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Differences in Levels of Human 1,3-β-D-Glucan from Bronchoalveolar Lavage (BAL) Fluid between The Immunocompromised and Immunocompetent Groups Patients with Suspected Lung Cancer

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Abstract

Background: Invasive candidiasis occurs in immunocompromised individuals as an opportunistic infection in patients with lung cancer. Although culture and histopathology remain the standard diagnosis of fungal infections, other tests are still needed to provide faster results. Human 1,3-β-D-Glucan (BDG) uses ELISA to detect candidiasis. β D-Glucan level from BAL fluid marked positive with cut-off value ≥130 pg/mL. This study aims to determine differences in the levels of Human 1,3-β-D-Glucan from Bronchoalveolar Lavage (BAL) fluid between the immunocompromised and immunocompetent groups in suspected lung cancer patients at RSU Dr. Saiful Anwar Malang.

Method: A cross-sectional study was conducted on 33 lung cancer patients who had risk factors for invasive candidiasis in Dr. Saiful Anwar Hospital Malang.

Result: The result shown significant differences in BAL levels of Human 1,3-β-D-Glucan between the immunocompromised and immunocompetent groups (P=0.009). In the different tests, there was a slight difference in the levels of BAL Human 1,3-β-D-Glucan but it was not statistically significant based on age and sex between the immunocompromised and immunocompetent groups (P=0.632, P=0.338, P=0.472, P=0.667).

Conclusion: Patients suspected of lung cancer have risk factors for invasive candidiasis with higher BDG levels due to immunoparalysis. There were significant differences in the BAL Human levels of 1,3-β-D-Glucan between the immunocompromised and immunocompetent groups. (J Respirol Indones 2022; 42 (2): 90–6)

Keywords: 1,3-β-D-Glucan, lung cancer, invasive candidiasis, immunocompromised.

Perbedaan Kadar Human 1,3-β-D-Glucan dari Cairan Bronchoalveolar Lavage (BAL) antara Kelompok Imunokompromais dan Imunikompeten Pasien Terduga Kanker Paru

Abstrak

Latar Belakang: Candidiasis invasif terjadi pada individu imunokompromais sebagai infeksi oportunistik pada pasien keganasan termasuk kanker paru. Meskipun kultur dan histopatologi tetap merupakan standar diagnosis infeksi jarur, masih diperlukan pemeriksaan lainnya yang dapat memberikan hasil lebih cepat. Human 1,3-β-D-Glucan (BDG) menggunakan ELISA sebagai salah satu pemeriksaan candidiasis, kadar β D-Glucan dari cairan BAL adalah positif bila cut off ≥130 pg/mL. Penelitian ini bertujuan mengetahui perbedaan kadar BAL Human 1,3-β-D-Glucan antara kelompok imunokompromais dan imunokompeten pada pasien yang terduga kanker paru di RSU Dr. Saiful Anwar Malang.

Metode: Studi cross sectional dilakukan pada 33 pasien kanker paru yang memiliki faktor risiko candidiasis invasif di RSUD dr Saiful Anwar Malang.

Hasil: Dari penelitian menunjukkan perbedaan bermakna kadar BAL Human 1,3-β-D-Glucan antara kelompok imunokompromais dengan imunokompeten (P=0,009). Pada uji beda didapatkan sedikit perbedaan kadar BAL Human 1,3-β-D-Glucan tetapi tidak signifikan secara statistik berdasarkan usia dan jenis kelamin pada kelompok immunocompromised dan immunocompetent (P=0,632, P=0,338, P=0,472, P=0,667).

