Received: 4 March 2021 | Revised: 8 April 2021 | Accepted: 10 April 2021

DOI: 10.1002/jcla.23806

RESEARCH ARTICLE

WILEY

Diagnostic value of serum human Galactomannan aspergillus antigen and 1,3-beta-D-glucan in immunocompromised patient suspected fungal infection

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Abstract

Background: The prevalence of fungal infection (FI) in developing countries is high, but the diagnosis of FI is still challenging to determine, so it is needed evaluation of biomarkers other than microbiological culture, because the culture has low sensitivity, high cost, not available in every laboratory and needs a long time. The detection of human galactomannan Aspergillus antigen (GAL) and 1,3-beta-D-glucan (BDG) on the fungal cell wall could be the promising biomarkers for fungal infection. Neutropenia, lymphopenia and CD4T cells in the immunocompromised patients are essential factors, but these cell associations with BDG and GAL levels have not been evaluated yet. The study aimed to evaluate GAL and BDG for detecting fungal infection and their association with total leucocyte count, neutrophil, monocyte, lymphocyte and CD4T cells.

Method: A cross-sectional study was conducted among 86 patient with suspected FI. Fungal infection established using EORTC/MSG criteria. Serology test performed using ELISA. Leucocyte cells were measured using a haematology autoanalyser, and CD4T cells were analysed using BD FACSPresto. Statistical analysis obtained using Spearman's correlation coefficient, ROC curve analysis and 2 × 2 contingency table.

Results: Serum Galactomannan and BDG had a significant correlation with CD4T cells and total lymphocyte count ($p < 0.05$). The cut-off OD GAL >0.3 had sensitivity 54.6%, specificity 87.5% and AUC 0.71; meanwhile, the BDG cut-off >115.78 pg/ mL had sensitivity 71.2%, specificity 52.4% and AUC 0.63 for detecting fungal infection.

Conclusions: The immunocompromised patients can undergo GAL for determining the diagnose of FI. The lower the CD4T cells and total lymphocyte count, the higher the GAL and BDG serum levels.

KEYWORDS
1,3-beta-d-glucan, galactomannan, fungal Infection, haematology parameters, immunocompromised

1 | INTRODUCTION

Fungi are a heterotrophic group of organisms mostly found as saprobes in soil and decaying organic matter. Fungi are eukaryotic organisms with various internal membrane systems, membrane-bound organelles and transparent cell walls consisting of polysaccharides (glucan, mannan) and chitin.[1]

Fungal infection or mycosis is defined as an invasion into the tissues caused by a fungus. Fungal infections are clinically classified into superficial mycosis (cutaneous and subcutaneous mycosis) and systemic mycosis that are often found in the lungs as a primary organ of infection and then spread to other organs.[2] Pulmonary mycosis or systemic mycosis in certain conditions, especially acute infections, can generally result in a high mortality rate of up to 50% or even up to 100%.[2]

The main factor that plays a role in increasing fungal infections is the host's immunity disorder, especially in immunocompromised individuals. Tao et al,[3] on immunocompromised patients, also mentioned host-related factors played significant roles in poor prognosis, while bacterial determinants had little effect on outcome. The entire immunocompromised population with invasive fungal infection had the mortality rate varies from 27% to 77%.[4]

Alveolar macrophages, neutrophils and monocytes are the first phagocytic defence mechanisms against inhaled conidia and hyphae.[5] Neutrophils are activated rapidly and attack the hyphae and envelop the hyphae within one hour, thus inhibiting yeast formation. Neutrophil extracellular traps (NETs) capture and kill hyphae and yeast.[6] In the study of Cordonnier et al,[7] there were significant differences in Galactomannan (GAL) levels in patients with neutropenia and non-neutropenia. Based on several studies, it has been shown that neutropenia is a considerable risk factor for fungal infection, although it is often found in non-neutropenic patients.[7]

The diagnosis of fungal infection mostly depends on the gold standard of microbiological culture.[8] The selection of specimens for culture and microscopy should be carried out under aseptic conditions, and samples should be sent to the laboratory immediately. Culture provides a definitive diagnosis of fungal infection, but the sensitivity is only less than 50%.[9] The results of the culture examination also take several days so that the patient's treatment is delayed. This condition raises an urgent need for a rapid diagnosis of fungal infection to be carried out. Moreover, at this time, candida therapy technology continues to develop, including nanoparticle therapy, hoping that it can reduce side effects and is more efficacious than the antifungal currently used.[10] The development of new treatments on candida is expected to be followed by the rapid diagnosis of fungal infections so that patients can be better managed.

