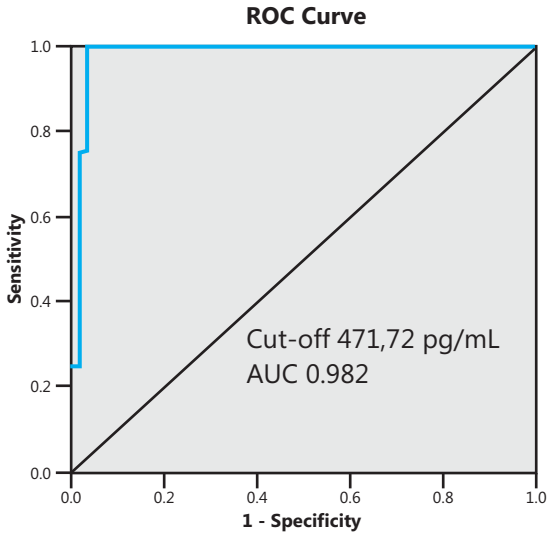

Figure 1. ROC curve

The most commonly found diagnosis of this study was ALL (88.3%). This result was different from the study by Bartlet *et al*., which found that the prevalence of IFI in AML malignancies was greater (28.2%) compared to ALL (10%) among all malignancies.[1] Acute leukemia is the most common malignancy in children. Based on data from acute leukemia visits in the Dr. Moewardi Hospital in 2018, it was found that the percentage of ALL patient visits was greater (73%) compared to AML (27%).

The maintenance phase of chemotherapy was most commonly found in this study (51.7%), followed by the induction phase (26.7%). This result was different from the study by Pagano *et al*., which stated that the highest risk of IFI in leukemia was in the induction phase of chemotherapy.[8] Based on Cancer Research 2018, the consolidation phase of chemotherapy in ALL is performed in the long term

Table 3. 2x2 table of the diagnostic test of serum (1,3) β-D glucan compared to fungal culture as a gold standard

Serum (1,3) β-D-Glucan Levels (pg/mL)	Fungal Blood Culture		Total
	Positive	Negative	
≥ 471,72 (positive)	4	2	6
< 471,72 (negative)	0	54	54
Total	4	56	60

Note pg/mL= picogram/milliliter

with small doses, accompanied by steroid administration for approximately two years and sometimes in conjunction with antibiotics.[9]

The highest risk factor for IFI in the patients in this study was the use of IV lines (50.7%) and neutropenia (50%). This data was following research by Lien *et al*., which showed that the condition of neutropenia in malignant patients with chemotherapy was twice more likely to suffer from IFI.[10]

The number of patients with negative fungal cultures was greater than the patients with negative fungal cultures. Clinical manifestations of IFI are difficult to prove with blood culture. Sampling for blood culture in this study was merely performed once. A one-time sampling strategy can produce many positive culture results, but taking more than one blood sample is sometimes also necessary.[11] Serum (1,3) β-D-glucan levels in this study ranged from 206,92-640,56 pg/mL. Median of serum (1,3) β-D-glucan levels in patients with positive fungal cultures was 482.87 (476,13-640,56) pg/mL, whereas patients with negative fungal cultures had mean±SD 298,68±114,39 pg/mL. Research by Azoulay *et al*. obtained serum (1-3) β-D-glucan levels of 144 (77-510) and 50 (30-125) pg/mL in a group of hematologic malignancy with IFI and the group without IFI.[12]

Serum levels (1-3) β-D-glucan had an excellent performance as a diagnostic biomarker of fungal infections in patients with acute leukemia on chemotherapy with excellent AUC values (> 0.9-1). Sensitivity and specificity of serum (1-3) β-D-glucan levels > 90% indicated that biomarkers could not merely be used as a diagnostic instrument but could also be used as screening instruments. Good diagnostic value was indicated by PLR > 10 and NLR close to 0.

CONCLUSIONS AND SUGGESTIONS

Based on the research involving 60 samples of acute leukemia patients on chemotherapy with suspicion of IFI, it could be concluded that the serum (1,3) β-D glucan has an excellent performance to diagnose fungal infections in acute leukemia patients on chemotherapy with comparison to fungal culture as a gold standard. Further research involving acute leukemia patients with suspicion of IFI, healthy control, and the use of other body fluid than serum was needed.

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