Kesimpulan: Pasien terduga kanker paru memiliki faktor risiko candidiasis invasif dengan kadar BDG lebih tinggi karena imunoparalisis. Terdapat perbedaan yang bermakna pada kadar BAL Human 1,3-β-D-Glucan pada kelompok imunokompromais dan imunokompeten. (J Respirol Indone 2022; 42 (2): 90–6)

Kata kunci: 1,3-β-D-Glucan; kanker paru; imunokompromais; kandidiasis invasif

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INTRODUCTION

Pulmonary mycoses are lung disorders, including the respiratory tract, caused by infection, fungal colonization, or hypersensitivity reactions to fungi. The frequency of pulmonary mycoses has increased in recent years along with the increasing number of patients with impaired immune systems such as malignancy patients, in this case, lung cancer, organ transplantation, HIV/AIDS infection, chronic systemic disease, corticosteroids, and invasive medical devices (e.g., mechanical ventilation and central venous catheters). Certain conditions, especially acute infections, pulmonary mycoses, or systemic mycoses in general, can result in high mortality rates reaching 50% or more or even up to 100%.1,2

One of the most studied immunological markers in patients with lung cancer is the CD4, CD8 count, and CD4/CD8 ratio. CD4 deficiency, quantitative lymphocyte changes, and the existence of other theories that suggest T cell dysfunction in lung cancer cause individuals with lung cancer to be usually immunocompromised, making it easy for infections to occur including fungal infections which can complicate healing.3–5

The morbidity and mortality associated with invasive fungal infections (IFI) caused by Candida albicans and non-albicans Candida spp., especially in immunocompromised hosts, is increasing. The diagnosis of pulmonary mycoses considered rather difficult often leads to delayed treatment. The diagnosis of pulmonary mycoses is based on a physical examination and supporting examinations in the form of imaging studies (radiology), certain clinical laboratory results, and mycological examinations. Direct microscopic examination of Bronchoalveolar Lavage (BAL) with 10% KOH can detect fungal elements in general in the form of spores and hyphae. However, the conventional identification of fungal species takes a long time, especially in fungal breeding.1,6,7

The mycological examination is an important diagnostic procedure for pulmonary mycoses, one of which is serological examination. Serological assays are classically used to detect host antibody reactions to fungal elements. Among the well-studied fungal antigens is 1,3-β-D-Glucan (BDG), which has been developed to diagnose IFI. BDG is a cell wall component of many fungi, including Aspergillus spp., Candida spp., Fusarium spp., Pneumocystis jirovecii, and mycoses.1,8–10

There is still controversy regarding the method of examining lung fungal infections from BAL specimens, especially in patients with lung cancer. In addition, at RSU, Dr. Saiful Anwar Malang has not provided a rapid diagnostic test for fungal infections. Thus, research is needed on the examination with 1,3–β-D-Glucan (BDG).

METHODS

There were 33 subjects from RSU Dr. Saiful Anwar ward was enrolled. This type of research is analytic observational with a cross-sectional approach. The subjects are suspected lung cancer patient hospitalized with a risk of fungal infection in the inpatient ward of Dr. Saiful Anwar Malang. Sampling in this study was carried out by consecutive sampling, namely every patient with suspected lung cancer who was hospitalized at Dr. Saiful Anwar during the research period and passed after judging from the inclusion criteria included: 1) Age ≥18 years; 2) Patients suspected of lung cancer from clinical and radiological results; 3) Fulfilling the requirements for FOB and patient BAL specimen collection, and 4) The patient and/or the patient's family are willing and sign the informed consent. Exclusion criteria were patients who had previously received antifungal therapy, albumin/IVIG (intravenous immunoglobulin) therapy, patients undergoing hemodialysis, patients diagnosed with invasive candidiasis.

Lung cancer is a malignant tumor originating from the bronchial epithelium or bronchial carcinoma confirmed by anatomic pathology results.3,1 Suspected lung cancer patients are those whom based on clinical and supporting examinations in the form of blood laboratories and chest X-rays that show the presence of a mass, either with a central picture of necrosis, or pulmonary parenchymal atelectasis, or
pleural effusion, all of which have been ruled out by other causes.