Among the available biomarkers, the examination of fungal antigens such as cryptococcus antigen, Galactomannan (GAL) or 1,3-beta-D-glucan (BDG) is the most useful for detecting fungal infections.[9] GAL is a polysaccharide that presents in almost all cell walls of Aspergillus sp.[8] The sensitivity to detection of GAL using ELISA reported to be 89% with a specificity of 92% in patients with haematopoietic stem cell transplantation. Another study found a lower sensitivity and varies between 40 and 50%, especially in the determination of prophylaxis and active antifungal therapy, but did not use serial samples for testing. BDG is a polysaccharide in the cell walls of various types of fungi. According to Boch et al, in invasive Aspergillus patients, BDG sensitivity was better than GAL (89%), and GAL specificity was better than BDG (97%).[11-13]

2 | MATERIAL AND METHODS

2.1 | Research subject

Subjects of study were in patients with a risk of fungal infection. This study was approved by the ethical committee of Dr. Saiful Anwar General Hospital, Malang, Indonesia (ethical number 400/109/K.3/302/2018). All patients included in this study were asked to sign a consent form (Informed Consent).

2.2 | Research design

We conducted a cross-sectional study with convenience series samples. The data were obtained from complete blood counts, fungal serology examination (human galactomannan Aspergillus antigen (GAL) and human 1,3-beta-D-glucan (BDG)), and fungal cultures. This study was conducted for six months, from July to December 2018. The GAL and BDG diagnostic value (sensitivity, specificity, positive predictive value and negative predictive value) were compared with the gold standard, referring to fulfil the proven or probable of the EORTC/MSG criteria. The criteria need the mycological test, such as the culture or direct microscopy or staining of fungi, which are positive. The inclusion criteria were patients 18–70 years old, with the risk of fungal infection (immunocompromised patients). The immunocompromised patients' criteria were HIV or AIDS infection, cancer, solid organ transplantation, sickle cell disease or chronic inflammatory conditions. Laboratory tests criteria of immunocompromised patients were CD4T cells <500 or absolute lymphocytes <1000 cells/μl for adults.[14] The exclusion criteria were patient who previously received antifungal therapy.

2.3 | Examination of haematological parameters and CD4T cells

The absolute values of leucocyte, neutrophil and lymphocyte count were measured by Sysmex XN 1000. CD4T-cell examination was done by immunofluorescence method with the BD FACSPresto instrument.

2.4 | Examination of serology and culture

Serological examination was done using Human Aspergillus Galactomannan (GAL) Antigen ELISA Kit (Sincere Biotech) and

human 1,3-beta-D-glucan (BAL) (Sincere Biotech). The microbiological examination was carried out by microscopic examination with KOH, VITEK2 staining and SDA (Sabouraud Dextrose Agar) medium culture.

2.5 | Statistical analysis

The data were analysed using IBM SPSS version 18.0 software. The normality data are carried out using the Kolmogorov–Smirnov, and the comparative data were analysed using t test or Mann-Whitney. The correlation between BAL or GAL with leucocyte cells was analysed using the Pearson test or Spearman non-parametric statistics. Diagnostic tests performed using the ROC curve, sensitivity, specificity, positive predictive values, and negative predictive values.

3 | RESULTS

A total of 86 subjects consisting of 44 fungal infections and 42 no fungal infections. Subjects aged 18–70 years old with a mean aged of 51 years for fungal infection patients and 54 years old for subjects with no fungal infection. Most of the sex is male. Of the 44 subjects with a fungal infection, 26 (59.0%) subjects had positive culture results. Of the 86 subjects who were immunocompromised patients, 20 (23.2%) got HIV, 33 (38.3%) subjects with cancer, and the remaining subjects had the other immunocompromised condition.