Immunocompromised status is one of the risk factors for invasive fungal infections, including candidiasis infection, and determined based on the examination of CD4 cut-off <500 cells/mm³.

Bronchoalveolar Lavage (BAL) fluid is a sample obtained from an examination carried out with a fiber optic bronchoscopy (FOB) procedure performed by performing flushing 50 mL of 0.9% saline solution. The technique of implementing BAL is done with a flexible bronchoscope that is inserted up to the subsegmental bronchus until the lumen is closed (wedged position).11,12 As much as 50 mL of 0.9% saline solution, which is warmed to 37°C, is inserted through a bronchoscope, then the liquid collected right away with suction from the bronchial tree with a negative pressure of 30 cmH₂O and inserted into the specimen holder. An ELISA procedure then follows the fluid resulting from BAL to check for BDG.

The required sample of BAL is about 10 ml, taken at the time of FOB, which is a diagnostic procedure for lung cancer. Samples from BAL were then centrifuged at 1000–3000 rpm for 10 minutes, and then the supernatant was transferred to an aliquot tube and stored at -80°C.

**Human Beta-D-Glucan** antigen is an antigen on the fungal cell wall which was detected using the Human Beta-D-glucan antigen ELISA kit (Sincere bio) and read with an ELISA reader according to the manufacturing procedure.

CD4+ examination was performed on blood samples from peripheral veins in lung cancer patients. The blood sampling results were examined for lymphocyte values and CD4+ count values.

The data obtained from the research results will be analyzed using IBM SPSS software version 25. All data variables are tabulated manually. Test the normality of the data using Shapiro-Wilk. The data included in the descriptive analysis were patient demographics, including age and gender, lymphocyte values, CD4 levels, and BDG levels from BAL results. The difference test is carried out using the T-independent test if the assumption of normality is met. If the normality assumption is not met, then the **Mann-Whitney** test is used.

**RESULTS**

The characteristics of the research subjects can be seen in Table 1. There were two age groups evaluated, less than equal to 45 years old subjects were 18.2% and more than equal to 45 years old subjects were 81.8%. The Mann Whitney test showed that there was no significant difference in the levels of Human 1,3-β-D-Glucan between the two age groups (P=0.451). The subjects were male dominant (87.9%) which Mann Whitney test showed no significant difference in Human 1,3-β-D-Glucan levels between gender.

![Table 1. Clinical Characteristics](image)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>%</th>
<th>Average BDG (pg/dl)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤45 years old</td>
<td>6</td>
<td>18.2</td>
<td>681.59±59</td>
<td>0.451</td>
</tr>
<tr>
<td>&gt;45 years old</td>
<td>27</td>
<td>81.8</td>
<td>893.81±810</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29</td>
<td>87.9</td>
<td>904.38±833.12</td>
<td>0.321</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>12.1</td>
<td>498.81±374.44</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1100</td>
<td>11</td>
<td>33.3</td>
<td>1147.67±1157.14</td>
<td>0.418</td>
</tr>
<tr>
<td>&gt;1100</td>
<td>22</td>
<td>66.7</td>
<td>709.00±517.90</td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;500</td>
<td>21</td>
<td>63.6</td>
<td>1099.06±887.30</td>
<td>0.009</td>
</tr>
<tr>
<td>≥500</td>
<td>12</td>
<td>36.4</td>
<td>428.50±337.62</td>
<td></td>
</tr>
<tr>
<td>Comorbid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPD</td>
<td>2</td>
<td>12.5</td>
<td>475.11±313.40</td>
<td>0.970</td>
</tr>
<tr>
<td>DM</td>
<td>3</td>
<td>18.8</td>
<td>602.36±352.07</td>
<td></td>
</tr>
<tr>
<td>Other Cancer</td>
<td>5</td>
<td>31.3</td>
<td>621.63±464.93</td>
<td></td>
</tr>
<tr>
<td>TB</td>
<td>3</td>
<td>18.8</td>
<td>1417.6±1578.26</td>
<td></td>
</tr>
<tr>
<td>HF</td>
<td>3</td>
<td>18.8</td>
<td>1178.21±1693.25</td>
<td></td>
</tr>
</tbody>
</table>

The cut-off point of lymphocytes count were 1100 cells/mm³, with subject less than equal to 1100 were less dominant (33.3%). The test result showed also no significant difference (P=0.418).

The immunological state was evaluated using CD4 counts. CD4 less than 500 subject group was 63.6% and more than equal to 500 subject group was 36.4% with a significant difference in the mean levels of Human 1,3-β-D-Glucan in CD4 group (P=0.009).

There were several comorbidities found from subjects such as chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM),
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tuberculosis (TB), heart failure (HF), and other cancer that were not normally distributed from the Shapiro-Wilk test. For the comorbidities a non-parametric test was performed, which conclude no significant difference in BDG in patients with different comorbidities ($P=0.970$). The Shapiro-Wilk test was performed in the data of patients with comorbidities, and the data on patients with comorbidities were not normally distributed, so the Kruskal Wallis test was performed. In the test, the $P=0.970$, it can be concluded that there is no significant difference. It can be concluded that age, gender, lymphocyte count, and comorbidities do not affect level of BDG. In the CD4 count, it was found that patients with low CD4 cells had significantly higher BDG levels than those with high CD4 cells.

Figure 1. Comparison of BAL BDG Levels in Different Immunity Status.

The immunocompromised study subjects had an average BAL BDG level of $1099.07\pm887.30$ pg/ml, while the immunocompetent study subjects were $428.51\pm337.62$ pg/ml as shown in Figure 1. There was a significant difference between the mean BAL BDG in the group with different immunity status ($P=0.009$).

In the group of immunocompromised subjects, there were 19 subjects aged $>45$ years, with an average BDG of $1067.61\pm885.24$ pg/ml. Meanwhile, two subjects aged 45 with an average BDG level of $1397.85\pm1201.25$ pg/ml, as shown in Figure 2B. There was no significant difference in BDG levels between different age groups ($P=0.632$).

Figure 2. A) Comparison of BDG levels in male and female gender in the Immunocompromised group; B) Comparison of BDG levels at the age of ≤45 years and >45 years in the Immunocompromised group.

In immunocompetent subjects aged ≤45 years old, the mean BDG was $323.46\pm281$ pg/ml, while in subjects >45 years old, the average was $481.03\pm381.54$ pg/ml as shown in Figure 3A. There was no significant difference in BDG in different age groups ($P=0.472$).

Figure 3. A. Comparison of BDG levels in male and female genders in the Immunocompetent group; B. Comparison of BDG levels at age >45 years and ≥45 years in the Immunocompetent group.
The immunocompromised group with the male gender had an average BDG level of 1151.64±910.76 pg/ml. In the female gender, the average BDG level was 599.62±513 pg/ml (figure 2A). There was no significant difference in BDG for men and women (P=0.338). The immunocompetent group was also divided by sex. There were 10 men with an average BDG of 434.60±355.20 pg/ml and 2 women with an average BDG value of 398±340.825 pg/ml (figure 3B). There was no significant difference between the BDG of the male and female groups (P=0.667).

DISCUSSION

In the characteristics of the research subjects, there was no significant difference in the age group, gender, lymphocyte count, and comorbidities on the average level of BAL Human 1,3-β-D-Glucan (P>0.05) between suspected lung cancer patients. This is in accordance with the literature, which states that Candida infection can be found in patients treated with lung cancer.13

Comorbidities found in this study included COPD, Diabetes Mellitus, tuberculosis, other cancers, and HF. These comorbidities may contribute to an increased risk of nosocomial invasive fungal infection in patients with suspected lung cancer. However, the comorbid data in this study yielded no statistically significant differences in BDG levels. Various literature states that pulmonary mycoses can occur in patients who previously had chronic lung disease as the underlying disease, such as COPD, lung and thoracic malignancies, pulmonary TB, and chronic systemic diseases such as Diabetes Mellitus.1,2,14