There was no significant difference in the total leucocytes count and neutrophil in the fungal infection and no fungal infection groups. Conversely, there were significant differences in the number of lymphocytes, monocytes, CD4T cells, BDG and GAL levels (Table 1).

The correlation between GAL and BDG serum levels with leucocytes, neutrophils and monocytes was statistically insignificant ($p > 0.05$). Meanwhile, the correlation between GAL and BDG serum levels with lymphocytes showed a significant correlation ($p < 0.05$, $r = -0.395$ and -0.348, respectively) with an opposing direction of correlation. These data indicate that the lower the lymphocyte levels, the higher the GAL and BDG serum levels. The correlation between GAL and BDG serum levels with CD4T cells indicated a negative correlation ($p < 0.05$, $r = -0.415$ and -0.495, respectively). The correlation between GAL with BDG serum levels had a significant correlation ($p = 0.000$ and $r = 0.630$) (Table 2 and Figure 1).

Human galactomannan antigen diagnostic test using a cut-off value >0.3 showed the sensitivity value of 54.6% and specificity value of 87.5% with an AUC of 0.71. The human 1,3-B-D-glucan diagnostic test using a cut-off value >115.78 pg/mL showed the sensitivity value of 71.2% and a specificity value of 52.4% with an AUC of 0.63 (Table 3 and Figure 2).

4 | DISCUSSION

Our study indicated that most subjects were a male gender. Kaur et al reported a study in 280 subjects with HIV and found that most subjects were 21–40 years old (77%) with a male gender predominantly with the ratio of 2.5:1 compared to women. This result was related to the population's productive age and the mostly male incidence. A study in Korea found different outcome, where opportunistic fungal infections were dominated by women 14 times higher than men with the highest age range of 30–39 years.[15]

We found most subjects in fungal infection group had lymphopenia, decreased monocyte count and CD4T cells <200 cells. According to the study of Kaur et al,[15] the group with probable fungal infection obtained lymphopenia; meanwhile, 280 subjects with HIV and opportunistic fungal infections, only 8% of subjects had CD4T cells >500, and 47.5% of subjects with CD4T cells <200 cells, and the rest <100 cells. Immunocompromised patients had defects in the production of cytokines IL-12, which were useful for the differentiation

TABLE 1 Characteristics data of subjects

	Fungal infection ($n = 44$)	No fungal infection ($n = 42$)	p Value
Age (years)	51.05 ± 2.59	54.08 ± 2.11	0.305
Men (n, %)	28 (63.6)	35(83.3)	0.046*
Women (n, %)	16 (36.4)	7 (16.7)	
Total (n)	44	42	
Leucocyte (/μL)	13,056.84 ±1788.79	13,892.89 ± 43	0.171
Neutrophil (/μL)	11,197.39 ± 1707.26	11,758.71 ± 1171.75	0.269
Lymphocyte (/μL)	734.68 ± 95.12	1013.36 ± 95.14	0.006*
Monocyte (/μL)	875.95 ± 144.97	899.84 ± 73.0	0.047*
TCD4 (cell)	134.72 ± 27.01	328.74 ± 35.39	0.000*
OD GAL	0.38 ± 0.02	0.20 ± 0.01	0.001*
BDG (pg/mL)	777.89 ± 141.79	371.47 ± 87.19	0.034*

Note: Data are presented as mean ± standard deviation (SD) and n (%).

Abbreviations: BDG, 1,3-B-D-Glucan; GAL, Galactomannan; OD, optical density.

*$p < 0.05$.

of CD4T cells to Th17. There were defects in Th17, so that they could not produce IL-17 to recruit neutrophils and monocytes.[5]

Neutrophils play a role in inhibiting the growth of hyphae. Hyphae growth occurs at a later stage of Aspergillus germination after conidial swelling. Patients with neutropenia lose the ability to prevent hyphal growth after swollen conidia. Neutrophils are thought to play a role in the late stages of germination.[16,17] The subjects in our study were immunocompromised patients with other infection sources that could not be excluded, so that it was difficult to find patients with only fungal infections. Hence, our study found many fungal infection patients with leukocytosis and neutrophilia.