The mean lymphocyte count ≤1100 cells/mm3 in this study showed an average value of BDG levels higher than the mean lymphocyte count >1100 cells/mm3. This may also influence the risk of invasive fungal infections. Decreased lymphocyte count due to the apoptosis process causes lymphopenia and increases the risk of nosocomial infections such as candida yeast infection in lung cancer patients.15 Although in this study, the lymphocyte count gave no statistically significant difference to the average BDG level.

This study showed a significant difference between the mean BAL BDG in groups with different immune statuses. So that the immune status is an important risk factor for BAL BDG levels. Previous studies found that the BALF BDG level may be an important predictor of mortality risk.16 For every 100 pg/ml increase in BALF BDG, the overall 90-day risk of death increased by 5%. Thus, a patient with a BALF BDG level of 1100 pg/ml may have a 50% higher risk of dying within 90 days after bronchoscopy when compared to a patient with a BALF BDG level of 100 pg/ml. They also found that BDG levels were significantly higher among those with candida growth in BALF culture and candida spp. growth. Indicates that the host’s immune system suppression may be associated with a higher probability of mortality risk.16,17

There was no significant difference in BAL BDG levels in patients suspected of lung cancer in the two different immune statuses based on the age groups >45 years and ≤45 years (P=0.632; P=0.472). However, age is a major risk factor for many cancers. Various genetic and food manipulations that slow down aging, such as studies conducted on mice, can reduce cancer incidence, and conversely, the susceptibility of host tissues to cancer increases with age. Exposure to risk factors early in life, such as infectious disease comorbidity factors, may have a role in the early life peak in cancer incidence.18 So that the above can also affect the occurrence of candidiasis in cancer patients at a younger age, and examine Human 1,3-D-Glucan meaningless by age group

In this study, showed there was no significant difference in BDG levels between male and female subjects with immunocompromised and immunocompetent status, although the mean and median were higher in the group of male subjects (P=0.338; P=0.667). The study of Lortholary et al., 2017 reported an equal chance of infection with Candida Albicans by gender in patients with malignancy.19 Differences in gender in severity, prevalence, and pathogenesis of infections caused by viruses, bacteria, parasites, and fungi, with men...
Men are generally more susceptible to these infections than women. These differences for acquired infectious diseases, observed through various routes such as individual, vector-borne, blood, food, and water, with gender differences in immunity, play a major role. Still, they may not be significant as they involve many factors. This could explain why candidiasis infection examined with Human 1,3-β-D-Glucan in immunocompromised patients with suspected lung cancer by gender could not be significant.

Patients with suspected immunocompetent lung cancer by age group and gender have in common the mean value of BAL Human 1,3-β-D-Glucan levels, which are lower than patients with suspected immunocompromised lung cancer. These results are consistent with the literature, which states that invasive fungal infections occur more frequently in the immunocompromised than in the immunocompetent. In a prognostic study, Jaijakul et al., 2012 reported that the negative slope of Human 1,3-β-D-Glucan levels had a good response to antifungal therapy with PPV=90% and with a positive slope after treatment failure (NPV=90%).

**CONCLUSION**

This study showed a significant difference in the levels of Human 1,3-β-D-Glucan from BAL fluid in the immunocompromised and immunocompetent groups. Although there was no significant difference in BAL Human 1,3-β-D-Glucan levels based on age and gender in different immune statuses, BDG levels based on age and gender in immunocompromised status had a higher positive value than immunocompetent. BDG assay as one of the diagnostic supports must be interpreted with clinical data by experienced doctors.

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https://www.klikparu.com/2013/03/bronchoalveolar-lavage-bal.html