Bongomin et al[5] showed a correlation between IL-17 and CD4T cells in patients with chronic pulmonary aspergillosis. These data are also consistent with Doffinger et al,[18] where there was impaired production of IL-17 and IL-12 in patients with chronic pulmonary aspergillosis. IL-12 stimulates T helper CD4 and CD8T cells for the production of IFN-γ, which is vital as a defence mechanism for pulmonary infection. The subjects in our study were immunocompromised patients with lymphopenia and decreased CD4T cells, so we found the negative correlation between GAL and BDG levels with lymphocytes and CD4T cells in this study. Besides that, aspergillus has many types of mycotoxins, one of which is gliotoxin (GT). GT can inhibit T lymphocytes' activation by specifically inhibiting NF-kB in inducing transcription of several inflammatory cytokines and hematopoietic growth factors.[16]

The other fungal infection study indicated that the sensitivity and specificity of BDG varied from 55%–100% and 71%–93% with cut-offs also ranging from 6 to 120 pg/ml. Our study showed BDG had sensitivity 71.2% and specificity 52.4%. The different results of this study from the other, because the kinetics of BDG were not yet

TABLE 2 Correlation between beta-D-glucan and Galactomannan with haematology parameters

	Beta-D-Glucan (BDG)		Galactomannan (GAL)	
Parameters	***r***	***p***	***r***	***p***
Leucocyte total	0.074	0.497	0.023	0.836
Neutrophil	0.059	0.646	0.013	0.975
Lymphocyte	−0.348	0.000	−0.395	0.000
Monocyte	0.030	0.780	−0.014	0.901
CD4T cells	−0.495	0.000	−0.415	0.000

Abbreviations: BDG, 1,3-B-D-Glucan; GAL, Galactomannan; OD, optical density.

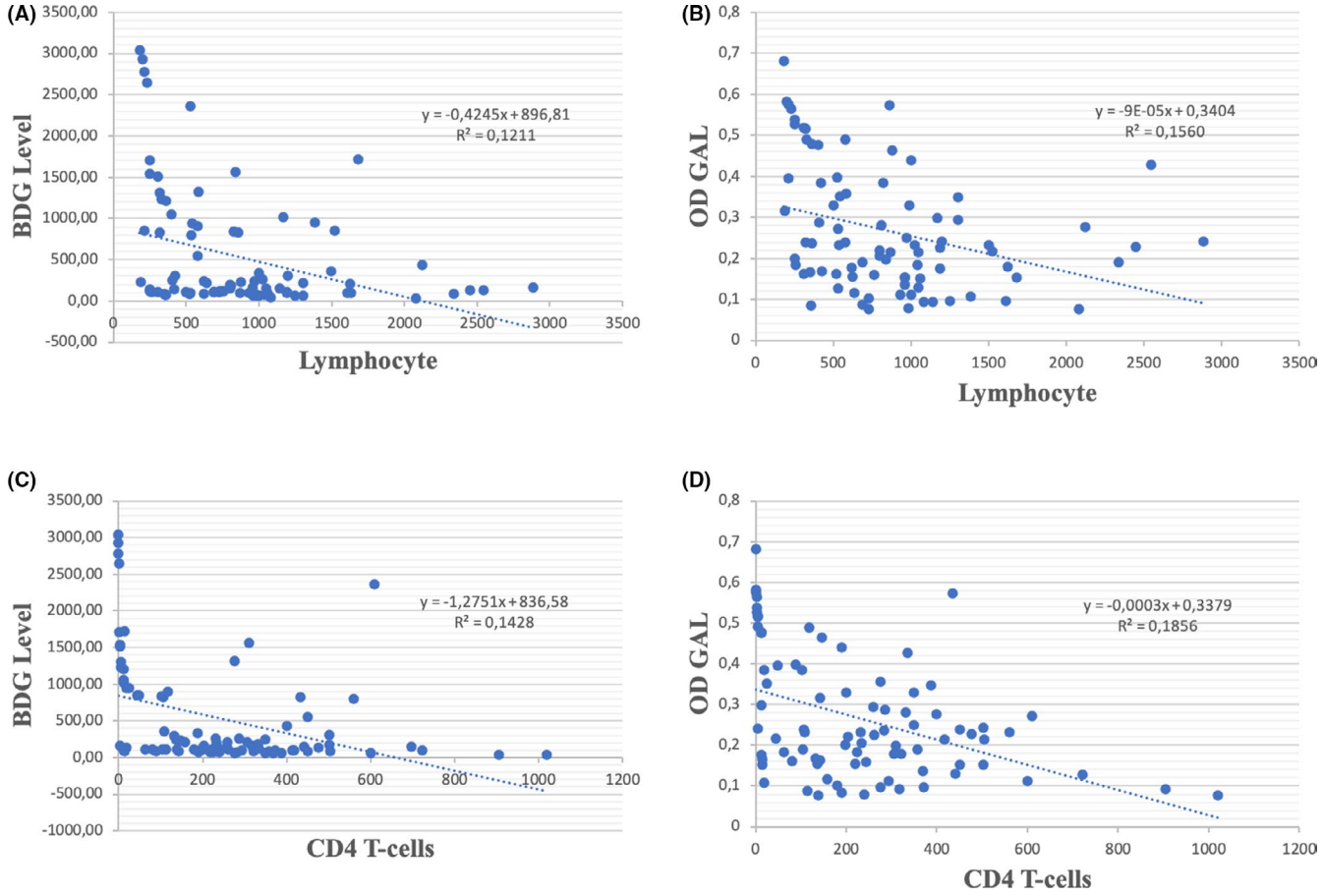


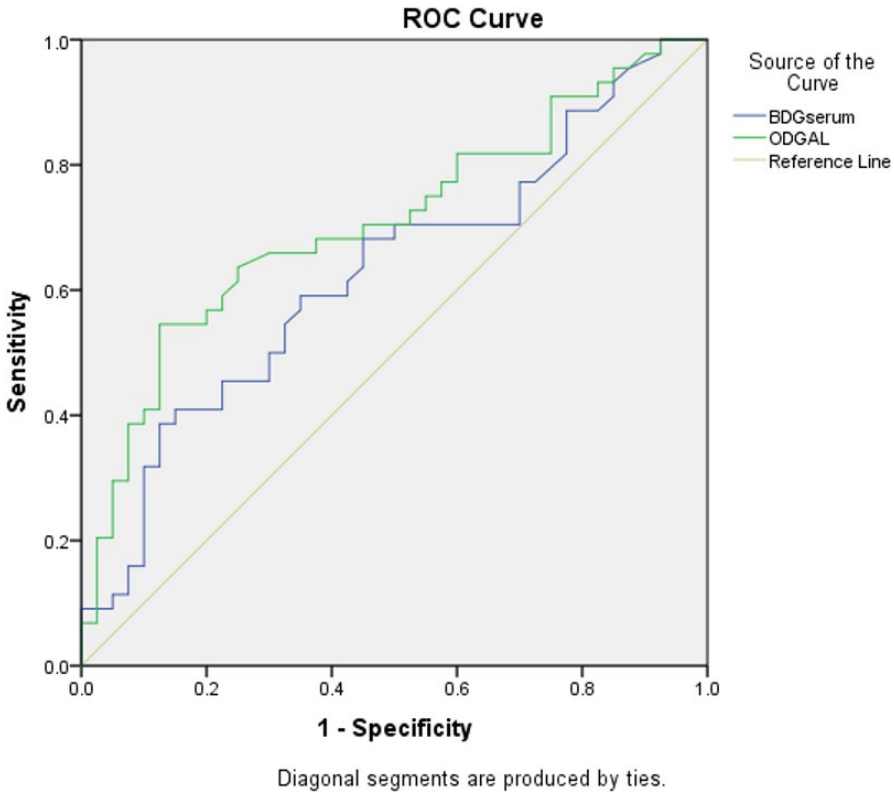
FIGURE 1 Correlation between BDG and GAL with lymphocyte and CD4T cells. (A) Correlation between BDG and lymphocyte ($r = -0.348$)*. (B) Correlation between GAL and lymphocyte ($r = -0.395$)*. (C) Correlation between BDG and TCD4 ($r = -0.495$)*. (D) Correlation between GAL and TCD4 ($r = -0.415$)*. *$p < 0.05$. Abbreviations: BDG, 1,3-B-D-Glucan; GAL, Galactomannan; OD, optical density

TABLE 3 Diagnostic value of beta-D-glucan and Galactomannan

Parameters	Cut-off	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
Beta-D-Glucan (BDG)	115.78 pg/mL	71.20%	52.40%	61.54%	62.86%
OD Galactomannan (GAL)	>0.3	54.60%	87.50%	82.8%	63.6%
Beta-D-Glucan (BDG) and OD Galactomannan (GAL)	115.78 pg/mL and >0.3	48.89%	90.48%	84.62%	62.30%
Beta-D-Glucan (BDG) and Lymphopenia	115.78 pg/mL and Lymphocyte count <1000 cells/μL	71.11%	54.76%	62.75%	63.89%
OD Galactomannan (GAL) and Lymphopenia	>0.3 and Lymphocyte count <1000 cells/μL	53.33%	88.1%	82.76%	63.79%

Abbreviations: BDG, 1,3-B-D-Glucan; GAL, Galactomannan; OD, optical density.

FIGURE 2 Roc curve of beta-D-glucan and Galactomannan



known . BDG is still questionable whether it could be used as a biomarker to monitor antifungal therapy . In patients with positive BDG candidemia, BDG remains positive after negative cultures, even in patients who respond to treatment.[11] BDG is a polysaccharide in various fungi types in the cell walls, but it was also found in several types of bacteria, algae and various kinds of plants.[11] This increases the risk of false positives on BDG serological examinations, resulting in low BDG test specificity. The same results were obtained in 31 study subjects who evaluated BDG as a diagnostic marker for fungal infections; the sensitivity and specificity results were not too good for the diagnosis of fungal infections with values of 80% and 82%, so it was concluded that BDG could be used as a screening tool for fungal infections.[16]

Our study indicated that GAL had sensitivity 54.6% and specificity 87.5%. A meta-analysis of GAL in proven and probable fungal infections groups showed low sensitivity and specificity in organ transplant patients who were predominantly patients with normal neutrophils. Several studies had demonstrated that neutropenia is a significant risk factor for in aspergillus infection.[6] Aspergillus culture alone provided a definitive diagnosis of fungal infection, but the sensitivity is only <50%.[9]

The combination of BDG and GAL examination with lymphocyte count is expected to increase the test's sensitivity and specificity. The combined test between BDG and GAL and the combined test between GAL and lymphopenia showed better specificity than BDG or GAL examination alone, but the sensitivity results are less profitable. Meanwhile, the results of BDG and lymphopenia did not

produce higher sensitivity and specificity values, so the examination using GAL alone had reasonable specificity.

The study's limitation that the number of samples was not much. It was challenging to find immunocompromised patients without other co-infections and neutropenia. Examination of culture that was done often got negative results due to limited detection tools so that the gold standard used only meet the Prove or Probable of EORTC/MSG criteria.

5 | CONCLUSION

Galactomannan had a better AUC and specificity value than BDG to be used for diagnostic purposes. Meanwhile, BDG had better sensitivity than GAL, so that it had potential value for screening test. There was a significant correlation between BDG and GAL with lymphocytes and CD4T cells. These data indicate that immunocompromised patients can undergo GAL for determining the diagnosis of fungal infection.

ACKNOWLEDGEMENTS

The authors would like to thank the Director of Saiful Anwar General Hospital Malang, the Dean of Medical Faculty of Brawijaya University and all participants of this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this publication.

AUTHOR CONTRIBUTIONS

HS and US conceptualized the idea and study design. LP and NK performed the literature search. US and TR managed the patient follow-up and data collection. HS and LP performed data analysis, interpretation and drafted the manuscript.

DATA AVAILABILITY STATEMENT

The database for the study can be acquired from the principal investigator, Hani Susianti, [hanisusianti.fk@ub.ac.id].

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How to cite this article: Susianti H, Parmadi L, Firani NK, Setyawan UA, Sartono TR. Diagnostic value of serum human Galactomannan aspergillus antigen and 1,3-beta-D-glucan in immunocompromised patient suspected fungal infection. *J Clin Lab Anal*. 2021;35:e23806. https://doi.org/10.1002/jcla.23806